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## STARCH AND ITS DERIVATIVES

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# STARCH

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*By*

J. A. RADLEY

M.Sc., F.I.C.

BEING VOLUME ELEVEN OF A SERIES OF  
MONOGRAPHS ON APPLIED CHEMISTRY

Under the Editorship of  
E. HOWARD TRIPP, Ph.D.

*SECOND EDITION*  
*(REVISED)*



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## EDITORIAL PREFACE

IN these days of intensive and extensive research, every worker in science or its applications knows how rapidly the contents of text-books and encyclopædias become out of date; and those who wish to see new work published know the difficulties which abnormal taxation and high labour costs offer to the realisation of their desire. The one obvious solution of the problem is the publication of monographs that would focus attention upon recent work, or upon new aspects of old work, and upon their theoretical implications. Such books are usually written by experts for other experts in related fields of science, or for the well-educated layman whose thirst for new knowledge has not been quenched by the more sensuous outpourings of the ephemeral press.

It is interesting at times to speculate upon what aspects of our civilisation the future historian will select as the most characteristic of our time. Scientific discoveries and their application to human welfare, we may be sure, will find a place; and to these many will add the growth of our sense of 'values'. The value of new work in science varies greatly: the golden grain is always accompanied by chaff, and there is no precious ore without country rock. Owing to the difficulty of assessing the value of work at the time of its production, we find that our scientific periodicals stand in danger of being swamped by the mass of second- and third-rate material that is thought to be worth publishing, but which posterity will decree would have been better left in manuscript form. It is the first duty of the monograph writer to estimate the value, either actual or potential, of recent work upon the subject of which he writes: he must pick out the plums to save others from the indigestion that follows eating the whole pie. Further, in addition to being accurate, his work must be presented in a form that is both assimilable and attractive; in other words, he must show that lucid exposition can be achieved by the use of few words, if



they are rightly chosen, and that attractive presentation is attained rather by clear thinking than by superficial display.

The present series of monographs has been designed with these objects and ideals in view. The task which the authors have been set is no easy one; so that should performance occasionally fall short of intention, the critical reader is asked to echo the words of Goethe that 'higher aims, even if unfulfilled, are in themselves more valuable than lower aims quite attained'.

E. HOWARD TRIPP.

## AUTHOR'S PREFACE

IT is hoped that this book will recommend itself to many scientific and technical workers without any lengthy apologia from the author. Like all the books that have appeared in this Series, this one seeks to summarise recent progress in an important domain of chemical industry; for starch, if not the food of the gods, is a fundamental necessity to all human beings. Though much of the text is descriptive, critical commentary has not been forgotten. The author hopes that his own experience of the industry has helped him to preserve a balance between the purely scientific and the technological, and to give just weight to diverse views on controversial points. As the manufacture of the various commercial kinds of starch has already been described at length in several useful books, this part of the subject has been treated in a broad, general way; *per contra*, the manufacture of dextrin, about which little has appeared in print, has been treated in detail. Notwithstanding the very great amount of work already done on starch and its derivatives (as the numerous references to the literature testify), the careful reader will not fail to discern many a gap in our knowledge; and it is hoped that he, whether he be a purely academic or a technical worker, will find in these pages some food for thought and some material upon which to base further investigations.

I have pleasure in acknowledging the help I have received from firms and individuals in compiling this volume. My thanks are due to Messrs. Imperial Chemical Industries, Ltd., for the generous permission to use their photomicrographical apparatus, and to Mr. E. Young for placing at my disposal his great micrographic skill; to Drs. S. H. Oakshott, R. J. W. Reynolds, Messrs. H. Blackshaw, J. Faulds, P. T. Gale, N. Strafford, and J. A. Kierman for their valuable criticisms; to the editors of various publications and to firms who have lent

blocks for diagrams and apparatus; to Prof. D. H. Cook for providing samples of tropical starches, and to other workers who have sent samples of starches or reprints of their publications. Finally, but by no means least, I thank the Editor of the Series and the Publishers for their valuable work in converting the original manuscript into the finished book.

J. A. RADLEY.

## AUTHOR'S PREFACE TO SECOND EDITION

THE need to commence an edition of this book only some eight months after the appearance on the market of the first edition is proof of the sustained interest in the subject of starch in all its various aspects. It is all the more interesting in view of the limitations of circulation due to the present state of the European and Asiatic starch-producing countries.

In this present edition of thirty-four chapters and an Appendix much new material has been included, comprising some eleven entirely new or completely re-written chapters and about 1,200 new references. The chapter on Physical Chemistry has been subdivided into some five shorter chapters as, with the addition of the new material, it would have been decidedly unwieldy. The new arrangement, it is hoped, will allow of easier reference. Many new photographs and diagrams have been added better to illustrate both the previous matter and the new material.

Many new papers on starch and its uses are constantly coming forward and many of the older observations have received fresh significance and explanation. A certain amount of the old work has been discarded although in many cases the references to it remain for those who wish to follow the subject through every phase of its development. The uncertain or non-arrival of papers from Germany and the occupied countries, and the pressure of war circumstances at every stage have all contributed to the difficulties of production of this edition, but it is hoped that it includes all the more important work published to the end of 1941.

The seven entirely new chapters on 'The Role of Starch in the Plant,' 'The Food Industry' and those on Amylase Action will, it is hoped, be of value and interest to workers in botany and biology, and those connected with baking, food, brewing and fermentation industries. It is an extremely difficult matter for one worker to deal with a number of different industries in one volume and avoid, equally, over-emphasis or under-statement

of the importance of various points, but it is hoped that the various sections are reasonably well-balanced.

I count myself particularly fortunate in having the chapter on 'The Structure of Starch' entirely re-written by Professor E. L. Hirst and Dr. G. T. Young, to whose researches the progress in this field owes so much. My best thanks are also due to Mr. G. V. Caesar of Stein Hall & Co., Inc., N.Y., for the chapter on 'The Hydrogen Bond in Starch,' to Professor F. F. Farley, who is head of the Department of Chemistry, University of Detroit, U.S.A., for kindly contributing the chapter on 'The Oxidation of Starch,' and to Mr. C. Paine for writing the Appendix dealing with the subject of patent references. My thanks are also due to Messrs. J. M. Faulds and L. Haddock for their assistance in proof-reading, to various workers who have kindly sent me reprints of their papers on Starch and many interesting letters, to the various publications who have allowed me to reproduce photographs and diagrams and, finally, to the Editor and Publishers for their valuable help in preparing the present edition.

J. A. RADLEY.

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## PART I

## THE STRUCTURE AND REACTIONS OF STARCH

## CHAPTER I

## HISTORICAL

THE ease with which starch may be prepared from tubers and grains of various plants would lead one to expect that the ancients were familiar with the substance, but it is impossible to decide with any degree of certainty when the substance now known as starch was recognised as a distinct entity.

The use of starch for the manufacture of paper and papyrus appears to have been known to the Egyptians, and traces of a starch adhesive have been found on documents dating about 3500 B.C. Documents dating 130 B.C. are mentioned by Pliny, who described the method in which the sizing of papyrus was carried out with a paste made from fine white flour and boiled with a weak solution of vinegar. Early specimens of paper bear clear indications of having been sized and weighted with a crude starch. J. Wiesner mentions a Chinese document clearly dated A.D. 312 which had undoubtedly been sized with starch. This worker<sup>1-5, 19</sup> has examined about 500 Oriental and European papers dating from the eighth century to the end of the nineteenth, and found that starch paste was the earliest size employed. Papers dating from the eighth and ninth centuries A.D. were all sized with starch, its use being discontinued, however, about the thirteenth or fourteenth century A.D.<sup>2-3</sup> In his examination of East Turkish and Tibetan manuscripts, all of which were made in the eighth century A.D., he found that the paper appeared to have been sized first with a fine paste to render it non-absorbent, and then a filling of unmodified starch applied to give weight and thickness. This procedure appears to have been adapted from the Chinese method.<sup>4</sup>

Rice and wheat starch were suspected to be present in heavily-pasted Arabian papers made towards the end of the eighth century. Samples of Arabian, Chinese, Indian, Persian and Turkish papers, 84 in all, showed that no starch had been used in those dated prior to the eighth century.<sup>5</sup> Oriental papers manufactured during the period A.D. 700 to 1300 were all heavily coated with starch paste. After the fourteenth century, the practice of sizing



papers with starch was abandoned until comparatively recent times.

The use of starch paste for sizing textiles does not seem to have been used by the ancients, although Pliny mentions its use for whitening cloth.

The earliest description of the preparation of starch appears to be that given by Cato (234-149 B.C.). The source of this starch was a cereal, which was steeped in water for ten days, the water poured off, and the starch stirred with clean water. The deposit was wrapped in a linen cloth, and after the creamy liquid had been pressed out into a vessel, it was levigated once more. The moisture was evaporated by solar heat. He adds that 'we employ starch to clean linen.' The preparation of starch was also described by Pliny,<sup>6</sup> according to whom the highest grade came from the Island of Chios, and inferior products from Crete and Egypt.

There appears little agreement among various writers as to the date on which starch was introduced into Northern Europe as a stiffening agent for linen. Its use for this purpose appears to have been known in the fourteenth century, and English documents of 1390 have also been found which contain references to it.

The introduction of starch into England to any appreciable extent appears to have occurred between 1561 and 1564, various coloured starches being imported during the last-mentioned year, which also marked the arrival from Flanders of Mistress Dingham van der Plassen as professor of laundry work. Later another Dutchwoman, Mistress Guilham, became superintendent of Queen Elizabeth's laundries.

Red, yellow and purple starches were used as cosmetics, as well as the uncoloured starch for powdering the hair, whilst a blue starch was employed by the Puritans, but the use of the latter was banned by the Queen in 1596. Yellow starch was for some time most fashionable; its use, however, fell into disrepute when Mrs. Turner, the poisoner, was publicly executed, and ascended the scaffold wearing a yellow-starched ruffle. The monopoly in starch-making was revoked in 1601 by the Queen. In 1607 James I reimposed a modified monopoly, but since good wheat was being used in contravention to the terms of the monopoly starch-making was again forbidden in 1610. The year 1622, however, saw the revival of the trade.

Samuel Newton and others took out a patent in 1707 for the manufacture of starch, and in 1841 Coleman patented a process for manufacturing rice starch by fermentation, which was followed by one granted to Brown & Polson in 1854.

In 1719 Leeuwenhoek<sup>7</sup> studied starch granules microscopically, and made accurate observations on the swelling phenomena shown by starch when heated in water. He suggested that the swelling starch grains contained an insoluble substance which escaped from the fissures he observed in the outer insoluble sac. Pomet,<sup>8</sup> 1725, considered that Parisian starch was the best grade then available, and mentions the poor colour of products dried by artificial heat.

The Universal Lexikon<sup>9</sup> described the manufacture of the starch then in use, and recommended the use of the residual gluten for feeding to domestic fowls. The 1744 edition gives an account of the use of starch for strengthening the warp threads in textile-sizing, and in 1780 the Académie des Sciences awarded a prize to Roux for his suggestion to use starch for dusting foundry moulds.

Adhesives with pure starch were described by Duhamel du Monceau<sup>10</sup> in 1771; these, he claimed, had greater strength than those prepared from flour. In the next year this worker<sup>11</sup> described the manufacture of wheat starch and Parmentier's method for manufacturing horse-chestnut and potato starches. The analysis of this starch was published in 1773. It is of interest to note that in 1800 Vauquelin,<sup>12</sup> when analysing the wash-water from factories working this method, detected the presence of alcohol, ammonia and acetic acid, but considered it uneconomic to extract them.

The next discovery of importance was by Kirchhoff<sup>13</sup> in 1811, who presented before the Académie des Sciences three flasks containing syrup obtained from vegetables like potatoes and wheat, sugar obtained from this syrup by desiccation, and sugar extracted from the syrup by water—the first time starch-sugar had been prepared.

Then, 20 years later,<sup>14</sup> according to well-authenticated reports, a fire broke out in a textile mill in Dublin. One of the workmen noticed that the starch that had been turned brown by the heat of the fire had altered greatly in physical characteristics, becoming readily soluble in water to give a thick adhesive paste. The roasting of starch was repeated, and the basis of the large industry of the manufacture of British gum was thus laid. As early as 1804, however, Roard<sup>15</sup> had mentioned that Bouillon-Lagrange had obtained gum substitutes from starch by a torrification process. The method of preparation was published by the latter worker in 1811.<sup>16</sup> In the same year Vauquelin<sup>17</sup> noted that starch may be transformed into a soluble gummy substitute by heating. These observations, however, do not appear to have been followed up as quickly as were those made after the Dublin fire.

Dextrin appears to have been made commercially by the acid process in Germany about 1860, but the first clear mention in patent literature concerns the patent granted to V. G. Bloede in America in 1867, in which starch was roasted after moistening with acid.

Probably the greatest use of starch prior to 1830 was as a hair powder, although it was used to a very limited extent in the textile and paper trades, also as a special food preparation, and for stiffening the ruffles of the gentry of the Renaissance and Cavalier periods.

## REFERENCES

1. J. WIESNER, *Kaiserl. Akad. Wissensch., Vienna*, 1911, 26.
2. ——— *Mittheilungen aus der Sammlung der Papyrus Erzherzog Rainer, Vienna*, 1887, **1**, 45.
3. ——— *ibid.*, 1887, **2-3**, 179.
4. ——— *Kaiserl. Akad. Wissensch., Vienna*, 1904, **148**, 26.
5. ——— *ibid.*, 1902, **72**, 583.
6. PLINIUS SECUNDUS, GAIUS, *Naturalis Historia*.
7. A. VAN LEEUWENHOEK, 'Epistolae Physiologicae super compluribus Naturae Arcanus,' *Epistolae* 26, 1719.
8. POMET, 'History of Drugs,' London, 1725, p. 11.
9. ANON, 'Universal Lexikon,' Vol. 1, 1733; Vol. 15, 1737; Vol. 39, 1744.
10. DUHAMEL DU MONCEAU, 'Description des Arts et Metiers,' 1771, Vol. 23.
11. ——— *ibid.*, 1772, Vol. 41.
12. VAUQUELIN, *Ann. Chim. Phys. Paris*, 1800, **38**, 248.
13. G. S. C. KIRCHHOFF, *Mémoires Acad. Imp. Sci. St. Petersbourg*, 1811, **4**, 27.
14. ANON, *Pulp and Paper Mag.*, Gardenvale, 1922, **20**, 879.
15. ROARD, *Ann. Chim. Phys. Paris*, 1804, **50**, 220.
16. BOUILLON-LAGRANGE, *Bull. Pharm. Paris*, 1811, **3**, 395.
17. VAUQUELIN, *ibid.*, 1811, **3**, 49.
18. STUBBS, 'Anatomie of Abuses,' 1583.
19. J. WIESNER, *Papierfabr.*, 1911, 886.

## CHAPTER 2

## 'THE ROLE OF STARCH IN THE PLANT'

A PLANT is made up of a more or less complex system of cells, the simpler fungi being unicellular plants. The cells vary in shape, size, distribution and function and consist of protein matter enclosed in and attached to a cellulosic membrane. As the cell grows cavities appear in the protein matter and fill with the plant sap, which is an aqueous solution of salts, sugars, etc. In the protein matter are colourless bodies known as leucoplasts, the chloroplasts, which contain chlorophyll, the green colouring matter of the plant and, finally, the chromoplasts, which contain colouring matter other than chlorophyll.

By irradiation with light the leucoplasts change to chloroplasts and in the dark the opposite effect is observed. The production of starch, both as a reserve material and in the transient state for current use, is somehow connected with the action of these bodies and the chlorophyll in the presence of air, light and sap.

Green plants in daylight breathe in carbon dioxide, give out oxygen, whilst starch makes its appearance in the chloroplasts in an amount depending on the intensity of the light. In the dark these processes are reversed, oxygen is taken in, carbon dioxide given out, starch disappears and the chloroplasts are converted to leucoplasts. The starch formed during the day is solubilised in the plant at night and transported to other parts of the plant as required by its metabolism. The temperature also has an influence on the amount of starch existing in either form, and again the periodic fluctuations in the amount and the form of starch present is largely dependent on the species of plant.

The plant sap contains mono-, di- and trisaccharides, and A. von Baeyer<sup>1</sup> suggested that the carbon dioxide taken in by the plant was converted to formaldehyde, with liberation of oxygen, and that successive polymerisations of formaldehyde to sugar and of sugar to starch then took place. It is interesting that only those wave-lengths of light absorbed by the chlorophyll are effective in producing starch. The starch appears in the form of granules and we cannot yet adequately account for its appearance in such a form. Both the leucoplasts and the chloroplasts produce starch, but the former must use a different mechanism from the latter. The leucoplasts work in the absence

of light and carbon dioxide and are responsible for the reserve supply of starch in the tubers, seeds and rhizomes, etc., of the plant. Some workers consider that the intermediate products between carbon dioxide and starch, i.e. the sugars, etc., are made and assimilated as required and that the starch is only elaborated in the cell when excessive nutriment is available.

There is no doubt that the so-called 'starch  $\rightleftharpoons$  sugar' balance of plant tissues is greatly influenced by the obscure mechanisms taking place in the plastids, possibly induced by the presence of enzymes, which Hanes has recently separated from peas and various tubers (see below).

S. Ruben, W. Z. Hassid and M. D. Kamen<sup>2</sup> have used radioactive carbon ( $C^{14}$ ) to follow the assimilation of carbon dioxide by the plant. By this method the carbon atoms are 'labelled' and their course throughout any reaction can be followed accurately. In experiments on barley plants exposed to 'labelled' carbon dioxide under conditions of light and darkness radioactive carbohydrates and chlorophyll (as phytochlorin and phytorhodin) were formed in the living leaf in both the presence and absence of light. When leaves are placed for some hours in the dark before exposure to the 'labelled' carbon dioxide, however, the formation in the absence of light of radioactive carbohydrates could not be detected. The chlorophyll contained radioactive carbon after exposure to the gas in light but not after exposure to it in the dark. The bulk of the radioactive matter was found to be water-soluble but is not carbohydrate, carbonate, keto-acids or pigments.

In a further paper these workers<sup>3</sup> find that, in the green algae *Chlorella pyrenoidosa*, a considerable fraction of the radioactive substance formed is also non-carbohydrate. These results seem to indicate that the cell mechanism is such that the active carbon dioxide reacts reversibly with certain substances present, in a non-photochemical process. With *Chlorella* the radioactive carbon appears to be in a carboxyl group. A considerable number of naturally occurring acids, e.g. formic, acetic, propionic, tartaric, malic, citric, ascorbic, etc., have been found to be inactive as well as water-soluble proteins and aldehydes, ketones, carbohydrates, hydrolysable polysaccharides, etc., containing a free carbonyl group. The molecular weight of the 'labelled' substance is large.

These workers, with others,<sup>4-6</sup> consider that the absorbed carbon dioxide does not combine with the chlorophyll but carboxylates a substance already present and this is followed by reduction to a hydroxyl body. This hypothesis avoids the necessity for

postulating formaldehyde formation and subsequent polymerisation, and the energy required by the former is very much less than that required for the latter reaction. These workers consider that the hydroxyl body may, in turn, be carboxylated and repeat the cycle to form a long carbohydrate chain. Processes in which carbohydrates are formed by interaction of accumulated reduction products, possibly by intervention of enzyme action, are not excluded. It would appear that this method of using 'labelled' carbon atoms has a wide scope in the study of plant metabolism.

A great many observations by numerous workers have been made on the effects of different factors such as light, water-content, presence of inorganic salts, temperature, sugar concentration, etc., on the starch-sugar balance, and one important point which emerges from all this work is that sucrose, fructose and glucose are the main substances produced when starch is broken down in the plant. Maltose and dextrans are rarely found and then only in minute quantities, and yet they are the main products formed when amylases act on starch outside the plant.

The appearance in the plant of sucrose and fructose raises an important problem as to the nature of the mode of action of the system which can facilitate the conversion of glucopyranose units of the polysaccharide into fructose.

The work of F. Laquer and P. Meyer <sup>7</sup> on the glycolytic system of muscle gives some suggestion as to the type of mechanism that may be involved. The glycolytic muscle system is able to produce lactic acid more readily from starch and glycogen than from glucose and fructose, whilst some glycolytic muscle systems are unable to act even on the sugars. J. K. Parnas and T. Baranowski <sup>8</sup> find that an intermediary during the conversion of glycogen to lactic acid is hexose diphosphate. Thus a probable mode of breakdown of the polysaccharide is that individual glucopyranose units are detached, accompanied by, or perhaps due to, the introduction of phosphoric acid residues and that an esterified fructofuranose molecule is liberated. This is an example of a molecular chain of  $\alpha$ -glucopyranose units liberating a fructofuranose derivative on degradation.

The investigation of several hexose phosphates isolated from yeast and muscle have contributed much towards the chemistry of the muscle and the complex carbohydrate metabolism of the animal body. Harden and Young <sup>9,10</sup> have shown that in the presence of phosphates the fermentation by yeast juice is accelerated and hexose diphosphoric acid is formed. Recent work

indicates that hexose phosphates are formed in the higher plants and probably play an important part in the carbohydrate metabolism of the plant (see also J. Bodnár<sup>15</sup> and W. W. Jones<sup>16</sup>).

Extracts of germinating peas, beans, etc., acting upon hexose diphosphate, give products characteristic of sugar breakdown in yeast or muscle,<sup>11-13</sup> and the fact that phosphates stimulate the respiratory sugar metabolism in higher plants suggests that the formation of phosphoric esters may occur as part of this process.

Bodnár<sup>14</sup> was first to demonstrate that inorganic phosphorus disappeared when added to ground peas and phosphorylation had probably occurred. Barrenscheen and Albers<sup>17</sup> found the acid-soluble phosphorus increased in irradiated *Elodea canadensis* and during the germination of rye. Later, Barrenscheen and Pany<sup>18</sup> showed that hexose phosphate occurred in *elodea* after irradiation in a dilute sugar solution containing inorganic phosphorus. Tánkó<sup>19</sup> isolated a mixture of hexose diphosphate and hexose monophosphate from a mixture containing inorganic phosphorus and a pea preparation, whilst J. Burkard and C. Neuberg<sup>20</sup> have shown that both glucose and fructose monophosphates occur in beet leaves. W. Z. Hassid<sup>21</sup> has also isolated a hexose phosphate from pea leaves which is apparently a mixture of glucose- and fructose-phosphates.

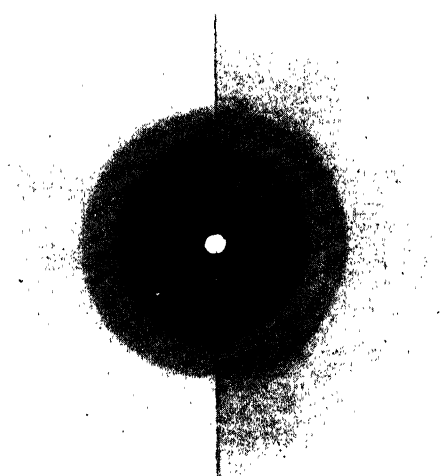
It is of interest to note that the 1 : 6 : diphosphoric ester of fructofuranose can, by the action of various phosphatases, give rise to an equilibrium mixture of 6-mono-phosphoric esters of glucose and fructofuranose.

C. S. Hanes<sup>22</sup> has carried this interesting subject still further. This worker extracted an enzyme system from the seeds of a wrinkled culinary variety of pea, which catalysed the formation of hexose phosphates from starch, dextrin and maltose. Glucose-1-phosphate is formed, apparently by the direct phosphorylytic cleavage of a terminal glucose unit from the non-aldehydic end of the chain-molecule of starch (see p. 16). Starch and iodine staining dextrans participate directly in the reaction as is shown by the decrease in iodine colour as the phosphorylytic action proceeds, and this decrease is of the type one would expect to occur during endwise degradation of the chain-molecules.

This degradation with the formation of glucose-1-phosphate is a reversible process catalysed in both directions by the enzyme 'phosphorylase.' An alternative reaction, catalysed by the phosphoglucose conversion system, which contains two or more enzymes, is the formation of reducing hexose monophosphate from glucose-1-phosphate, while with non-dialysed preparations fructose diphosphate is formed.







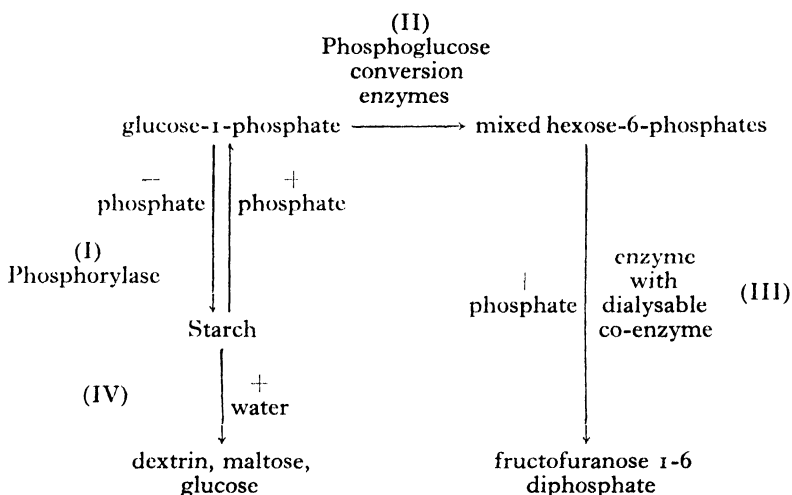
*[Photograph by W. T. Astbury, reproduced by courtesy of 'Nature'.*

Potato starch.                      Hanes' synthetic starch.  
X-ray photograph.

*[Facing p. 9.*

FIG. 1.

Hanes<sup>23</sup> next prepared phosphorylase by fractional precipitation of extracts from pea-meal using ammonium sulphate and obtained it free from enzymes which catalysed the alternative reaction, Nos. II and IV in the diagram. This product reversibly catalysed the conversion of glucose-1-phosphate to starch and free mineral phosphate. A particularly suitable source of the enzyme was later found to be crude potato tuber juice. The schematic diagram below (Hanes) makes clear the alternative reactions which can take place.



A state of equilibrium is reached in the reaction starch + phosphate  $\rightleftharpoons$  glucose-1-phosphate when the ratio of free phosphate to glucose-1-phosphate attains a certain value which is dependent on the pH value of the reaction mixture. More starch is formed at pH 5.4 than at pH 6.4, and it appears in the form of rounded granules with a maximum diameter of 6-8  $\mu$ , surrounded by loose floccules of protein which make it difficult to purify. The 'synthetic' starch appears to have all the properties of natural starch but appears to be more resistant to solution and to retrograde more rapidly than the common starches. The photograph opposite shows that the X-ray diagram of the synthetic starch is of the B-type, similar to that given by potato starch shown in the left half of the photograph.

In the above reaction the *effective* concentration of starch does not appear to be proportional to its *total* concentration, as additions of even considerable amounts of starch do not measurably affect the equilibrium. In acting upon the glucose-1-phosphate

with phosphorylase there is a pronounced induction phase, and Hanes noted that the addition of even small amounts of starch, maltose or certain other substances eliminated this lag and had the striking effect of increasing the initial velocity of reaction by as much as fifteen-fold in the case of starch additions.

Using the preparation containing the coenzyme (which is removable by dialysis) hexose-6-phosphates and free phosphates give fructofuranose 1:6:diphosphate. It is too early yet to attempt any detailed evaluation of the part played by the above system in the metabolism of the plant, but it will undoubtedly throw light on the known peculiarities of the so-called 'starch-sugar' balance in plants. Further work on phosphorylases from different sources is dealt with on page 35.

## REFERENCES

1. A. VON BAEYER, *Ber.*, 1870, **3**, 68.
2. S. RUBEN, W. Z. HASSID and M. D. KAMEN, *J. Amer. Chem. Soc.*, 1939, **61**, 661.
3. — and D. C. DE VAULT, *Science*, 1939, **90**, 570.
4. R. EMERSON, *Ergeb. Enzymforsch.*, 1936, **5**, 305.
5. H. GAFFRON and K. WOHL, *Naturwissenschaften*, 1936, **24**, 81, 103.
6. K. V. THIMANN, *Science*, 1938, **88**, 50c.
7. F. LAQUER and P. MEYER, *Hoppe-Seyl. Z. physiol. Chem.*, 1923, **124**, 211.
8. J. K. PARNAS and T. BARANOWSKI, *Compt. rend. soc. biol.*, 1935, **120**, 307.
9. A. HARDEN and W. J. YOUNG, *Proc. Roy. Soc. London*, 1908, **380**, 299.
10. — *Biochem. Zeit.*, 1911, **32**, 173.
11. T. BABA, *ibid.*, 1935, **275**, 248.
12. C. NEUBERG and M. KOBEL, *ibid.*, 1930, **229**, 433.
13. — *ibid.*, 1934, **272**, 445.
14. J. BODNÁR, *ibid.*, 1916, **73**, 193.
15. — *ibid.*, 1925, **165**, 1.
16. W. W. JONES, *Plant Physiol.*, 1936, **11**, 565.
17. H. K. BARRENSCHEEN and W. ALBERS, *Biochem. Zeit.*, 1928, **197**, 261.
18. — J. PANY, *ibid.*, 1930, **219**, 364.
19. B. TÁNKÓ, *Biochem. J.*, 1936, **30**, 692.
20. J. BURKARD and C. NEUBERG, *Biochem. Zeit.*, 1934, **270**, 229.
21. W. Z. HASSID, *Plant Physiol.*, 1938, **13**, 641.
22. C. S. HANES, *Proc. Roy. Soc. London*, 1940, **B128**, 421.
23. — *Nature*, 1940, **145**, 348.

## ADDITIONAL REFERENCE

- P. OSTERN, J. A. GUTHKE and B. UMSCHWEIF, *Enzymologia*, 1937, **3**, 5.  
(Enzymatic phosphorylation of starch.)

## CHAPTER 3

## THE STRUCTURE OF STARCH FROM CHEMICAL EVIDENCE

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THE lack of definite knowledge concerning the structure of the starch molecule bears evidence to the difficulties which attend the investigation of polysaccharides in general, and of starch in particular. One factor responsible for this slow rate of progress was the misleading result of attempts to determine the molecular weight of polysaccharides and their derivatives by cryoscopic methods, which appeared to indicate that starch, and also cellulose, were built up by the association of elementary units of no more than two or three glucose residues.<sup>1</sup> The reason for this abnormal cryoscopic behaviour of colloidal polysaccharides is still not clear, but it indicates the caution which must be exercised even in the application of well-established methods to such complex systems. A second obstacle lay in the extreme readiness with which the starch molecule is decomposed, which rendered doubtful any conclusions drawn from the reactions of its decomposition products. As will be seen later, it was not until 1928 that this difficulty was overcome by the application of the process of methylation, which had previously proved valuable in the investigation of simple carbohydrates.

Since, in this chapter, the object is to present the evidence upon which modern views concerning the structure of the starch molecule have been based, it is proposed to deal with the chemistry of starch and its derivatives from the structural point of view only. Because of their commercial importance, much work has been carried out on a large number of starch derivatives, and the relevant literature has grown to such proportions that it would be quite impossible to give here even the briefest reference to the majority of the papers. Readers who are interested will find an account of various aspects of starch chemistry, together with several thousands of references and abstracts, in Walton's book, 'A Comprehensive Survey of Starch Chemistry' (New York, 1928), which reviews the subject up to 1925.

One of the most characteristic and important properties of

starch is its occurrence in granular form. The size and shape of the granules are typical of the source from which they have been obtained and are often used as an indication of the origin of a particular starch (see Chap. 1, Pt. IV). These granules are composed almost entirely of polysaccharide material which, on hydrolysis, yields glucose, but, in addition, there are small amounts of nitrogenous matter, phosphorus-containing substances and other inorganic residues. Something will be said later concerning these impurities (see Chap. 6, Pt. I), but it is now clear that they have no significance in connection with the general chemical structure of the starch molecule.

As regards the carbohydrate portion of the granules, there has been much controversy over the question of homogeneity. Whilst there is general agreement that the sole product of hydrolysis is glucose, from an early period many investigators distinguished between two components of the starch granule. A. Meyer<sup>2</sup> and Maquenne<sup>3</sup> found that one portion, 'amylopectin,' was resistant to the action of malt extract, whilst the other portion, which they named 'amylose,' was readily hydrolysed. Later workers reported corresponding differences in the physical properties of the two fractions. Differences in solubility were used to effect a separation, Gruzewska<sup>4</sup> using alkali, and Ling and Nanji<sup>5</sup> ice-cold water, to dissolve the amylose. Large variations in the relative amounts of the two constituents were observed, and the amylose appeared to assume, in the course of time, the insolubility and high viscosity characteristic of amylopectin. It has since been shown<sup>6</sup> that both amylose and amylopectin (prepared by Ling and Nanji's method) contain an identical repeating unit of glucose residues, and the difference most probably lies in the degree of aggregation. Ultra-centrifuge measurements<sup>7</sup> indicate average particle weights of 60,000 for amylose and 300,000 for amylopectin. More recently K. H. Meyer<sup>8</sup> and his collaborators have reported the separation of an 'amylose' fraction from maize and potato starch, corresponding to about 5 per cent. of the total weight in the case of potato starch, by extraction with various solvents (e.g. hot water and chloral hydrate). Chemical examination appears to indicate an unbranched chain structure of this amylose, in contrast with that of the residual starch which possesses branched chains (see below). The question of definition arises in that it is not clear whether the material termed 'amylose' by Meyer in this instance refers to the same substance as that examined by earlier workers. For instance the 'amylose' described by K. H. Meyer appears to resemble more closely the 'amylo-amylose' (prepared by

digestion of starch with water in an autoclave) of other workers. This material gives maltose quantitatively with  $\beta$ -amylase and is not identical with Ling and Nanji's 'amylose.'

Because of the confusion which tends to arise from use of a nomenclature which badly needs reform, it may be useful to anticipate results described below and summarise the present position as regards this problem. It is probable that starch granules contain at least two components. The major portion, which gives maltose (60 per cent.) and  $\alpha$ -amylopectin on treatment with  $\beta$ -amylase, consists of a branched chain structure built up of repeating units containing each some 24-30 glucose residues united by 1:4  $\alpha$ -*D*-glucosidic links. It is this portion which has hitherto been the subject of most of the structural investigations, and in order to save repetition, this material will be referred to in the following paragraphs as 'starch,' although it is recognised that natural starch contains to some small extent other and structurally different components. The latter are present in small amount, and because of this, and since most of the structural work has been carried out with derivatives (e.g. acetates and methyl ethers) which can be fractionated and purified, the structural conclusions reached are valid for the major component of starch, even though complete separation of the components was not possible initially.

The other component which has been isolated yields maltose quantitatively with  $\beta$ -amylase, and consists of long unbranched chains of length greater than 100 glucose residues, united by 1:4  $\alpha$ -glucosidic links. Furthermore, on the evidence at present available, it seems to be this latter component of native starch which has recently been synthesised by C. S. Hanes.<sup>54</sup> The position is rendered still more complex by virtue of the effects which traces of phosphoric acid and proteins have in profoundly modifying the colloidal properties of starch.

The presence of traces of nitrogen and phosphorus in certain starches has been referred to above and has been the subject of discussion for many years, more especially in connection with the amylose-amylopectin controversy (see Chap. 6, Pt. I). Typical values for the nitrogen and phosphorus-content of various starches are given in Table 1. It seems probable that the nitrogen is due to the presence of protein impurities—further evidence being thus provided of the extraordinary difficulty of obtaining pure and homogeneous specimens of this polysaccharide. The significance of the phosphorus is, however, more controversial. Following Fouard's observations,<sup>9</sup> Samec<sup>10</sup> carried out extensive investigations upon the colloidal properties of starches, in which

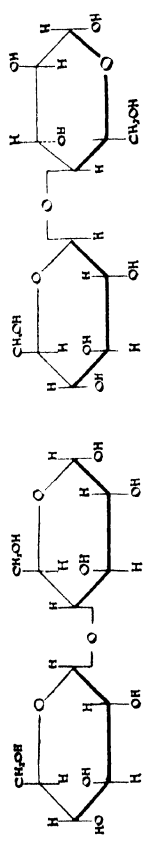
he related the phosphorus-content to the physical properties associated with amylopectin and separated phosphorus-free and phosphorus-rich components by electro-dialysis (see Chap. 6). Karrer and Kraus,<sup>11</sup> however, were unable to correlate the phosphorus-content with other characteristics of starch fractions. The situation appears to have been clarified in a paper by Posternak,<sup>12</sup> who isolated, by controlled hydrolysis of sago, arrowroot and potato starches, 6-glucose monophosphate. From corn and maize starch he could obtain only glycerophosphates, and he concludes that in rhizome starches the phosphorus is present as the 6-monophosphoric ester of one of the glucose residues, but in cereal starches (where the amount present is very much smaller) it is an impurity, possibly in the form of

TABLE I  
NITROGEN AND PHOSPHORUS CONTENTS OF TYPICAL STARCHES

<i>Starch.</i>	<i>Nitrogen. Per cent.</i>	<i>P<sub>2</sub>O<sub>5</sub>. Per cent.</i>
Potato . . .	—	0·2
Rice . . .	0·05	0·04
Maize . . .	—	0·03
Waxy maize . . .	—	0·01
Wheat . . .	0·09	0·10
Horse-chestnut . . .	0·06	0·05
Banana . . .	—	0·05

lecithin, since it may largely be removed by exhaustive extraction with solvents. The fundamental structure of starch appears, therefore, to be unaffected by the presence of phosphorus—a conclusion which is substantiated by the chemical similarity of derivatives of starches irrespective of the phosphorus-content.

Early workers were much impressed by the effect of enzymes upon starch, and many attempts were made to gain an insight into the chemical structure by an examination of the various dextrans obtained by the action of ferments. Although the original aim of obtaining structural evidence was only partially achieved, owing to the complexity of the reactions involved, these classical researches provided some of the earliest data of enzyme chemistry, and the products isolated are still providing difficult structural problems for the present-day organic chemist. Reference may be made in passing to the work of Musculus,<sup>13</sup> to the isolation of erythro-dextrin and achroo-dextrin by O'Sullivan,<sup>14</sup>



MALTOSE  
( $\alpha$ -LINKAGE)

CELLOBIOSE  
( $\beta$ -LINKAGE)

FIG. 2.

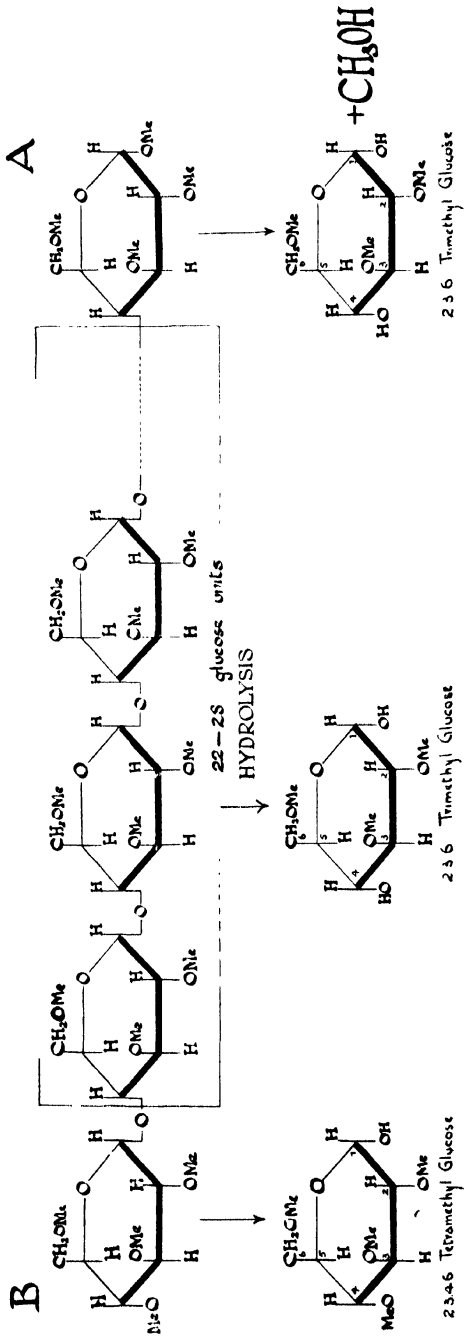


FIG. 3.—The hydrolysis of methylated starch.



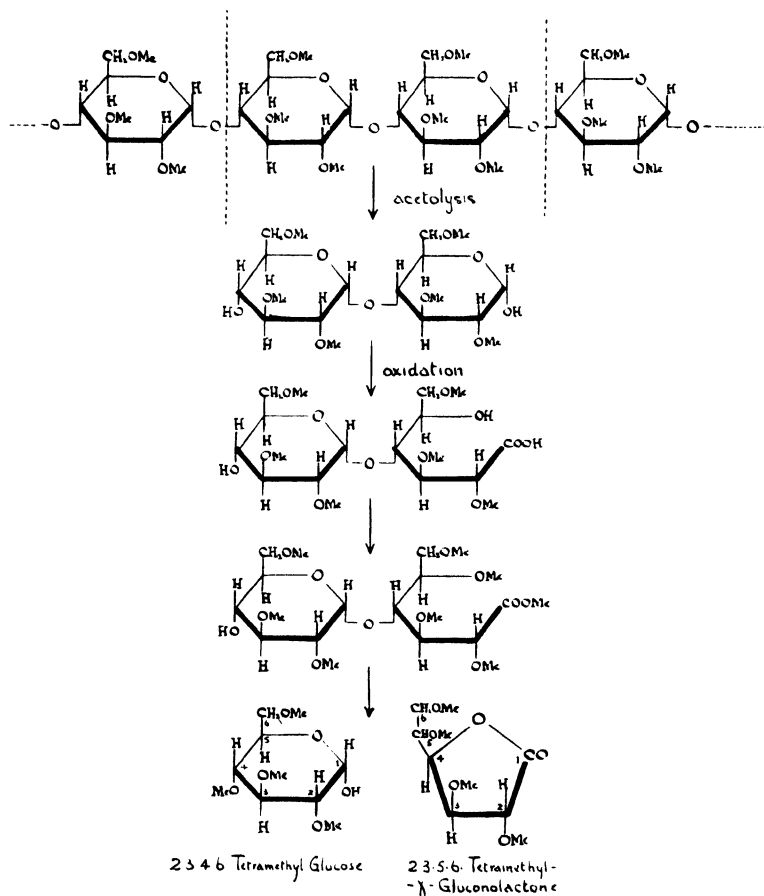


FIG. 3A.—The acetolysis of methylated starch.

[Facing p. 15.]

to the work of Brown and Heron<sup>15</sup> and Lintner and Düll<sup>16</sup> on diastatic hydrolysis, to the isolation of Schärdinger's dextrin<sup>17</sup> and, finally, to the recent work of Myrbäck on diastatic hydrolysis and the nature of the 'Limit Dextrin.'<sup>18</sup> The absence of definite knowledge concerning the mode of action of enzymes renders evidence concerning the structure of the starch molecule obtained from these experiments unreliable, and, as will be seen later, the present position is rather that the results of chemical investigations help to interpret the mechanism of enzymic reactions.

It has long been known that the main product of the hydrolysis of starch by acid is *d*-glucose, which may be recovered quantitatively as such, or as  $\alpha$ -methyl *d*-glucoside. The manner in which these glucose molecules are combined in starch was suggested by early experiments<sup>14</sup> in which maltose was isolated from starch by the action of the enzyme,  $\beta$ -amylase. Maltose was shown by Haworth and Peat to have the formula given in Fig. 2.<sup>19</sup> The glucopyranose units in maltose are linked by an  $\alpha$ -bond from the glucosidic (reducing) hydroxyl group on C<sub>1</sub> of one glucose residue to the hydroxyl group on C<sub>4</sub> of the second.

Further structural evidence was obtained from a study of the acetylation and methylation of starch by Haworth, Hirst and Webb.<sup>20</sup> By repeated treatments with dimethyl sulphate and alkali, all the hydroxyl groups are replaced by methoxyl, giving a methyl derivative which may be purified by fractional precipitation from solvents, and is characterised by optical rotation and viscosity measurements. Hydrolysis of methylated potato starch gave an almost quantitative yield of 2 : 3 : 6-trimethyl glucose (Fig. 3). The isolation of 2 : 3 : 5 : 6-tetramethyl glucono-lactone from the products of acetolysis of methylated starch<sup>21</sup> provided final proof that successive units are united by 1 : 4- $\alpha$ -linkages (Fig. 3A)—a fact which does not necessarily follow from the isolation of maltose from starch by the action of  $\beta$ -amylase. Since positions 1 and 4 are concerned in the junction of successive glucose residues, the free hydroxyl group on C<sub>5</sub> must therefore have been involved in ring formation of the glucose molecule, indicating that the glucose is present in starch as the stable 6-membered pyranose ring.

These and other facts indicate that starch contains chains of glucopyranose residues, united by 1 : 4- $\alpha$ -linkages, in distinction to cellulose, where the junctions are of the 1 : 4- $\beta$  type. It is, however, apparent that if the chain is finite in length, two end-glucose units should be distinguishable in the methyl derivative. The first, the 'reducing' end-group (A in Fig. 3),

on hydrolysis will yield 2 : 3 : 6-trimethyl glucose, since the methoxyl group on C<sub>1</sub> is glucosidic in character and removed by dilute acid. The second, the non-reducing end-group B, on hydrolysis will yield 2 : 3 : 4 : 6-tetramethyl glucose. Estimation of the proportion of this non-reducing end-group will give a measure of the length of the chain. Examination of the hydrolysis products of methylated potato starch by Hirst, Plant and Wilkinson<sup>6</sup> revealed the presence of about 4 per cent. of 2 : 3 : 4 : 6-tetramethyl glucopyranose, corresponding to a chain-length of some 25 glucose units. Since then, specimens of methylated starch prepared by various methods and from many plant sources have been examined in this way, and all have been found to contain one such 'end-group' in every 24-30 glucose

TABLE 2  
THE REPEATING UNIT OF STARCHES FROM VARIOUS SOURCES

<i>Source.</i>	<i>Chain-Length of Repeating Unit (No. of Glucose Residues).</i>	<i>Reference.</i>
Potato : . . .	24-30	6, 39
Waxy maize . . .	26-29	26
Maize . . . . .	24-30	25
Rice . . . . .	28-32	28
Wheat . . . . .	24-26	29
Horse-chestnut . . .	28	29
Canna . . . . .	27	27

units. The method by which this estimation is carried out has been subjected to certain criticisms by Hess<sup>22</sup> but these have been shown to be invalid,<sup>23, 24</sup> and refinements now available allow of great accuracy in the determination. On the other hand, it has been shown that the method of end-group assay suggested by Hess is liable to errors<sup>24</sup> and it has recently been modified.<sup>58</sup> From the examination of a large number of starches the interesting fact emerges that starches from potato,<sup>6</sup> maize,<sup>25</sup> waxy maize,<sup>26</sup> canna,<sup>27</sup> rice,<sup>28</sup> wheat<sup>29</sup> and chestnut<sup>29</sup> all have the same fundamental chemical unit, despite the wide difference in plant species. These results are shown in Table 2.

It should be mentioned here that besides 2 : 3 : 6-trimethyl glucose and 2 : 3 : 4 : 6-tetramethyl glucose, about 5 per cent. of dimethyl glucose is always obtained on hydrolysis of methylated starches. The significance of this product will be discussed later.

The simplest, but by no means exclusive, conclusion from these facts would be that the starch molecule is a straight chain of 24-30 glucopyranose units, corresponding to a molecular weight of 4000-5000. Physical investigations require modification of this view, however, and it has repeatedly been emphasised by the workers concerned that this simple picture of the structure is inadequate. (See, e.g., Hirst, Plant and Wilkinson;<sup>6</sup> Haworth, Hirst and Oliver;<sup>30</sup> Haworth, Hirst and Isherwood,<sup>59</sup> who discuss the 'laminated' structure of Fig. 4; Haworth<sup>69</sup> and Hirst and Young.<sup>28</sup> The high molecular weight of starch has been stressed also by Staudinger,<sup>32</sup> Hess,<sup>22</sup> Freudenberg<sup>38</sup> and K. H. Meyer<sup>8</sup> whose formula is identical in principle with Fig. 6.) Although cryoscopic data are unreliable, the accuracy of osmotic pressure and ultra-centrifuge determinations of molecular weights has been well established, and there is little doubt that the particle size of starch and its derivatives is considerably greater than

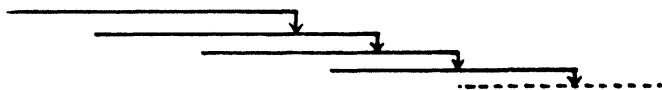


FIG. 4.—A diagrammatic representation of Haworth and Hirst's formula.

Each horizontal line represents a chain of 24-30 glucose residues. The arrows represent junctions from the reducing end-group of each chain to a glucose unit in an adjacent chain.

5000. The measurement of the osmotic pressure of starch solutions is a peculiarly difficult task, owing to the reactivity of the hydroxyl groups and the colloidal nature of the material. Among the pioneer work in this direction, that of Samec<sup>10</sup> must take a high place. In an extensive series of experiments, he found values for the molecular weight of starch specimens of *ca.* 100,000. Since then acetyl and methyl derivatives of starch have been examined by Staudinger and his school,<sup>32</sup> and by Carter and Record,<sup>31</sup> and results of a similar order have been obtained. To the former authors is due the valuable method of determining molecular weights from viscosity data. Preliminary observations on the polyoxymethylenes showed that measurement of the viscosity of these chain-like compounds under standard conditions gives values which bear a simple relationship to the molecular weight, determined by osmotic pressure measurements and by chemical 'end-group' assays. The application of this procedure to polysaccharides has been a more complex task, but a large number of polysaccharide derivatives have been examined

and the relationship between viscosity and molecular weight established in each case. In the course of this work, conclusive proof has been provided that, under appropriate conditions, starch may be converted into the acetyl and then into the methyl derivatives without appreciable change in molecular weight, and from the acetyl derivative the starch may be regenerated unchanged.

Another powerful method of molecular weight measurement has been provided by the use of the ultra-centrifuge. Besides confirming the previous estimates of the order of the molecular weight, this method of attack affords valuable information on the degree of homogeneity of the products. -

This overwhelming mass of physical evidence makes it therefore necessary to regard each chain of 24-30 glucose residues as a repeating unit, the starch molecule being composed of a number of such units or bricks, united together by some bond (the nature of which will be discussed later) to give a structure of molecular weight 100,000 to 200,000. Fig. 5 represents a formula of this type. (See plate facing p. 24.)

The picture of the starch molecule which we thus obtain has received support and amplification, directly and indirectly, from numerous other investigations which may now be considered.

The estimation of the proportion of tetramethyl glucose in the hydrolysis products of methylated starch measures the frequency of the non-reducing end-group (B in Fig. 3). That the proportion of reducing end-groups (A) is very much smaller is shown by the low reducing power of starch itself. The difficulties in estimation which this involves are increased by the fact that the reducing power of oligosaccharides is not directly proportional to the chain-length. Comprehensive investigations in this field have, however, been carried out by Richardson and co-workers,<sup>33</sup> who have evolved an accurate method for determining the reducing power of starch products of high molecular weight. Taking the reducing power of maltose as the basis for the calculation, the values obtained by Richardson for unmodified starches correspond to molecular weights which vary between 74,000 and 240,000 (460 and 1470 glucose units respectively). Acid-modified starches are of lower molecular size, the reducing power increasing with the length of acid treatment. It is therefore apparent that the reducing hydroxyl group which terminates each chain of 24-30 glucose residues is involved in some combination which masks its reducing power. Attempts to detect other groupings such as lactones and anhydro rings, which might terminate the repeating unit and deprive

it of reducing power, have failed, and it is now generally assumed that the reducing hydroxyl group takes part in the junction of successive repeating units to form the macromolecule. The gradual increase in reducing power during hydrolysis by acid is to be ascribed to the fission of the molecule, either between successive repeating units or within the repeating units, to liberate reducing hydroxyl groups.

Attempts have been made by other methods to estimate the proportion of reducing end-group and to follow the increase which occurs during hydrolysis. Caldwell and Hixon,<sup>34</sup> have utilised Jackson and Hudson's periodic acid oxidation<sup>35</sup> of starch. This remarkable reaction results in fission of the glucopyranose rings whilst still retaining the oxygen bridge and the glucosidic links between adjacent glucose units in the chain. Assuming that the reducing end-group of the chain will, under these conditions, yield three molecules of formic acid and one of formaldehyde, the molecular weight may be estimated. The values for starch dextrans were found to agree well with those calculated from the reducing power. More recently, however, specimens of cellulose have been found<sup>60</sup> to give comparatively large amounts of formaldehyde and further investigation appears to be required.

Another interesting method of attack is due to Wolfrom.<sup>57</sup> Methylated potato starch is hydrolysed and simultaneously the liberated reducing groups are mercaptalated. Determinations of the combined sulphur in the product are carried out during the operation, and extrapolation to zero time then gives an estimate of the original chain-length. The values obtained for this particular sample of methylated potato starch indicated a molecule containing at least 150 glucose residues.

The results of these three lines of investigation, due to Richardson, Caldwell and Hixon, and Wolfrom, confirm the view that the starch molecule is of high molecular weight, formed by the junction of a number of repeating units of 24-30 glucose residues. Further evidence for this theory will now be considered.

Whilst under appropriate conditions starch may be converted into a methyl derivative of extremely high molecular weight, it had been noticed by Haworth and his co-workers that repeated methylation under vigorous conditions gradually reduces the molecular weight.<sup>36</sup> The proportion of non-reducing end-group remains, nevertheless, unchanged. Thus, the molecular weight of a specimen of methylated maize starch was reduced to 38,000 in the course of seventeen methylations, the chain-length of the repeating unit being the same (*ca.* 30 units) before and after

treatment.<sup>37</sup> Vigorous conditions of methylation also lead to products of lower molecular weight but unchanged repeating unit, and Table 3 gives typical results in the case of rice starch. Similar results have been reported by Hess<sup>22</sup> and by Freudenberg,<sup>38</sup> who completed the methylation of partially methylated starch by treating with methyl iodide and metallic sodium in solution in liquid ammonia. The substitution of these last hydroxyl groups was invariably accompanied by a decrease in

TABLE 3

THE MOLECULAR WEIGHT AND REPEATING UNIT OF RICE STARCH DERIVATIVES \*

<i>Derivative.</i>	<i>Method of Preparation.</i>	<i>Molecular Weight of Fractions.†</i>	<i>Molecular Weight of Repeating Unit.‡</i>
Acetate A .	Acetic anhydride in pyridine .	720,000	—
Methylate A .	Methylation of acetate A at 55°	670,000	—
Methylate B .	Direct methylation of starch at 20° . . . . .	520,000 385,000 250,000	5900
Methylate C .	Direct methylation of starch at 55° . . . . .	190,000 250,000 205,000	6700
Methylate D .	Direct methylation of starch at 20° in atmosphere of N <sub>2</sub> .	95,000 560,000 525,000 400,000	6100
Methylate E .	Disaggregation of methylate D followed by remethylation	20,000 §	6300

\* See reference <sup>28</sup>.

† Estimated by viscosity measurements.

‡ By the end-group assay method <sup>24</sup>. A molecular weight of 6100 corresponds to a chain-length of 30 glucose units.

§ Confirmed by osmotic pressure and ultra-centrifuge measurements.

molecular weight, indicated by viscosity determinations. It is clear that these observations are difficult to account for on any hypothesis of random hydrolysis of long unbranched chains such as have been considered by Richardson for the structure of starch. If that were the case, the proportion of end-group would be expected to increase with the decrease in molecular weight, as indeed it does during the more vigorous hydrolysis which leads to dextrin formation.

Further evidence concerning the presence of repeating units

in the starch molecule has been obtained by the present authors in recent experiments<sup>28</sup> in which the molecular size of a sample of methylated rice starch has been reduced from some 500,000 to 23,000 by hydrolysis with oxalic acid in aqueous methyl alcohol. After methylation of the degraded product to protect any hydroxyl groups exposed during the 'disaggregation,' the proportion of end-group was found to be identical with that in the original specimen, corresponding to a repeating unit of 30-31 glucose units. The contrast between this 'disaggregation' reaction, occurring at the junction of repeating units and the normal random hydrolysis by acid, which results immediately in the formation of short chain-length dextrans, is apparent. It is confirmed by

TABLE 4

THE PROPERTIES OF DISAGGREGATED METHYL RICE STARCH \*

	<i>Typical Normal Methyl Derivative.</i>	<i>Disaggregated Derivative.</i>	<i>Typical Methylated Dextrin.</i>
Molecular weight . . . . .	> 200,000 (1000 glucose units)	20,000 (100 glucose units)	2000-4000 (10-20 glucose units)
Repeating unit (by end-group assay) . . . . .	29-32 glucose units	31 glucose units	6-12 glucose units
$[\alpha]_D^{20}$ in $\text{CHCl}_3$ . . . . .	+ 210°	+ 224°	+ 180°-200°
Solubility in chloroform, acetone, etc. . . . .	Difficultly soluble	Very soluble	Very soluble
Melting-point . . . . .	ca. 200° decomp.	130° decomp.	

\* See reference<sup>28</sup>.

the fact that whilst during the latter process the rotation falls rapidly, during 'disaggregation' it remains constant and on subsequent methylation increases slightly. The properties of a sample of 'disaggregated' methylated starch, prepared in this way, are compared in Table 4 with those of normal samples of methylated starch and of methylated dextrans.

These results support the view that the starch molecule is built up of repeating units, containing some 30 glucose residues, each repeating unit being joined by a glycosidic linkage to another such unit, forming branched chains. On the other hand, they cannot be reconciled with the long unbranched chain structure of Richardson,<sup>33</sup> or with the formulæ suggested by Staudinger<sup>32</sup> (Fig. 6) and by Hess,<sup>22</sup> which involve a main chain with short, glucosidically linked side chains. In the latter case, 'disaggregation'



should yield a non-homogeneous product, the main portion of which should be of short chain-length.

It is interesting to compare the chemical process of 'disaggregation' with the action of an enzyme, 'amylophosphatase,' isolated by Waldschmidt-Leitz and Mayer.<sup>40</sup> This enzyme causes an immediate fall in the viscosity of starch pastes, and the reducing power rises finally to a value corresponding to a chain-length of 36 glucose residues. This would appear to indicate that hydrolysis

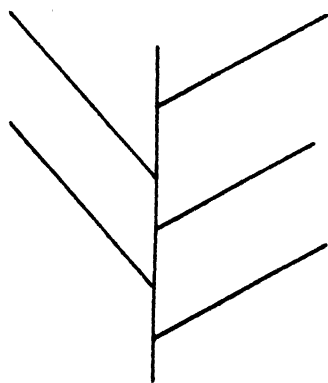


FIG. 6.—A diagrammatic representation of Staudinger's formula.

Branched chains of *ca.* 20 glucose residues are attached to alternate glucose units in the main chain (vertical line). The junction is to C<sub>3</sub> or C<sub>4</sub> of the glucose units.

The variations in the viscosity of starch pastes with small changes of conditions, the retrogradation of such pastes and the known power of the hydroxyl group to co-ordinate, appeared to favour the view that the chains were held together by the association of adjacent hydroxyl groups (see p. 121). Although other compounds where hydrogen bonds are known to occur (e.g. acetic acid) are dissociated in solvents such as water and alcohol, yet the large number of free hydroxyl groups in each repeating unit of the starch molecule might confer exceptional stability upon such a structure. On the other hand, we have seen that the work of Staudinger has shown that starch may be converted into acetyl and methyl derivatives without decrease in molecular size, and this author therefore concluded that the bond is covalent and similar in type to the glucosidic linkages within the chains.

is occurring between the repeating units. Phosphorus can, however, play no part in the 'disaggregation' of methylated rice starch, in which it is absent. It may be noted here that so far the actual repeating unit of 24-30 glucose residues has not been isolated chemically. Under the conditions so far examined, side reactions appear to occur if the 'disaggregation' reaction is continued below a molecular weight of 20,000, and a larger 'repeating unit' containing some 75-100 glucose residues with, respectively, 3-4 non-reducing end-groups could, therefore, be reconciled with the facts at present available.

From the first, the manner of junction of successive repeating units to form the macromolecule has been a subject of discussion.

Since the methyl derivative still retains the high molecular weight of the original starch, consideration of the kinetics of the 'disaggregation' reaction described above provides an estimate of the strength of the bond between the repeating units in starch, as it is this bond which must be broken during the process. From this it appears certain that the linkage is of the normal, covalent glycosidic type.<sup>41</sup> In the first place, the rate of 'disaggregation' is some six times slower than the rate of hydrolysis of methylated inulin to 1 : 3 : 4-trimethyl fructose. In the latter case it is known definitely that the hydrolysis occurring is that of normal anhydro bonds between successive fructo-furanose units.<sup>42</sup> Secondly, measurements of the velocity of 'disaggregation' at two temperatures enables the energy of activation to be calculated, and this is found to be *ca.* 21,000 cals., of a similar order to that for the hydrolysis of disaccharides such as sucrose and for some methylated glucosides. Now the activation energy required for the dissociation of polymers held together by a hydrogen bond is *ca.* 5000-8000 cals., and although several such bonds might co-operate to give activation energies greater than this, it is known from chemical evidence (considered below) that not more than one free hydroxyl group per repeating unit is available in fully methylated starch. It appears, therefore, that specimens of methylated starch of molecular weight not greater than 200,000 are composed of repeating units joined together by normal glycosidic bonds. Methylated starch of higher molecular size breaks down abnormally rapidly until this point is reached, possibly owing to aggregation of units of molecular weight 200,000 by weaker bonds.

The conclusion that a chemical bond is responsible for the junction of repeating units raises the question of the position of the hydroxyl groups involved. From the evidence previously discussed concerning the reducing power of starch and from the fact that the reducing power increases gradually during 'disaggregation,' it is fairly certain that one of the groups taking part is the glycosidic hydroxyl group of the end glucose unit of the chain (A in Fig. 3). The position of the glucose residue in the adjacent repeating unit to which the junction is made is not yet known, and provides one of the next problems of starch chemistry. It has, however, been shown simultaneously by Freudenberg and Boppel<sup>43</sup> and by the present authors<sup>44</sup> that, whatever glucose residue is involved, it is the primary alcoholic group on C<sub>6</sub> which is employed in the linkage. This fact follows from an examination of the hydrolysis products of fully methylated starch. It has been mentioned previously that besides

2:3:6-trimethyl glucose and 2:3:4:6-tetramethyl glucose, small amounts (*ca.* 5 per cent., roughly equivalent to the amount of end-group present) of dimethyl glucose are found in the fission products of methylated starch. The difficulty of ensuring complete methylation made it possible that this last material resulted from the presence of free hydroxyl groups, but it has since been isolated from fully methylated specimens of high methoxyl content, obtained in one case by repeated treatments of viscous material with methylating reagents and in the other by the methylation of a partially disaggregated product, which proceeds readily. The dimethyl glucose has been identified as being essentially 2:3-dimethyl *d*-glucose. Since C<sub>1</sub> and C<sub>4</sub> are involved in the junction to the neighbouring glucose residues of the repeating unit and C<sub>5</sub> in the pyranose ring formation, it follows that C<sub>6</sub> takes part in the bond to the adjacent repeating unit (see Fig. 7), which is therefore a 1, 6-glucosidic linkage, similar to that in gentiobiose. Whether it is an  $\alpha$ - or  $\beta$ -linkage is not yet known. In this connection it is interesting that the work of Freudenberg *et alii*<sup>45</sup> upon the optical rotation of starch and its degradation products leads to the conclusion that less than one in thirty linkages in the starch molecule can be of the  $\beta$ -type.

The conditions (especially temperature) of acid hydrolysis of starch may be expected to decide whether the molecule is broken between or within the repeating units, or both. The first type of breakage leads to 'disaggregation' described above in the case of methylated starch. It is remarkable, however, that similar treatment leads to the irregular breakdown of free starch, indicating the effect of methylation upon the stability of the glucosidic linkages. Products of the second type, in which the linkage between the repeating units has been retained, have been isolated from methylated potato starch,<sup>46</sup> and it is possible that such a process may account for previous reports of the occurrence of gentiobiose among the hydrolysis products of starch<sup>47</sup> (see p. 217). Products in which both types of linkage have been broken include the normal starch dextrans, obtained by heating with acid or with glycerol. These have been examined chemically<sup>48</sup> by Haworth, Hirst and Plant, and the chain-lengths do not differ greatly from those calculated from the molecular weight measured by physical methods.

Mention may be made here of the interpretation of the mechanism of enzyme action suggested by Hanes<sup>49</sup> in view of the picture of the starch molecule which emerges from the work described above.  $\alpha$ -Amylase yields products very similar to

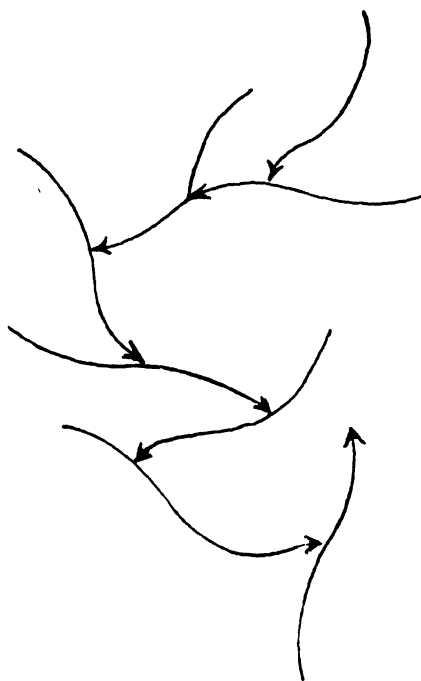


FIG. 5.—Diagrammatic representation of a portion of the starch molecule.

(Each arrow represents a repeating unit of 24-30 glucose residues, the head of the arrow being the reducing end of the chain. Some 40 repeating units combine to form a starch molecule.)

[See p. 18.]

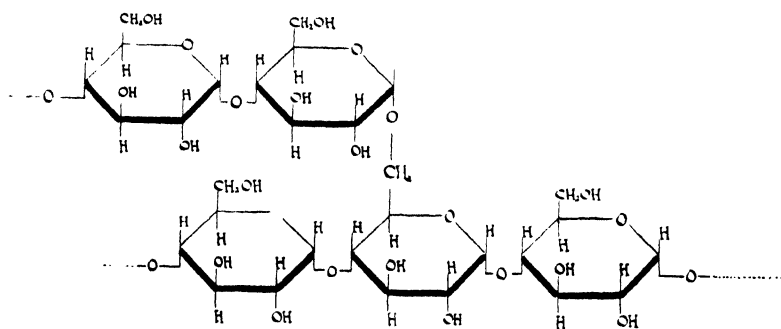


FIG. 7.—The linkage between repeating units in the starch molecule.

The position of the glucose residue in the lower chain to which the junction is effected is not yet known.

[Facing p. 24.]



those obtained by the action of hot dilute acid, and results in the formation of dextrans, with an average chain-length of about six. The proportions of reducing and non-reducing end-groups correspond to the molecular size as determined by osmotic pressure, and in all respects they appear to be straight chain fragments, obtained by fission of both types of linkage in the molecule. On the other hand,  $\beta$ -amylase appears to hydrolyse the 1, 4 linkages within the repeating units, yielding maltose, but the progress of this attack along the chain is impeded by the 1, 6 linkage to the adjacent repeating unit, which it is unable to break, and after some 60 per cent. of the molecule has been converted into maltose its attack ceases. If, however, this restriction is removed by heat or by acid,  $\beta$ -amylase will continue its action, and by successive repetitions of this process all but 3 per cent. of the starch may be hydrolysed to maltose<sup>50</sup> (see p. 466). The residual product of the initial attack,  $\alpha$ -amylo-dextrin, is of great interest. End-group assay<sup>51</sup> indicates a repeating unit of 12 glucose residues but its molecular weight, by osmotic pressure and by ultra-centrifuge<sup>52</sup> measurements, is of the order of 20,000 to 80,000, corresponding to some 120-480 glucose residues. The rotation is high ( $+190^{\circ}$ - $200^{\circ}$ ), in contrast to the low rotation of the dextrans formed by  $\alpha$ -amylase. Since the final yield is independent of the concentration of the enzyme,<sup>53</sup> and since in some preparations it has retained the whole of the phosphorus-content of the starch,  $\alpha$ -amylo-dextrin appears to be a genuine fraction of the starch molecule, and further investigation of this product may be of great help in elucidating the fine structure of starch. One of the most important of recent developments in the chemistry of starch arises from the work of C. S. Hanes<sup>54</sup> which has resulted in the enzymic synthesis of a component of natural starch. This author investigated the properties of an enzyme obtainable from potato tuber juice and from pea seeds, and which, under appropriate conditions, converts starch into glucose 1-phosphate. On the other hand, glucose 1-phosphate can give rise, under the influence of this enzyme, to granules which appear to have many of the properties of natural starch (see p. 8).<sup>61</sup> It most closely resembles the material known as 'amylo-amylose,' obtained by autoclaving starch with water.<sup>64</sup> It gave a deep blue colour with iodine, and was hydrolysed quantitatively to maltose by  $\beta$ -amylase. The chemical examination of Hanes' preparation has been carried out by Haworth, Heath and Peat,<sup>62</sup> and a similar product has been examined by Hassid.<sup>63</sup> The extremely interesting fact emerges that this synthetic substance consists of a long unbranched chain of 1-4-linked *d*-glucopyranose

residues, with a chain-length greater than 100 residues. These investigations are still in their early stages, but it is possible that Hanes' synthetic material is identical with the substance recently found by K. H. Meyer to be a component, in small amount, of potato and other starches (see p. 12). It follows that a synthesis of the major component of starch, having a branched chain structure built up of small repeating units, remains to be achieved. In the paper by Haworth, Heath and Peat, consideration is given to the interesting and difficult problem as to why the enzyme which attacks the branched-chain structure with formation of glucose 1-phosphate, acts synthetically upon the latter substance *in vitro* with production of long unbranched chains of glucose residues.

The facts outlined in this brief review lead to the conclusion that the main constituent of the starch molecule is composed of some 40 repeating units of 24-30 glucose residues, linked together successively by 1-6 glucosidic bonds. Such a structure would have a molecular weight of *ca.* 200,000. In the starch granule, however, it is clear that the particle size is still greater (*ca.*  $10^6$ ), and it is possible that hydrogen bonds are responsible for the aggregation of the molecules (see Chap. 8, Pt. I) and for the ordered structure of the granule. Observations by Reich and Damansky<sup>55</sup> and by Sutra<sup>56</sup> indicate that under very mild conditions of acetylation, one hydroxyl group of each glucose unit is not attacked, and this may indicate some such interaction of hydroxyl groups in adjacent chains. The elucidation of the fine structure of the starch granule is one of the tasks which still await the investigator in this field.

## REFERENCES

1. H. PRINGSHEIM *et al.*, *Ber.*, 1922, **55**, 1428, 1433; 1923, **56**, 1520; 1926, **59**, 2058. See also Pringsheim, 'The Chemistry of the Saccharides'.
2. A. MEYER, *Bot. Zeit.*, 1881, **39**, 841, 857.
3. L. MAQUENNE, *Compt. rend.*, 1895, 1307.
4. Z. GATIN-GRUZEWSKA, *ibid.*, 1906, 540.
5. A. R. LING and D. R. NANJI, *J. Chem. Soc.*, 1923, **123**, 2666.
6. E. L. HIRST, M. M. T. PLANT and M. D. WILKINSON, *ibid.*, 1932, 2375.
7. O. LAMM, *Kolloid-Zeit.*, 1934, **69**, 44; T. SVEDBERG, *Ber.*, 1934, **67A**, 117.
8. K. H. MEYER *et al.*, *Helv. Chim. Acta*, 1940, **23**, 845 *et seq.*; 1941, **24**, 378.
9. E. FOUARD, *Inst. Pasteur*, **21**, 475.
10. See M. SAMEC, 'Kolloidchemie der Stärke' (Dresden and Leipzig); also, *Kolloidchem. Beih.*, 1938, **47**, 387.
11. P. KARRER and E. VON KRAUS, *Helv. Chim. Acta*, 1929, **12**, 1144.
12. T. POSTERNAK, *ibid.*, 1935, **18**, 1351.

13. F. MUSCULUS, *Ann. Chim. Phys.*, 1860, **60**, 203; *Compt. rend.*, 1862, **54**, 194.
14. C. O'SULLIVAN, *Chem. Soc. Trans.*, 1872, **25**, 579; 1876, **1**, 478; 1876, **2**, 125; 1879, **35**, 770.
15. H. T. BROWN and J. HERON, *ibid.*, 1879, **35**, 596.
16. C. J. LINTNER and G. DÜLL, *Ber.*, 1893, **28**, 2533; 1895, **28**, 1522; *Zeit. ges. Brauw.*, 1894, **17**, 339; *Chem. Ztg.*, 1897, **21**, 737; 1892, **15**, 6.
17. F. SCHÄRDINGER, *Wien. Klin. Woch.*, 1904, No. 8; *Zentr. Bakt. Parasitenk.*, 1908, **11**, 22, 98; 1911, **11**, 29, 188.
18. K. MYRBÄCK *et al.*, *Biochem. Zeit.*, 1937, **293**, 107; 1938, **297**, 160, 172; *Skand. Arch. Physiol.*, 1938, **80**, 334, 340; *Svenskkem. Tidskr.*, 1938, **50**, 284.
19. C. J. A. COOPER, W. N. HAWORTH and S. PEAT, *J. Chem. Soc.*, 1926, **129**, 876; J. C. IRVINE and BLACK, *ibid.*, 1926, 862. See also Haworth and Peat, *ibid.*, 1926, 3094.
20. W. N. HAWORTH, E. L. HIRST and WEBB, *ibid.*, 1928, 2681. See also (Sir) J. C. IRVINE and J. MACDONALD, *ibid.*, 1926, 1502.
21. W. N. HAWORTH and PERCIVAL, *ibid.*, 1931, 1342.
22. K. HESS and K. H. LUNG, *Ber.*, 1938, **71B**, 815.
23. F. J. AVERILL and S. PEAT, *J. Chem. Soc.*, 1938, 1244.
24. E. L. HIRST and G. T. YOUNG, *ibid.*, 1938, 1247.
25. W. N. HAWORTH, E. L. HIRST and F. J. AVERILL, forthcoming publication.
26. W. N. HAWORTH, E. L. HIRST and M. D. WOOLGAR, *J. Chem. Soc.*, 1935, 177.
27. W. Z. HASSID and W. H. DORE, *J. Amer. Chem. Soc.*, 1937, **59**, 1503.
28. E. L. HIRST and G. T. YOUNG, *J. Chem. Soc.*, 1939, 1471.
29. — *ibid.*, 1939, 951.
30. W. N. HAWORTH, E. L. HIRST and E. OLIVER, *ibid.*, 1934, 1917.
31. CARTER and RECORD, *ibid.*, 1939, 660, 664.
32. E.g. H. STAUDINGER and E. HUSEMANN, *Annalen*, 1937, **527**, 195; *Ber.*, 1938, **71B**, 1057. See also, 'Die Hochmolekularen Organischen Verbindungen, Kautschuk und Cellulose,' Berlin, 1932.
33. W. A. RICHARDSON, R. S. HIGGINBOTHAM and F. D. FARROW, *J. Text. Inst.*, 1936, **27**, 131T; *Text. Res.*, 1936, **6**, 410; W. A. RICHARDSON and R. S. HIGGINBOTHAM, *J. Soc. Chem. Ind.*, 1938, **57**, 234.
34. C. G. CALDWELL and R. M. HIXON, *J. Biol. Chem.*, 1938, **123**, 595.
35. JACKSON and HUDSON, *J. Amer. Chem. Soc.*, 1937, **59**, 2049.
36. See W. N. HAWORTH, *Monatsh.*, 1936, **69**, 314.
37. F. J. AVERILL, quoted by CARTER and RECORD, *loc. cit.*
38. K. FREUDENBERG, H. BOPPEL and M. M. DELIUS, *Ber.*, 1938, **71**, 2505; *Naturwiss.*, 1938, **26**, 123.
39. D. K. BAIRD, W. N. HAWORTH and E. L. HIRST, *J. Chem. Soc.*, 1935, 1201.
40. E. WALDSCHMIDT-LEITZ, M. REICHEL and A. PURR, *Naturwiss.*, **20**, 254.
41. C. E. H. BAWN, E. L. HIRST and G. T. YOUNG, *Trans. Faraday Soc.*, 1940, **36**, 880.
42. W. N. HAWORTH and H. R. L. STREIGHT, *Helv. Chim. Acta*, 1932, 609.
43. K. FREUDENBERG and H. BOPPEL, *Naturwiss.*, 1940, **28**, 264.
44. C. E. H. BAWN, E. L. HIRST and G. T. YOUNG, *loc. cit.*; BARKER, E. L. HIRST and G. T. YOUNG, *Nature*, 1940, **147**, 296.



45. K. FREUDENBERG, *Ber.*, 1936, **69**, 1252, 1258.
46. BARKER, E. L. HIRST and G. T. YOUNG. Unpublished work.
47. T. C. TAYLOR and LIPSCHITZ, *J. Amer. Chem. Soc.*, 1932, **54**, 1054.
48. W. N. HAWORTH, E. L. HIRST and M. M. T. PLANT, *J. Chem. Soc.*, 1935, 1214.
49. C. S. HANES, *New Phytol.*, **36**, (2) 101 ; (3) 189.
50. HOPKINS, COPE and GREEN, *J. Inst. Brew.*, **39**, 487.
51. W. N. HAWORTH, E. L. HIRST, KITCHEN and PEAT, *J. Chem. Soc.*, 1937, 791.
52. BECKMANN and LANDIS, *J. Amer. Chem. Soc.*, 1939, **61**, 1495.
53. G. A. VAN KLINKENBERG, *Proc. Acad. Sci. Amst.*, **34**, 893.
54. C. S. HANES, *Nature*, 1940, **145**, 348 ; *Proc. Roy. Soc.*, 1940, **B128**, 421.
55. W. S. REICH and A. F. DAMANSKY, *Bull. Soc. Chim. Biol.*, **19**, (i) 518 ; (ii) 357.
56. R. SUTRA, 'La Constitution de l'Amidon,' Hermann, Paris.
57. M. L. WOLFROM and D. R. MYERS, *J. Amer. Chem. Soc.*, 1941, **63**, 1336.
58. HESS, GRIGORESCU, STEURER and FRAHM, *Ber.*, 1940, **73B**, 505.
59. W. N. HAWORTH, E. L. HIRST and F. A. ISHERWOOD, *J. Chem. Soc.*, 1937, 577.
60. G. F. DAVIDSON, *J. Text. Inst.*, 1941, **32**, T. 109.
61. W. T. ASTBURY, F. O. BELL and C. S. HANES, *Nature*, 1940, **146**, 558.
62. W. N. HAWORTH, R. L. HEATH and S. PEAT, *J. Chem. Soc.*, 1942.
63. HASSID and MCCREADY, *J. Amer. Chem. Soc.*, 1941, **63**, 2171.
64. M. SAMEC, *Kolloidchem. Beih.*, 1921, **13**, 272.

## ADDITIONAL REFERENCES

Reviews of the progress of investigations upon the structure of starch include the following :—

65. G. F. DAVIDSON and W. A. RICHARDSON, *Science Progress*, 1936, **31**, 68.
66. H. STAUDINGER, *Ber.*, 1936, **69B**, 819 ; *Naturwiss.*, 1937, **25**, 673.
67. W. N. HAWORTH, *Ann. Rev. Biochem.*, 1936, **5**, 81.
68. ——— *Monatsh.*, 1936, **69**, 314.
69. ——— *J. Soc. Chem. Ind.*, 1939, 917.
70. C. S. HANES, *New Phytol.*, **36** (2), 101 ; (3) 189.
71. M. SAMEC, *Kolloidchem. Beih.*, 1938, **47**, 371.
72. R. SUTRA, *loc. cit.*, reference <sup>56</sup>.

## CHAPTER 4

## SOME PHYSICAL PROPERTIES OF STARCH

PURE starch, as distinct from commercial starch, is a white, odourless, tasteless, neutral powder, insoluble in cold water or organic solvents. In the well-dried state it is hygroscopic; when air-dried, starch contains appreciable amounts of water (see p. 382). The colour of commercial starches may vary from white to a shade of grey or brown, their reaction may be either alkaline or acid. The odour of a commercial starch is often characteristic; it is especially noticeable when the starch is treated with hot water to form a paste. The densities of the more important starches are all approximately 1.625, although that of potato starch is somewhat higher.

The study of the physical properties of starch is a fascinating one, and leads to information explaining a number of peculiarities that may often be observed. Why, for example, should one batch of starch be more readily convertible to dextrin than another? or give a stiffer paste than a previous delivery? or require more heat to form a paste when treated with water? Again, why should one paste give more adhesive or stable mucilages than another? or take longer to reach the same viscosity when converted to dextrin by enzymes? Physical chemistry often gives the answer to such questions.

Starch is insoluble in cold water but swells to some extent when placed in it, and shrinks to its original size when dried to its previous water-content. Starch containing 42 per cent. of water appears as a fluid, but the slightest pressure on it causes it to solidify and appear as a damp solid cake. The paste flows very slowly, but if attempts are made to pour it out of a container quickly it solidifies. By the action of heat on a starch suspension, a stage may be reached which passes that of true swelling, and the starch gelatinises. The granules swell to many times their original size and some starches, e.g. potato starch, burst, releasing an amount of starch material from the inner portion of the granule which goes into solution. A starch paste as usually made therefore consists of granules in various stages, some partially swollen, and some burst, together with soluble material and debris of the outer portions of the burst granules. Starch pastes as usually prepared are therefore not homogeneous.

The actual structure of the granules of starch has received

much attention. Nägeli<sup>1</sup> assumed that they are built up from rounded or oval micellar units arranged symmetrically. Later, A. Meyer<sup>2, 3</sup> postulated that the granules are built up of dendritic crystals of starch substance which are densely packed in concentric layers; intermediate layers separate these and consist of the crystals in a less densely-packed form. The water field in the starch was considered by this worker to be distributed between the crystals in such a manner that the less densely-packed layers contained the greater proportion of it. The effect of density of packing and moisture-content on the appearance of the granules is held by Meyer to account for the striations which some starches show under the microscope.

Katz<sup>4</sup> and Samec<sup>5</sup> have shown that the structure suggested by Nägeli is probably the correct one, and this view is supported by O. A. Sjostrom,<sup>6</sup> who has studied the microscopical appearance of starches, both untreated and of the 'soluble' type, and has obtained excellent photo-micrographs of the various granules swelling in water. In some of the photo-micrographs the micellar structure is clearly shown. A. E. Hanson and J. R. Katz<sup>7</sup> found strong evidence in Lintnerised wheat starch of orderly concentric and radial arrangement of globular units of small dimensions, the concentric connections being stronger than the radial ones, as will be seen by an examination of Sjostrom's photographs. In aqueous glycerol the successive layers peel off like the layers of an onion, and in completely dextrinised starch the entire granule from the surface to the centre appears to be made up of these concentric layers, showing that the micellar arrangement is the same throughout the granule. The photograph by Radley and Young shows these layers quite plainly.\* However, N. P. Badenhuizen<sup>22</sup> considers that the block structure described by the above workers is not preformed in starch grains, but arises during swelling and comes from homogeneously resistant layers of the granule. After swelling different starches with various agents, the individual blocks cannot be separated from each other by a micro-needle, but appear to be fastened together with a soft swollen substance. Badenhuizen acted on starch with chromic acid to make the blocks more apparent, and found them to be about  $1 \mu$  in size.<sup>23</sup>

A. Frey-Wyssling<sup>35</sup> considers the double refraction shown by starch granules to be due to strains, possibly in a radial direction, and not to the structure of the starch. The structure of modified starches has been examined by R. Haller<sup>97</sup> who, by treating them with gold chloride or ammoniacal silver salt solutions followed by copper-oxide-ethylenediamine solution, was able

\* Photomicrograph No. 13, facing p. 377.

to follow the dissolution of the starch material leaving lamellar, concentric membranes behind where the gold or silver salts had collected.

O. E. Stamberg<sup>43</sup> has calculated the surface area of seventeen types wheat flour from the dimensions recorded by Grewe and Bailey.<sup>44</sup> The average is 2004 sq. cm. per gm. and individual samples range from 1780 to 2339 sq. cm. per gm. The granules below  $7\ \mu$  in diameter constitute some 82 per cent. of the total number, but the bulk of the mass, some 93 per cent., is due to granules larger than  $14.8\ \mu$ , which contribute some 76.4 per cent. of the total surface area. The surface area of potato, wheat, corn and rice starches are 853, 1907, 3077 and 8000 sq. cm. per gm., respectively, giving a rough ratio of 1 : 2 : 3 : 8.

These surface areas and the average particle size are of importance industrially, e.g. in the manufacture of the so-called 'Baking Powders' (see p. 302). The power of starch to adsorb various substances does not appear to depend on the surface area presented by the various starches (*vide infra*).

**Adsorption by Starch.**—Dried, but not moist, starch granules adsorb from 5 to 6 times their volume of carbon dioxide, which is only incompletely removed in a vacuum although treatment with boiling water removes it completely.<sup>45</sup> T. J. Schoch<sup>114</sup> has pointed out that if dried starch is confined over various liquids the vapour adsorption is dependent on the hydrophilic nature of the liquid.

Cuprammonium solution swells starch granules<sup>46</sup> but they do not pass into colloidal solution, potato and wheat starches being affected differently by the cuprammonium solution.<sup>47</sup> The starches appear blue after treatment and contain some 12 per cent. of copper oxide. On washing with ammonia the copper content gradually decreases and the starch finally dissolves. A. V. Rakovski<sup>48</sup> has studied this phenomenon and C. E. Guignet notes that the ammonia present in the blue starch-copper compound is driven off at  $40^{\circ}\text{C}$ .<sup>49</sup>

W. Traube<sup>50</sup> noted that solutions of alkali or ethylenediamine containing starch dissolve larger amounts of cupric hydroxide than when starch is absent. A cupra-ethylenediamine solution affects starch in a similar manner to that shown by cuprammonium solution. After repeated washing the blue ethylenediamine-copper-starch substance still contains nitrogen.

The adsorption of acids<sup>51, 52</sup> and bases<sup>53, 54</sup> by starch has been investigated by several workers, and it has been found<sup>52</sup> that adsorption is not a function of the granule surface per unit weight but that starches vary in their adsorption capacity. With

hydrochloric acid the ordinary adsorption isotherm is followed for solutions up to 0.4 N with all the common starches except maize. The isotherm is also followed at ordinary temperatures using butyric and isovaleric acids.<sup>55</sup>

T. Pfeiffer and B. Tollens<sup>56</sup> have studied the sodium and potassium compounds of starch. E. Fouard<sup>54, 57</sup> found that the conductivity of solutions of ammonia and piperidine is diminished by the addition of starch and ascribes this to adsorption and not salt formation which would increase the conductivity. A Reyhler,<sup>58</sup> however, considers that in these cases compounds analogous to the alcoholates are formed.

The adsorption by starch of barium sulphate,<sup>59</sup> alkaline earth and lead carbonates,<sup>60, 61</sup> sodium chloride and copper sulphate<sup>62</sup> has been examined by several workers. G. Carriere<sup>63</sup> finds that barium ions are adsorbed by potato starch or soluble starch suspensions but not by the colloidal solutions. A. V. Rakovski<sup>64</sup> considers that starch adsorbs little or no acids or salts from solution but that aqueous alkalis and hydroxides of heavy metals in aqueous ammoniacal solution are strongly adsorbed, and this worker has presented data on the adsorption of bases by starch.<sup>65</sup>

The author has treated starch in suspension with strong solutions of zinc, aluminium and magnesium sulphates, and after washing free from any trace of the salts has treated with acid and tested for the metals, using the fluorescence micro-tests described in the book by the author and J. Grant.<sup>66</sup> In each case the test showed the presence of the metal. The starch treated with one of the metallic salts mentioned, washed free from the salt and then immersed in a different salt solution showed, after washing free from the second salt solution, the presence of the metal of the second but not the first salt when treated with acid. The starch, therefore, appeared to behave like a zeolite, one metal replacing another in the 'adsorption complex.' The amounts adsorbed were not large but gave very definite reactions.

Starch treated with a solution of a calcium salt adsorbs the calcium or, if treated with sodium chloride solution, the sodium. The 'sodium starch' swells much more than the 'calcium starch,' showing that the metal present exerts a very definite effect (see Richardson and Higginbotham, p. 71). If oxidised starches are used the same differences in swelling power are shown. Now, potato starch contains phosphoric acid, the smaller granules containing a larger proportion of the acid and swelling more than the large granules. The phosphoric acid content

therefore plays a part in the swelling behaviour of starch and thus has an influence on the viscosity.<sup>70</sup> By preparing samples of starch of approximately uniform granule size (e.g. by sedimentation) and forming the calcium, sodium, etc., starches from them, measuring the degree of gelatinisation in distilled water, the influence of the metal may be shown. A repeat experiment, but using a saline solution, would show the effect of chain-length on the swelling powers, as here the effect of metallic ions and of the phosphate radicle is eliminated.

The base exchange capacity is small and would appear to be in the neighbourhood of 3 milli-equivalents of metal per 100 gm. of starch.

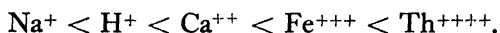
Besides water, liquid ammonia, liquid hydrogen cyanide, formamide and formic acid are excellent gelatinising media for starch. It will be noted that all these liquids have a high dielectric constant, i.e. are capable of dissociating any hydrophilic cohesion between the starch molecules. The preferential adsorption of alkalis, e.g. sodium hydroxide, on to the OH groups of the starch (see p. 97) likewise brings about dissociation by neutralising intermolecular attraction.<sup>114</sup> That the alkali is merely adsorbed is shown by the fact that it can be completely removed by repeated precipitation with methanol. If insufficient free water is present to hydrate the starch, as in saturated sodium hydroxide solutions, the starch does not gelatinise.

As will be discussed (see p. 50) gels may be obtained by the action of a Hofmeister series of salts but, as in the case of sodium hydroxide, the product does not approach the solution state and the internal granule structure is not completely disrupted. Peptising ions carrying a large inert residue, e.g. the quaternary bases, produce a much greater disruption and trimethyl benzyl ammonium hydroxide will give, in the cold, a limpid solution of low viscosity. It may be that the function of the quaternary ammonium ion is to act as a wedge and assist the prying apart of the molecules. Morpholine, trimethylamine and ethylene diamine act similarly in hot solution, in these instances apparently without the alkaline decomposition which is apparent on heating starch gels made with sodium hydroxide solution. In these cases it may be the hydrophilic amine group which associates with the starch by a process similar to hydration.

**Other Properties of Starch.**—The birefringence of starch has already been mentioned. W. I. Nasarov<sup>67</sup> has noted that a suspension of potato starch in benzene displays, by side illumination, different colours according to its moisture-content; with 16 per cent. moisture it appears reddish-yellow, with

21 per cent. reddish-violet, and with 24 per cent. violet, the colours changing when the solution is warmed. It would be interesting to see if a rapid approximation of the moisture-content could be obtained by this method, using the Guild Colorimeter. B. Hošpes and B. Dmitrijev<sup>68</sup> have used a Lange's photocolorimeter to characterise starch samples according to the granule sizes present. They find that the maximum light absorption by the starch-milk is dependent on the concentration of the suspended dry starch. The values with starches of small granule size are more than those of large granules. The rate of decrease in absorption by a suspension on keeping depends on the granule size, so that periodic observation of, e.g., 10 gm. starch in 100 ml. of liquid readily gives a means of characterising the starch.

A. M. Shkodin<sup>69</sup> has determined the distribution curves for water suspensions of four different grades of potato starch and showed that the size of most of the granules lies between 40 and 80  $\mu$ . The velocity of sedimentation of starch increases with increase in the concentration of the electrolyte present. Hydrochloric acid, sodium and calcium chlorides, lactic acid and calcium lactate were used, the last three at concentrations up to 5 mol. per l. In the presence of ferric chloride the velocity of sedimentation reached a sharp maximum at 0.2 equiv. per l. Electro-ösmostic measurements showed a decrease of  $\zeta$  potential in the presence of electrolytes. The effect of

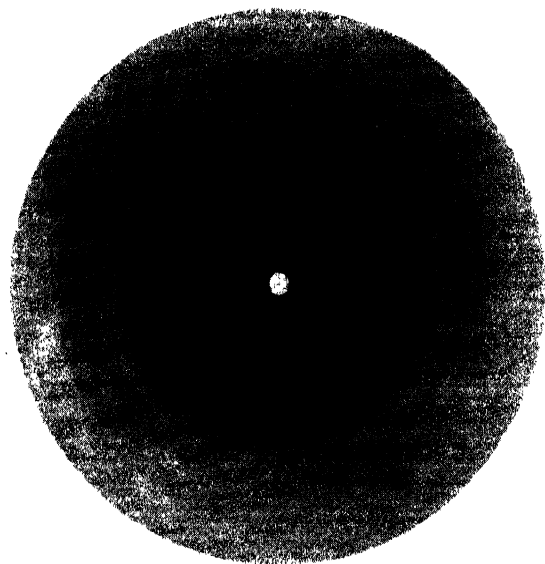


The maximum of the velocity of sedimentation in the presence of ferric chloride corresponded to the isoelectric point of the starch on the  $\zeta$  potential curve. In the presence of electrolytes the starch precipitated in the form of aggregates containing 2 to 3 or more granules. The protective action of dextrin and starch on gold sols and Congo blue sols has been studied by W. Pauli and J. Szper,<sup>71</sup> who found their protective effect inferior to that of gum-arabic.

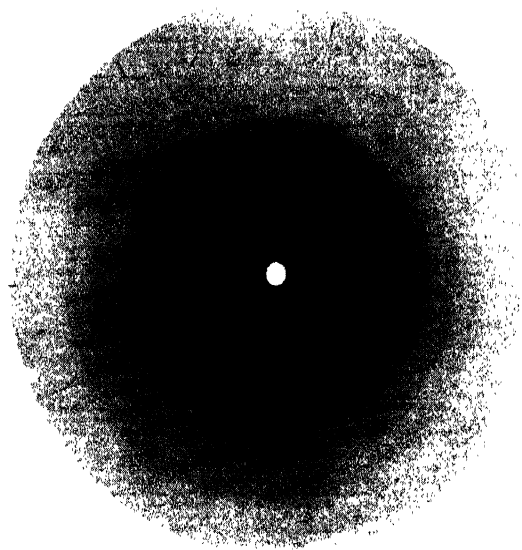
**X-Ray Examination of Starch.**—A number of workers<sup>98-102</sup> have examined starch by means of X-rays but the results have not been encouraging, and no conclusive quantitative results have been obtained. The diagrams yielded in these investigations may be either an amorphous halo or show a few interference rings which show there is some crystalline or micellar structure prevailing in the sample. No attempt at quantitative evaluation of these diagrams has so far been successful, chiefly because no oriented sample could be obtained which would allow a reliable calculation of the elementary cell. One can measure and evalu-







A-type.  
X-ray photograph of rice starch.



[Photographs by W. T. Astbury and reproduced by courtesy of 'Nature'.  
B-type.  
X-ray photograph of potato starch.  
[Facing p. 35.]

FIG. 8.

ate a few diffuse rings but indications of the dimensions and symmetry of the lattice are absent. A number of workers<sup>98-102, 115</sup> have shown that natural starch gives definite X-ray patterns (see especially X-ray photographs by W. T. Astbury, facing p. 9 and opposite). Four principal types of diagram are obtainable and are known as the A-, B-, C- and V-types, characteristic of the plant source or the mode of preparation of the starch. The diagrams opposite illustrate the A- and B-types whilst the C-type appears to be almost certainly a mixture of A- and B-types. The V-type is given by samples obtained by alcoholic precipitation from solution. W. T. Astbury, F. O. Bell and C. S. Hanes<sup>115</sup> found that a specimen of amylo-amylase precipitated by alcohol after electrophoretic separation gave a V-type photograph but using the synthetic starch prepared by Hanes (see p. 9) precipitation with alcohol gave the B-type of photograph. It appears that the phosphorylase present in the plant (see p. 8) may have some influence on the manner of deposition of the starch and hence its X-ray diagram, but the above workers are more inclined to the tentative view that the conditions of deposition may be the deciding factor, and this view is also supported by R. S. Bear and C. F. Cori.<sup>116</sup>

R. S. Bear and C. F. Cori<sup>116</sup> prepared synthetic polysaccharides with muscle, heart and liver phosphorylase.<sup>118</sup> The polysaccharide obtained using muscle phosphorylase was able to exhibit both the B- and V-types of X-ray diffraction pattern. It had a blue iodine reaction and retrograded readily and was thus similar to plant starches. The similarities in the X-ray diagrams of plant starches and this synthetic polysaccharide included ring positions and relative intensities of the rings and also the alterations brought about in the intensities of the rings by wetting or drying. The polysaccharides formed with liver and heart phosphorylases<sup>117</sup> behave like glycogen in a diffuse pattern characteristic of an amorphous material having a red-brown iodine reaction and not retrograding. The interesting problem now to be settled is why muscle phosphorylase produces starch *in vitro* and glycogen *in vivo* and why it differs in this respect from liver, heart and brain phosphorylase which produce glycogen both *in vitro* and *in vivo*.

Lampitt and co-workers<sup>103</sup> find one interference line present in both retrograded and non-retrograded wheat starch fractions prepared by milling (see below), and consider that the fundamental structural unit postulated to give rise to this line is present in both retrograded and non-retrograded portions. J. R. Katz,<sup>37-41</sup> who with co-workers distinguishes between a crystalline and an

amorphous diagram and has shown how various treatments of the starch affects the diagram obtained. Katz and his co-workers from their work consider that air-dried starches contain water of crystallisation and that the staling of bread may be accompanied by crystallisation of the amorphous starch present.<sup>41</sup>

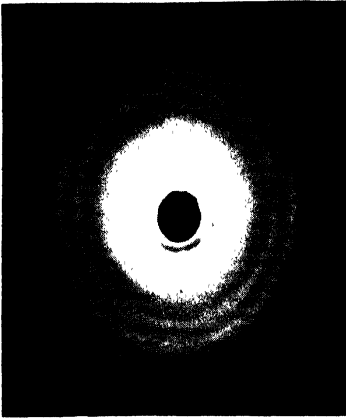
When starch is ball-milled or ground to destroy the granule structure the X-ray diagram is of the amorphous type.<sup>102</sup> L. H. Lampitt, C. H. F. Fuller and N. Goldenberg<sup>103</sup> have recently examined the changes in the X-ray spectrum of wheat starch which are produced by ball-milling the starch. Prior to grinding the interplanar spacing, 'd,' was found to be similar to the values found by Katz<sup>105</sup> and Naray-Szabó.<sup>104</sup> Every preparation examined by these workers exhibited a 'd' value which is of the same order as that experimentally determined for the thickness of the molecules of three different trimethyl- $\beta$ -methyl glucosides by Cox, Goodwin and Wagstaff.<sup>113</sup>

According to Meyer *et al.*<sup>106</sup> starch subjected to a pressure of 20,000 atmospheres gives an amorphous X-ray pattern presumably because the disruption of the inner arrangement of the crystallites in the granule structure.

As already mentioned the presence of a certain amount of water, probably of crystallisation, is necessary in order to get an X-ray diagram which is not amorphous, and from this it may be concluded that water is an essential factor in the structural formation of the starch granule.<sup>107, 108</sup> Katz and Weidinger<sup>109</sup> have shown that starch heated to 180-200° C. no longer gives the crystalline pattern, and it should be remembered that at this temperature not only is the loosely-held water present driven off but also that water which is not removed by maintaining starch at a temperature of 110° C. for a long time. The evolution of this moisture from well-dried starch is described in the chapter on Dextrin.

Lampitt *et al.* by extracting milled starch have obtained several fractions, differing in water solubility and described on p. 76. The H.W.S. and the C.W.S. fractions gave different but definite X-ray patterns and if precipitated from their solutions by alcohol the resulting X-ray pattern differed from that given by the solids obtained by heat-drying. They thus conclude that the structure of the starch is greatly dependent on the way in which the starch is dried, and in the case of both the H.W.S. and C.W.S. fractions the X-ray pattern appears to be independent of the molecular weight of the fraction.

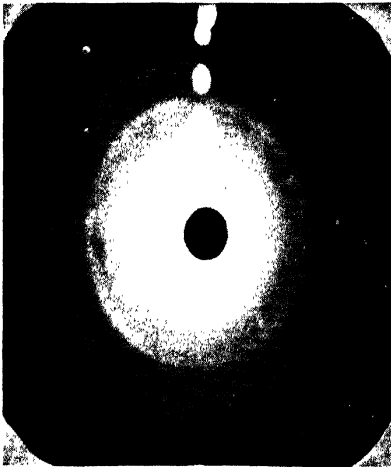
The X-ray pattern obtained with starch milled from 0-120 hours appears to be due to the H.W. insoluble fraction but



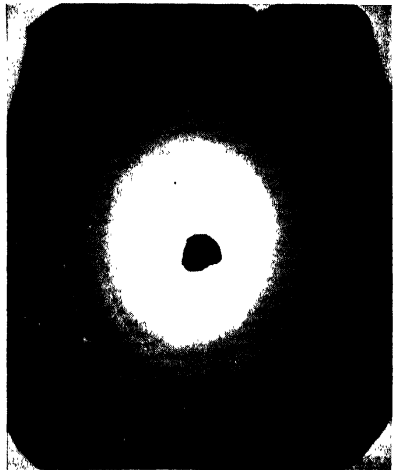
Unground wheat starch.



B  
Starch ground for 120 hrs.



C  
H.W. insol. 300 fraction (heat dried).

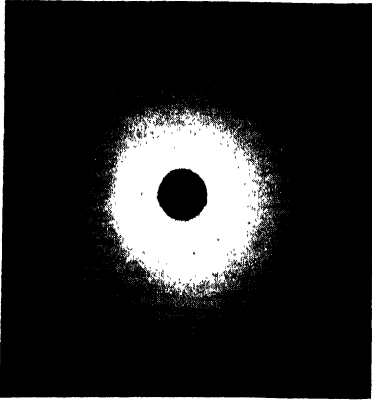


D  
Ash from the H.W. insol. 300 fraction (C).

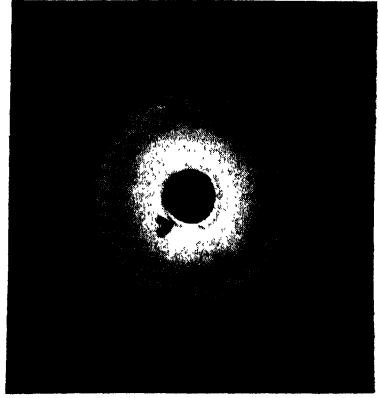
[Reproduced by courtesy of 'J. Soc. Chem. Industry'.

FIG. 9.

[Facing p. 36.

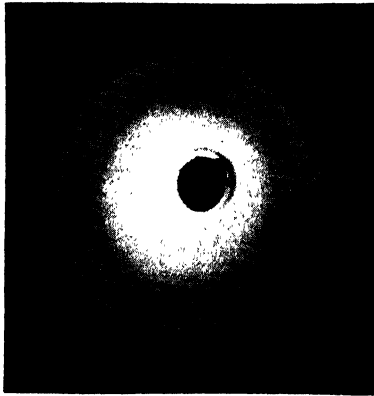


H.W. sol. 120 fraction (heat dried).



F

H.W. sol. 300 fraction (alcohol-precipitated).



G

H.W. sol. 470 fraction (alcohol-precipitated).

*[Reproduced by courtesy of 'J. Soc. Chem. Industry']*

FIG. 9 (continued).

beyond this time the contamination from the mill plays an increasingly important part until, after 1200 hours milling, any pattern obtained is due entirely to the silicates or other inorganic material from the mill. Between 300 and 470 hours' milling a change in the structure of the H.W.S. fraction takes place, as shown by the definite change in the X-ray pattern.

Figures 9 A and B show the change brought about by grinding whilst C and D show that part of the X-ray pattern of the H.W. insoluble fraction is due to the presence of inorganic contamination by the mill materials. E and F show the differences between X-ray patterns of heat-dried and alcohol precipitated H.W.S. fractions, and if F and G be compared it will be seen that the X-ray pattern of the H.W.S. fraction, 470 hours' milling, differs from those of the other H.W.S. fractions.

The starch separating from solutions of H.W.S. and C.W.S. fractions had a much more definite pattern than that remaining in solution, thus confirming the observations of previous workers<sup>108-112</sup> and supporting their conclusions that starch pastes increase their crystalline character on ageing. This phenomena is further discussed in Chap. 7, Pt. I.

It would appear, however, that most of the evidence derived from X-ray examination of starch is mostly negative in character. As fibre diagrams are not obtained it would indicate that apparently there are no long unbranched chain-molecules present in starch. Here the evidence is in the same direction as that furnished by much stronger and recent chemical considerations. Hirst and Young (see p. 23) have concluded that starch consists of a highly branched chain system, the branches having a molecular weight of about 4500. Although X-ray diagrams cannot supply additional quantitative knowledge to this view they are not contrary to it.<sup>42</sup>

**Amylose and Amylopectin.**—The study of the swelling and gelatinisation of starch has greatly increased our knowledge of the structure of the granules and led many workers to the conclusion that they contain two different forms of the same substance upon which the physical behaviour of the starch depends. Foremost in these studies were Maquenne and Roux,<sup>8</sup> Gatin-Gruzeska,<sup>9</sup> and Nägeli.<sup>10</sup> They postulate that the inner portion of the starch granule consists of a substance termed 'amylocellulose,' 'amylose,' or 'granulose,' which is the soluble portion, and the outer portion, called 'amylopectin,' which gives a viscous paste with water, and to which the paste-forming properties of starch are due.

As will be discussed later, according to some workers an essential

difference between these two substances appears to be the presence of phosphoric acid in the molecule of the amylopectin, although recent work goes to show that one is a polymer of the other and that the two forms are readily interchangeable (see p. 103 and below). Fouard<sup>11, 12</sup> has shown that some of this phosphoric acid may be removed from the starch by acids, but that a certain amount is retained very tenaciously and is probably combined with the starch, a conclusion upheld by the work of Samec; but Fouard maintains that only one substance is present in starch. There does not appear to be a sharp line of demarcation between the chemical constitution of these two substances, but a gradual transition from one to the other.<sup>3, 13</sup> The paste-forming properties of starch are attributed by some workers to the amylopectin and the deep blue coloration given by iodine is ascribed to the amylose portion.

L. Gatin-Gruzewska<sup>9</sup> has separated the two constituents by dissolving out the amylose portion with alkali and considers that amylopectin forms some 40-60 per cent. of the solid matter. C. Tanret<sup>14</sup> obtains different results, his figures for amylopectin varying from 67 per cent. in chestnut starch to 79.5 per cent. in banana starch, whilst Samec and von Hoeft<sup>15-17</sup> obtain figures in the neighbourhood of 83 per cent. W. S. Reich and A. F. Damansky find the amylopectin to be present to the extent of 82 per cent.<sup>24</sup> T. C. Taylor and R. P. Walton<sup>18</sup> have found the ratio of amylose to amylopectin to be 76 : 24 in wheat starch and 83 : 17 in tapioca starch, whilst the first-named worker, in conjunction with H. A. Iddles<sup>19</sup> and C. O. Beckmann,<sup>25</sup> has obtained the following ratios: maize and rice starches, 81-89 per cent. amylose and 12-19 per cent. amylopectin; potato starch, 97-98 per cent. amylose and 1.8-2.9 per cent. amylopectin (a similar figure being obtained by F. H. Thurber<sup>26</sup> for sweet potato starch).

O. Dahl,<sup>23</sup> using an electrophoretic method, obtained 16-18 per cent. of amylose from wheat, maize and potato starches and a 9 per cent. yield from rice starch. A similar figure for wheat starch was obtained by O. E. Stamberg and C. H. Bailey,<sup>34</sup> who further found no difference in the amylopectin-content of large or small granules. J. L. Sarin and R. L. Sehgal,<sup>72</sup> by electrophoresis of water calthrop starch, obtained a figure of 4.27 per cent. for the amount of  $\alpha$ -amylose present. A. Eckert and A. Marzin<sup>20</sup> separated the amylopectin portion by treating the starch with 0.1 N hydrochloric acid solution in methyl alcohol, which dissolves the amylose, and obtained the following figures for percentage content of amylopectin: potato starch 83, arrowroot starch 80, maize starch 75, wheat starch 63, rice starch

80. Pringsheim and Wolfsohn obtained 66 per cent. amylopectin from potato starch using the method of Ling and Nanji, and K. H. Meyer and co-workers<sup>110</sup> consider that maize starch contains 10 per cent. of amylose. The method employed by Taylor and Iddles with maize and rice starches was to swell the starch by means of ammonium thiocyanate, followed by ultrafiltration or electro dialysis of the solution. Samec has employed electro dialysis of the starch solutions<sup>15-17</sup> and a simple electro dialyser has been described in detail by S. Redfern.<sup>73</sup> Taylor has also ground starch in a pebble mill until soluble, and used the solution to separate the amylopectin, and a very thorough study of milled wheat starch fractionation has been made by L. H. Lampitt, C. H. F. Fuller and N. Goldenberg. The method of extracting with alkali appears to yield only about half the amylose present, the remainder being retained in association with the amylopectin. A. R. Ling and D. R. Nanji<sup>21</sup> employed chemical and physical methods, including the use of enzymes, and consider the ratio of amylose to amylopectin in wheat, rice, maize and barley starches to be practically constant at 2 : 1. It will thus be seen that widely varying results have been obtained by different workers for the amounts of amylopectin and amylose present in the different starches.

The use of enzymes by Ling and Nanji is open to grave objections, and the depolymerisation that may take place under the influence of enzymes has been investigated by a number of workers<sup>27-32</sup> and is described in fuller detail in Section V. K. H. Meyer<sup>36</sup> from his work on enzymes concludes that amylose has a straight chain and amylopectin a branched chain-molecule.

One method of attack which has been used by a number of workers<sup>78-84</sup> is based on the separation of fractions of varying solubility if the starch is first ball-milled, or ground, in the dry state. Few of the operators using this method have attempted much in the way of quantitative examination, but recently Lampitt, Fuller and Goldenberg<sup>85</sup> have investigated the method very fully. They examined the three fractions, arbitrarily selected by their solubility in cold and hot water, with respect to viscosity, reducing power, X-ray patterns of the dried fractions and their behaviour towards the addition of alcohol to their solutions, separation from solutions on ageing at various temperatures and concentrations and certain other properties (see also p. 76).

Their work is important in that it has shown that the proportions of amylose and amylopectin found in any particular sample of starch depends on the severity of the methods of treatment



to separate the two fractions. Whether the amylopectins or amyloses separated by the various methods are really identical is difficult to judge owing to the lack of criteria for these substances. By the grinding technique it does seem possible to convert practically the whole of the amylopectin present into amylose, and that in this depolymerisation only lateral links between the chain-molecules are ruptured but no fission of the repeating units occurs. After extensive milling (4000 hours) the product was essentially starch-like in character.

To sum up: 'amylose' and 'amylopectin' has been separated by purely (a) mechanical means, e.g. grinding followed by extraction,<sup>74-85</sup> (b) chemical, including enzymatic processes, and precipitation by suitable reagents from solutions made with the aid of starch peptising agents,<sup>17, 21, 86-94</sup> and (c) physical methods, such as electro dialysis of autoclaved or chemically peptised starch.<sup>17, 19, 20, 95, 96</sup> Owing to the lack of methods for characterising these substances and the gradual transition between the chemical or physical constitution of amylose and amylopectin, the establishment of the identity of products made by different methods has not been easy. Recent work has, however, put the examination of these substances on a surer basis. Amylose and amylopectin are interconvertible and this is responsible for many of the vagaries of starch in technical practice.

From the work discussed in these pages it is clear that the amylose and amylopectin fractions separated by the different methods are by no means identical, for example *all* fractions prepared by methods (a) and (b) contain phosphorus to varying degrees although the amylose fractions generally have a smaller phosphorus-content than the amylopectin fraction, but instances have been cited where the phosphorus-content is identical in both fractions. Method (c) gives amylose very poor in phosphorus and an amylopectin very rich in phosphorus. L. H. Lampitt, C. H. F. Fuller and N. Goldenberg<sup>119</sup> have pointed out sources of error in method (c), and one cannot do better than quote their opinion verbatim. They say, 'Method (c), originally put forward by Samec, has been widely used to prepare  $\alpha$ - and  $\beta$ -amyloses.<sup>73, 82, 94, 120-123</sup> There is a concentration at the anode of the phosphorus-bearing fraction of the starch, attributed to the increased negative charge due to its greater phosphorus-content.<sup>82, 124-125</sup> Accordingly, two fractions are obtained—one comparatively rich in phosphorus and the other comparatively poor in phosphorus, the liberated phosphorus dialysing out in the process.<sup>33-34, 82, 94, 123-124, 126, 130</sup> It should, however, be emphasised that the separation of the starch solution into two

phases during electro dialysis may take a very considerable time<sup>10</sup> and during this period retrogradation will take place to an extent depending on the experimental conditions.<sup>7</sup> As the dialysis cell is vertical, with the anode at the bottom,<sup>33-34, 73, 82, 94, 123-130</sup> the starch fraction migrating to the anode will contain some retrograded starch, the physico-chemical properties of which may be different from those of the fraction separated by electrophoresis. Since retrograded wheat starch has a relatively higher phosphorus-content (as shown by these workers in their paper<sup>119</sup>—*J.A.R.*), any contamination of the anodic fraction by retrograded starch will also increase its phosphorus-content. Further, the physico-chemical properties of the starch may be altered as a result of local concentration of acid or alkali at the electrodes and the effect thereof on the dissolved starch.'

## REFERENCES

1. C. W. NÄGELI, 'Die Stärkekörner,' 1858.
2. A. MEYER, *Botan. Zeit.*, 1881, **39**, 841 and 857.
3. — 'Untersuchungen über die Stärkekörner,' G. Fischer, Jena, 1895.
4. J. R. KATZ, 'Abderhalden's Handbuch der Biologischen Arbeitsmethoden,' 1934, Abt. II, Teil 3, Heft 6.
5. M. SAMEC, 'Kolloidchemie der Stärke,' T. Steinkopff, Dresden and Leipzig, 1927.
6. O. A. SJOSTROM, *Ind. Eng. Chem.*, 1936, **28**, 63.
7. A. E. HANSON and J. R. KATZ, *Zeit. phys. Chem.*, 1934, **168A**, 341.
8. L. MAQUENNE and E. ROUX, *Compt. rend.*, 1905, **140**, 1303.
9. L. GATIN-GRUZEWSKA, *ibid.*, 1908, **146**, 540.
10. C. W. NÄGELI, *Liebig's Ann. d. Chem.*, 1874, **173**, 218.
11. E. FOUARD, *Compt. rend.*, 1908, **146**, 813.
12. — *Bull. Soc. chim. France*, 1908, **3**, 1170.
13. L. MAQUENNE, *Compt. rend.*, 1903, **137**, 88, 797 and 1266; 1908, **146**, 542.
14. C. TANRET, *ibid.*, 1914, **158**, 1353; **159**, 530.
15. M. SAMEC and F. VON HOFFFT, *Kolloidchem. Beih.*, 1912, **4**, 132.
16. — *ibid.*, 1913, **5**, 141.
17. — *ibid.*, 1914, **6**, 23 and 291.
18. T. C. TAYLOR and R. P. WALTON, *J. Amer. Chem. Soc.*, 1929, **51**, 3431.
19. T. C. TAYLOR and H. A. IDDLES, *Ind. Eng. Chem.*, 1926, **18**, 713.
20. A. ECKERT and A. MARZIN, *J. prakt. Chem.*, 1932, ii, **133**, 110.
21. A. R. LING and D. R. NANJI, *J. Chem. Soc.*, 1923, **123**, 2666.
22. N. P. BADENHUIZEN, *Protoplasma*, 1937, **28**, 293; 1937, **29**, 246.
23. — *Zeit. phys. Chem.*, 1936, **175**, 383.
24. W. S. REICH and A. F. DAMANSKY, *Bull. Soc. Chim. Biol.*, 1937, **19**, 158.
25. T. C. TAYLOR and C. O. BECKMANN, *J. Amer. Chem. Soc.*, 1929, **51**, 294.
26. F. H. THURBER, *Ind. Eng. Chem.*, 1933, **25**, 565.
27. H. POTTEVIN, *Ann. Inst. Pasteur*, 1899, **13**, 665.
28. S. NISHIMURA, *Biochem. Zeit.*, 1928, **200**, 81.

29. H. PRINGSHEIM and A. BEISER, *ibid.*, 1924, **148**, 336.
30. U. OLSSON, *Zeit. phys. Chem.*, 1923, **126**, 29.
31. E. WALDSCHMIDT-LEITZ and K. MAYER, *Zeit. physiol. Chem.*, 1935, **286**, 168.
32. J. BLOM, A. BAK and B. BRAAE, *ibid.*, 1937, **350**, 104.
33. O. DAHL, *ibid.*, 1940, **263**, 39, 81.
34. O. E. STAMBERG and C. H. BAILEY, *Cereal Chem.*, 1939, **16**, 309.
35. A. FREY-WYSSLING, *Naturwiss.*, 1940, **28**, 78.
36. K. H. MEYER, *Arch. Sci. phys. nat.*, 1940, **22**, Suppl. 19.
37. J. R. KATZ and J. C. DERKSEN, *Zeit. physik. Chem.*, 1933, **166A**, 27 ; 1934, **168A**, 321 ; 1936, **175A**, 383 ; 1937, **180A**, 405.
38. J. R. KATZ and A. WEIDINGER, *Zeit. physik. Chem.*, 1937, **180A**, 423.
39. M. SAMEC and J. R. KATZ, *ibid.*, 1937, **180A**, 436 ; *Rec. Trav. Chim.*, 1937, **56**, 776.
40. J. R. KATZ and J. SEIBERLICH, *Zeit. physik. Chem.*, 1938, **183A**, 146.
41. J. R. KATZ, *Rec. Trav. Chim.*, 1937, **56**, 766, 785.
42. H. MARK, *Chem. Reviews*, 1940, **26**, 184.
43. O. E. STAMBERG, *Cereal Chem.*, 1939, **16**, 769.
44. GREWE and C. H. BAILEY, see *Brit. Cotton Inst. Res. Ass. Summary*, 1927, **8**, 63.
45. J. BOEHM, *Botan. Zeit.*, 1883, **41**, 522, 538, 554.
46. A. PAYEN, *Compt. rend.*, 1859, **48**, 67.
47. C. W. NÄGELI, *Königliche Bayer. Akad. Wissensch.*, 1863, **2**, 272 ; via *Chem. Zentr.*, 1865, 494.
48. A. V. RAKOVSKI, *J. Russ. Phys. Chem. Soc.*, 1914, **46**, 246 ; via *J. Chem. Soc.*, 1914, v. 106, part 2, p. 434.
49. C. E. GUIGNET, *Compt. rend.*, 1889, **109**, 528.
50. W. TRAUBE, *Ber.*, 1921, **54**, 3220.
51. F. ROBINSON, *Proc. Cambridge Phil. Soc.*, 1910, **15**, 548.
52. H. LLOYD, *J. Amer. Chem. Soc.*, 1911, **33**, 1213.
53. E. DEMOUSSY, *Compt. rend.*, 1906, **142**, 933.
54. E. FOUARD, *Bull. Soc. chim. France*, 1909, Série 4, **5**, 828.
55. A. LASNITSKI and L. F. LOEB, *Biochem. Zeit.*, 1924, **146**, 96.
56. TH. PFEIFFER and B. TOLLENS, *Ann.*, 1881, **210**, 285.
57. E. FOUARD, *Bull. Soc. chim. Belg.*, 1910, **24**, 105.
58. A. REYCHLER, *ibid.*, 1909, **23**, 378.
59. MECKLENBERG, *Zeit. physik. Chem.*, 1913, **83**, 609.
60. R. MARC, *ibid.*, 1913, **81**, 641.
61. O. ARENDT, *Kolloidchem. Beih.*, 1915, **7**, 212.
62. K. SHERINGA, *Chem. Zentralbl.*, 1921, **1**, 131.
63. G. CARRIERE, *Chem. Weekbl.*, 1939, **36**, 612.
64. A. V. RAKOVSKI, *J. Russ. Phys. Chem. Soc.*, 1912, **44**, 586.
65. — *ibid.*, 1913, **45**, 7 and 13.
66. J. A. RADLEY and J. GRANT, 'Fluorescence Analysis in Ultra-violet Light,' 3rd Ed., 1939, Chapman & Hall, London.
67. W. I. NASAROV, *Colloid J. (U.S.S.R.)*, 1938, **4**, 439 ; via *Chem. Zentr.*, 1939, **1**, 4404.
68. B. HOŠPES and B. DMITRIJEV, *Zeit. Spiritusind.*, 1939, **82**, 115.
69. A. M. SHKODIN, *Colloid J. (U.S.S.R.)*, 1939, **5**, 411 ; *Khim. Referat Zhur.*, 1939, No. 9, 11.
70. I. A. VESELOVSKIĭ, *Khlopchato-Bumazhnaya Prom.*, 1935, No. 5, 45 ; via *Chem. Abstr.*, 1940, **34**, 1871.
71. W. PAULI and J. SZPER, *Trans. Farad. Soc.*, 1939, **35**, 1316.
72. J. L. SARIN and R. L. SEHGAL, *Current Sci.*, 1940, **9**, 185.

73. S. REDFERN, *Cereal Chem.*, 1938, **15**, 712.
74. C. L. ALSBERG, *Ind. Eng. Chem.*, 1926, **18**, 190.
75. — and E. E. PERRY, *J. Biol. Chem.*, 1925, **63**, lxvi.
76. — *Proc. Soc. Exp. Biol. Med.*, 1924, **22**, 60.
77. — and E. P. GRIFFIN, *Cereal Chem.*, 1925, **2**, 325.
78. — and FIELD, *J. Amer. Chem. Soc.*, 1926, **48**, 1299.
79. FIELD, *Proc. Soc. Exp. Biol. Med.*, 1928, **25**, 71.
80. C. O. BECKMANN and LANDIS, *J. Amer. Chem. Soc.*, 1939, **61**, 1495.
81. T. C. TAYLOR and KERESZTESY, *Ind. Eng. Chem.*, 1926, **28**, 502.
82. — and T. J. SCHOCH, *J. Amer. Chem. Soc.*, 1933, **55**, 4248.
83. — and C. O. BECKMANN, *ibid.*, 1929, **51**, 294.
84. — and R. SALZMANN, *ibid.*, 1933, **55**, 264.
85. L. H. LAMPITT, C. H. F. FULLER and N. GOLDENBERG, *J. Soc. Chem. Ind.*, 1941, **60**, 1.
86. E. L. HIRST, M. M. T. PLANT and M. D. WILKINSON, *J. Chem. Soc.*, 1932, 2375.
87. H. C. SHERMAN and J. C. BAKER, *J. Amer. Chem. Soc.*, 1916, **38**, 1885.
88. M. E. BALDWIN, *ibid.*, 1930, **52**, 2907.
89. P. KARRER and E. VON KRAUS, *Helva Chim. Acta*, 1929, **12**, 1144.
90. M. SAMEC, *Biochem. Zeit.*, 1928, **195**, 72.
91. — and HAERDTL, *Kolloid. Beih.*, 1920, 281.
92. — and MAYER, *ibid.*, 1921, 272.
93. —, MINAEFF and RONZIN, *ibid.*, 1924, 203.
94. — and M. BLINC, *ibid.*, 1938, **47** 371.
95. BAIRD, W. N. HAWORTH and E. L. HIRST, *J. Chem. Soc.*, 1935, 1201.
96. FREUDENBERG and RAPP, *Ber.*, 1936, **69**, 2041.
97. R. HALLER, *Helv. Chim. Acta*, 1940, **23**, 596.
98. J. KATZ, in 'Comprehensive Survey of Starch Chemistry,' edit. by R. WALTON, New York, 1928.
99. O. L. SPONSLER, *Science (N.S.)*, 1925, **62**, 547.
100. — *Amer. J. Bot.*, 1922, **9**, 471.
101. — *J. Gen. Physiol.*, 1923, **5**, 757.
102. OTT, *Physikal. Zeit.*, 1926, **27**, 174.
103. L. H. LAMPITT, C. H. F. FULLER and N. GOLDENBERG, *J. Soc. Chem. Ind.*, 1941, **60**, 69 and 183.
104. NARAY-SZABÓ, *Ann.*, 1928, **465**, 299.
105. J. KATZ, *Zeit. phys. Chem.*, 1930, **150A**, 37.
106. K. H. MEYER, HOPF and MARK, *Ber.*, 1929, **628**, 1103.
107. CENTOLA, *Atti R. accad. Lincei*, 1936, **23**, 617.
108. K. H. MEYER, P. BERNFELD and E. WOLFF, *Helv. Chim. Acta*, 1940, **23**, 854.
109. J. KATZ and A. WEIDINGER, *Zeit. phys. Chem.*, 1939, **184A**, 100.
110. K. H. MEYER and W. BRENTANO, *Arch. Sci. phys. nat.*, 1936, 111.
111. — and VAN DER WYK, *Helv. Chim. Acta*, 1937, **20**, 1331.
112. — W. BRENTANO and P. BERNFELD, *ibid.*, 1940, **23**, 845.
113. COX, GOODWIN and WAGSTAFF, *J. Chem. Soc.*, 1935, 1495.
114. T. J. SCHOCH, *Cereal Chem.*, 1941, **18**, 121.
115. W. T. ASTBURY, F. O. BELL and C. S. HANES, *Nature*, 1940, **146**, 558.
116. R. S. BEAR and C. F. CORI, *J. Biol. Chem.*, 1941, **140**, 111.
117. G. T. CORI, C. F. CORI and G. SCHMIDT, *ibid.*, 1939, **129**, 629.
118. — *ibid.*, 1940, **135**, 733.
119. L. H. LAMPITT, C. H. F. FULLER and N. GOLDENBERG, *J. Soc. Chem. Ind.*, 1941, **60**, 231.

120. M. SAMEC, *Cereal Chem.*, 1936, **13**, 592.  
 121. HOPKINS, STOPHER and DOLBY, *J. Inst. Brew.*, 1940, **46**, 426.  
 122. O. DAHL, *Svensk. Kem. Tids.*, 1929, **51**, 219.  
 123. O. E. STAMBERG, *Cereal Chem.*, 1940, **17**, 372.  
 124. M. SAMEC, *Kolloid. Beih.*, 1931, **33**, 269.  
 125. — *Kolloid-Zeit.*, 1938, **85**, 247.  
 126. — *Trans. Faraday Soc.*, 1935, **31**, 395.  
 127. TANNER and ENGLIS, *Food Res.*, 1940, **6**, 563.  
 128. DUMANSKI and BARVINOK, *Kolloid. Shurn.*, 1938, **4**, 181.  
 129. EULER *et al.*, *Naturwiss.*, 1938, **48**, 790.  
 130. H. THURBER, *J. Assoc. Off. Agric. Chem.*, 1940, **23**, 126.  
 131. L. H. LAMPITT, C. H. FULLER and N. GOLDENBERG, *J. Soc. Chem. Ind.*, 1941, **60**, 175.

## ADDITIONAL REFERENCES

- M. DE SMET, *Natuurwet. Tijdschr.*, 1936, **18**, 118. (Effect of starch on charcoal suspensions.)  
 B. N. SASTRI and A. KRISHNAMURTHI, *Proc. Soc. Biol. Chem. India*, 1936, **1**, 3. (Fractionation of starch.)  
 J. A. VAN DER HOEVE, *Congr. intern. tech. chim. ind. agr., Compt. rend., 5th Congr.*, 1937, **2**, 250. (General.)  
 L. A. GRANSKAYA and N. E. SACKUN, *Colloid J. (U.S.S.R.)*, 1937, **3**, 117. (Hydration of starch.)  
 Z. P. CHESHEVA, *ibid.*, 1937, **3**, 121. (Vapour tension and bound water in starch.)  
 O. KRATKY and B. SCHNEIDMESSER, *Ber.*, 1938, **71B**, 1413. (X-ray investigation of Schardinger's  $\alpha$ -dextrin.)  
 S. M. STREPKOV and C. K. KURAMSCHIN, *J. prakt. Chem.*, 1938, **150**, 186. (Properties of eleven different starches tabulated.)  
 A. TYCHOWSKI, *Biochem. Zeit.*, 1937, **291**, 247. (Potato starch contains 42 per cent. amylopectin and 58 per cent. amylose.)  
 J. DEDEK, B. JELINEK and I. KULCICKYJ, *Ann. Ferm.*, 1936, **2**, 79. (Cations present influence colloidal properties of starch.)  
 W. NOSSIAN, *J. prakt. Chem.*, 1861, **83**, 41. (Examined 7 starches. Wheat starch most and acorn starch least hygroscopic.)  
 H. RODEWALD, *Landw. vers. Stat.*, 1894, **45**, 201. (Heat of wetting of wheat starch studied.)  
 — *Zeit. phys. Chem.*, 1897, **24**, 193. (Heat of wetting of starch studied thermodynamically.)  
 — *ibid.*, 1900, **33**, 593. (Deduces mathematical expression for heat of wetting of starch.)  
 — *ibid.*, 540. (Specific heat of wheat starch containing 0 to 33.6 per cent. water varies but not uniformly from 0.2697 to 0.3054.)  
 E. PAROW, *Zeit. Spiritusind.*, 1907, **30**, 432. (Specific gravity of wheat, rice, maize and potato starches.)  
 H. GAUDECHON, *Compt. rend.*, 1913, **157**, 209. (Heat of wetting of starch by 16 different liquids measured.)  
 M. W. BEIJERINCK, *Konink. Acad. Wetensch.*, 1915, **13**, 305. (Deposits from cooled solutions of certain starches are crystalline.)  
 W. L. STOCKHAM, *Agr. Coll. North Dakota Exp. Stat., Bull.* **120**, 1917. (Moisture capacity of starch increased by hydrolysis but decreased by dextrinisation.)  
 R. O. HERZOG and W. JANCKE, *Naturwiss.*, 1921, **9**, 320. (Amylodextrin is crystalline, starch acetate amorphous.)

- R. O. HERZOG and W. JANCKE, *Umschau*, 1921, **25**, 53. (Note that starch has crystalline form.)
- SPROCKHOFF, *Zeit. Spiritusind.*, 1922, **45**, 217. (Specific heats of starches and dextrins.)
- R. FUERTH, *Ann. Phys.*, 1923, **70**, 63. (Dielectric constant of starch is 11.6, of dextrin 8.0.)
- E. H. HARVEY, *Amer. J. Pharm.*, 1924, **96**, 752. (Many physical constants of corn, potato and tapioca starches given.)
- C. L. ALSBERG, *Plant Physiol.*, 1938, **13**, 295. (Discussion on structure of starch granule. 112 references.)
- K. HESS and B. RABINOWITSCH, *Kolloid-Zeit.*, 1926, **39**, 300. (Swollen grains have certain amount of inner structure.)
- N. P. BADENHUIZEN, *Protoplasma*, 1937, **29**, 246. (Contradicts Hess and R. concept of granule structure.)
- H. HALL, *Cereal Chem.*, 1930, **7**, 270. (Wheat starch heated one hour at 100° C. with 50 per cent. water remains a white powder but granules swollen.)
- C. B. FALL, *Chem. Age*, 1923, **31**, 37. (Photomicrographs of the more common starches.)
- J. REILLY, P. P. O'DONOVAN and H. MURPHY, *Proc. Roy. Dublin Soc. Sci.*, 1934, **21**, 37. (Mol. wt. determination on amylose separated from frozen potato-starch paste.)
- M. SAMEC, *Biochem. Zeit.*, 1930, **218**, 249. (Discussion of micellar structure of starch.)
- in 'Colloid Chemistry, Theoretical and Applied,' edited by J. Alexander, Chem. Catalog Co., New York, 1932. (Summary of the colloidal chemistry of starch.)
- A. WIELER, *Protoplasma*, 1938, **31**, 370. (Mechanism of the formation of starch granules discussed.)
- L. H. PULKKI, *Cereal Chem.*, 1938, **15**, 749. (Particle size and structure of wheat-starch granules.)
- W. I. NASAROV, *J. Appl. Chem. Russ.*, 1939, **12**, 1745. (Temperature of dissolution of potato starch lowest after 1 day's action of 2N HCl. Further treatment raises it.)
- P. A. PCHELIN, *Colloid J. (U.S.S.R.)*, 1939, **5**, 741; via *Chem. Abstr.*, 1940, **34**, 924. (The contact angles between liquids and starch gels measured.)
- E. H. ZUBASCHENKO, *Khim. Referat Zhur.*, 1939, **11**, 12. (Effect of various compounds on size of contact angle between solution and starch film.)
- M. SAMEC, *Chem.-Ztg.*, 1939, **63**, 204. (General.)
- N. V. IVANOV, M. M. KURTGATNIKOV and V. A. KIRSANO, *Enzymologia*, 1938, **4**, 163. (Structure of starch in same species varies with conditions of growth.)
- W. W. LEPESCHKIN, *Protoplasma*, 1938, **30**, 309. (Discussion of chemical structure of the starch grain.)
- M. SAMEC, *Kolloid-Zeit.*, 1940, **92**, 1. (X-ray, colloidal and physico-chemical aspects of starch.)

## CHAPTER 5

## THE SWELLING AND GELATINISATION OF STARCH

**The Swelling of Starch.**—Starch swells when treated with cold water, and the swelling is reversible. If the water be heated, the granules swell to many times their original volume and gelatinise. Thus on treatment with water starch exhibits two distinct phenomena:—

(a) Real swelling in cold water, in which the absorption or loss of hygroscopic water is attended by swelling or shrinkage, and

(b) Gelatinisation in hot water above certain temperatures, whereby the starch cannot be recovered from the suspension in its original form.

Ordinary air-dried starch contains a varying and considerable amount of moisture, depending on the origin of the starch and the humidity of the air. Wheat starch, on the average, contains about 13 per cent. moisture, whereas potato starch contains about 20 per cent. If starch is intensely dried, small fissures are sometimes observed in the granules, and the author has noted that these are very pronounced if the starch is dried in a vacuum. Samec has noted that such intensely dried starches are more susceptible to the influence of hydrolytic agents than ordinary unprocessed starch, and this may be ascribed to the easier access to the granule interior that these small fissures allow.

The reason why starch is insoluble in cold water but disperses in hot water appears to be connected with its crystalline structure. The crystals are arranged axially from the hylem but, although they are not touching, free access of water is probably prevented owing to the formation of oxygen bridges between the nearly parallel crystals. These bridges may be too strong to be broken by cold water, but raising the temperature may bring about the breaking of a few, allowing the starch to swell and thus throwing a heavier strain on the adjacent oxygen bridges which are then easily broken. Ling and Mehta have postulated the presence of a thin membrane of hemicellulose around each granule which may tend to restrict swelling and enzyme action (see p. 305). Gortner and Hamalainen,<sup>87</sup> from enzymatic studies on raw starch, also consider a membrane, but of protein, may surround the granules.

The hygroscopic water is very difficult to expel. Bloch<sup>1</sup> states that a temperature of 155-160° C. is required to remove the last traces of water, and gives a method of estimating water based

on the fact that a given weight of starch occupies a definite volume at maximum hydration.<sup>2</sup> If starch be left in a sufficiently damp atmosphere, it may take up as much as 36 per cent. water. When the vapour pressure of a substance which swells in water is measured and plotted against the amount of water present, the curve obtained is of the  $f$  type, and thus a ready means of studying the swelling of starch is available. With starch the curves so obtained on hydration and on dehydration do not lie one on the other, but show a lag behind the true amount of water present, i.e. the curves show hysteresis. The same phenomenon is shown by starch films, and can be examined by the technique of Farrow and co-workers.<sup>35, 36</sup>

F. Ullick<sup>3</sup> noted a rise of 3° C. when air-dried starch was mixed with its own weight of water, and a rise of 13.8° C. for a sample previously dried at 120° C. C. A. Winkler and W. F. Geddes<sup>4</sup> have also studied the heat of hydration or water-absorption of wheat starch and found that samples prepared from different wheats gave essentially the same heats of hydration. They separated potato starch into two portions, one containing all the large granules, the other all the small, and found that there was no significant difference between the heats of hydration of the two samples. They consider that the water actually permeates the granules of the starch. The heats of hydration of rice, wheat and potato starches were measured by these methods and found to vary widely at the same water-content and to increase in the order given. The critical moisture-holding content of tapioca starch has been shown by S. B. Etorina<sup>48</sup> to be 19 per cent.

The amount of heat liberated by wetting dried starch with water varies with the amount of water used, and if the heat evolved is plotted against the amount of water added a parabolic curve is obtained. From measurements carried out to ascertain the entropy changes in such a system, it may be shown that the first water added to starch is taken up in a regular manner as a hydrate, i.e. in the same manner as water of crystallisation, and this has led J. R. Katz and his co-workers<sup>5</sup> to suggest that this water is bound as in a zeolite, a suggestion which they also support with evidence obtained by their work on the X-ray spectroscopy of starch. G. Centola,<sup>49</sup> from his studies on the hydration of starch, considers that the molecules must have a zig-zag structure.

However contradictory the above work may appear, we may safely conclude that starch consists of two substances, one of which gives viscous pastes with water, and the other gives clear mobile solutions. These constituents are further differentiated in that one is more resistant to the action of acids and enzymes



than the other. Both are present in varying proportions in the different starches, and the relationship between them has already been discussed. We may now pass on to an examination of the behaviour of starch when treated with hot water.

**The Gelatinisation of Starch.**—On heating with water above certain temperatures, the various starches form viscous pastes. The ease of formation of these pastes often varies with the previous treatment of the starch, i.e. whether during manufacture it has been subjected to the action of acid or alkali in excessive amounts, or for excessive periods of time. The viscosity of such pastes is generally lower than normal, and allows of a more thorough agitation and dissemination of heat when the pastes are being made. The fact is of interest where the pastes are intended as adhesives, or for sizing yarn, or dressing textiles. A further factor in determining the viscosity of a starch paste is the drying-treatment accorded to the starch during manufacture. One which has been dried at a very low temperature generally shows a greater viscosity when made into a paste than one which has been quickly dried at an elevated temperature.<sup>6</sup>

When granules of potato starch are suspended in water and the temperature of the suspension slowly raised, they first swell slowly, then, at a certain point, a small hole appears at the hilem. This hole does not make its appearance simultaneously in all the granules. The hole increases in diameter as the heating is continued, and the granule swells up to many times its original volume, and loses its birefringence. The granule now appears as a gelatinous sac or vesicle filled with an aqueous solution of amylose, which can be stained with iodine solution. The Brownian movement can be observed in the interior of the sac, and the solution gradually diffuses through the gelatinous wall. This is demonstrated by adding a concentrated tannin solution to a fresh preparation, when a precipitate is formed inside the sac, but a preparation several days old does not show this. When the heating is still further continued the sac bursts. The latter effect does not happen with all starches, but can readily be observed in potato starch, which gives a solution containing amylose and particles of the sac in suspension. Granules of wheat starch do not appear to burst or lose their identity; the paste made from this starch remains full of large gelatinous vesicles, and thus explains to some extent why wheat-starch pastes do not penetrate textiles but tend to lie on the surface. It also explains the variations in viscosity obtained from starch pastes, especially from potato starch, on stirring; the structure of the granules is being broken down, and so they take up less space and the viscosity falls. To observe the swelling of

starch when heated with water under the microscope, a suspension of one part of starch in two hundred parts of water is very convenient. The examination may be carried out by heating a suspension and examining withdrawn drops under the microscope, or the suspension on the microscope slide may be heated electrically,<sup>7</sup> or by means of a current of hot water<sup>8</sup> in contact with the underside of the slide. Different workers give varying temperatures at which the various starches gelatinise; this is not only due to the different conditions of experiment, but also because some workers take the gelatinising point as that at which anisotropy disappears from all the granules, whilst others take it to be when the majority of the granules show loss of anisotropy. L. Heintz keeps suspensions of starch at different temperatures and then filters. When the filtrate shows a blue iodine colour he considers the starch has commenced to gelatinise. Thus he finds rice starch starts to gelatinise at 55° C. and that the process is complete at 75° C. Furthermore, starches from different varieties of the same species of plant often show variations in their gelatinisation temperature—thus Alsberg and Rask<sup>9</sup> found this to be the case with wheat and maize starches, and their observations on the latter starch have been confirmed by Dox and Roark,<sup>7</sup> who examined thirteen varieties and obtained gelatinisation temperatures ranging from 64.1° to 71.1° C. S. G. Willimott<sup>50</sup> has studied the gelatinisation temperatures of maize, potato and colocasia starches. Alsberg also found the viscosity of the mucilages prepared from the different varieties to vary considerably. It should be emphasised that every granule of starch has its own gelatinisation temperature, which may be called its 'specific gel temperature,' at which it is fully swollen and no longer shows anisotropy. In the same starch suspension the larger granules appear to swell at a lower temperature than the smaller granules, and it is interesting to note that, other factors being equal, a delivery of starch with a smaller average particle size than normal generally requires a somewhat higher temperature for conversion to dextrin and gives a more opaque paste unless boiled for a considerable time, whilst a delivery having a great majority of larger sized granules than usual is more readily converted. This may be due to the ratio of amylose to amylopectin being different in the large granules from that in the small granules, but the subject has not been sufficiently studied as yet; it will be of interest when a precise method of estimating this ratio has been evolved (see pp. 37, 89).

**The Use of Swelling Agents to Study Gelatinisation.**—Instead of heating a starch solution to observe the swelling, use

may be made of the effect of certain salts and alkalis which lower the specific gel point so much that at ordinary temperatures the effect can readily be obtained and observed. The following compounds,<sup>10</sup> in the percentage concentrations stated, can be used for observations at normal temperature (see also p. 374): sodium hydroxide 0.53, potassium hydroxide 0.75, potassium thiocyanate 12-15, potassium iodide 26-28, ammonium nitrate 30-35, silver nitrate 29, chloral hydrate 55. Other salts that may be used are calcium nitrate or chloride, sodium or potassium bromide, or ammonium thiocyanate. Use has already been made commercially of the properties of these substances to produce special starches for various purposes, and further reference will be made to them in the appropriate sections. The addition of soap to the water used for swelling starch raises the gel point progressively, and if sufficient is added it inhibits the swelling completely. Penick and Ford<sup>38</sup> have patented the use of soaps, sulphonated tallow, vegetable oils, and hydrogenated phenols, as inhibitors of swelling, to obtain a modified starch. The starch is heated in water containing the inhibitor until the anisotropy of the granules disappears. The swelling of starch and of gelatine also has been studied by J. R. Katz,<sup>11-13</sup> using swelling agents of the organic type, i.e. thio-urea, resorcinol, benzene sulphonic acid or chloral hydrate, etc. He finds that compounds containing two hydrophilic groups show a somewhat weaker swelling action than those containing only one, and ascribes this to the weaker adsorption of the former. In the presence of a single hydrophilic group the swelling action on potato starch is in the order phenol > aniline > benzoate > benzene sulphonic acid. The length of the molecule also appears to play some part in the swelling action where two compounds of the same empiric formula, the same molecular weight and containing the same hydrophilic group, are concerned. Thus para-diphenyl sulphonate exerts a much greater swelling effect than naphthalene-sulphonic acid. With alkylamine sulphates and bromides a 'lyotropic' series of the alkylamine cations is obtained, and the curves show that the effect is stronger the longer the alkyl group; and the preventive effect of the sulphate is weakened, the greater the number and lengths of the alkyl groups in the amine. The effect of the amine-group is additive to that of the sulphate. The greatest effect ever observed by Katz and co-workers<sup>39</sup> is given by triheptylamine bromide.

J. R. Katz and J. Seiberlich<sup>70</sup> have examined the influence of mixtures of lyotropic agents on the pasting temperature and showed that, with one or two exceptions, the curve for a mixture came between those for the components. The clear, viscous

solution obtained by the action of formamide on starch has been studied by several workers.<sup>72</sup>

The endothermal effects during the gelatinisation of potato starch suspensions in sodium and potassium chloride solutions and in various acids have been studied by V. I. Nazarov and A. V. Nikolaev.<sup>71</sup>

Starch appears to follow the same rules as gelatine and the proteins as regards swelling brought about by the presence of salts. The influence of the anion on the swelling is marked and unmistakable, some anions merely producing a slight swelling whilst others act so powerfully as to cause the starch to swell and gelatinise in the cold. C. E. Mangels and C. H. Bailey<sup>14</sup> used the hydroxides, thiocyanates, iodides, and chlorides of potassium and sodium, and some of the alkaline-earth salts, and followed the swelling by determinations of the viscosity of the suspensions, using the Ostwald-pipette type of viscometer. In general, the swelling of the starch increases with the concentration of the salt present and a considerable variation in the power of the different reagents may be noted. The anions fall into a Hofmeister lyotropic series, being arranged in the order:  $\text{Cl} < \text{Br} < \text{I} < \text{CNS} < \text{salicylate} < \text{OH}$ . If the effect of the cations be examined, it is found that they exhibit some effect other than that shown by a lyotropic series. The presence of small amounts of electrolytes, even in such small amounts as are present in tap water,<sup>15</sup> affects the viscosity of starch pastes adversely; and E. Wiegler<sup>16</sup> has also shown the disproportionate effect on the viscosity of these pastes exercised by minute amounts of electrolytes.

J. R. Katz and co-workers<sup>47</sup> have recorded the relationship between the pasting temperatures of potato starch and the molar concentrations of electrolytes and various acids added to the water. They examined the chlorides of  $\text{NH}_4$ , Li, Na, K, Be, Mg, Ca, Sr, Ba, Al, and those of their sulphates which are soluble, and the thiocyanates. Beryllium chloride was without effect in the range examined (0.5-3.5 molar), but all the other chlorides depressed the pasting temperature. All the sulphates increased the pasting temperature, but the thiocyanates had a remarkable depressing effect.

Depression of the pasting temperature sets in at the following molar concentrations of acid: with nitric and hydrobromic about 0.5; hydrochloric, about 0.8; phosphoric, about 1.7; and sulphuric, about 4.5. All the organic acids cause depression; the effect of citric, tartaric and acetic acids was slight, but a strong effect was noted with the chloroacetic acids which increased with the number of chlorine atoms.

As will be discussed later, on heating a starch suspension to the boiling-point, and maintaining it at that temperature for some time, a rise in viscosity to a maximum is observed, followed by a fall to a minimum, but ultimately rising to a final value on cooling it. R. A. Gortner<sup>17</sup> has studied the gelatinisation of starch, and considers that the continual boiling greatly reduces the hydration of the starch, thus leading to a fall in viscosity. He thinks that this explains why the viscosity of potato-starch pastes falls more rapidly than that of wheat starch. It must be remembered, however, that the gelatinised vesicle of potato starch is more fragile than wheat starch, and therefore much more readily broken down, leading to a decrease in viscosity. The above worker considers that gelatinisation in the cold, using swelling agents, differs from gelatinisation brought about by heating, but that the mechanism of the cold swelling is the same whichever agent he used. The gelatinisation involves at least three stages according to this worker. At low concentrations of the chemical no hydration takes place, but as the concentration is raised there is a rapid increase in the swelling of the granules, and finally rupture takes place followed by the peptisation of the particles thus produced with a concomitant increase in volume. As the concentration is still further increased, a point is reached where it causes a slow osmotic dehydration.

**Methods of Following the Course of Gelatinisation of Starch: Optical Methods.**—When a starch granule gelatinises four phenomena occur: swelling, loss of anisotropy, the power of injured granules to take up certain dyestuffs,<sup>18</sup> and solubilisation. Several workers have explained anisotropy by postulating that there are small crystals in starch which are subjected to internal strains, and that these strains are relieved when the granule gels, so that anisotropy disappears.

Swelling, loss of anisotropy, and staining of the granules with dyestuffs, such as Congo red, can be followed visually, but as about 300 granules have to be examined at each reading, and such readings are not continuous, points like these constitute a drawback to visual methods. Such methods have, however, shown that each granule has its own temperature of gelatinisation, and therefore it is not sufficient to obtain the gelatinisation point of a sample of starch in order to differentiate between different starches.

If the temperature of a starch suspension be increased slowly at a constant rate, samples may be withdrawn at successive increments of time, and the temperature need not be measured directly. If such heated samples be examined under a microscope fitted with crossed Nicol prisms, those grains which have lost their

anisotropy can readily be seen except where they are too small to show this effect. Carrying such an experiment to its end-point, we should finish with all the granules gelatinised, and if the number of granules gelatinised in each sample is plotted against the time of heating of the sample, and hence against the temperature, since this increases at a constant rate, an *f* shaped curve is obtained, as shown in Fig. 10.

At the point A on this curve only the very largest granules have just started to gelatinise, whilst at the point B the largest of

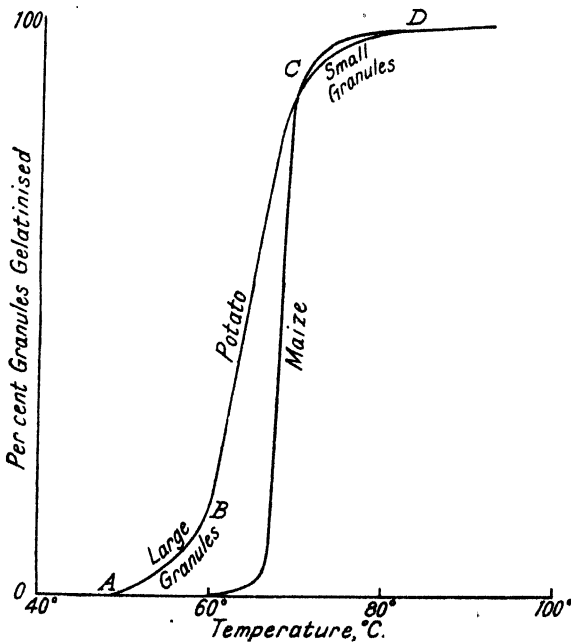


FIG. 10.

the granules of average size have begun to lose their anisotropy. From point B to C the curve shows that within a short period of time, or a narrow temperature range, the granules of the average size forming the bulk of the mass have gelatinised, and from C to D the smaller granules are affected. The temperature at C corresponds to the gelatinisation point, as recorded by those workers who take the point at which the anisotropy disappears from the majority of the granules.

This curve gives some information about the previous history of the starch, and it will be found that although tapioca starches

from different factories give the same form of curve, the gradients are different. A starch that is separated by sedimentation and of first quality will show a steep gradient, whereas a starch separated by centrifugal methods has a less steep gradient, and is spread out more. Fig. 10 shows the *type* of curve for starches of this kind. This may be expected, as a centrifuged starch contains all the granules of all sizes, each having its own gelatinisation point, whereas many of the granules of a first-class sedimented starch are large and the size is more uniform, so that they all gelatinise within a narrow range of temperature.

As the size of the granules appears to influence the speed of dextrin formation in the roasting process, this curve also gives information about the ease with which roasting can be brought about; the steeper the curve the less complicated the process.

As injured starch granules can be stained with Congo red, this property can be utilised to follow the gelatinisation, instead of using the loss of anisotropy as the criterion, and the same type of curve is obtained as before.

Another method of following the gelatinisation of starch is to suspend 5 gm. of starch in 100 ml. water, heat at a definite rate to a definite temperature, cool, make up the volume of the suspension to 250 ml., and then allow the suspended matter to settle at 15° C. in a graduated cylinder. When settlement is complete, the volume of the settled material is read and plotted against the temperature. This is repeated with a number of samples and curves similar to those shown in Fig. 11 may be obtained. The curve marked A represents the type given by potato starch, and it will be seen that it rapidly reaches its maximum volume, and therefore at this point the viscosity of the paste is in the region of the maximum obtainable for this figure. The curve B, which shows the type given by wheat starch, is much more extended, and conforms with the fact that pastes of this starch take longer to reach their maximum viscosity.

Another method but recently introduced by Cook and Axtmayer<sup>65</sup> makes use of a photoelectric cell to measure the increase in the amount of light passing through a starch suspension as it is gradually heated.\* A 60-watt tungsten lamp is used as the source of light, fed from a Delta transformer to smooth out line voltage fluctuations. The light is focused by a condenser lens on to a flask containing a suspension of the starch under examination. The flask is wide-necked, fitted with a thermometer and a stirrer, and is immersed in water contained in a litre pyrex beaker, which is heated by a three-stage hot plate. The beaker has around it black paper in which are cut two holes,

\* See p. 57.

diametrically opposed, of 3.75-5 cm. diameter, through which the light enters and leaves. On leaving, the beam is caught by a copper oxide or photronic type of photoelectric cell, connected directly to a microammeter reading from 0.50 microamperes.

To make a determination the water in the beaker is heated to about 40° C., 1-2 gm. air-dried starch suspended in 25 ml. water is poured into the flask, and the temperature adjusted so that it rises 0.5° C. per minute. Observations are taken from this point until the readings of the microammeter have become constant or boiling-point is reached.

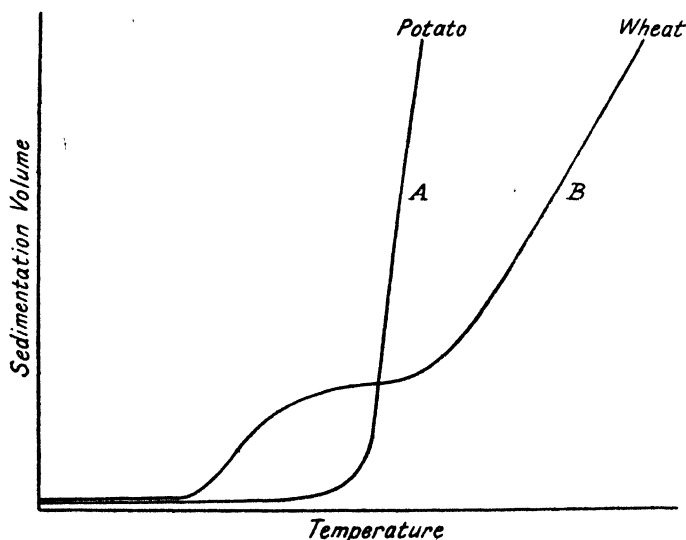


FIG. 11.

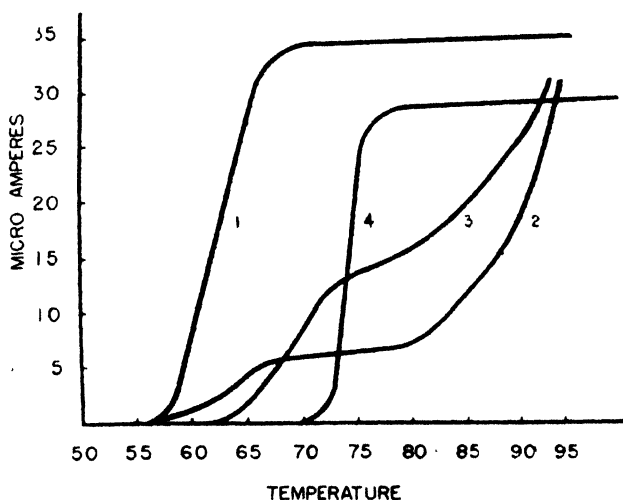
The readings may be plotted to give smooth curves (see Fig. 12), since the current in the cell is a linear function of the light transmitted, and the authors call the temperature at which an increase in transmission of light is shown the 'transition temperature.' This point and the rate of gelatinisation, as shown by the curves, are reproducible and characteristic of particular samples of starch. They may be used to identify the starch if the same process of manufacture is used for the sample and the standard, but treatment of a particular starch with acids or alkalis, or drying over desiccating agents, or at 50-60° C., modifies the temperature of transition and the slope of the curve.

A constant rate of heating should be observed, as these workers find that this factor affects the transition temperature but not the



slope of the curve; thus by changing the rate of heating from 6.3 to 0.8 minutes per degree rise in temperature, the transition temperature of a cassava starch was raised 3° C. The swelling of starch may therefore lag behind the temperature when this is raised too quickly; the author has also found this to be the case, more especially with maize and rice starches.

Yantfa starch was separated into two fractions having an average particle size of 4 and 12 microns respectively, but no difference was observed between the two curves obtained from these fractions. The above workers consider, however, that small differences might be shown by the curves obtained from the large and small granules



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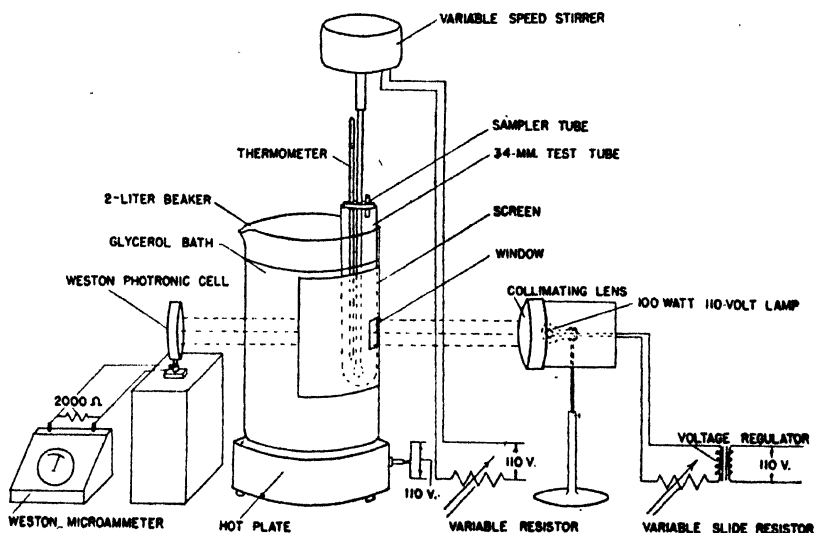
FIG. 12.

of wheat starch. The fact that taro starch, with an average particle size of 2 microns, gives exactly the same type of curve as edible canna starch is held by these workers to indicate that the granule size has no influence on the rate of gelatinisation. The large and small granules may have different temperatures of transition, but apparently they gelatinise and rupture at the same rate.

Another point of interest in this work is that tropical starches were found to differ characteristically from northern starches. Northern or temperate-zone starches showed a transition temperature between 55° and 65° C., and gave gently rising curves showing a moderate increase in transmission, followed by a latent period or slowing down of the rate of increase of transmission between 65° and 85° C., and finally exhibiting an increased rate

of transmission up to  $90^{\circ}$  to  $95^{\circ}$  C. On the other hand, tropical starches show a transition temperature between  $60^{\circ}$  and  $78^{\circ}$  C., with curves rising fairly steeply until the transmission becomes constant, which occurs most often at a temperature below  $85^{\circ}$  C. Figure 12 shows in a diagrammatic way the types of curves obtained with arracacha (1), wheat (2), potato (3) and taro (4) starches when examined by this method.

Cook and Axtmayer confirm the observations previously made by Sjostrom,<sup>32</sup> Taylor, and Schoch,<sup>31</sup> that granules of tropical starches rupture completely below  $85^{\circ}$  C., but that with white



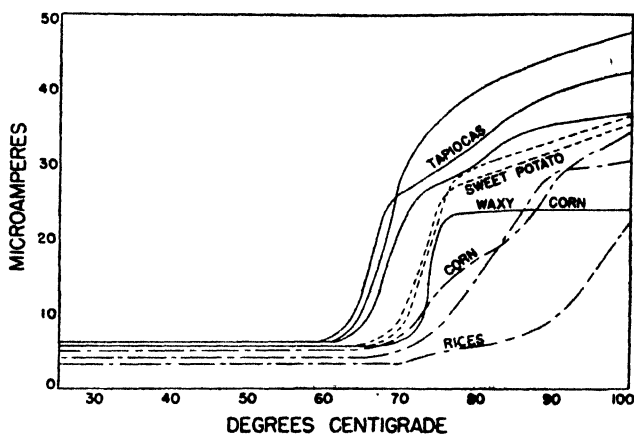
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FIG. 13.—Photopasting apparatus.

potato, wheat, barley, maize, and rice many swollen but unruptured granules may be observed in their pastes even at temperatures between  $90^{\circ}$  and  $95^{\circ}$  C., a peculiarity which they attribute to the fragility of the outer sac of tropical starches. They found that a plant can acclimatise itself in such a way that the starch giving one type of curve tended to give the other type when grown in a different zone, so that a variety of potato grown in the north yielded starch which gave a curve of the temperate-zone type, but when the same variety was cultivated in a tropical zone the starch obtained from it gave a curve tending towards the tropical zone type.

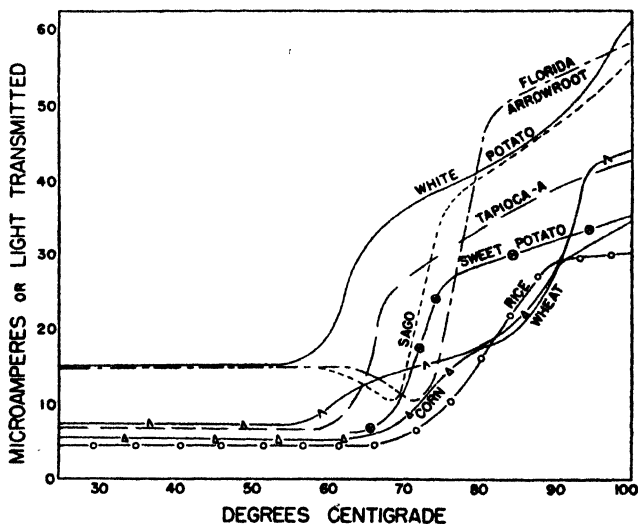
W. L. Morgan has developed Cook and Axtmayer's method, and Fig. 13 shows the apparatus used. The test requires

only 0.33 grm. of sample, the starch being suspended in 65 c.c. of water contained in the large test tube, the stirrer and the



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FIG. 14.—Variations in raw starches.



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Pasting begins at: white potato, 55°; wheat, 56°; tapioca A, 61°; rice, 63–70°; corn, 64°; sweet potato, 64–67°; sago, 69.5–70°; Florida arrowroot, 72° C.

FIG. 15.—Pasting of raw starches.

glycerol-heater started and the rate of heating adjusted to give a rise of 2.5° C./min., so that a test is completed in about 30 minutes.

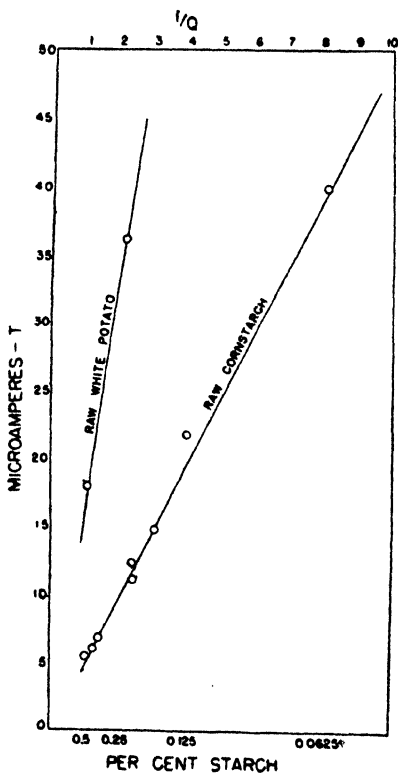
The various raw starches give characteristically different transparency/temperature curves (see Fig. 14), and Morgan considers that easy and positive identification of raw starches is possible owing to the characteristic shape and location of the curves. The larger grained starches at the low dilution of 0.5 per cent. are initially less opaque than the smaller-grained cereal starches (Fig. 15). Just before the onset of pasting the breaking apart of small grains of sago and arrowroot into the individual granules causes a small increase in opacity and this behaviour is characteristic of these and other sticky types of starch. Tuber starches show steeper curves than grain starches, owing to the shorter gelatinising range, and they show the greater transparency characteristics of pastes from this type of starch.

Wheat starch has two types of granule, the large readily pasted type and the small more resistant type, and this is brought out by the pasting curve for wheat starch. According to Fig. 14 the grain size distribution of raw rice starches has a definite effect on the pasting curves, which is what might be expected.

Duplicate tests by this method give curves in very close agreement. 'Waxy' corn starch gelatinises completely over a very narrow temperature range below 80° C.,

showing that it is very desirable for certain types of work, and its curve shows that in paste characteristics it is very similar to sweet potato and tapioca starches. The pasting curves of the several grades of raw tapioca starches show variations from each other although they are of the same type.

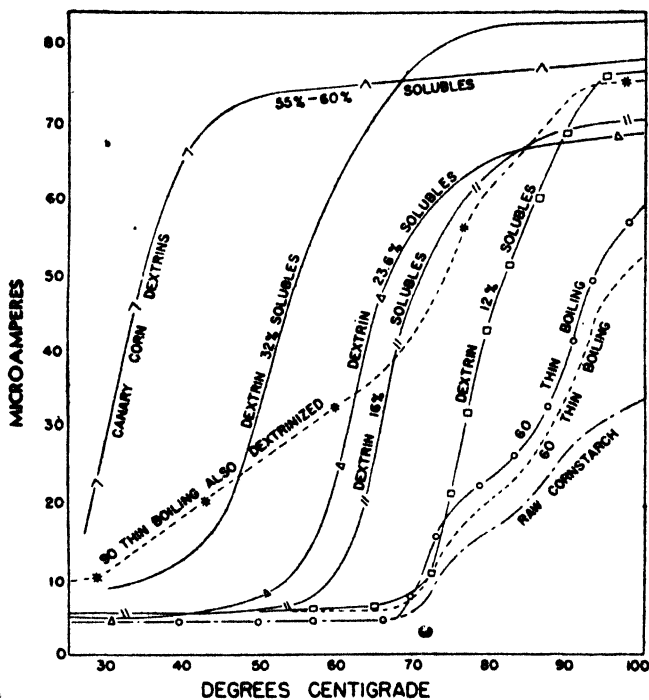
Fig. 16 shows the relationship of transparency to the amount



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FIG. 16.—Opacity of unpasted starches.

of unpasted starch in suspensions of different concentrations, and it will be seen that it is a hyperbolic or inverse ratio function. This also holds for the simpler pasted starches. Acid thin-boiling starches (Fig. 17) give the same form of curve as raw starch but the initial pasting temperature and general location of the curve have, however, shifted upwards. Lower initial pasting temperatures and greater final transparency is, however,



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FIG. 17.—Corn dextrans and thin boiling starches.

given by dextrans, the shifts altering progressively with increase in the degree of conversion. It is interesting to note that the 90 thin-boiling starch, which has also been dextrinised, gives a curve indicative of both treatments. Tapioca dextrans and oxidised starches (Figs. 18, 19, 20) likewise give progressive shifts in the location of the curves as the modification is increased. Fig. 21 illustrates the use of this method to determine the approximate composition of a mixture of starches. It is assumed that the curve for the sample is the additive resultant of the components,

and as the reciprocals of the light transmission are proportioned to the amounts of starch present, the composition can be calculated from these quantities. The corn and wheat curves cross at 80° C., i.e. have the same transmission at this point, so that they may be regarded as a single component so as to calculate the amount of tapioca starch present. The curve of the sample will, therefore, deviate from this point in a manner depending on the amount

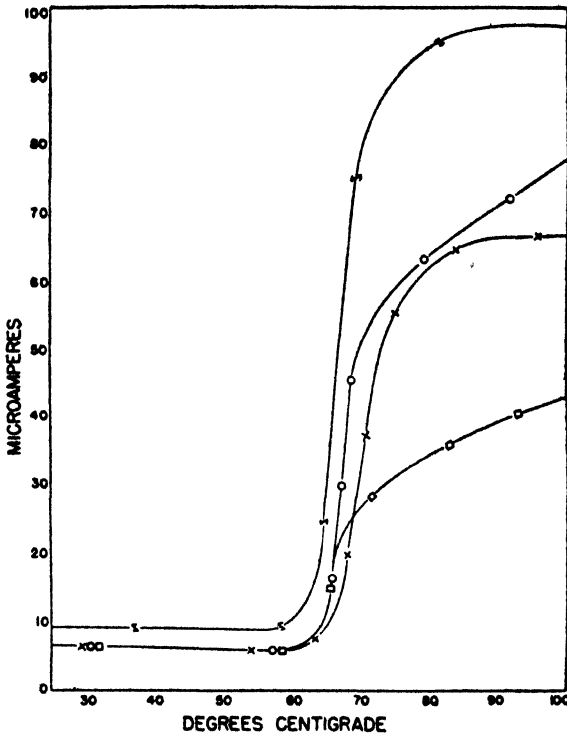
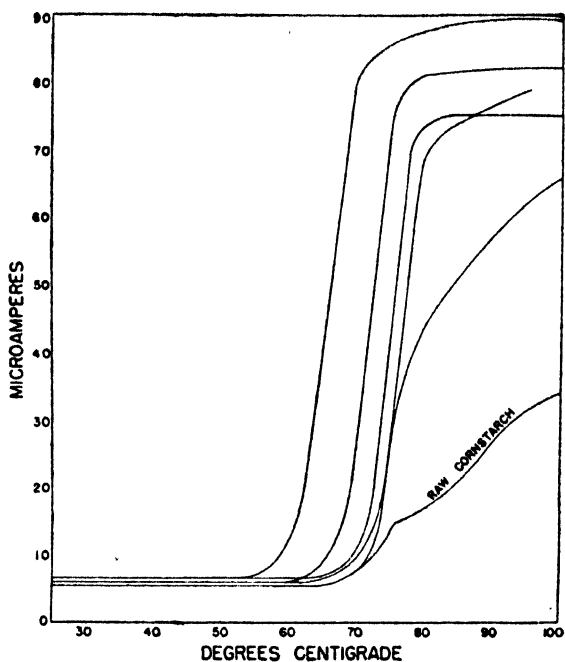


FIG. 18.—Pasting of tapioca dextrins.

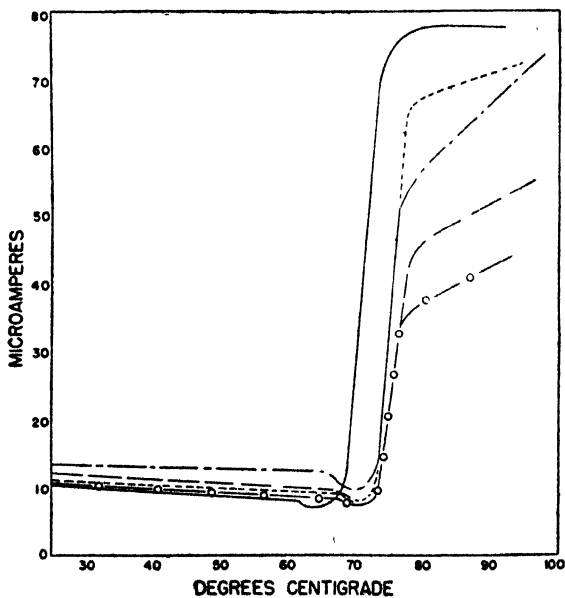
of tapioca starch present. The difference in the reciprocals of transmission at 80° C. allows one to estimate the percentage of tapioca starch, this being related to the extreme difference in reciprocals of the corn-wheat point at 80° C.

Thus a figure of 43 per cent. tapioca is indicated by the transmission values at 80° C., or if the point at 89.50° C. is taken, where wheat or corn curves again show the same transmission, a value of 45 per cent. is obtained. In the same manner at 66° C. the curves for tapioca and the unknown sample cross, and the



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FIG. 19.—Pasting of oxidised corn starches.

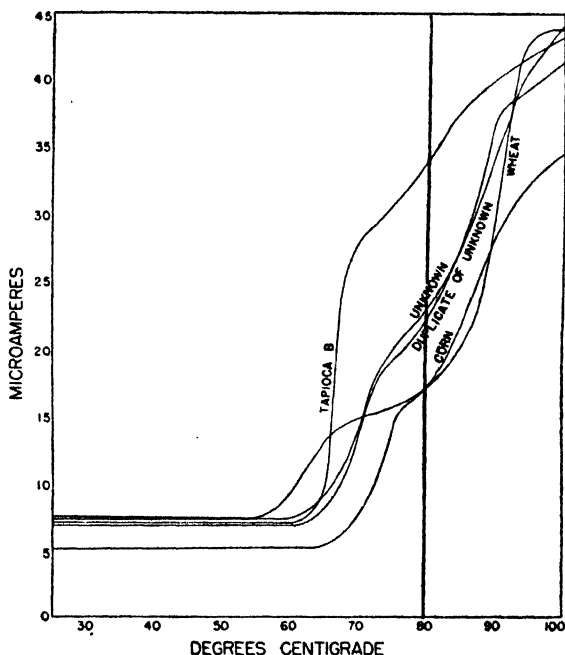


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FIG. 20.—Pasting of oxidised tapiocas.

maize can be calculated and a figure of 40 per cent. is obtained. The curve of a mixture of 40 per cent. tapioca, 40 per cent. corn and 20 per cent. wheat starch is close enough to show the validity of the above method for matching unknown samples.

The method is only applicable where swelling but not solution or break up of the granules is encountered, as in the case of



At 80° C.	T	1/T	
Raw wheat }	17.0 ma.	0.0588	0.0588
Raw corn }			
Raw tapioca	33.0	0.0303	
Extreme difference		0.0285	
Unknown sample	21.5	0.0465	0.0465
Lower difference			0.0123
$\% \text{ tapioca} = \frac{0.0123}{0.0285} = 43\%$			
Duplicate of unknown: 20 raw wheat, 40 raw corn, 40 raw tapioca			

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FIG. 21.—Analysis of mixed starches.

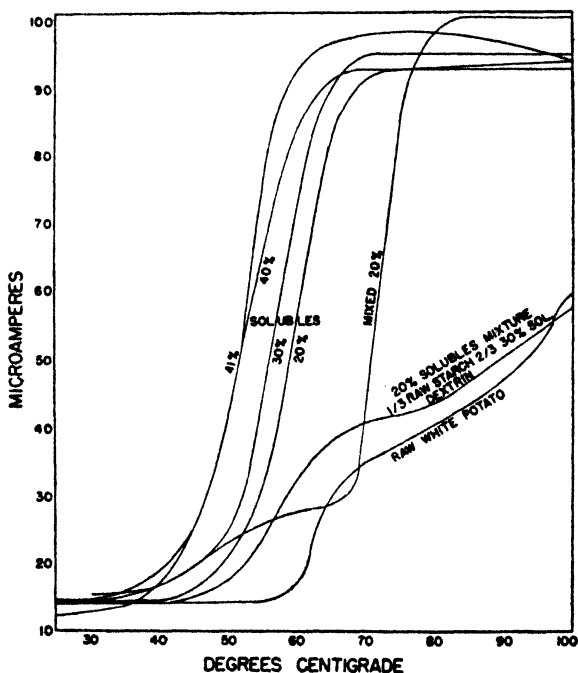
dextrins. Fig. 22 shows that true dextrin of 20 per cent. solubility is easily located, but a mixture of a higher converted dextrin with raw starch does not lend itself satisfactorily to the calculation of composition.

A photometric method of measuring changes in the opacity of starch gels has been used by C. A. Glabau and P. F. Goldman <sup>40</sup>



in their studies on the staling of bread. A. J. Ophof<sup>41</sup> considers that the transparency of cassava-starch gels depends on the grain-size as well as on the concentration, and that the temperature of swelling depends on the concentration.

A. Küntzel and K. Doehner<sup>63</sup> devised an apparatus which automatically records the temperature and the light absorbed, and consider that paste formation takes place in two phases. At the first temperature they consider that a 'melt' is formed of



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FIG. 22.—White potato dextrins.

the starch crystallites together with deformation of the 'fibre' molecule. At the second temperature the hydration of the starch molecules, discharged from their lattice formation, sets in and swelling occurs. They also measured the heat liberated when starch pastes were formed and for potato starch this was  $-36.75$  calories per gm. of dry starch. It includes the heat produced during the 'melting' and deformation stage (endothermic) amounting to  $-39.35$  cals., and that liberated during hydration (exothermic) amounting to  $+2.6$  cals. The heat formed during the swelling of dried starch prior to paste formation was  $17.47$

cal. Katz and Seiberlich<sup>62</sup> consider that in the formation of a paste the starch, when containing a definite excess of moisture, is transformed into another modification which then absorbs water and swells, and X-ray evidence has been obtained by these workers to support this suggestion. W. W. Lepeschkin<sup>64</sup> also supports the two-stage swelling theory, but suggests that hydrates are first formed and these then swell.

**Methods Depending on Viscosity.**—When starch granules swell and gelatinise they occupy a much larger volume in the system than before this process took place, and hence the viscosity alters more or less with the swelling. Einstein<sup>45</sup> deduced a formula for the viscosity of a suspension of rigid spheres in any liquid in terms of the viscosity ( $\alpha$ ) of the pure liquid and the ratio ( $f$ ) of the volume of the suspended matter to the total volume. This was

$$\alpha_1 = \alpha(1 + kf),$$

where  $k$  is a constant, which Einstein first took to be equal to 1, but afterwards made equal to 2.5. A similar formula was deduced by Hatschek<sup>46</sup> for cases in which the suspended material occupies less than 40 per cent. of the total volume. In this case  $k = 4.5$ . This formula was tested by Harrison<sup>19</sup> for swollen starch and water, and found to hold fairly well up to 30 per cent. concentration. The viscosity depends only on the volume of dispersed phase and not on the degree of dispersion.

Harrison<sup>19</sup> has examined the connection between the volume occupied by the swollen granules and the viscosity of the paste or solutions so obtained, working on lines similar to those previously mentioned. He carefully heats a 1 per cent. suspension of starch to a few degrees above the point of gelatinisation of the sample, care being taken not to disturb or break down the structure of the suspension by shaking or vibration. After cooling to 16° C., 15 ml. of the liquid are transferred to a graduated centrifuge-tube, and after centrifuging the volume of the granules is read off. The viscosity of a separate portion, compared with water at 16° C., is also determined, and Harrison finds that the viscosity is equal to 4.75 times one-fifteenth the volume of the granules, as read off the centrifuge-tube, plus unity. Harrison found that maize, wheat and rice starches show a slight increase in viscosity with the length of time of heating, sago starch showing a slight decrease and potato starch, after rapidly acquiring its maximum viscosity, a decrease which takes place in an irregular manner, according to the amount of stirring during the preparation. As the vesicles of the potato starch are comparatively fragile and easily broken, the determination should be repeated several times. This method

serves very well for determining the comparative stiffening powers of different samples of the same starch, but he considers that it cannot be used for comparing different starches, owing to the different times of heating, the different temperatures required to obtain the pastes, and the effect of heating on the viscosity.

H. Geinitz<sup>69</sup> has studied the times of flow, through a 'Tsuda' capillary tube viscometer at 20° C. and with various applied pressures, of 0.2 per cent. solutions of various types of the potato starch and potato starch products. He finds that soluble starch solutions obey the Hagen-Poiseuille law, their viscosity not changing with aging but that chemically treated starch deviates somewhat from the law whilst the greatest deviation is shown by untreated starch. The two latter types decrease in viscosity with age and finally reach a steady value.

G. V. Caesar<sup>20</sup> has studied the problem of the viscosity of starch pastes in a different way, and his results are of especial interest to the practical man dealing with pastes containing 10-20 per cent. of starch. The determination with any degree of accuracy of the viscosities of pastes of these concentrations at normal temperature is limited, and Caesar's work is of interest in that it follows the various changes in the viscosity of starch paste throughout the whole heating up, boiling and cooling processes to which many starches are subjected commercially.

Briefly, he utilises the amount of work done in stirring a starch paste as the measure of viscosity of that paste. The starch suspension is heated carefully, and slowly, up to the boiling-point, and is stirred by a stirrer attached to an electric motor. The electrical input into this motor is a function of the work done by the stirrer, i.e. of the resistance of the paste to stirring. The electrical current is measured by a wattmeter, and in his paper Caesar plots the current consumed against the time, and therefore the temperature of heating. Figs. 23-26 illustrates the type of curve obtained.

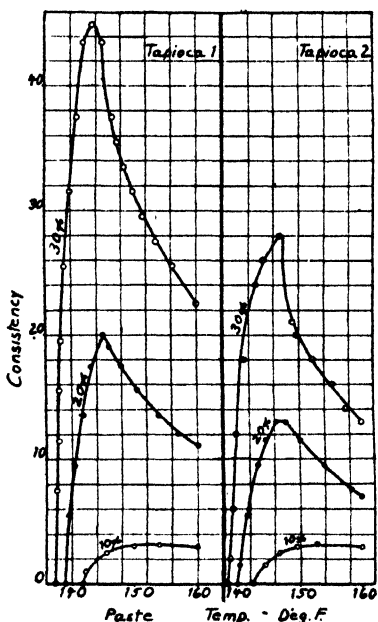
At first the current consumed remains constant until some of the granules begin to gelatinise, when it increases to a maximum and then falls to a minimum during the boiling-period, but again rises as the paste cools down. In Fig. 23, which shows the effect of concentration of tapioca starch on the viscosity of the paste and illustrates only a part of the complete curve, a notable displacement of the peaks will be observed. These displacements are only small, but they are real and significant. With a concentration of 20 per cent. starch, the swollen granules are closely packed, and the shearing strains imposed on them are therefore high; hence there results a quicker breakdown at a lower temperature than with a 10 or 15 per cent. paste.

Another significant feature is the appearance of a dip in the curves after the peak has been passed, which is especially noticeable on curves obtained with the higher concentrations of starch. These are probably due to the delayed rupture of the smaller granules, which are the last to swell, offsetting the effect of breaking down the structure of the paste brought about by heating and stirring. A quicker falling off in viscosity is noted for the higher concentrations than for the lower, due no doubt to the greater disintegration brought about by the higher internal strains set up in the higher concentrations.

When this method is applied to different starches (Figs. 23-26) certain similarities are brought out by the curves. The curve for potato (Fig. 26) resembles that of tapioca, having the same form, and it is seen that wheat, potato and tapioca starches all begin to gelatinise at the same time, but wheat starch gelatinises more slowly than the other two. This may be because the vesicles of potato and tapioca are less resistant to damage than those of wheat starch.

As maize and wheat starch pastes give rather brittle gels on cooling, the curves for these two starches in this region are not the true curves, otherwise the curve for maize starch should cross that of wheat starch and finish well above it. Another factor contributing to this error is the slow rate at which these pastes cool. The cooling-curves for potato, tapioca and sago starches are practically linear, but that for wheat is curved, showing a short-flowing paste.

As the peak values show the swelling power of the starches, Caesar considers that these values can be used to compare different samples of starch. The course of the curves in this case will show the stability to heat and agitation of different samples, factors which may be influenced by the previous history of the starch. One starch may, for example, show a lower peak value



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FIG. 23.—Consistency Record of tapioca-flour pastes at 10 to 30 per cent. concentration.

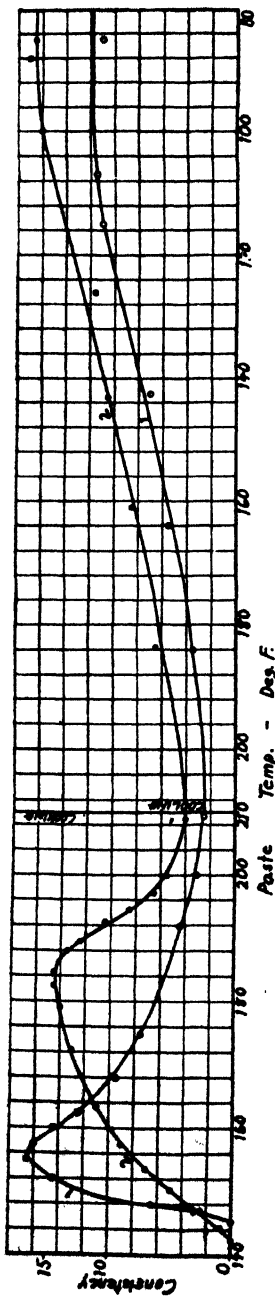


FIG. 24.—Consistency Record of 20 per cent. wheat (1) and sweet-potato (2) pastes.

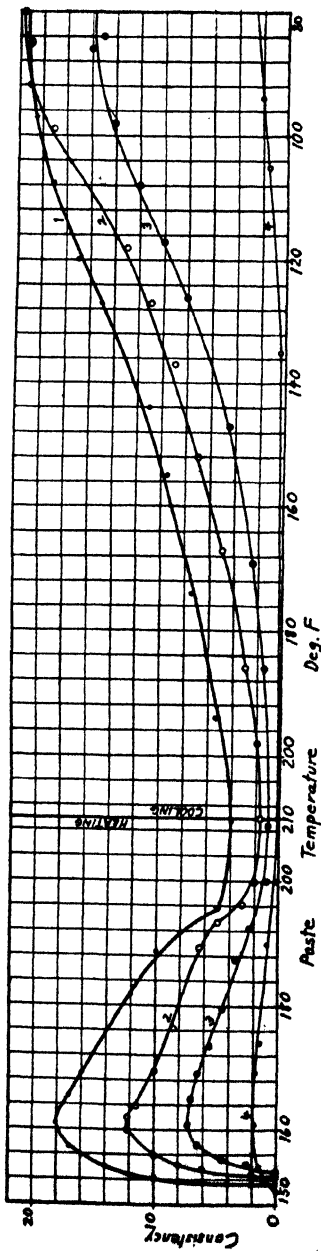


FIG. 25.—Consistency Record of 20 per cent. corn-starch pastes.  
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than another, but the rest of the curve may indicate that it is more stable to heat and agitation. The swelling action of sodium hydroxide on starch has also been followed by Caesar by this same method.

The author has used a similar method to that of Caesar, only keeping the current in the motor constant and counting the r.p.m. Plotting r.p.m. against temperature gave curves similar in shape to those obtained by Caesar, but inverted. The rate of heating greatly influenced the inflection points in the curves, which were not readily duplicated. Maintaining a steady temperature ( $\pm 0.25^\circ \text{C.}$ ) for 20 minutes around the gel temperature for the mass of granules, often gave a series of discontinuous drops in the r.p.m., indicating that some granules either took an appreciable time before they started swelling and then gelled rapidly, or that they were coated by the already gelatinised starch, making the free water difficult of access.

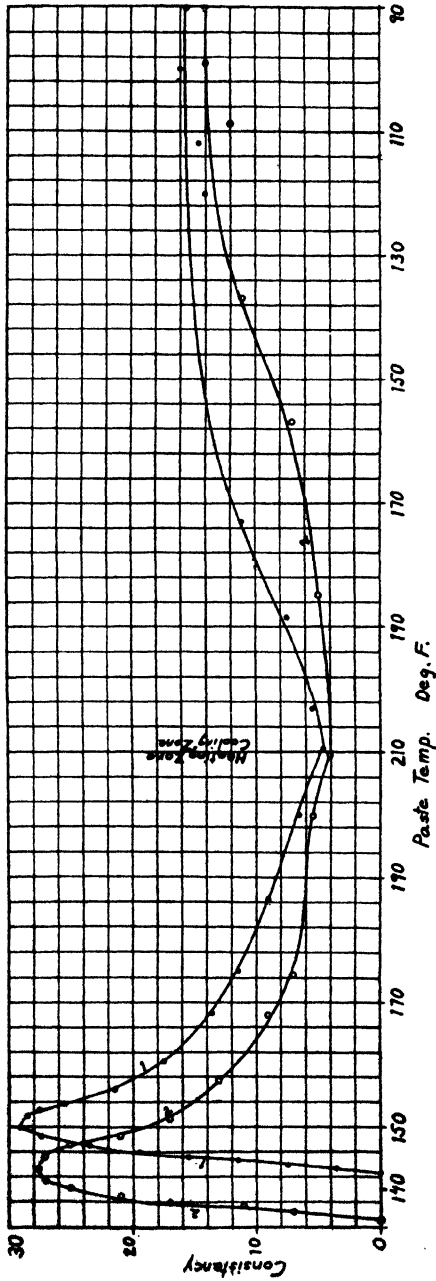


FIG. 26.—Consistency Record of 20 per cent. potato-starch pastes. 1. Imported. 2. Domestic. [Reproduced by courtesy of 'Industrial and Engineering Chemistry'.

It may be noted that H. Herschel and C. Berquist<sup>42</sup> find the Bingham and Green plastometer the best instrument for determining the consistency of starch and dextrin pastes. The rigidity and yield-shear values, as given by this instrument, indicate the 'body,' spreading power, and gel-forming power of starch or dextrin preparations, and should be of interest in the textile and adhesive trades. These results are confirmed by other workers.

Curves for the gelatinisation of several different starches have been given by W. S. Reich,<sup>21</sup> and the influence of particle size on the viscosity of pastes has been studied by several workers. E. Szego<sup>22</sup> found that the best-quality potato starches contain 1.5-6.0 per cent. by weight of the granules having a diameter less than  $20\mu$ , and that 43-44 per cent. by weight of the granules have a diameter exceeding  $40\mu$ . On separating the small granules and determining the viscosity of the paste obtained, it was found to be greater than that given by a paste of the larger granules. It was also noted that such pastes had a lower acidity than those obtained from large granules. J. Grüss<sup>23</sup> made similar observations on barley starch, and found the ratio of the viscosity of the paste given by the original starch to that of the small granules to be 1 : 3 approximately, thus agreeing with the amounts of amylopectin present in the samples as determined by this worker, viz. 12.8 and 34.8 per cent. The specific gravity of the large granules was found by these workers to be 1.526, and that of the smallest granules 1.144. J. Janicki<sup>24</sup> confirms E. Szego's finding that the small grains of potato starch yield more viscous pastes than the large grains. He considers that neither the total phosphorus present nor the amount of non-dialysable phosphorus of the starch influences the viscosity of the paste when potato starches from different sources are under consideration. He determined the amylopectin-content according to the method of Samec, obtained figures for different starches varying from 84.3-89 per cent., and found that the value obtained for any one starch was independent of the size of the granules. A. Fernbach, however, claims that the smaller grains of starch are richer in phosphorus than the larger granules.<sup>25</sup> Sprockhoff and Parlow<sup>33</sup> are other workers who have found that pastes made from potato starch are more viscous when the granules are very small. They point out, however, that it is essential that the starch should be rapidly manufactured and used soon after making. The inferior tenacity and viscosity of smaller grained starch, found in practice is due to the longer treatment required for its preparation and separation. Not only is a more prolonged enzymatic action permitted to take place

at its surface but this area is relatively great in the case of the smaller starch granules.

W. A. Richardson and R. S. Higginbotham<sup>67</sup> separated one sample of potato starch into various fractions. Each fraction contains granules of uniform size but varied from other fractions in the mean radius of the granules. They found the phosphorus-content to increase with decreasing mean radius while the copper reducing powers, specific viscosities in 30 per cent. calcium thiocyanate solution, the solubilities of the fractions in 0.01 M. phosphate buffer solution, pH 7, are almost identical and the differences in swelling capacity in the same buffer solution are small. It would therefore appear that the average chain-lengths are the same and that the granules have the same structure.

The swelling was much higher in distilled than in salt water and the differences in swelling between the various fractions are not due to differences in the volume/surface area ratio of the granules.<sup>29</sup> As the swelling of wheat starch is unaffected by the above factors it is evident that esterified phosphoric acid affects the swelling to an extent dependent upon the amount present, but only when the average chain-length and structure of the granules are the same and no free electrolyte is present.

Janicki also thinks that the amylopectin-content of starch is independent of the total of the non-dialysable phosphorus, and that the non-dialysable phosphorus is not identical with amylophosphoric acid. From this he deduces that the major portion of the phosphorus (91.7-99.7 per cent.) is adsorbed on the starch as colloidal phosphates of Ca, Mg, K, Fe and Na, the rest being undoubtedly held in the form of an ester. He considers that the decrease in the viscosity of starch pastes on ageing cannot therefore be explained by the saponification of the organic phosphoric ester, but is induced by changes in the degree of hydration of the starch and of the colloids present. He is thus opposed to Samec's views (see p. 90), and holds that the question whether starch is homogeneous or not is still unanswered.

**The Effect of Injury to Starch Granules before Gelatinisation on the Properties of the Pastes.**—From what has already been said about the lowering of the viscosity of starch pastes by mechanical treatment, owing to the more or less organised structure of the paste first formed being destroyed, it might be expected that severe injury to the outer portions of the starch granules before making the pastes might have some effect on their viscosity, as the swollen granules would not occupy the same space as those of an uninjured starch. Observations on this point show that these expectations are fulfilled.



C. L. Alsberg and E. P. Griffing<sup>26</sup> have noted that over-grinding of flour resulted in the appearance of injured starch granules which disperse in contact with sufficient water. Scheffer<sup>27</sup> notes that such injured granules are easy to detect with the polarising microscope, or by staining them with Congo red and examining under the microscope in the usual manner. L. H. Lampitt and co-workers<sup>76</sup> consider Congo red to be more suitable for this purpose than iodine as the detail is not obscured using the former (see also p. 372). They note that after 50 hours' ball-milling the granules of wheat starch did not appear to be injured but Congo red then stained them a clear red. Maquenne<sup>28</sup> has found that the soluble matter from the raw starch when acted upon by amylase rises from 2 per cent. for uninjured starch granules to about 94 per cent. for starch which has been severely ground. However, Alsberg and Griffing<sup>75</sup> find little difference in the rate of formation of reducing substances from badly damaged starch and gelatinised starch when these are subjected to the action of enzymes. W. W. Lepeschkin<sup>37</sup> has made the interesting observation that if starch granules are cut up under cold water swelling does not take place. It would appear possible, therefore, that the heat generated by grinding may bring about some change which assists swelling upon subsequent immersion in water. Lampitt and co-workers,<sup>76</sup> however, suggest that the swollen granules obtained by milling wheat starch for 200 hours and then suspending it in water are substantially in the same state as those swollen by heating in water and that gelatinisation, therefore, depends on the appearance of water-permeability of the granules, which has probably been brought about by some change in structure, and is not due essentially to the action of heat. Definite proof of the absence or presence of an outer membrane on the starch granules, which has been suggested by some workers<sup>87</sup> (see p. 309), would be of interest in this connection, and whether it is protein<sup>87</sup> or hemicellulose as suggested by Ling. The observed dependence of the gelatinisation temperature on thermal pre-treatment of the granules supports the conclusions of Lampitt and co-workers.

These workers have made an extensive investigation on the various fractions obtained by ball-milling starch. They obtained three fractions which were arbitrarily fixed by their solubility in cold water (the C.W.S.F.), in hot water (the H.W.S.F.) and a fraction insoluble in hot water (H.W.I.F.). When solutions of the cold or the hot water-soluble fractions were dried the resultant material, in each case, showed lower solubilities upon attempting to redissolve them. Samec<sup>77</sup> and also Friese and Smith<sup>78</sup> have

observed the same behaviour with starches, and Hanes and Catle<sup>79</sup> and Haworth and co-workers<sup>80</sup> with high molecular weight dextrans. The strength of such films appears to decrease with decrease in molecular weight of the film-forming material.<sup>76, 81</sup>

Lampitt, Fuller and Goldenberg find that on ball-milling the X-ray pattern disappears quickly, the organised structure is destroyed and also considerable depolymerisation takes place. The C.W.S.F. increases quite slowly after the X-ray pattern has entirely disappeared and continues to increase, and after 2000 hours' milling wheat starch is almost completely soluble in cold water. It retains, however, the essential characteristics of starch even when milled for 4000 hours. These facts point to the conclusion that depolymerisation and destruction of the granule structure are distinct from each other, and from the observations of these workers on the reducing powers of the various fractions it appears that the milling only ruptures the lateral links between the starch chain-molecules but does not induce breakdown of the repeating units.

During the course of milling the rate of formation of the hot-water soluble fraction from the insoluble portion appears to be very similar to the rate of transition of the former into the cold-water soluble fraction. It is possible that, as suggested by Heiniger,<sup>82</sup> some further depolymerisation takes place during the extraction with hot water. The H.W.I.F. has the characteristics of amylopectin, the H.W.S.F. of amylose and the C.W.S.F. of soluble starch. This work is highly important as it shows the ease with which amylopectin can be depolymerised to amylose, and agrees with the results of Svedberg,<sup>83</sup> Lamm,<sup>84</sup> Richardson<sup>85</sup> and others,<sup>82, 86</sup> which indicate that the methods of extraction and fractionation used to prepare amylose and amylopectin determine the relative proportions of these two compounds in any given sample; the greater the severity of the process the more amylose is obtained. Delffs<sup>51</sup> has utilised the solubility of severely ground starch to prepare solutions of starch.

Advantage has been taken of the fall in the viscosity of pastes from ground starch to obtain the cold-water dispersible starches (see p. 253) commercially, and apparently the greater the degree of severity of grinding to which the starch is subjected the better the dispersion obtained in cold water. The viscosity of the pastes made from mechanically injured starch falls as the extent of the injury is increased.<sup>29</sup> If potato starch is ground in a pebble mill, and samples withdrawn from time to time are made into pastes, their viscosity is found to fall in a fairly regular manner, and

Katz <sup>61</sup> has suggested that such a ground potato starch might give special technical effects if used for sizing yarns.

Starch granules injured by grinding do not show the black cross when examined with a polarising microscope, but they are still birefringent.<sup>30</sup> Solutions of these preparations in cold water deposit a flocculent precipitate on standing in the same manner as a solution made by boiling untreated starch. After grinding wheat starch for several days in a pebble mill about 60 per cent. of it disperses in cold water, and by successive grinding of the residue and extracting it with cold water practically all the starch can eventually be dispersed. This part of the subject is of especial interest to bakers and millers, and has been studied from this point of view by several workers.

Amylose can be separated from amylopectin by means of the grinding technique, the sample being ground until the viscosity of its solution in cold water is practically constant and the solution dialysed. This yields a gel of amylopectin and a solution of amylose. The point at which the viscosity has fallen to the minimum value is readily reached with the gramineæ starches, but the process is more protracted with others. Woodruff and MacMasters <sup>44</sup> point out that starch prepared from frozen corn sometimes gives lower viscosity in solution than starch from unfrozen corn, and indicate that added chemicals affect maize starch more than wheat starch.

S. Ono <sup>68, 88</sup> has made some interesting observations of the effect of ultrasonic waves on starch paste. The disintegration of potato and wheat starch pastes by waves of 470 and 800 kc. per second was followed by observation of the changes in specific volume and microscopically. Potato starch is affected quicker than wheat starch and the disintegration is greater the higher the frequency used. The reduction in specific volume is comparable with that caused by heating to 150° C. Gases dissolved in the paste appear to have little effect and Ono considers that the disintegration takes place due to the mechanical action of pulsating bubbles which, by their adiabatic compression, cause a rise in temperature which, in turn, effects hydrolysis of the starch. The frictional forces caused by the vibration of medium-sized particles, and the changes in kinetic energy of the paste granules appear to have no effect in the early stages of the disintegration but some depolymerisation accompanied by oxidation may occur.

W. Seck and G. Fischer have studied the behaviour of starch solutions at high rates of shear and find that after treatment at 70° C. in a colloid mill the viscosity of the pastes fell by 56-98 per cent. (see p. 254).

**Viscosity and Structure.**—The viscosity of colloidal solutions is influenced by the number and, what is important from our point of view, the shape of the colloidal particles.<sup>89</sup> Abnormally high 'specific viscosities' (see below) are characteristic of colloidal solutions having thread-like micelles or macromolecules present. These macromolecules can form an irregular, ever-shifting network, and the effective length of the molecules and therefore the molecular weight will decide the viscosity, those with the highest molecular weight and greatest length exerting a disproportionate effect on the viscosity compared with the effect contributed by a much larger number of those of lowest molecular weight. In starch solutions where a mixture of polymerides is present this state of affairs is particularly true. As the average molecular weight of the polymerides decreases the abnormal specific viscosity becomes less marked owing to the fact that a network resisting flow becomes more and more difficult to form as the length of the chains present decrease. A critical value will be reached below which any further decrease in the molecular weight will cease to have any effect on the viscosity. The increase in reducing power, which is dependent on the terminal aldehydic groups of the starch molecule, will, however, continue steadily with decreasing molecular weight. Thus, providing a homogeneous solution is used, viscosity determinations can be used to characterise sols of starches and dextrans but only when the molecular weight is high and, as Lampitt *et al.*<sup>90</sup> point out, they should prove valuable where the reducing power is unconnected with molecular weight as in oxidised starches<sup>91</sup> and where the terminal aldehyde groups have been destroyed.

From his work on viscosity Staudinger<sup>89, 92-95</sup> concludes that the starch molecule occupies an intermediate shape between the thread-like molecules of cellulose and the spherical ones of glycogen, and the work of Lampitt, Fuller and Goldenberg (*vide infra*) appears to confirm this conclusion.

Staudinger has found that solutions containing thread-like molecules obey the empirical equation  $\eta_r - 1 = K_m CM$  where  $\eta_r$  is the viscosity of the solution relative to that of the solvent (for starch solutions—water; for rubber—benzene, etc.),  $K_m$  is a constant dependent on the solvent,  $C$  the concentration of the solution and  $M$  the molecular weight. Thus  $(\eta_r - 1)/C$  or  $\eta_s/C$  would be directly proportional to the molecular weight. Staudinger and co-workers,<sup>92, 94, 96-98</sup> Mark and Simha,<sup>99</sup> Hirst and Young,<sup>100</sup> and others<sup>101</sup> find the equation to hold for solutions of starch esters in organic solvents, and Samec<sup>102</sup> has verified

it for aqueous solutions of  $\beta$ -amylose and dextrans of high molecular weight which gave practically the same values for  $K_m$ , as also did potato and wheat starch acetates in chloroform.  $K_m$  was obtained by osmotic pressure data but a number of workers<sup>103-106</sup> have pointed out that this only measures the 'number average,' but that the viscosity of a mixture of polymerides of differing molecular weights will depend on the weight-average molecular weight. For the comparison to be strictly valid only solutions containing material of homogeneous molecular weight can be considered. Samec's value of  $K_m$  cannot be accepted without further experimental data since his data show that the dextrans and  $\beta$ -amylose he used contained polymerides of varying molecular weight.

It is therefore necessary to obtain a value of  $K_m$  by comparing the viscosity data with that obtained using material of homogeneous molecular weight or by comparison with data obtained by the ultracentrifuge in which the weight-average molecular weight is determined.

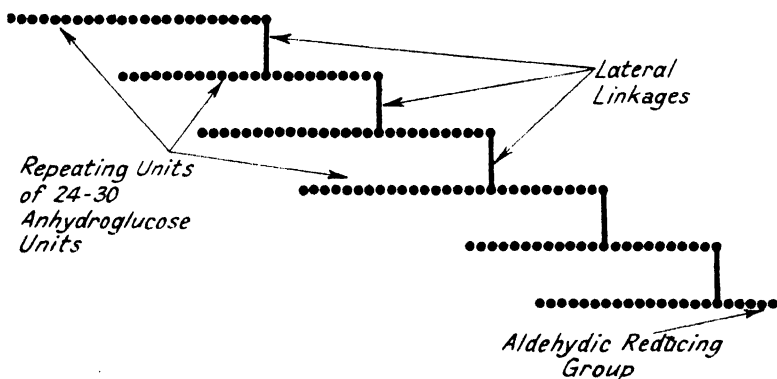
In the absence of any other values for  $K_m$  for aqueous starch solution Lampitt, Fuller and Goldenberg<sup>90</sup> used the expression  $\eta_s/C_g$ , which is directly proportional to the molecular weight for the data obtained on the viscosities of wheat starch fractions prepared by dry milling. They concluded that the molecular weight of the cold-water soluble fraction (C.W.S.F.) apparently remains constant, whilst that of the hot-water soluble fraction (H.W.S.F.), calculated from both viscosity and reducing power data, starts and continues to fall after 50 hours' milling, and that it obeys Staudinger's equation. Although the values of  $\eta_s/C_g$  are constant for the C.W.S.F., the molecular weight, calculated from reducing power data, falling continuously with continued grinding. With one fraction (300 hours' grinding) the reducing power increased slightly on boiling the solution but the viscosity was not altered. This illustrates the point made above that the molecules in this case were no longer thread-like<sup>107</sup> and therefore their influence on the viscosity has ceased to be significant.

The spherical macromolecules of glycogen in solution do not obey Staudinger's rule,<sup>93, 95, 108-110</sup> and solutions of the C.W.S.F. appear to behave similarly. The H.W.S.F., on the other hand, appears to behave like the thread-like molecules of cellulose, which follow the rule.<sup>111</sup> These facts are in accordance with Staudinger's conclusion that the starch macromolecule is midway between glycogen and cellulose in its properties.<sup>92-95</sup>

Fig. 27<sup>112</sup> shows diagrammatically that 'high-molecular' starches would have a definite thread-like character, i.e. the width is small

compared with the length, and that the viscosities would follow Staudinger's rule. With the C.W.S.F., however, as the macromolecules have a much shorter average length,<sup>76</sup> the width becomes comparable to the length, and the Staudinger rule ceases to hold as the molecules are no longer thread-like.

Fig. 28 shows why an aqueous solution of a high molecular dextrin can follow Staudinger's rule.<sup>102</sup> The starch macromolecule is diagrammatically represented as having 11 repeating units, each being a chain of 30 anhydroglucose groups, each unit being connected by a lateral link. In the diagram of the dextrin (a 'high-molecular' dextrin) the repeating units have been shortened from 30 to 12 anhydroglucose units but the lateral linkages are intact. Its molecular weight would thus be about



### Structure of the Starch Macromolecule

FIG. 27.

40 per cent. that of the starch, but it would be more thread-like in nature than starch and would be expected to show a similar relationship between the reducing power and the viscosity of its solutions as has been found for the H.W.S.F. starches by Lampitt *et al.* If the lateral linkages are broken, as shown in the last diagram, then the breadth becomes comparable with the length, and Staudinger's rule is not followed. The inverse relationship, observed by Lampitt and co-workers, between the viscosity of the H.W.S. starch fractions and their reducing power has also been noted by other workers for starches<sup>113</sup> and yellow dextrins.<sup>114</sup> Other workers have shown that viscosity is a function of the molecular weight, or of the properties connected with it in the case of starch.

**The Rigidity of Starch Pastes**—The properties of elasticity and rigidity are very closely related (for definition see E. C.

Bingham<sup>52</sup>), and in the case of starch pastes the determination of these values gives interesting results. S. M. Neale<sup>53</sup> has

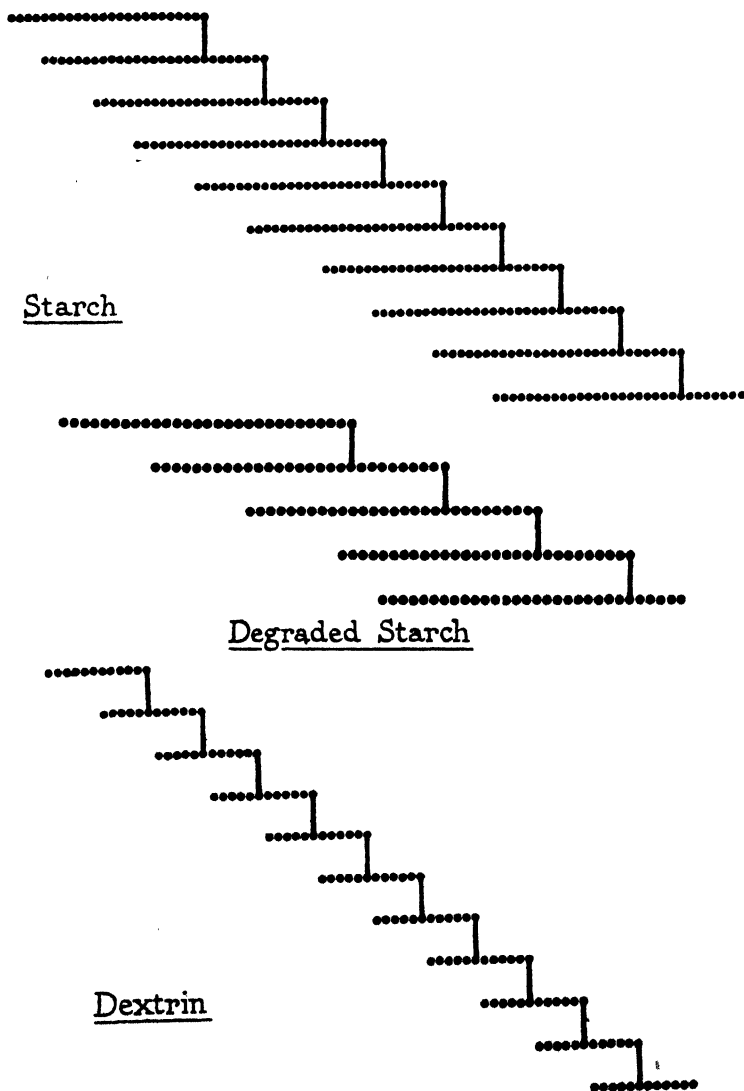


FIG. 28.

measured the elasticity of air-dried films of starch. F. T. Peirce<sup>54</sup> has applied some sensitive and non-destructive tests of rigidity

to films of potato starch, and such tests are also applicable to the testing of yarns and fabrics so as to determine the effect of sizes or finishing processes. He finds that there is a rectilinear relationship between the change in torsional rigidity and the humidity. The rate of change of flexural rigidity is nearly the same, from 30-80 per cent. R.H., whilst the effect of humidity on Young's modulus is expressed by sigmoid curves. The torsional and flexural rigidities vary together over most of the range, 34-65 per cent. R.H., and he concludes that the test of resistance to twisting provides adequate evidence of the effects of moisture on the elastic properties generally. The amount of swelling is different in different directions, and the thickness of the film increases at a rate 70 per cent. greater than the length. This tends to show that the water goes between rather than into the layer parallel with the surface. G. E. Porst and M. Moskowitz<sup>43</sup> attempted to measure the 'yield shear value' by extrapolation of flow-shear curves but obtained indefinite results owing to the gradual slope of the curves obtained. They found the results are considerably influenced by the temperature at which the pastes are prepared, but that the plastometer is of value as an aid to matching submitted products. F. D. Farrow, G. M. Lowe and S. M. Neale<sup>54</sup> observed the flow in starch pastes at rates of shear below Bingham's theoretical yield value. Farrow and Lowe<sup>73</sup> had previously shown that the rate of flow of starch pastes, when driven through a capillary tube, is not proportional to the pressure but increases more rapidly than the pressure increases. Newton's postulate describing the flow of most liquids at low speeds was found not to hold for starch pastes by Farrow, Morris and Neale.<sup>54</sup> The equation which the latter workers have introduced contains more than one constant characteristic of the liquid and appears to describe the flow of starch pastes better than any other two-constant equation despite the fact that it does not hold, even approximately, over a wide range.

These workers point out that since in most viscometers the shearing stress is different at different points in the liquid the distribution of shear will be different from that shown by liquids obeying Newton's postulate. Their expression is evolved in terms of stress and strain which allow the measurements to be expressed independent of the type of viscometer and allow comparison of results of different workers using different types of viscometers.

The work was carried out over a total range of shear of 300,000-fold, and they found no evidence of discontinuity of the



flow-pressure curve such as would be required to satisfy the yield-value hypothesis of Bingham that no flow takes place below a certain value of the applied stress. The stress-shear curve for starch pastes either passes through or terminates, near to the origin.

U. Ebbecke and R. Haubrich<sup>74</sup> find a great increase in the viscosity of starch solutions when pressures of 400-800 atmospheres are employed.

S. Woodruff and M. M. MacMasters<sup>60</sup> measured the relative viscosity and gel-strength of starch pastes from different varieties of corn, or the same variety of corn treated in different ways, and found viscosity differences were very small compared with differences in gel-strength. The gel-strength fluctuated widely with the variety of corn from which the starch was made and with the growing conditions of the corn. Commercial starches gave weaker gels than samples carefully prepared in the laboratory. These workers used the Tarr and Baker<sup>55</sup> jelly tester to determine gel-strength, whilst a Stormer viscometer was used for viscosity determinations. Champion White Pearl, Sutton White Dent, Pioneer Hi-Bred 305A, Reid Yellow Dent and a Reid starch gave gels in that order of strength, the latter giving a gel as weak as the commercial starch samples. Corn grown under poor conditions or frozen in the ear gave weak gels, the viscosity, however, was affected but little in comparison to gel-strength.

As the gel-strength of certain starches for use in foodstuff industry, e.g. for blanc-mange powders, etc., or in the adhesive industry is important, this work emphasises the importance of the determination of gel-strength, which is a better guide than viscosity data for the selection of starches to be used for certain purposes.

C. M. McDowell and F. T. Usher<sup>56</sup> measured the rigidity of non-aqueous suspension of raw starch, but their work is of no interest to us here. G. P. Arcay and Etienne<sup>57</sup> include starch in the substances whose gels they examined for the presence of rigidity. It should be pointed out that consistency as measured by Caesar (*v.s.*) is partly conditioned by viscosity, plasticity, thixotropy and rigidity. B. Brimhall and R. M. Hixon<sup>58</sup> have modified a MacMichael viscometer<sup>66</sup> into an apparatus resembling that of Schwedoff,<sup>59</sup> and they obtained no correlation between the viscosity and the rigidity curves, which indicates that these values measure different properties of the pastes; and they consider that rigidity is dependent upon the condition of the granule membrane.

These workers<sup>115</sup> have more recently introduced a gelometer for measuring breaking strength and elasticity of starch gels which embodies the following principles: Suction is used to deform

the gel, the volume of deformation being measured by hydrostatic means. 'Skin effects' and the influence of the containing walls are both eliminated and the deforming force changes uniformly and at a constant rate. This apparatus is suitable for starch concentrations of 6.5 to 8.5 per cent., but for lower concentrations the modified MacMichael instrument mentioned above is preferable.

## REFERENCES

1. BLOCH, *Compt rend.*, 1894, **118**, 146.
2. — *ibid.*, 1854, **39**, 969.
3. F. ULLICK, *Zeit. ges. Brauw.*, 1891, **14**, 565.
4. C. A. WINKLER and W. F. GEDDES, *Cereal Chem.*, 1931, **8**, 455.
5. J. R. KATZ and J. C. DERKSEN, *Zeit. phys. Chem.*, 1930, **150A**, 100.
6. H. T. BROWN and J. HERON, *Chem. Soc. Trans.*, 1879, **35**, 614.
7. A. W. DOX and G. W. ROARK, *J. Amer. Chem. Soc.*, 1917, **39**, 742.
8. C. K. FRANCIS and O. C. SMITH, *J. Ind. Chem. Eng.*, 1916, **8**, 509.
9. C. L. ALSBERG and O. S. RASK, *Cereal Chem.*, 1924, **1**, 107.
10. A. REYCHLER, *Bull. Soc. chim. Belg.*, 1920, **29**, 118.
11. J. R. KATZ, *Trans. Faraday Soc.*, 1933, **29**, 300.
12. — *Biochem. Zeit.*, 1933, **262**, 355.
13. J. R. KATZ, A. WEIDINGER and F. J. F. MUSCHTER, *ibid.*, 1933, **263**, 31.
14. C. E. MANGELS and C. H. BAILEY, *J. Amer. Chem. Soc.*, 1933, **55**, 1981.
15. L. EYNON and J. H. LANE, 'Starch,' Heffer & Sons Ltd., Cambridge, 1928.
16. E. WEIGL, *Zeit. Spiritusind.*, 1933, **56**, 62.
17. R. A. GORTNER, *Cereal Chem.*, 1933, **10**, 298.
18. H. HUSS, *Arkiv. f. botan.*, 1922, **18**, 1, 23.
19. W. HARRISON, *J. Soc. Dyers Col.*, 1911, **27**, 84.
20. G. V. CAESAR, *Ind. Eng. Chem.*, 1932, **24**, 1432.
21. W. S. REICH, *Compt. rend. Soc. Biol.*, 1933, **113**, 1496.
22. E. SZEGO, *Bull. ass. chim. suc. dist.*, 1931, **48**, 268.
23. J. GRÜSS, *Wochschr. Brau.*, 1932, **49**, 389.
24. J. JANICKI, *Roczniki Chem.*, 1932, **12**, 402 (in French).
25. A. FERNBACH, *Compt. rend.*, 1904, **138**, 428.
26. C. L. ALSBERG and E. P. GRIFFING, *Cereal Chem.*, 1925, **2**, 325.
27. SCHEFFER, *Chem. Zentralblatt*, 1919, **4**, 749.
28. L. MAQUENNE, *Compt. rend.*, 1904, **138**, 375.
29. C. L. ALSBERG, *Ind. Eng. Chem.*, 1926, **18**, 190.
30. C. L. ALSBERG and E. E. PERRY, *J. Biol. Chem.*, 1925, **63**, lxvi; *Proc. Soc. Expt. Biol. Med.*, 1924, **22**, 60.
31. T. C. TAYLOR and T. J. SCHOCH, *J. Amer. Chem. Soc.*, 1933, **55**, 4248.
32. O. A. SJOSTROM, *Ind. Eng. Chem.*, 1936, **28**, 63.
33. SPROCKHOFF and PARLOW, *Zeit. Spiritusind.*, 1930, **53**, 62.
34. F. T. PEIRCE, *J. Text. Inst.*, 1928, **19**, 237T.
35. F. D. FARROW and E. SWAN, *ibid.*, 1923, **14**, 465T.
36. URQUHART and WILLIAMS, *ibid.*, 1924, **15**, 559T.
37. W. W. LEPESCHKIN, *ibid.*, 1938, **30**, 309.
38. PENICK and FORD, E.P. 488,672, 1937.

39. J. R. KATZ, J. SEIBERLICH and A. WEIDINGER, *Biochem. Zeit.*, 1938, **297**, 412.
40. C. A. GLABAU and P. F. GOLDMAN, *Cereal Chem.*, 1938, **15**, 451.
41. A. J. OPHOF, *Chem. Weekbl.*, 1936, **33**, 91.
42. H. HERSCHEL and C. BERQUIST, *J. Ind. Eng. Chem.*, 1921, **13**, 703.
43. G. E. PORST and M. MOSKOWITZ, *ibid.*, 1922, **14**, 49.
44. S. WOODRUFF and M. M. MACMASTERS, *Trans. Ill. State Acad. Sci.*, 1936, **29**, 107.
45. EINSTEIN, *Drude's Ann.*, 1906, **19**, 289.
46. E. HATSCHKE, *Zeit. Koll.*, 1910, **7**, 301.
47. J. R. KATZ, J. SEIBERLICH and A. WEIDINGER, *Biochem. Zeit.*, 1938, **298**, 320, and 323.
48. S. B. ETORMA, *Philippine J. Agric.*, 1936, **7**, 409.
49. G. CENTOLA, *Atti Accad. Lincei*, 1936, **23**, 617.
50. S. G. WILLIMOTT, *Cyprus Agric. J.*, 1936, **31**, 94.
51. W. DELFFS, *Pogg. Ann.*, 1860, **109**, 648.
52. E. C. BINGHAM, *J. Rheol.*, 1930, **1**, 511.
53. S. M. NEALE, *J. Text. Inst.*, 1924, **15**, 443T.
54. F. D. FARROW, G. M. LOWE and S. M. NEALE, *ibid.*, 1928, **19**, 18T.
55. L. W. TARR, *Del. Agr. Exp. Sta. Bull.*, **142**, 1926.
56. C. M. McDOWELL and F. T. USHER, *Proc. Roy. Soc.*, 1931, **131A**, 409.
57. G. P. ARCAJ and ETIENNE, *Compt. rend.*, 1927, **185**, 701.
58. B. BRIMHALL and R. M. HIXON, *Ind. Eng. Chem. (Anal. Ed.)*, 1939, **11**, 358.
59. T. SCHWEDOFF, *J. Phys.*, 1889, **8**, [2], 341.
60. S. WOODRUFF and M. M. MACMASTERS, *Illinois Agr. Expt. Sta. Bull.*, **445**, 1938.
61. J. R. KATZ, *Text. Res.*, 1939, **9**, 61, 114.
62. — and J. SEIBERLICH, *Zeit. phys. Chem.*, 1938, **183A**, 146.
63. A. KÜNTZEL and K. DOEHNER, *Kolloid-Zeit.*, 1939, **86**, 124, 130, 254 and 258.
64. W. W. LEPESCHKIN, *Bot. Abst.*, 1923, **13**, 865.
65. D. H. COOK and J. H. AXTMAYER, *Ind. Eng. Chem. (Anal. Ed.)*, 1937, **8**, 226.
66. R. F. A. MACMICHAEL, *J. Ind. Eng. Chem.*, 1915, **7**, 961.
67. W. A. RICHARDSON and R. S. HIGGINBOTHAM, *Nature*, 1940, **146**, 234.
68. S. ONO, *Rev. Phys. Chem. Japan*, 1940, **14**, 25.
69. H. GEINITZ, *Kolloid-Zeit.*, 1940, **90**, 58.
70. J. R. KATZ and J. SEIBERLICH, *Rayon Textile Monthly*, 1940, **21**, 42.
71. V. I. NAZAROV and A. V. NIKOLAEV, *Compt. rend. Acad. Sci., U.S.S.R.*, 1939, **24**, 263. (In English.)
72. ANON, *Kolloid-Zeit.*, 1939, **87**, No. 3, 308.
73. F. D. FARROW and G. M. LOWE, *J. Text. Inst.*, 1923, **14**, 414T.
74. U. EBBECKE and R. HAUBRICH, *Biochem. Zeit.*, 1939, **303**, 242.
75. C. L. ALSBERG and E. P. GRIFFIN, *Wheat Studies*, 1935, **11**, 246.
76. L. H. LAMPITT, C. H. FULLER and N. GOLDENBERG, *J. Soc. Chem. Ind.*, 1941, **60**, 1.
77. M. SAMEC, *Kolloidchem. Beih.*, 1937, **46**, 134; 'Kolloidchemie der Stärke,' 1927, P41, T. Steinkopff, Dresden and Leipzig.
78. FRIESE and SMITH, *Ber.*, 1928, **61**, 1975.
79. C. S. HANES and M. CATTLE, *Proc. Roy. Soc.*, 1938, **125B**, 387.
80. W. N. HAWORTH, E. L. HIRST and WAINE, *J. Chem. Soc.*, 1935, 1299.
81. S. M. NEALE, *J. Text. Inst.*, 1924, **15**, T443; JAMBUSERWALA, *ibid.*, 1939, **30**, T85; 1940, **31**, T1.

82. HEINIGER, *Kolloidchem.*, 1932, **35**, 1.  
 83. SVEDBERG, *Nature*, 1938, **141**, 1000; *Ind. Eng. Chem. (Anal. Ed.)*, 1938, **10**, 113.  
 84. LAMM, *Kolloid-Zeit.*, 1934, **69**, 44.  
 85. W. A. RICHARDSON, R. S. HIGGINBOTHAM and F. D. FARROW, *J. Text. Inst.*, 1936, **27**, 131T.  
 86. REICH and DAMANSKY, *Bull. Soc. chim. biol.*, 1937, **19**, 158 and 357.  
 87. GORTNER and HAMALAINEN, *Cereal Chem.*, 1940, **17**, 378.  
 88. S. ONO, *Rev. Phys. Chem. Japan*, 1940, **14**, 101. (In English.)  
 89. STAUDINGER, *Compt. rend. Trav. Lab. Carlsberg*, 1938, **22**, 494.  
 90. L. H. LAMPITT, C. H. FULLER and N. GOLDENBERG, *J. Soc. Chem. Ind.*, 1941, **60**, 47.  
 91. W. A. RICHARDSON, *Chem. and Ind.*, 1939, 464.  
 92. STAUDINGER, *Naturwiss.*, 1937, **42**, 673.  
 93. — *Nature*, 1937, **140**, 1071.  
 94. — *Ber.*, 1937, **70B**, 1451.  
 95. — *Annalen*, 1937, **580**, 1.  
 96. — and HUSEMANN, *ibid.*, 1937, **527**, 195.  
 97. — and EILERS, *Ber.*, 1936, **69B**, 819.  
 98. — and HUSEMANN, *ibid.*, 1938, **71B**, 1057.  
 99. MARK and SIMHA, *Nature*, 1940, **145**, 571.  
 100. E. L. HIRST and G. YOUNG, *J. Chem. Soc.*, 1939, 1471.  
 101. BECKMANN and LANDIS, *J. Amer. Chem. Soc.*, 1939, **61**, 1495.  
 102. M. SAMEC, *Zeit. physiol. Chem.*, 1940, **263**, 17.  
 103. GEE, *Trans. Faraday Soc.*, 1940, **36**, 1171.  
 104. W. A. RICHARDSON, *Chem. and Ind.*, 1937, 353.  
 105. NEALE, *ibid.*, 1936, 602.  
 106. DAVIDSON, *J. Text. Inst.*, 1936, **27**, 144.  
 107. PHILPOT, *J. Chem. Soc.*, 1939, 1481.  
 108. H. N. HAWORTH, E. L. HIRST and SMITH, *ibid.*, 1939, 1914.  
 109. — *Chem. and Ind.*, 1939, 923.  
 110. CARTER and RECORD, *J. Chem. Soc.*, 1939, 670.  
 111. STAUDINGER, *Trans. Faraday Soc.*, 1933, **29**, 18.  
 112. BAWN, E. L. HIRST and G. YOUNG, *ibid.*, 1940, **36**, 880.  
 113. JAMBUSERWALA, *J. Text. Inst.*, 1940, **31**, T1; 1938, **29**, T149; 1939, **30**, T85.  
 114. HOPPLER, *Gel. Leim u. Klebstoffe*, 1939, **7**, 75.  
 115. R. M. HIXON and B. BRIMHALL, *Ind. Eng. Chem. (Anal. Ed.)*, 1941 **18**, 193.

## ADDITIONAL REFERENCES

- W. GALLAY, *Can. J. Res.*, 1937, **14B**, 360, 382, 391 and 409. (Flow and texture of starch pastes and factors altering them.)  
 A. K. KUHLMAN, *Zeit. ges. Getreide, Mühl. Bäckerwesen*, 1936, **23**, 128. (Good wheat distinguished from poor by viscosity and swelling methods.)  
 A. SCHULERUD, *Mühlenlab.*, 1926, **6**, 177. (The physical chemistry of bread-making from rye.)  
 S. N. GLARUM, *Text. Res.*, 1937, **8**, 56. (Viscosity of printing thickeners.)  
 H. KUKL, *Mühlenlab.*, 1937, **7**, 95. (Gelatinising starch in presence of phosphates.)  
 M. BLINC and M. SAMEC, *Cong. intern. tech. chim. ind. agr., Compt. rend., 5th Congr.*, 1937, **2**, 214. (Viscometers for starch reviewed.)

- E. SWAN, *J. Text. Inst.*, 1923, **14**, 465T; 1926, **17**, 527T'. (Adsorption of water by dried films of boiled starch.)
- S. WOODRUFF, *44th Ann. Report Illinois Agric. Exp. Sta.*, 1935, 252. (Starch gels precipitated with alcohol can be re-gelled repeatedly.)
- M. YOSHINO, *J. Soc. Chem. Ind.*, 1924, **43**, 144B. (Arrhenius' formula applied successfully to starch pastes.)
- C. BERQUIST, *J. Phys. Chem.*, 1925, **29**, 1264. (Plasticity of starch pastes.)
- W. HERTER, *Textilber.*, 1920, **1**, 8 and 31. (Gel-temperatures of wheat and rye starches given.)
- J. C. RIPPERTON, *Ind. Eng. Chem. (Anal. Ed.)*, 1931, **3**, 151. (Calcium salts in hard water have marked effect on viscosity of starch pastes.)
- G. M. MOFFETT, in 'A Comprehensive Survey of Starch Chemistry,' edited by R. P. Walton, Chem. Catalog Co., New York, 1927. (Sulphurous acid in steep water causes maize starch thus prepared to gel at lower temperature than starch separated by purely mechanical means.)
- M. SAMEC and F. ULM, *Kolloidchem. Beih.*, 1936, **43**, 287. (Chlorine dioxide has similar effect to sulphur dioxide on the gelatinisation of potato and wheat starches.)
- S. WOODRUFF and L. R. WEBER, *J. Agric. Res.*, 1933, **46**, 1099. (Photomicrographs of wheat starch at different stages of gelatinisation.)
- and H. HAYDEN, *ibid.*, 1936, **52**, 233. (Photomicrographs showing effect of freezing on physical and microscopical characteristics of corn and wheat starch gels.)
- and L. NICOLI, *Cereal Chem.*, 1931, **8**, 243. (Starch pastes must be heated to higher temperatures than that at which marked translucency is noted before the full gel-strength of the paste is developed.)
- O. W. CHAPMAN and J. H. BUCHANAN, *Iowa State Coll. J. Sci.*, 1930, **4**, 441. (Neither rate nor period of heating affect rigidity or syneresis of starch gels.)
- J. C. RIPPERTON, *Ind. Eng. Chem.*, 1931, **3**, 1; *Hawaii Agr. Exp. Stat. Bull.*, 1931, **63**, 48. (Measures swelling power of edible canna and potato starches.)
- M. S. FURRY, *W.S. Dept. Agr. Tech. Bull.*, 1932, **284**, 18. (Physical properties of starch pastes which affect their stiffening power on fabrics discussed.)
- S. RASK and C. L. ALSBERG, *Cereal Chem.*, 1924, **1**, 7 and 107. (Gelatinisation and viscosity of wheat and maize starches.)
- K. HESS and B. RABINOWITSCH, *Kolloid-Zeit.*, 1933, **64**, 257. (Cinematograph used to photograph swelling of potato starch.)
- K. LINSBAUER, *Bot. Centbl.*, 1935, **53A**, 172. (Gelatinisation followed by hot stage microscope.)
- M. SAMEC, *Cereal Chem.*, 1935, **12**, 592. (The nature of the paste-forming starch fractions.)
- W. SECK and G. FISHER, *Kolloid-Zeit.*, 1940, **93**, 207. (Gelation properties after various treatments.)
- R. M. HIXON and G. F. SPRAGUE, *Ind. Eng. Chem.*, 1942, **34**, 959. (Viscosity and gelling properties of starches from waxy maize, rice, sorgham and barley discussed.)
- B. BRIMHALL and R. M. HIXON, *Cereal Chem.*, 1942, **19**, 425. (The various methods for interpreting viscosity measurements as applied to starch pastes reviewed.)

## CHAPTER 6

THE ROLE OF THE MINOR CONSTITUENTS  
OF STARCH

**The Role of Phosphorus in Starch.**—As we have seen, the changes in the viscosity of starch pastes may be due in a large measure to the breaking down of the organised structure of the jelly by mechanical means, and that the viscosity and paste-forming ability of starch solutions are considered by some workers to be due to the amylopectin portion of the starch. We may now examine the physical behaviour of this substance in more detail, and especially the work aimed at correlating this behaviour with its chemical constitution.

Amylopectin may be distinguished from amylose in that it gives gelatinous and viscous solutions with water, and that these solutions with iodine give a bluish-red to colourless reaction. Further, amylopectin is much more resistant to enzyme action than amylose, and contains phosphorus which is not readily removable.

Starch which has been thoroughly washed with distilled water and then made into a suspension with a further quantity of water does not liberate an appreciable amount of electrolytes, which is in contrast to its behaviour in the dissolved state. Certain cations which are present in the untreated starch are readily removable by a thorough washing with dilute hydrochloric acid followed by a thorough washing with water. According to G. Malfitano and A. N. Moschkoff,<sup>6</sup> when a potato-starch solution is frozen and allowed to thaw, the liquid separating contains most of the electrolyte present in the original starch; if the mass be well washed with water the ash of this portion consists almost entirely of phosphoric acid and they were able to reduce the ash-content of potato starch in this manner to less than 0.2 per cent.

A. Coehn<sup>1</sup> appears to have been the first worker to note that starch in solution or in aqueous suspension has a negative electric charge. Working with potato starch, F. Bottazzi and C. Victorow<sup>2</sup> noted that amylose shows no electrical migration, whereas under the influence of an electric current the amylopectin fraction is transported to the anode. F. Bottazzi<sup>3</sup> observed that in an acid solution the starch migrates towards the cathode, and in an alkaline solution towards the anode, no migration occurring in neutral solution. Z. Gruzewska<sup>4</sup> also noted the anodic migration

of the amylopectin fraction of potato starch, and E. Fouard<sup>5</sup> observed the phenomenon in ultra-filtered solutions, using the ultra-microscope.

A. W. Thomas<sup>8</sup> gave potato starch an extended treatment with 7 per cent. hydrochloric acid and obtained a product, containing less than 0.02 per cent. phosphorus, that gave a true colloidal solution from which it was not precipitated by the addition of alcohol. H. C. Sherman and J. C. Baker<sup>9</sup> heated potato starch in suspension in 0.001 M sodium chloride solution and in water at 85° C., and separated the amylopectin from the amylose by centrifuging the solution. They found 0.156 and 0.147 per cent.  $P_2O_5$  in the amylopectin fractions and 0.023 and 0.023 per cent.  $P_2O_5$  in the soluble portions, respectively. M. Samec<sup>10</sup> obtained an amylopectin fraction containing 0.185 per cent. and an amylose fraction containing 0.007 per cent. of  $P_2O_5$ , figures which are in fairly good agreement with those of Sherman and Baker.

Samec has employed<sup>11-14</sup> extensively the electro dialysis of starch solutions to effect a rapid and efficient separation of the two fractions, the amylopectin giving a jelly from which the clear mobile solution of the amylose is readily removed. By this method he obtained an amylopectin fraction from potato starch, having 0.175 per cent.  $P_2O_5$  and an amylose fraction quite devoid of phosphorus. Samec's method of preparing his starch solutions is to heat the starch and water under pressure to give a temperature of 120° C. for not more than three hours which gives a solution which separates sharply into two well-defined and readily separated layers. The lower or jelly-like layer of amylopectin obtained in this way contains about 7 per cent. of its weight of dry matter. This method<sup>20</sup> cannot be employed with soluble starches or starch derivatives which have smaller micelles, and to obtain a separation in such cases one must resort to precipitation with metallic salts and alcohol.

E. L. Hirst<sup>15</sup> and his co-workers have investigated the separation of the two components in starch, and P. Karrer and E. von Kraus<sup>16</sup> have extracted starch with successive amounts of hot water and examined the various dissolved fractions so obtained but obtained no correlation between the phosphorus-content and other characteristics.

According to A. R. Ling and D. R. Nanji,<sup>56</sup> the ratio of amylose to amylopectin in wheat, rice, maize and barley starches is approximately the same. The appearance of the different amylopectins from these starches, their conductivity in solution, behaviour on electro dialysis and the phosphorus-content, however,

all show differences, although the amount present in the starch always appeared to these workers to be the main factor determining the viscosity of the solutions.

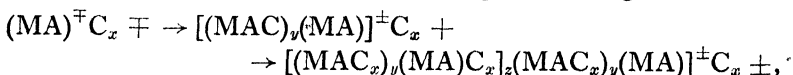
A. Fernbach<sup>57</sup> early showed that the phosphorus in some starches could not be removed by washing with water or by treatment with dilute acids, and both Tryller<sup>18</sup> and Fouard<sup>17</sup> have shown that although the cations are removed by dilute acids, the latter have but little effect on the phosphorus-content. Samec,<sup>19</sup> however, finds that starch solutions, after thorough dialysis, lose a portion of the phosphoric acid, and that the longer a starch solution is heated under pressure the greater is the amount of phosphoric acid which is lost upon dialysis. The resulting increase in acidity and hydrolysis on heating is attributed by A. Tychowski and S. Mesior<sup>62</sup> to the liberation of phosphoric acid, which is almost complete after heating for 10 hours at 120° C. The phosphorus-content of canna<sup>97</sup> and potato<sup>15</sup> starches are unaffected by acetylation, methylation or subsequent saponification. C. Tryller<sup>18</sup> has also shown that by washing with appropriate salt solutions and then with water until free from electrolyte the metallic atoms can be interchanged (see p. 32). It may be stated at this point that the mode of occurrence of phosphorus in starch appears to vary according to the variety of starch. It appears reasonably well established, as discussed below, that in the root starches, such as potato and arrowroot, and in sago the phosphorus occurs as a phosphoric ester of a polysaccharide, but in cereal starches such as maize and wheat it is present as a glycerophosphoric ester, possibly as a phosphatide.<sup>93</sup> It has been shown<sup>88</sup> that 90 per cent. of the phosphorus and 75 per cent. of the nitrogen in wheat starch can be removed by washing with alcoholic potash but potato starch retains its phosphorus when submitted to this treatment. Wheat starch pastes or wheat starch pre-treated with alkali is dephosphorylated by alcohol<sup>88</sup> but potato starch and intact wheat starch granules are unaffected.<sup>88</sup> Lampitt and co-workers<sup>96</sup> find that the hot-water soluble, phosphorus-rich fraction from milled wheat starch liberates some of its combined phosphorus when a solution of this fraction is precipitated by alcohol, and they consider that this is accompanied by some depolymerisation as the precipitated material is soluble in cold water.

Four theories have been put forward to account for the presence of phosphorus in association with the carbohydrate matter, and to explain its influence on the behaviour of the starch. These theories, which we will now examine briefly, may be termed :—



1. The formation of 'Werner Complexes'.
2. The adsorption theory.
3. The amylophosphoric acid theory.
4. Coacervation theory.

**The Formation of 'Werner Complexes'.**—G. Malfitano<sup>21</sup> regards a clear starch solution as a colloidal solution in which the colloidal particles consist of a phosphate ion surrounded by starch molecules. He also holds that the system starch-water never attains the state of a true solution, but that it is always a hydrosol or hydrogel.<sup>22</sup> According to this worker, the molecules of  $C_6H_{10}O_5$  form Werner complexes around the phosphate ion and are externally compensated by hydrogen, alkali or alkaline-earth cations.<sup>23</sup> The complexes have the power of forming still higher complexes, and these complexes yet higher ones again, thus:



these representing complexes of the first, second and third orders respectively, where M is the non-electrolyte portion, A the phosphate ion, and C a cation. On this theory the phosphate ion would be masked entirely by the carbohydrate and the cation partially so.<sup>24</sup> It follows from this theory that removal of phosphorus can only be accomplished by previously destroying the micelle or complex to liberate the phosphate ion, but Samec<sup>25</sup> has shown that the phosphoric acid may be removed by heating with water under pressure, followed by electro dialysis of the solution, although the degree of dispersion is not changed appreciably. Further, unless we assume that the carbohydrate phosphoric acids of low molecular weight, obtained by several workers, have the constitution of a complex compound, the hypothesis does not explain the linkage of phosphorus and amylose in potato or other starches, and such an assumption does not appear to be warranted.

The phosphate ions in Malfitano's complexes are completely masked, but in a complex of the first degree they should still be able to act electrochemically. The cations in these complexes are masked only to the extent of about 50 per cent. for a complex of the second degree, and 66 per cent. for a third degree of complexity. These cations, being masked, should be difficult to remove, e.g. by washing with acid, but in the case of potato starch Samec,<sup>25</sup> by repeated washing with 1 per cent. hydrochloric acid, was successful in removing 94 per cent. of the cations present, and this worker has also shown that the hydrogen ions in potato starch are not masked.

**The Adsorption Theory.**—We have previously mentioned (p. 71) that J. Janicki<sup>58</sup> considers that the major portion of the phosphorus in starch is adsorbed on the starch in the form of colloidal phosphates, although from 0.0 to 9.0 per cent. of the total phosphorus may be held as an ester. He considers that changes of viscosity of starch solutions on ageing may be explained, not by the saponification of the ester, but by the changes in the degree of hydration of the starch micelles and of the other colloids present. M. Schoen<sup>26</sup> is another worker who supports this theory.

If this theory is correct, colloidal solutions of starch should have a strong selective adsorption for phosphate ions, with a consequent change in the colloidal properties of the solution. On peptisation of the starch, or on its hydrolysis, the adsorption of the phosphate ion should gradually weaken, but so far the first condition has not been observed, and the second is opposed to the observation of Malfitano<sup>21</sup> that starch solutions, dispersed so that they can be ultra-filtered through collodium membranes, contain organically combined phosphorus. It is to be remembered that when washed with dilute acids it is always the cation which is removed from the starch and not the anion,<sup>18</sup> and by the adsorption theory this could be explained by assuming that the phosphate ion is much more strongly adsorbed, whereas the cations are only adsorbed normally. This assumption would be quite in keeping with certain observations that in some cases of adsorption of salts the anions are much more strongly held than the cations. However, with starch the phosphate ion is not adsorbed from solution. Lampitt and co-workers<sup>96</sup> find that, with ball-milled wheat starch, the cold-water soluble fraction contains phosphorus-free starch but in the hot-water soluble fraction, there are, on the average, about two atoms of non-dialysable phosphorus to each starch macromolecule. They also note that starch retrograding from both cold- and hot-water soluble fractions contains a relatively high phosphorus-content whilst the amount of the dialysable phosphorus left in solution in each case is not significantly affected. Much more non-dialysable phosphorus is found in the hot-water soluble fraction than in any other.

**The Amylophosphoric Acid Theory.**—If the starch is broken down in a certain manner, the phosphorus-content is still retained. Thus Samec<sup>27</sup> was able to obtain dextrans which contained from 0.264 to 0.511 per cent. of  $P_2O_5$ , i.e. a larger proportion than in the original starch, although the starch had undergone hydrolysis. Similar results have been obtained by H. Pringsheim<sup>28</sup> and his co-workers, who used pancreas extract to bring about the hydrolysis. T. Posternak<sup>29</sup> has prepared a

biose-phosphoric acid, whilst Northrop and Nelson<sup>7</sup> obtained a compound of low molecular weight containing 5 per cent. phosphorus by the prolonged action of 10 per cent. hydrochloric acid on potato starch, similar results being obtained by Samec,<sup>20</sup> who also peptised starch by the action of ultra-violet light and obtained a product containing 3.26 per cent.  $P_2O_5$ . Lampitt and co-workers<sup>96</sup> from their work on ball-milled wheat starch have shown that a phosphorus-rich fraction is present. M. Samec and F. von Hoefft<sup>59</sup> hold that a dibasic amylophosphoric acid is present in starch in which the amylose and the phosphoric acid are connected through an ester linkage and in this Posternak<sup>83</sup> agrees.

He assigns the formula  $\text{St}-\text{O}-\text{PO} \begin{matrix} \diagup \text{OR}_1 \\ \diagdown \text{OR}_2 \end{matrix}$  in which the St is

a glucose unit in the starch chain and  $R_1$  and  $R_2$  are metal or hydrogen atoms. This compound is hydrolysed by heating with water under pressure, and the longer the duration of heating the greater the loss of phosphoric acid by the amylopectin.<sup>11</sup>

The colloidal behaviour of amylopectin would be greatly influenced by the presence of such a group, as it would tend to increase the ionic hydration of the micelles and thus lead to an enhanced viscosity in solution as compared with the phosphorus-free carbohydrate. When the phosphoric acid is split off, the viscosity of the solution falls and the number of micelles travelling to the anode when a potential is applied is decreased. Thus, the hydrolysis of the ester is considered by these workers to be a fundamental cause of the fall in the viscosity of the solution, but other minor causes may also contribute to this effect.<sup>60, 13, 31-32</sup>

Samec<sup>59</sup> separated amylopectin from potato starch, and after saponifying it to remove the phosphorus-content, obtained a product which differed in some respects from the amyloses of Maquenne. This product<sup>13</sup> was then re-esterified according to the method of Neuberg<sup>33-34</sup> by treating it with phosphorus oxychloride in the presence of calcium carbonate. The resulting calcium amylophosphate appears to lose its calcium ions on electro dialysis,<sup>12</sup> and gives a product closely resembling the original amylopectin, except for an enhanced viscosity and a higher phosphorus-content. The phosphorus-content was raised from 0.0 per cent. to above 2 per cent. by this method, and the acidic nature and electrochemical behaviour of the synthetic amylopectin are similar to those of the naturally occurring product in starch. J. B. Kerb<sup>35</sup> has also prepared the amylophosphoric acid by the action of phosphorus oxychloride on a suspension of amylose and calcium carbonate in chloroform.

A further important point is that Samec<sup>36</sup> was able to obtain similar results with amylopectin derived from different starches, whilst H. Pringsheim<sup>37</sup> also obtained a gelatinous phosphoric ester by the action of phosphoryl chloride, in the presence of pyridine, on the polyamyloses obtained by the action of *Bacillus macerans* on starch.

The amylophosphoric acid obtained from potato starch gives a practically transparent jelly similar to the potassium salt which, according to H. Tryller,<sup>38</sup> occurs in the granule. If the starch is washed with hard water, the potassium is replaced by the calcium in the water. The barium and calcium salts give cloudy pastes with water, as they have but very slight solubility, and the pastes are therefore much less viscous than those obtained from the potassium salt or the free acid.

In view of the similarity between the viscosity/time curves of tapioca and potato starches it is interesting to note that the amylopectins in these starches are acidic and the phosphorus-content very similar. The amylopectins of the cereal starches are practically neutral, whilst the synthetically prepared amylopectins are acid and resemble potato and tapioca amylopectins in behaviour.

According to Samec, conductivity and viscosity measurements indicate that the amylopectin is not greatly hydrolysed by the action of takadiastase, so that when the achroo-dextrin stage of the hydrolysis is reached, at which no iodine reaction is given, some phosphorus has been split off, but a dextrin residue is obtained which contains over 1 per cent.  $P_2O_5$ . When synthetic amylophosphoric acid is used, the concentration of the diastase must be greatly increased to obtain any action, and still a small amount of residue is obtained as before which has a high phosphorus-content.

The influence of storage and presence of electrolytes on the viscosity of starch pastes has been studied by Samec,<sup>60</sup> who noted that a decrease in the viscosity of the solution was accompanied by an increase in its conductivity. The same changes that take place on ageing occur on heating, but at a much greater rate. As certain physical properties, e.g. optical rotation of the solution, do not alter in such cases, the changes cannot be due to degradation by hydrolysis or to peptisation.

Concurrently with the fall in the viscosity of starch solutions on ageing or on heating, a decrease occurs in the amount of material that can be precipitated by alcohol, and also a corresponding decrease in the amount of substance migrating under the action of electric current. As previously mentioned, demineralisation by freezing and thawing of starch solutions leads to the same

effect as ageing or heating under pressure. A 1 per cent. potato-starch solution made by heating starch under pressure has a high initial viscosity which is decreased if acid is added. The addition of a small amount of alkali causes a slight increase in the viscosity, but as the concentration of alkali in the solution is increased, the viscosity falls to a minimum and then rapidly increases again.

From potentiometric measurements Samec<sup>36</sup> considers that a 1 per cent. solution of potato amylopectin separated by electro-dialysis contains  $P = 25 \times 10^{-5}$  gram atoms of phosphorus per litre, and has obtained characteristic curves connecting the  $pH$  of the solution and the amount of alkali added. During the neutralisation of the first hydrogen atom the viscosity increases slightly at first, decreases to a minimum at a concentration of one equivalent of alkali corresponding to 1 gram-equivalent of phosphorus, and on continued addition of alkali the viscosity increases in a discontinuous manner with the increase in  $pH$  value. The curves for sodium carbonate and sodium hydroxide are similar in character, and amino-acids or albumins have also been used.

From these facts Samec concludes that the solution of amylo-phosphoric acid has a structure consisting of hydrated jelly-like particles, which increase in size owing to increased hydration on the addition of the first portion of the alkali with a consequent increase in viscosity of the solution. When the first hydrogen atom is neutralised, the particles pass into colloidal solution, and then the hydration of the dispersed phase increases as the addition of the alkali is continued. Thus the decrease in the viscosity to a minimum at the first end-point and the subsequent increase in viscosity may be explained.

In studying the decrease of viscosity of starch solutions on ageing, at constant temperature, the ratio between the initial and final viscosities is the same for solutions containing different concentrations of starch, and once the change has taken place the original viscosity cannot be restored by further treatment of the solution. This would suggest that some sort of irreversible reaction takes place, and as the ratio of the original to the final viscosity is constant for a range of concentrations, it would appear to be monomolecular.

Richardson and Higginbotham<sup>84</sup> have studied the influence of the combined phosphoric acid on the swelling of granular starch. They point out that in some starches, e.g. potato, the phosphoric acid exists in the starch as an ester but consider that in other starches, e.g. wheat, part of the phosphorus-containing impurity is removable by extraction with cold dilute acid or methanol (see O. Rask<sup>70</sup> and Schoch<sup>81</sup>) (see p. 98).

The metals combined with the phosphoric acid play an important part in swelling phenomena, for example, the swelling ability and the viscosity of a 3 per cent. paste of sodium starch are greater than those of pastes made from the corresponding calcium starch. In the presence of salt, however, these differences are not observed, and the above workers suggest this is due to the greater tendency of the sodium atoms to ionise and diffuse into the water. They found no simple correlation between the phosphorus-content of starches and the swelling behaviour after washing with the same salt solution. If the average chain-lengths of the starch molecules were the same<sup>85</sup> as they were in eight starches examined, such a correlation might be expected. They were therefore led to the conclusion that there may be differences in sub-microscopical structure which would mask the effect of the phosphorus on the swelling in pure water.

From the foregoing we see that the amylophosphoric-acid theory accounts for the mobility of the cations and the retention of phosphorus on washing, the chemical reactivity of the unswollen granules, and a number of other observations, but not for the behaviour of phosphorylated amylose, which has been prepared but which shows no increase of viscosity in solution.

Much of the work relating to the presence of a combination between the starch and phosphoric acid is confusing. Myrbäck and Ahlborg<sup>72</sup> consider it unlikely that there is any carbohydrate-phosphorus complex in starch at all. The weight of evidence, however, is against this. Haworth and co-workers<sup>73</sup> have prepared an  $\alpha$ -amylopectin of molecular chain-length of 16-17 glucose units, and consider the phosphorus to be chemically combined and present to twice the normal amount for potato starch.

Karrer<sup>74</sup> considers that the phosphorus in potato starch is distributed throughout all fractions of the starch, and is supported in this by Taylor and Schoch,<sup>50</sup> but Baldwin<sup>76</sup> and Samec<sup>75</sup> maintain that it is held in the amylopectin portion (see also Lampitt and co-workers,<sup>96</sup> p. 89). More recently Samec<sup>79</sup> has prepared amylophosphoric acids by a number of different methods, such as washing potato and similar starches with acids, prolonged heating of starch pastes, severe grinding, exposure to ultra-violet light, and suitable hydrolysis by diastase. In the solutions thus prepared he believed that amylophosphoric acids are present having micelles of different sizes and different phosphorus-content, and further, that the paste-formation depends upon having a proper balance between the tendency to associate and to hydrate. Malfitano and Catoire<sup>77</sup> and other workers<sup>78</sup>

also place considerable significance on the phosphorus-content of the various alleged amyloses.

To sum up the present position: Samec<sup>99</sup> considers the presence of phosphorus, in the form of an amylophosphoric ester, is partially responsible for the viscometric behaviour of starch, and some force is lent to his views by the discovery by Richardson and Higginbotham<sup>84</sup> that the degree of swelling of different sized starch granules can be correlated with the phosphorus-content of potato but not wheat starch. Other workers<sup>98, 100-102</sup> attribute the viscosity of starch pastes to the relative volume of the gelatinised granules in the paste. The two theories thus link up for potato starch, but more work is required before a theory is put forward to cover the viscometric behaviour of all starches.

Y. Nakamura<sup>80</sup> has estimated the contents of phosphorus and nitrogen of eleven different varieties of starch after acid treatment. It has been known for some years that nitrogen is present as an integral part of some starches and the rôle of nitrogen in the behaviour of starch may therefore be briefly reviewed.

**The Significance of Nitrogen in Starch.**—Both wheat and potato starches can be freed from cations by treatment with dilute hydrochloric acid, followed by a thorough washing with water, and from the sols of starches treated in this manner the amylose and amylopectin fractions can be obtained. The properties of the amylose fractions from the various starches are very similar, but a comparison of the properties of the different amylopectins show that distinct differences exist in their behaviour.

The starch of gramineæ is relatively rich in phosphorus, and after a very careful purification of the amylopectin fraction and an estimation of the phosphorus present in it, some measure of its conductivity and hydrogen-ion activity in solution should be predictable. In practice, however, the values obtained do not correspond to those which might be expected if the amylophosphoric-acid theory is correct. In attempting to explain the divergency, Samec estimated the phosphorus and nitrogen in both wheat and potato starches, using samples that had been exhaustively purified, and from his results he infers that in wheat starch the amounts of phosphorus and nitrogen exist in definite stoichiometric proportions. Further, he found that in certain solutions of wheat-starch amylopectin, this substance possessed a negative charge, whilst in other fractions there was a tendency for it to migrate to the cathode.

Samec suspected at first the presence of phytovitelines, which are thought by some workers to contain phosphorus. These

compounds are soluble in ammonia, so Samec then treated the wheat-starch amylopectin with ammonia in order to remove these products, if present. The treatment did not remove any phosphorus, the negative charge on the amylopectin was found to have increased, the substance itself resembled the amylopectin prepared from potato starch more closely than before the treatment, and the viscosity of its solutions had increased.

The results obtained by Samec thus point to the absence of phytovitelines, and it appears feasible that the nitrogen was present as protein matter. Wheat-starch amylopectin was therefore treated with pepsin and hydrochloric acid, or merely with potassium hydroxide solution, to remove any proteins present, and the products so obtained possessed the physical properties and acidity resembling those of potato-starch or tapioca-starch amylopectins.

Samec<sup>19-20</sup> therefore explains the differences between the physical properties of gramineæ amylopectins and those obtained from tuber starches by suggesting that the former is a protein-amylophosphoric complex in which the protein inactivates the phosphorus of the amylopectin electrochemically, and also to some extent chemically, whilst the tuber amylopectins are metallic salts of amylophosphoric acid. Posternak<sup>83</sup> and Nottbohm and Mayer<sup>90</sup> consider the phosphorus and nitrogen to be present as a phosphatide, and the latter workers find some 70 per cent. of the phosphatide-content of wheat flour (calculated from the choline-content) to be present as an adsorbate in starch. As they, and other workers,<sup>91-93</sup> point out, phosphatides can form stable complexes with carbohydrates, and thus wheat starch may possibly contain a carbohydrate-phosphatide complex, a conclusion supported by Samec in further papers.<sup>40, 86-89</sup> In conjunction with Waldschmidt-Leitz<sup>94-95</sup> he found that amylophosphatase from ungerminated barley liberates phosphorus from potato but not wheat starch, free reducing groups being formed concurrently in the former. With kidney phosphatase, free from amylase, both starches are dephosphorylated, accompanied, in the case of wheat starch, by the liberation not of reducing groups but of free amino groups, thus showing the close connection between the phosphorus and nitrogen present in wheat starch.

**The Coacervation Theory.**—Samec first suggested that the protein was bound 'salt-like' to the amylophosphoric acid, but later he<sup>80</sup> himself showed this view to be unsatisfactory. P. Koets<sup>41</sup> suggests that coacervation between the protein and the amylophosphoric acid takes place. Coacervation is the name



given to the separation of droplets from solutions of hydrophilic colloids caused by the difference in sign of two colloids present, causing the micelles to approach, with the result that their double layers partially discharge each other. The more loosely-bound water of hydration of the micelles is lost when the partial discharge takes place, and when the force of attraction between the micelles, due to the difference in sign, is balanced by the resistance to displacement of the more rigidly bound water of hydration. A conglomerate, consisting of positive and negative micelles which remain together but, as individuals, still retaining a portion of their water of hydration, is thus obtained.

If this theory is correct it might be expected that potato amylopectin would move cataphoretically to the anode, as the negatively charged amylophosphoric acid is present to a greater extent than the positively charged protein, giving a resultant negative charge. In practice this is observed. Samec and Antonovic<sup>42</sup> have shown that excess amylophosphoric acid was present in certain fractions of wheat amylopectin which moved to the anode, whereas in other fractions in which the protein was in excess there was a tendency to move to the cathode.

Koets points out that the amylopectin made by Samec was prepared by removing the phosphoric acid from a sample of amylopectin and then re-esterifying the amylose so obtained. Amylopectin prepared by the esterification of pure amylose, however, does not show the high viscosity of the amylopectin prepared by Samec's method, and is, indeed, little more than that of the amylose from which it was prepared. This, according to Koets, may probably be due to the presence of a small amount of protein matter in Samec's original amylopectin which was liberated when the phosphoric acid was removed, but which was still present in the amylose obtained, so that when this was re-esterified a fresh coacervate was formed between the amylophosphoric acid formed and the protein matter present. It has also been shown<sup>38-43</sup> that by increasing the amount of positively charged protein matter coacervating with the amylophosphoric acid from potato starch, a product is obtained which closely resembles the amylopectin obtained from wheat starch, which is known to contain a much higher proportion of protein matter.

Samec found that the colour given by an iodine-potassium iodide solution with amylopectin passes from blue to red the greater the nitrogen-content of the sample, and Koets finds that the same behaviour is shown by coacervates of amylophosphoric acid and protein, so that the facts fit in quite well with the theory.

From viscosity measurements in the presence of sodium

hydroxide, Koets and H. R. Kruyt<sup>63-64</sup> conclude that the OH-groups at the surface of the amylose micelles dissociate and function as centres of electric charges and hydration, the extent of dissociation being governed by the  $pH$  value of the solution. In neutral or weakly acidic solutions they consider the dissociation and surface charges to be very small, neither being strong enough to overcome the crystallite cohesion of the molecules.

In alkaline media the dissociation, and therefore the density, of the surface charge increases, leading to the formation of a double surface-layer about the particles, and attaining a maximum at approximately 0.1 N caustic soda. In contrast with other lyophilic colloids, e.g. gum-arabic, which contain highly ionised surface groups, a higher  $pH$  value is necessary in the case of amylose to bring this about. As the amount of sodium hydroxide is increased, an exchange takes place between the H and Na ions, and the addition of a small amount of alkali causes the solution to gel owing to surface dehydration of the swollen micelles. This takes place in solutions of 1.5-3.0 N alkali. At concentrations above 3.5 N the alkali itself brings about dehydration. Amylophosphoric acid possesses an electric layer which increases with the number of phosphoric-acid groups introduced. A mixture of amylophosphoric acid and amylose reacts with the positive component of proteins to form a complex, but amylose alone does not have this property.

Samec's explanation is considered to be unsatisfactory by W. N. Haworth and E. L. Hirst,<sup>69</sup> who point out that pure amylose fractions can be prepared containing a full complement of phosphorus. Amylopectin, moreover, reacts chemically as if composed entirely of amylose, whilst amylose cannot be kept as such since it retrogrades to amylopectin through a continuous range of intermediate stages (see p. 40). From their studies of methylated starch, these workers consider that these two substances differ in the condition of aggregation and hydration, the tendency of the molecules towards aggregation and interlocking of the molecules being occasioned by the stereochemical conformation of the macromolecules produced in consequence of the  $\alpha$ -glucosidic linkages. The colloidal properties of starch and the micellar character of starch solutions they consider to be fully explained by this view.

**Fatty Acids Present in Certain Starches.**—In 1885 L. Sostegni<sup>44</sup> hydrolysed rice starch with hydrochloric acid, and noted that the insoluble portion of the starch remaining after this treatment contained a substantial amount of an ether-soluble fatty material which melted at 47-48° C. Ren-Ichiro Aoi<sup>45</sup> examined

rice starches made both by the Japanese and by the Chinese methods, and found 0.56 and 0.66 per cent. of fatty matter respectively.

T. C. Taylor and L. Lehrman,<sup>46</sup> examining the 'refinery mud,' which is the insoluble matter left after the acid hydrolysis of maize starch in the commercial production of glucose, found that it contained fatty matter. L. Lehrman<sup>47</sup> has identified palmitic, oleic, linoleic and linolenic acids in the fatty matter obtained in a similar manner from cassava starch.

T. C. Taylor<sup>48-49</sup> and his co-workers have purified various starches with alcoholic hydrochloric acid, gelatinised the treated starch with ammonium thiocyanate solution and, after complete dispersion had been obtained, precipitated the starch with alcohol. Suspensions of the precipitated products were prepared and the amylopectin separated and purified by electrophoresis and dialysis. With maize and rice starches the amylopectin fraction contained about 2 per cent. of fatty matter. In addition to his work on rice, wheat and maize starches,<sup>65-66</sup> Lehrman, with E. A. Kabat, has shown that potato starch contains no combined fatty acids,<sup>67</sup> and that banana starch contains 0.2 per cent. of combined fatty matter<sup>68</sup> and a small amount of phyto-sterol which had not before been detected in starch.

The fatty matter must be present in the starch in some combined form, as extraction with ether for about 90 hours does not remove it, although after it has been separated from the starch it is quite soluble in this solvent.

T. C. Taylor and T. J. Schoch<sup>50</sup> think that this fatty matter plays an important part in the behaviour of certain starches, and that the phosphorus-content of the starch is distributed at random. They find that the starches from horse-chestnut, tapioca, maize and rice all appear to contain fatty matter in a combined form. Potato starch appears to contain no combined fatty matter, and it is interesting to note that the phosphorus-content of this starch is comparatively high, whereas the phosphorus-content of the above starches is comparatively low, varying, according to Samec,<sup>11</sup> from 0.012-0.039 per cent. These starches contain 0.56, 0.12, 0.73, and 0.83 per cent. of fatty matter, respectively.

Taylor and J. H. Wertz<sup>51</sup> have further shown that removal of the fatty acids without destroying the carbohydrate portion of the complex gives a product which is insoluble, and does not migrate when potentials up to 220 volts are applied, although the palmitic ester of amylose does so readily under these conditions, but its methoxy-compounds do not. Taylor and Walton<sup>61</sup> consider that although the esterified fatty acids present in starch

do not contain any directly ionic groups, they may cause an increase in the electric charge carried by the starch. Taylor therefore concludes that there are two complexes present in starch, one of which carries the fatty material. He also concludes that in cereal starches the carbohydrate material in the amylose and amylopectin portions is different, either in chemical behaviour or structurally.

O. S. Rask and J. K. Phelps<sup>70</sup> found that heating corn starch with alcoholic ammonia removes the fatty acids quantitatively, but Taylor and Werntz<sup>51</sup> have since shown that eight successive treatments are necessary before the fatty-acid content becomes negligible. T. S. Schoch<sup>81</sup> also has recently expressed the opinion that the fatty matter in starch is distributed extraneously as an impurity in the starch granules but his statement has been contested by L. Lehrman.<sup>82</sup> Taylor and R. T. Sherman<sup>71</sup> also claim that the unsaturated fatty acids present in corn starch are more readily hydrolysed away from the amylose than the saturated acids.

**Other Acids Present in Starch as Esters.**—Certain commercial maize starches have acid properties, and these are ascribed by Samec<sup>52</sup> to the presence of a sulphonic-acid group, which he thinks may be introduced into the molecule when the manufacture of starch involves the purification or bleaching with sulphurous acid. He finds that starch may be readily sulphonated with sulphuryl chloride, and that the product has acid properties similar to those acquired when the phosphoric ester is formed.

The acidity of roast dextrins,<sup>53</sup> soluble starches,<sup>54</sup> and starches that swell cold water,<sup>55</sup> is ascribed by Samec to the formation of carboxyl groups in the carbohydrate molecule, especially in those processes which involve the use of oxidising agents. The introduction of carboxylic acid groups into starches of low phosphorus-content by oxidation enhances the swelling of the granular starch (Richardson: see ref. 67, Chap. 4). J. A. Radley has noticed that the acidity of roast dextrin appears to increase a little after the dextrin has been re-moistened, which may be due to acid formed from anhydrides present, or to oxidation of aldehydic compounds formed during the roasting process.

#### REFERENCES

1. A. COEHN, *Zeit. Electrochem.*, 1897, 4, 63.
2. F. BOTTAZZI and C. VICTOROW, *Reale accad. Lincei, Atti, Rome*, 1910, 19, 7.
3. — *ibid.*, 1909, 18, 87.
4. Z. GRUZEWSKA, *J. physiol. pathol. gén.*, 1912, 14, 7.

5. E. FOUARD, 'L'etat colloïdale de l'amidon et sa constitution physicochimique,' p. 9, Laval, 1911.
6. G. MALFITANO and A. N. MOSCHKOFF, *Compt. rend.*, 1910, **150**, 710; **151**, 817.
7. J. H. NORTHPRO and J. M. NELSON, *J. Amer. Chem. Soc.*, 1916, **38**, 472.
8. A. W. THOMAS, *Biochem. Bull.*, 1913, **3**, 403.
9. H. C. SHERMAN and J. C. BAKER, *J. Amer. Chem. Soc.*, 1916, **38**, 1885.
10. M. SAMEC, *Kolloidchem. Beih.*, 1914, **6**, 23.
11. M. SAMEC and H. HAERDTL, *ibid.*, 1920, **12**, 281.
12. M. SAMEC and A. MAYER, *Compt. rend.*, 1921, **172**, 1079; **173**, 321.
13. — *Kolloidchem. Beih.*, 1922, **16**, 89 and 91.
14. — *ibid.*, 1921, **13**, 272.
15. E. L. HIRST, M. T. PLANT and M. D. WILKINSON, *J. Chem. Soc.*, 1932, **54**, 2375.
16. P. KARRER and E. VON KRAUS, *Helv. Chim. Acta*, 1929, **12**, 1114.
17. E. FOUARD, *Compt. rend.*, 1907, **144**, 501.
18. H. TRYLLER, *Chem.-Ztg.*, 1920, **44**, 833, 845.
19. M. SAMEC, *Kolloidchem. Beih.*, 1914, **6**, 34.
20. — *ibid.*, 1931, **33**, 449.
21. G. MALFITANO, *Compt. rend.*, 1906, **143**, 400.
22. G. MALFITANO and A. N. MOSCHKOFF, *Bull. Soc. chim. France*, 1912, **11**, 606.
23. G. MALFITANO, *Compt. rend.*, 1913, **126**, 1681.
24. — *Kolloid-Zeit.*, 1928, **46**, 3.
25. M. SAMEC, *Biochem. Zeit.*, 1930, **218**, 249.
26. M. SCHOEN, *Bull. Soc. chim. Biol.*, 1930, **12**, 1033.
27. M. SAMEC, *Kolloidchem. Beih.*, 1914, **6**, 49.
28. H. PRINGSHEIM, H. BARCHARDT and R. LEVY, *Naturwiss.*, 1933, **21**, 299.
29. T. POSTERNAK, *Compt. rend.*, 1933, **197**, 1157.
30. M. SAMEC, *Kolloidchem. Beih.*, 1934, **40**, 449.
31. — *Zeit. gesamt. Getreide, Mühlen u. Bäckereiwesen*, 1933, **20**, 4.
32. — *Roczniki Chem.*, 1933, **13**, 607.
33. C. NEUBERG and H. POLLAK, *Biochem. Zeit.*, 1911, **36**, 5.
34. C. NEUBERG and KRETSCHMER, *ibid.*, 1911, **36**, 5.
35. J. B. KERB, *ibid.*, 1919, **100**, 3.
36. M. SAMEC, M. MINAEFF and N. RONZIN, *Kolloidchem. Beih.*, 1924, **19**, 203.
37. H. PRINGSHEIM and K. GOLDSTEIN, *Ber.*, 1923, **56**, 1520.
38. H. TRYLLER, *Chem.-Ztg.*, 1920, **44**, 833.
39. M. SAMEC, *Biochem. Zeit.*, 1927, **186**, 337.
40. — *Kolloidchem. Beih.*, 1931, **33**, 95.
41. P. KOETS, *J. Phys. Chem.*, 1936, **40**, 1191.
42. M. SAMEC and ANTONOVIC, *Kolloidchem. Beih.*, 1926, **23**, 377.
43. PRZYLECKI and DOBROWOLSKA, *Biochem. Zeit.*, 1932, **245**, 388, **248**, 16.
44. L. SOSTEGNI, *Gazz. chim. ital. Palmero*, 1885, **15**, 376.
45. REN-ICHIRO AOI, *J. Chem. Soc. Tokio*, 1923, **44**, 755.
46. T. C. TAYLOR and L. LEHRMAN, *J. Amer. Chem. Soc.*, 1926, **48**, 1739.
47. L. LEHRMAN, *ibid.*, 1932, **54**, 2527.
48. T. C. TAYLOR and H. A. IDDLES, *Ind. Eng. Chem.*, 1926, **18**, 713.

49. T. C. TAYLOR and J. M. NELSON, *J. Amer. Chem. Soc.*, 1920, **42**, 1726.
50. T. C. TAYLOR and T. J. SCHOCH, *ibid.*, 1933, **55**, 4248.
51. T. C. TAYLOR and J. H. WERNITZ, *ibid.*, 1927, **49**, 1584.
52. M. SAMEC, *Monats. Chem.*, 1929, **53**, **54**, 852.
53. M. SAMEC and M. FORSTER, *Kolloidchem. Beih.*, 1934, **39**, 464.
54. M. SAMEC, *ibid.*, 1928, **28**, 155.
55. M. SAMEC and M. BLINC, *ibid.*, 1933, **38**, 40 and 48.
56. A. R. LING and D. R. NANJI, *J. Chem. Soc.*, 1923, **123**, 2666.
57. A. FERNBACH, *Compt. rend.*, 1904, **138**, 428.
58. J. JANICKI, *Roczniki Chem.*, 1932, **12**, 402. (In French.)
59. M. SAMEC and F. VON HOFFFT, *Kolloidchem. Beih.*, 1913, **5**, 141.
60. — *ibid.*, 1912, **4**, 132.
61. T. C. TAYLOR and R. P. WALTON, *J. Amer. Chem. Soc.*, 1929, **51**, 3431.
62. A. TYCHOWSKI and S. MESIOR, *Biochem. Zeit.*, 1937, **291**, 218, 399; *ibid.*, 1937, **292**, 141.
63. P. KOETS, *Proc. Kon. Akad. Wet.*, 1935, **38**, 63.
64. P. KOETS and H. R. KRUYT, *Kolloidchem. Beih.*, 1937, **47**, 100.
65. L. LEHRMAN, *J. Amer. Chem. Soc.*, 1929, **51**, 2185.
66. — *ibid.*, 1930, **52**, 808.
67. L. LEHRMAN and E. A. KABAT, *ibid.*, 1933, **55**, 850.
68. — *ibid.*, 1937, **59**, 1050.
69. W. N. HAWORTH and E. L. HIRST, *Trans. Faraday Soc.*, 1932, 16.
70. O. S. RASK and J. K. PHELPS, *Ind. Eng. Chem.*, 1925, **17**, 187.
71. T. C. TAYLOR and R. T. SHERMAN, *J. Amer. Chem. Soc.*, 1933, **55**, 258.
72. K. MYRBÄCK and K. AHLBORG, *Svensk. Kem. Tid.*, 1937, **49**, 216.
73. W. N. HAWORTH, E. L. HIRST and WAINE, *J. Chem. Soc.*, 1935, 1299.
74. P. KARRER, *Helv. Chim. Acta*, 1929, **12**, 1144.
75. M. SAMEC, *Kolloidchem. Beih.*, 1912, **4**, 132; 1913, **5**, 141; 1914, **6**, 23; 1916, **8**, 33.
76. M. E. BALDWIN, *J. Amer. Chem. Soc.*, 1930, **52**, 2907.
77. G. MALFITANO and M. CATOIRE, *Compt. rend.*, 1923, **177**, 1309.
78. H. C. SHERMAN and J. C. BAKER, *J. Amer. Chem. Soc.*, 1916, **38**, 1885.
79. M. SAMEC, *Cereal Chem.*, 1936, **13**, 592.
80. Y. NAKAMURA, *J. Fac. Hokkaido Imp. Univ.*, 1935, **38**, 2.
81. T. J. SCHOCH, *J. Amer. Chem. Soc.*, 1938, **60**, 2824.
82. L. LEHRMAN, *ibid.*, 1939, **61**, 212.
83. POSTERNAK, *Helv. chim. Acta*, 1935, **18**, 1351.
84. W. A. RICHARDSON and R. S. HIGGINBOTHAM, *Nature*, 1940, **146**, 234.
85. — and FARROW, *J. Text. Inst.*, 1936, **27**, 131T.
86. M. SAMEC, *Kolloid-Zeit.*, 1938, **85**, 247.
87. — *Biochem. Zeit.*, 1927, **186**, 337.
88. — and BENIGER, *Kolloidchem. Beih.*, 1931, **33**, 95.
89. — and M. BLINC, *ibid.*, 1938, **47**, 371.
90. NOTTBOHM and MAYER, *Zeit. Unters. Lebens.*, 1934, **67**, 369.
91. PRZYLECKI and MAJMIN, *Biochem. Zeit.*, 1935, **280**, 413.
92. MEYER, *Compt. rend. Soc. Biol.*, 1937, **124**, 1288.
93. MACLEAN, 'Lecithin and Allied Substances,' Longmans, Green & Co. London, 1918.
94. WALDSCHMIDT-LEITZ and A. MAYER, *Zeit. physiol. Chem.*, 1935, **236**, 168.
95. — M. SAMEC and A. MAYER, *ibid.*, 1937, **250**, 193.

96. L. H. LAMPITT, C. H. F. FULLER and N. GOLDENBERG, *J. Soc. Chem. Ind.*, 1941, **60**, 231.
97. HASSID and DORE, *J. Amer. Chem. Soc.*, 1937, **59**, 1503.
98. GALLAY and BELL, *Canad. J. Res.*, 1936, **14**, 360.
99. M. SAMEC, 'Kolloidchemie der Stärke,' Dresden u. Leipzig, 1927, pp. 17-35.
100. GALLAY and BELL, *Canad. J. Res.*, 1936, **14**, 381.
101. — *ibid.*, 1936, **14**, 391.
102. W. HARRISON, *J. Soc. Dyers and Col.*, 1911, **27**, 84.

## ADDITIONAL REFERENCES

- I. MARKEVITSCH and M. KHOLODOVA, *Colloid J. (U.S.S.R.)*, 1938, **4**, 387. (Effect of time of storage of potatoes on the capacity of the starch to form complex compounds with proteins.)
- W. PAULI and ST. SZPER, *Kolloid-Zeit.*, 1938, **82**, 335. (Acidic groups in phosphorus-free dextrans attributed to uronic-acid groups.)
- P. KOETS and H. R. KRUYT, *ibid.*, 1938, **82**, 315. (Characteristic properties of cellulose, starch and dextrin can be fully explained by considering combined water to be attached between micelles and not forming a distended envelope.)
- V. I. ZARAROV, *Upsekhi Khim.*, 1937, **6**, 1105; *Chem. Zentr.*, 1938, **1**, 3776. (A review of the colloidal chemistry of starch.)
- M. SAMEC and M. BLINC, *Kolloid.-Beih.*, 1939, **49**, 75. (Review of amylase action on starch.)

## CHAPTER 7

## THE RETROGRADATION OF STARCH

J. SCHOCH<sup>61</sup> has recently applied the term 'retrogradation' to all cases where starch shows a tendency to revert to a less soluble form, including such processes as the precipitation of starch from solution by alcohol, or by removal of a peptising electrolyte where the starch is dissolved in it, the insolubilisation of starch on freezing, or drying as in the skin formation on pastes, and its deposition from aseptic solutions on keeping. Hirst and co-workers<sup>61</sup> have applied it to insolubilisation of starch on drying, and S. Woodruff and co-workers,<sup>56, 82-84</sup> and Tanner and Inglis<sup>85</sup> have applied it to the changes occurring on freezing or alcoholic precipitation. Lampitt, Fuller and Goldenberg<sup>69</sup> have, with some justice, restricted the use of the term to those physico-chemical changes of state taking place in solutions, gels and pastes of hydrophilic colloids on ageing. As will be seen below there are some very definite distinctions in some of the properties of, for example, starch separated by freezing and that deposited on ageing. Except where the term is used under specific headings below this is the meaning attached to it in this book.

L. Maquenne<sup>1</sup> noted that homogeneous starch pastes deposited solid matter on standing which is not coloured by iodine, or readily attacked by malt extract,<sup>53, 64</sup> and which, although soluble in caustic potash solutions, is but slowly acted upon by dilute mineral acids. The substance is deposited more readily at lower temperatures,<sup>53, 61, 63-65</sup> and its formation is favoured by the presence of mineral acids,<sup>2</sup> whilst the amount and speed of the retrogradation is dependent on the concentration of the paste or solution.<sup>8, 61-65</sup> The deposited substance is not soluble in water at ordinary temperatures, and requires to be heated under pressure before solution is again obtained. In this case it is slightly soluble at 120° C., but has to be heated to about 150-155° C. before it is completely dissolved.<sup>53, 61</sup> Meyer and co-workers,<sup>68</sup> however, found that retrograded maize  $\beta$ -amylose redissolves in warm water, Lampitt and co-workers<sup>69</sup> found retrograded wheat starch readily redissolves in water near the boiling-point, whilst S. Woodruff<sup>70</sup> observed that retrograded  $\beta$ -amylose readily redissolves in water at 95° C. It should be noted that Professor Woodruff uses the term 'retrogradation'



here as applying to the deposition on ageing although her paper deals with the freezing of starch pastes.

The solution so obtained from the deposited substance is readily attacked by malt extract, and gives the characteristic blue colour with iodine solution. In Maquenne's<sup>3</sup> view, foreign matter present in the starch paste is largely responsible for the formation of this deposit, and in the absence of this foreign matter the deposition would not take place. Solutions of starch made by heating it under pressure with water to 130-150° C. show this phenomenon, and also solutions of dextrans made by the dry roasting method. In the latter case it is termed in the adhesive trade 'set-back' or 'pastiness,' and is regarded as an undesirable feature, as it denotes the presence of unconverted starch, and is often shown by dextrans which give solutions that thicken on storage, thus leading to mechanical difficulties when used on machines. On the other hand, Schoch<sup>61</sup> finds that starches which have been modified by oxidation, dextrinisation or ethylation show little tendency to revert, but in each case the modification of the starch must have passed some sort of 'diffuse limit,' as a lightly oxidised or dextrinised starch will retrograde. Schoch is, of course, using the term 'retrogradation' in the sense described above. Several workers find amyloamylose to retrograde rapidly, and the ability of a dextrin to retrograde appears, from the work of Hanes and Cattle,<sup>66</sup> and of Freeman and Hopkins<sup>67</sup> to depend on the molecular weight, the lower this value the less the tendency to retrograde. Other workers claim that this also applies to starch.<sup>68, 71</sup> Starch pastes of low concentrations behave similarly to starch solutions, but at higher concentrations they set to a gel on cooling and later show signs of syneresis. At still higher concentrations syneresis is absent.<sup>53, 54</sup> The X-ray patterns of starch, 'soluble' starch and dextrin solutions and pastes, as well as that of bread, are all sharper in definition after ageing and retrogradation<sup>77-79</sup> but this is not observed with glycogen or non-retrograding dextrans.<sup>80</sup>

J. Wolff and A. Fernbach<sup>4-5</sup> discovered an enzyme in the grain and in other portions of cereals which, when added to starch pastes, very greatly increased the rate of deposition of the white substance. This enzyme they named amylocoagulase. They found that the addition of malt extract increased the formation of the deposit, and that once the precipitation had been initiated in this manner, heating the mixture to 120° C. to destroy the activity of the malt diastase had no effect in stopping the deposition. A malt extract previously heated to 120° C. before adding it to the starch paste was without effect on the rate of for-



Photomicrograph No. 1.



Photomicrograph No. 2.



Photomicrograph No. 3.



Photomicrograph No. 4.

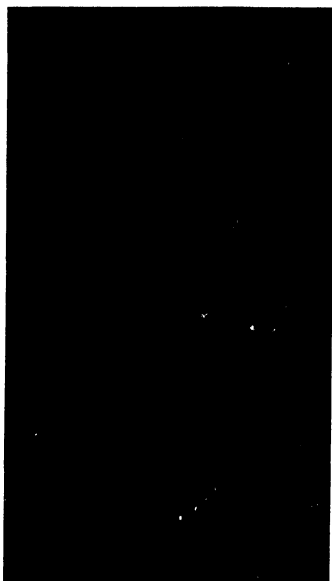
Nos. 1 and 2. Spierer photographs of corn starch partially swollen in water at 70° C., showing luminous particles.

No. 3. Same as 1 and 2, but particles here are showing tendency to concentric arrangement.

No. 4. Acid-treated wheat starch, showing radial cracks and concentric arrangement.

[Facing p. 104.]

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Photomicrograph No. 5.



Photomicrograph No. 6.



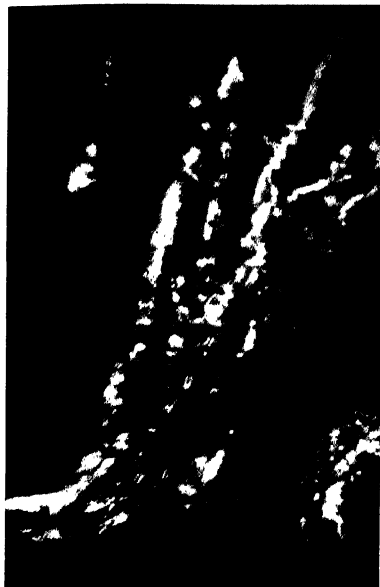
Photomicrograph No. 7.



Photomicrograph No. 8.

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- No. 5. Particles in active Brownian Movement on rim of partially swollen wheat-starch granule photographed with Spierer lens.
- Nos. 6 and 7. Corn starch fully swollen at 95° C. then frozen, showing reticulation and Spierer lines (see p. 64).
- No. 8. Wheat starch heated at 70° C. in water then frozen. Spierer lines just beginning to form.



Photomicrograph No. 9.



Photomicrograph No. 10.



Photomicrograph No. 11.



Photomicrograph No. 12.

Nos. 9 and 10. Same field of frozen wheat-starch gel ; (9) with Spierer lens, (10) with ordinary lens.

Nos. 11 and 12. Dense luminous particles from starch taken with (11) Spierer lens and (12) with ordinary lens.

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mation of the deposit. They also found <sup>6</sup> that to obtain the above effect with malt diastase it was necessary first to obtain a good dispersion of the starch, such as is observed in pseudo-solutions of starch prepared by heating it with water under pressure, or obtained by the action of certain liquefying enzymes free from amylocoagulase on starch pastes. Such solutions readily show this deposition upon the addition of an infusion of raw barley, rye, or wheat, which are without effect on pastes prepared in the normal manner.<sup>7</sup>

The action of amylocoagulase is favoured by the presence of alkali, and solutions of the enzyme are de-activated by heating to 63° C., although dry powder preparations containing it may be heated to higher temperatures before the enzyme is destroyed.

Maquenne and Roux <sup>9-11</sup> came to the important conclusion that two substances exist in starch, one of which slowly undergoes condensation with itself, giving the white matter which is deposited from solution. This portion of the starch they termed 'amylocellulose' or 'amylose.' To the other constituent, 'amylopectin,' they ascribed the gelatinising power of the starch, since amylose when dissolved in water gives mobile solutions devoid of gelatinising power. By the action of malt diastase on starch pastes they obtained maltose corresponding in amount to about 80 per cent. of the starch, and they further observed that on allowing malt extract to stand,<sup>12</sup> or by adjustment of the *pH* value with acid,<sup>13</sup> a larger amount of maltose was obtained, and they considered the amylopectin was a maltosan-like amylose.<sup>14</sup>

Retrogradation is considered by E. Fouard <sup>15</sup> to be due to the fixation of the phosphoric acid by the starch molecule (see below). In his work he found <sup>16</sup> that reversion is unaffected by the presence of weak acids, but that strong acids favour the action up to a limiting *pH* value and, further, that reversion is retarded by alkalis to the same extent when used at concentrations giving the same amount of hydroxyl ions. It should be noted that although the amount of material deposited increases progressively with decrease in *pH* value below *pH* 3, there is a falling off in the amount retrograding owing to the acid hydrolysing the starch. L. Berczeller,<sup>17</sup> however, considers that the phenomenon is due to the aggregation of colloidal particles of amylose present, and that the action of amylase in speeding up the deposition is merely due to its action in reducing the viscosity of the solution by liquefying the amylopectin, thus allowing a freer passage of colloidal particles to one another and permitting the larger aggregates thus formed to precipitate immediately. The homologues of amylose present in natural starch are readily soluble in water,

unlike the highly condensed amylose present in the reversion product, and so it has been suggested that the lower homologues may act as a protective colloid for those higher in the series, and that when the protective compounds are removed or aggregate themselves the insoluble amylose precipitates from the solution. H. Sallinger<sup>18</sup> has shown that ptyalin can also effect reversion, which he attributes to the conversion of the protective colloid into non-protective sugar, whereby reversion is brought about. Should this be so, then it is unnecessary to assume the existence of a coagulating enzyme. A. Fernbach and J. Wolff,<sup>19</sup> however, injected extract of malt subcutaneously into rabbits, and found that the blood serum appeared to contain an anti-amylocoagulase; this they regard as proof of the presence of amylocoagulase in malt extract.

Using starch pastes containing about 10-20 per cent. of starch, or solutions of certain dextrans containing about 40 per cent. of solid matter, retrogradation may be noted after about 48 hours have passed, whereas a paste made by heating starch with an equal weight of water at 100° C. for 1 hour can readily be broken off short if allowed to stand for the same time at normal temperatures. From this J. R. Katz<sup>20-21</sup> concludes that the swelling and stiffening of starch in water is an equilibrium process, and that the limiting condition thus depends on the temperature and the relative amount of water present (see below). This phenomenon is not characteristic for one variety of starch, but is shown by potato, rye, wheat, barley, maize, and other starches. The retrogradation of wheat starch ground in a ball-mill for varying lengths of time has been thoroughly studied by L. H. Lampitt, Fuller and Goldenberg.<sup>69</sup>

**'Retrogradation' of Starch by Freezing.**—S. Woodruff and her co-workers have studied the effect of freezing on the retrogradation of starch gels and have obtained a number of interesting results. Markedly different results were produced by freezing at -2° C. and freezing at -70° C., the gel in the latter case on thawing retained most of the slimy consistency of a newly gelatinised starch, and had a 'brittle' microscopic appearance, whereas the former had a spongy, fibrous structure showing heavy strands under the microscope. It was found that there was a transition temperature at -25° C. below which the gel after thawing had the 'brittle' microscopic appearance, whereas the coarser microscopical appearance was always obtained by freezing above this temperature.

When material frozen at temperatures higher than -25° C. was examined microscopically between crossed Nicols, strands of

anisotropic material which had not existed before freezing could be seen, and when the granules were not completely gelatinised before freezing small anisotropic particles were seen which tend to form chains during freezing. When more completely gelatinised paste is used the phenomenon is not readily obtainable.

Gels 'retrograded' by precipitation with alcohol differed markedly from those 'retrograded' by freezing, in that between crossed Nicols they were luminous throughout and granules in the gels appeared to have been dehydrated but not re-oriented into strands, as by freezing. Re-gelatinising this substance gives a gel very similar to the original.

It should be noted that, as judged by the X-ray pattern, the changes effected by freezing of starch pastes are distinct from those taking place during retrogradation, as generally understood when applied to physico-chemical changes which take place on ageing of starch pastes and solutions.<sup>75</sup>

S. Woodruff<sup>56</sup> has also used the Spierer lens in this work, and the strands of reticulated, anisotropic material appear with even greater distinctness. For convenience she has termed these 'Spierer lines.' They are parallel, somewhat discontinuous, alternating bright and dark bands which follow the strand in the frozen structure. Amylose which has been allowed to retrograde for some months to a white flocculent material showed a 'brush-heap' or 'fish-egg' structure, but after dissolving at 95° C. and freezing, the material precipitated was fibrous, having similar Spierer lines and strands as the frozen starch gels. Amylose and amylopectin are indistinguishable by the reticulation shown on freezing. The Photomicrographs 1-12, taken by Professor S. Woodruff, illustrate some of the results obtained.

Small rounded particles were observed in slightly swollen starch granules or in the fluid surrounding them. They were separated and many showed anisotropy. Woodruff suggests that these particles may lose water of hydration during freezing and re-orient themselves to give the luminous Spierer lines, but her experimental work directed to proving this has, so far, proved unsuccessful.

**'Retrogradation' by Solvents.**—The precipitation of starch from solution by alcohols may be considered as involving a type of retrogradation, as it is in marked contrast to that of crystalloids where there is a definite solubility for each alcohol/water mixture. Methanol slowly added to an autoclaved starch solution produces no change until some 40 per cent. has been added, when the solution goes opaque, and when 45 per cent. has been added the starch has flocculated completely. T. J. Schoch<sup>61</sup> gives the



following percentages for the concentration of solvent to initial flocculation and points out that they are significantly in line with the dielectric constants: acetone or diacetone, 32; methyl or ethyl alcohol, 38-40; methyl cellosolve or dioxan, 53-56; glycerol or ethylene glycol, above 70. Owing to the very narrow limits of concentration necessary for initiating the precipitation and completing it, attempts to separate possible starch components by fractional precipitation have been unsuccessful.

The precipitated starch cannot be redissolved to give anything approaching its former degree of dispersion, even by autoclaving, but it can be dissolved in alkali although not easily. Since alkali peptised starch does not retrograde on freezing or on standing, nor does it show the skinning effect observed with ordinary starch pastes, this behaviour might be expected.

Certain polar compounds having a heavy hydrophobic loading will also precipitate starch, but by a mechanism different from retrogradation.<sup>61</sup> In the case of such compounds, e.g. certain wetting agents, fatty acid, long-chain alcohols, soaps, turkey-red oil and cyclohexanol, they are adsorbed by association of the polar group with the OH groups of the starch, thus producing an oriented, waterproof film of fatty acid, or other radicles. Some of the physical differences between the tuber and cereal starches are ascribed by T. J. Schoch<sup>61</sup> to the presence of polar fatty acids in the latter. Removal of the fatty acids by solvent extraction in the case of maize and rice gives products which give more transparent and glutinous gels than the untreated starches. Tapioca pastes becomes 'short' and opaque, thus moving in the opposite direction, when a sulphonated oil is added. On removal of these compounds from the starch, e.g. steam distilling of cyclohexanol from the starch which it has precipitated from the paste, the starch readily redissolves in water showing retrogradation has not occurred. Much use is made of this effect in the adhesives industry, the properties of the pastes being readily altered to suit the type of work for which it is to be used (Chap. I, Pt. 3).

We see from the foregoing work that the affinity of starch for water is opposed by the more powerful forces of association between the starch molecules, and consequently hydration proceeds generally only to the gel stage and only with difficulty to the stage of giving a colloidal solution. Such solutions are metastable and readily revert to the more stable insoluble state. More complete dissociation can be brought about by the presence of a substance having a greater ability than water to satisfy the intermolecular forces is necessary. The diagrammatic repre-

sentation by T. J. Schoch readily shows the various degrees of starch dispersion and the influence of various factors upon them :

MONOMOLECULAR DISPERSION

↑ Heat	↑ Dextrins and modified starches
↓ Time	Solutions in quaternary bases
↓ Concentration	Autoclaved pastes
↓ Acidity	Caustic alkali gels
	Thiocyanate gels
	Boiled pastes
	Raw starch
	Frozen starch
	Alcohol-precipitated starch
	Starch 'skins'

**Complete Retrogradation.**—The above worker has recently suggested that there may be considerable foundation for the older concept of two fractions existing in starch having different chemical configuration, physical characteristics and chemical reactivity. He has obtained a semi-crystalline material in the form of minute spheroids or needles at the interface between autoclaved starch solution and butyl or amyl alcohol. It is quite different from the amorphous floc of retrograded or methanol-precipitated starch, and dehydration with alcohol renders it completely insoluble. If not dehydrated but separated by centrifuging and treating with boiling water it gives a clear, thin syrup even at 20 per cent. concentration, the solution showing exaggerated tendencies to retrogradation. Cooling sets this solution to a rubbery gel which cannot be liquefied by heating. The other portion of the starch gives clear stable syrups. The semi-crystalline material produced by this method has a much greater alkali lability than the parent raw starch, whilst the soluble fraction is correspondingly more alkali stable. The further examination of these fractions from the point of view of structural differences will be of interest.

**The Explanation of Retrogradation.**—Two suggestions as to the cause of retrogradation brought about by enzymes have already been discussed briefly above. Zsigmondy<sup>86</sup> and Samec<sup>87</sup> have observed aggregation in starch solutions on ageing by means of the ultra-microscope, and the latter worker,<sup>82</sup> and similarly Pringsheim,<sup>63</sup> considers that dehydration and aggregation are large contributory factors to this phenomenon. Staudinger and Husemann<sup>71</sup> consider the ageing effects to be due to the presence of phosphorus as they do not appear if this is removed by

acids. Richardson and co-workers<sup>88, 89</sup> consider that this treatment would cause depolymerisation and suggests that the starch separating consists of the larger starch macromolecules present. C. H. F. Fuller<sup>54</sup> ascribes it to the reduction, with time, of the hydration capacity of the starch, and J. R. Katz considers that there is an equilibrium between two phases present. His work, more particularly directed towards the examination of the causes and prevention of bread staling, is discussed below. Meyer and co-workers<sup>68</sup> consider that immediately after preparation starch solutions are supersaturated and retrogradation is the result of the solution attaining equilibrium. Alsberg<sup>53</sup> tends to support this view and suggests that the property is common to all supersaturated solutions and gels, whilst further indirect support also comes from the work of Jackson and Hudson<sup>90</sup> (see p. 19) who found that colloidal solutions which deposited matter on ageing could be obtained from starch oxidised with periodic acid. In this interesting reaction the pyranose rings are split between the C<sub>2</sub> and the C<sub>3</sub> positions, the two hydroxyl groups being converted to aldehydic groups but the linkages between the pyranose rings are unaffected. Work on other colloids such as gelatin, agar, pectin, Irish moss tends to support Alsberg's contention and the work of Kruyt<sup>91-92</sup> on various colloids falls also into line with this suggestion. The latter worker points out that when the concentration of a colloid is too low for it to form a network of hydrated micelles, i.e. to gel, the particles of dissolved phase are precipitated, probably due to aggregation of the colloidal particles. As the property of retrogradation tends to disappear with fall in mean molecular weight it is interesting to note that the power of setting to a gel is affected in a similar manner.

#### **Practical Significance of Retrogradation in Industry.—**

The change in starch solutions leading to the formation of retrogradation products has a certain practical importance in industry, and has been used to explain several observed facts.

Some soluble starches have been put on the market to be used for textile purposes, and are claimed not only to dissolve in cold or warm water, but to give clear solutions that are practically permanent. Such starches have generally undergone an oxidation process, and certain substances may be present to inhibit the retrogradation.

In connection with the bakery trade, much interest has been shown by various workers<sup>22-26</sup> in the so-called 'staling' of bread. The early work on this subject is well known, and has been summarised by W. Platt<sup>52</sup> and C. L. Alsberg.<sup>53</sup>

As is well known, on keeping bread under normal conditions, it changes in texture from a soft to a hard granular state. True staling is not a mere hardening process due to loss of moisture, and early workers found that the staling process could be reversed by heating the bread for a short time. They also found that staling is accompanied by a loss of the swelling power of the crumb in water and by a reduction in the amount of water-extractable polysaccharides. The changes in starch pastes on ageing and the staling process in bread were also connected by some of these workers.

J. R. Katz, between 1912 and 1916, made contributions of major importance to this subject, and extended the previously held conceptions. With Verschaffelt<sup>26</sup> he examined fresh and stale bread microscopically, and found that the granules of starch in stale bread are sharply outlined, due to a very thin layer of air surrounding the granules, whereas in fresh bread no such break occurs between the starch and the matrix of gluten. Katz took as criteria of staling (1) changes in hardness of the crumb, (2) changes in the amount of amylose extractable by water, and (3) changes in the swelling power of the crumb when suspended in water. He pointed out that these were in the same direction in both bread and in starch pastes of similar water-content, and further, that retrogradation in starch pastes and the staling of bread both take place more readily at lower temperatures. Katz therefore attributes staling to the retrogradation of the starch present.

Against this view must be cited the observation that bread, which contains a high proportion of starch, begins to stale a few hours after it has been made, whereas a starch paste containing a high proportion of starch does not begin to show retrogradation until after 24 to 48 hours. It is a well-known fact that stale bread can be freshened by heating for a short time in an oven at relatively moderate temperatures, but retrograded starch cannot be redissolved or obtained in the form of a gel at the temperature used to freshen stale bread, and is indeed very much more resistant to solution. It is generally held that stale bread is more digestible than new bread, but it has been found that retrograded starch is much more resistant to enzymes and the actions of acids than newly-prepared starch pastes or solutions.

Ostwald,<sup>57</sup> although agreeing that staling is caused by a change in the starch, believed it to be due to syneresis caused by the change in its hydration capacity. Kuhlmann and Golosowa<sup>55</sup> followed the amount of 'bound water' in bread throughout the process of baking, cooling, and staling. The 'water-binding capacity' was found to be greatest at the conclusion of the baking process,

and to become less during the process of cooling and subsequent staling.

C. H. F. Fuller<sup>54</sup> used the Farinograph, which is essentially a dynamometer that records in graphical form the work done during the mixing of a bread dough at a fixed temperature, to determine the absorption of water by breadcrumb at various stages during the staling process. He found that staling is rapid for the first few hours, when the bread is kept at normal temperatures, and that it still takes place, although slowly, at 60° C. At this temperature, according to Katz, bread would remain fresh indefinitely. He further found that on keeping at temperatures between 60° and 100° C. the bread crumb toughens, the change being relatively rapid at 100° C. The loss of absorption by breadcrumb at high temperatures is not reversible, and may not be directly connected with the normal staling change.

Fuller points out that in certain cases, e.g. when bread is made from a formula containing diastatic malt extract or a similar component, the results obtained by his absorption method disagree with those obtained by Katz's swelling-power method and with compressibility determinations, in that abnormally low values for absorption are given by the fresh crumb, and these values do not decrease as the bread stales. The swelling-power method of Katz and the compressibility show the normal decrease on staling, and so far no explanation has been put forward to account for the above findings.

Katz applied the swelling-power test and the determination of soluble amylose to starch gels, and found changes similar to those obtained with bread. Fuller, using the Farinograph, finds the same behaviour, and further supporting evidence has been obtained by Katz from X-ray studies.

**The Prevention of Retrogradation.**—The amount of retrogradation in starch solutions appears to be greater in very dilute acids and bases than in water, and on increasing the amount of alkali present in a progressive manner retrogradation is partially and then completely inhibited. According to Samec<sup>82</sup> acid salts and basic salts behave in a similar manner to acids and bases respectively. In general, it appears that agents which peptise starch, such as urea, acetamide, phenol, polyphenols or amines (see below, and p. 50) retard or prevent retrogradation. Samec and Benkovic<sup>72</sup> have shown that ammonium thiocyanate or chloride, phosphates, glucose, glycerol and borax also partly inhibit the retrogradation. Agents tending to coagulate starch such as ethyl ether, or acetate, the water-miscible monohydric alcohols and ammonium sulphate increase retrogradation.<sup>72-74</sup>

In order to prevent the staling of bread, Katz has suggested keeping it at about  $60^{\circ}$  C., which would inhibit staling for 12 to 24 hours. This method would, however, cause excessive drying out, a loss of the volatile flavouring constituents, and a toughening of the bread crust, all of which would detract from its practical value.

A further suggestion due to Katz is that the bread should be kept at  $-20^{\circ}$  C., when there would be no free water except in the solid form and no retrogradation of the starch. At  $0^{\circ}$  C. the rate of staling is at a maximum, so that this point must be passed rapidly when the temperature is reduced to the lower limit, and on thawing out the deposition of moisture on the crust of the bread must be prevented. Bread has been kept in the fresh condition<sup>54</sup> for two to three weeks by enclosing the loaf in a thin rubber membrane, contact between loaf and bag being ensured by exhausting the air from the container and then rapidly freezing in a brine bath. When required for use, the bread is allowed to regain room-temperature before the bag is removed.

J. R. Katz has carried out some interesting work on prevention of staling in bread by adding various substances to it, and he has also studied the liquefaction of starch and gelatine, using capillary active substances. Some of his observations form the basis of processes used commercially to obtain liquid glues and starch products.

If a globule of mercury is placed in water, the metal assumes a negative charge and the water a positive charge. Substances which contain polar groups, when dissolved in the water, are adsorbed on the mercury by virtue of the charge carried by the polar group of the substance. A. Frumkin<sup>27</sup> has measured the adsorption of various compounds at the mercury/water interface, and found that substances showing positive polar adsorption have the power of liquefying gels of gelatine or of lowering the gel-point of gelatine. Among these compounds are urea, thiourea, thioacetamide and other organic substances. Positively adsorbed compounds also prevent the retrogradation of starch and, used in a sufficient concentration, can effect its liquefaction. Such substances appear to be adsorbed on the surface of the starch micelles.

Katz has found that aldehydes, such as acetaldehyde or propionaldehyde, and strongly basic substances, such as pyridine, exert a strongly retarding effect on the retrogradation of starch solutions. Unfortunately, such compounds cannot be used in bread or other foodstuff preparations, but suitable compounds can be, and are, used in commercial preparations which are destined for

other purposes. Ketones were found by Katz to be inactive in this respect, although aldehydes as a class were the most effective of all the compounds tried. Basic substances are somewhat less active in preventing the staling of bread, pyridine and dipropylamine being the most active.

Acetaldehyde (10 per cent. by volume) will prevent staling, and under the microscope its marked effect on the hydration capacity of the starch at room-temperature can be seen in its prevention of swelling. The iodine reaction of soluble starch is not altered by its presence, and if added to a starch gel the loss in intensity of the blue colour which normally occurs on ageing does not take place, but if the acetaldehyde is removed the normal decrease in intensity of the blue colour begins at once.

Katz showed that, judged by the swelling-in-water criterion, bread remained fresh for many days. The amount of soluble starch at this period had, however, fallen to approximately the same amount as present in normally staled bread. If the added substance be removed by passing a current of air through the bread, the staling process starts at once and follows its normal course, although prior to the removal the texture and appearance left nothing to be desired.

The action of the retarder is not clear, and it is possible that some type of addition product is formed between it and the hydroxyl-groups in the starch, so that aggregation through interaction between these groups to produce the less soluble form of starch is inhibited. In this case it is, of course, necessary to assume that the addition product so formed would not be capable of aggregating or crystallising. It is very difficult to visualise a colloidal chemical action which is limited only to aldehydes and strongly basic substances, and it is just as difficult to visualise an addition compound between these substances and starch, so loosely bound that the mere passage of air is sufficient to remove the volatile component.

The problem is a complex one, and in bread the system contains other substances which would doubtlessly influence the behaviour of the whole. It may be that, as suggested by Whymper,<sup>25</sup> the discrepancies shown in the behaviour of the two systems, bread and starch pastes, are due to some sort of protective action exerted on the starch by the gluten present in the former, but so far no completely satisfactory solution of the problem has been put forward. The gluten appears to play quite a definite part, probably by absorbing water from the starch gel and altering its structure. This is confirmed by the fact that the addition of fully hydrated gluten during bread-making results in a loaf which

keeps fresh for a long period.<sup>59</sup> There appears to be a definite equilibrium between the two forms of amylose at any particular temperature; and in the case of bread, storing at an elevated temperature affects this equilibrium in such a manner as to yield bread having preferred properties, but a number of difficulties would be encountered in the practice of this method. The same end is achieved by altering the state of hydration of the starch by freezing, and although so far the only substances which can be added to prevent staling are noxious, it is probable that at some future date an innocuous chemical will be found to serve the purpose. Hutchinson<sup>58</sup> in a comprehensive study of staling distinguishes between chemical and physical staling. The former can be followed by the X-ray spectra, decrease in sedimentation volume of the powdered crumb and decrease in extractable polysaccharides. These tests show a maximum reaction in about 48 hours but the staling continues after this without much further indication being given by the tests. The further progress in staling is marked by loss of flavour and aroma and the bread becoming brittle and crumbly.

D. W. Kent-Jones<sup>59</sup> sums up some of the reasons for bread staling more rapidly than it should normally do. They are:—

1. Doughs made too tight or too slack.
2. Over-mixing of the doughs.
3. Doughs made too warm.
4. Doughs used in an under-ripe or over-ripe condition.

Another problem in which the retrogradation of starch probably plays an important part is in the manufacture of certain articles of confectionery, especially those known as the 'clear gum' type. These hard gums are generally made by using gum-arabic, glucose, etc., and are practically transparent. Substitution of the gum-arabic by starch gives what at first appears to be an apparently good gum, but on storage it becomes perfectly opaque, due to the retrogradation of the starch, such behaviour precluding the use of untreated starch for this kind of work (see p. 303).

The action of formaldehyde on starch has received much attention commercially, and work has been directed mainly towards producing substances for use as adhesives, textile agents or anti-septic agents. In light of the considerations discussed above, it may be of interest to examine this work briefly.

**Reactions with Formaldehyde.**—According to Classen, when starch or dextrin is treated with formaldehyde, compounds of constant composition are formed<sup>28</sup> which are comparatively stable to heat, and which may, or may not, form gels, according to the



conditions of preparation. Upon treating with dilute acid or alkali, free aldehyde is liberated. These white compounds have been suggested for use as antiseptic surgical dressings by the above worker<sup>29-31</sup> and others.<sup>32, 33</sup>

C. Longard<sup>34</sup> held that the compounds formed are mechanical mixtures of starch and formaldehyde, but further work tends to show that a chemical entity is concerned, very possibly by cross-linkage, via a methylene group, between two hydroxyl groups in different molecular chains.

Potato starch and amyloextrin, on treatment with 40 per cent. formaldehyde for two months,<sup>35</sup> give no reaction with iodine, and yield compounds that rapidly decompose on heating with liberation of formaldehyde. These compounds are hydrolysed slowly by water and rapidly by acids, the product of hydrolysis resembling amyloextrin. The temperature of gelatinisation is stated to decrease with increase in concentration of formaldehyde and the length of time of the action.<sup>36</sup>

Removal of the formaldehyde from the white compound regenerates the starch practically unchanged,<sup>37, 38</sup> and addition of ammonium acetate to 'amyloform' solution<sup>39</sup> causes the starch to give the normal blue colour with iodine, whilst if alcohol be added to the amyloform solution, practically unchanged starch is precipitated. Formaldehyde is also regenerated by treatment with phenylhydrazine and alcohol.<sup>41</sup> W. von Kaufman and A. Lewite<sup>40</sup> consider that the formaldehyde produces a reversible physical change in the state of aggregation of the starch, although G. Woker and H. Maggi<sup>42-46</sup> contend that the starch is hydrolysed by formaldehyde.

According to H. Sallinger,<sup>47</sup> amyloextrin, after digestion with formaldehyde, showed no change in its optical activity, and soluble starch converted to amyloform and then regenerated showed no increase of reducing power. To explain why he found hydrolysis and degradation to occur, whilst other workers obtained unaltered starch, G. Woker<sup>43, 46</sup> suggests that under the conditions used by the other workers resynthesis of the degradation products occurred, and he cites as an analogous phenomenon the digestion of egg albumin by papain.

M. Samec<sup>48</sup> has studied the reaction at various temperatures, and finds that a loose addition- and highly-hydrated compound is formed, giving no iodine reaction and having about the same molecular weight and dialysable fraction, considerably higher viscosity, slightly higher specific gravity, and slightly lower optical rotation than starch itself. He considers that formaldehyde is incapable either of degrading starch or of resynthesising

its degradation products. The action of formaldehyde appears to be the same on both amylose and amylopectin.

Although the compound formed when 38 per cent. formaldehyde is added to starch in slight excess gels in the cold, it becomes insoluble in boiling water. Potato starch gives a clear gel, cassava starch a slightly cloudy gel some time later, whilst wheat and maize form opaque pastes. If a large excess of formaldehyde be used, especially with starch previously gelled, a type of product soluble in cold water is obtained, a still different type being produced by the action of formaldehyde when present in less than the theoretical amount.

If a soluble starch giving a limpid solution is used, the product no longer disperses and fluid solutions in hot water are not obtainable; instead, it swells up at the temperature of gelatinisation of the original untreated non-soluble starch. An interesting product is that obtained by the action of formaldehyde on dry non-soluble starch, which gives a thick viscous solution at 55° C. It may be prepared<sup>51</sup> by heating 100 pts. of starch with 1.8 pts. of formaldehyde for 1 hour at 76° C. in a closed vessel with stirring. By varying the conditions of reaction, the temperature of gelling and the nature of the solution can be considerably altered.

For example, if the above product is pasted with 1½ times its weight of water at 55° C., dried at 80° C. and powdered, a cold-water compound is obtained which with 12-14 times its weight of water gives a highly adhesive transparent jelly.

E. Blumer<sup>49</sup> treats starch with diluted alkali hydroxide of 5° Bé. and formaldehyde for several hours, and then heats for a short period; after washing the product free from formaldehyde and neutralising with dilute acetic acid, he dries it at a temperature not exceeding 50° C. Other patents<sup>60</sup> describe the production of a starch product resistant to water by the action of formaldehyde and an acid on dehydrated starch. The colour, opacity and adhesiveness of the products vary according to the conditions of the reaction and the type of starch employed. G. J. Leuck uses anhydrous starch to prepare formaldehyde starch for use as a plywood adhesive where the presence of the minimum amount of water is desirable.<sup>93</sup>

The action of formaldehyde on starch has been summarised by F. Beltzer.<sup>50</sup>

The above facts throw little light on the results obtained by Katz on the prevention of staling of bread. It would appear, however, that the role of gluten in the process of staling is an important one, which must be taken into consideration when postulating a theory to account for the phenomenon.

## REFERENCES

1. L. MAQUENNE, *Compt. rend.*, 1903, **137**, 88.
2. — *ibid.*, 1903, **137**, 797.
3. — *ibid.*, 1903, **137**, 1266.
4. J. WOLFF and A. FERNBACH, *ibid.*, 1903, **137**, 718.
5. — *ibid.*, 1904, **138**, 819.
6. — *ibid.*, 1904, **139**, 1217.
7. — *J. Fed. Inst. Brew.*, 1904, **10**, 216.
8. L. MAQUENNE, *Compt. rend.*, 1904, **138**, 213.
9. E. ROUX, *ibid.*, 1906, **142**, 95.
10. L. MAQUENNE, *ibid.*, 1905, **140**, 1303.
11. — *Bull. Soc. chim.*, 1905, **33**, 723.
12. L. MAQUENNE and E. ROUX, *Compt. rend.*, 1906, **142**, 1387.
13. — *ibid.*, 1906, **142**, 126.
14. — *ibid.*, 1906, **142**, 1057.
15. E. FOUARD, *ibid.*, 1907, **144**, 501.
16. — *ibid.*, 1907, **144**, 1366.
17. L. BERCELLE, *Biochem. Zeit.*, 1917, **84**, 37.
18. H. SALLINGER, *Kolloid-Zeit.*, 1919, **25**, 79.
19. A. FERNBACH and J. WOLFF, *Woch. f. Brau.*, 1906, **23**, 743.
20. J. R. KATZ, *Verslag Akad. v. Wetenschappen, Amsterdam*, 1914, **23**, 652.
21. — *ibid.*, 1914, **23**, 655.
22. DILL, *Cereal Chem.*, 1925, **2**, 1.
23. L. LINDET, *Bull. Soc. chim.*, 1902, **27**, 634.
24. E. ROUX, *Compt. rend.*, 1894, **118**, 1356.
25. R. WHYMPER, *Third Report Comm. Coll. Chem., Brit. Ass. Advanc. Sci.*, 1920, p. 60.
26. J. R. KATZ, *Zeit. f. physiol. Chem.*, 1915, **95**, 104, 136 and 147.
27. A. FRUMKIN, *Coll. Sympos. Ann.*, 1930, **7**, 89.
28. A. CLASSEN, *Therapeut. Monatsh.*, 1897, 33.
29. — *Pharm. Zeit.*, 1896, **41**, 615.
30. — G.P. 92,259, 1896. (Lapsed.)
31. — G.P. 94,282, 1896. (Lapsed.)
32. A. GOTTSTEIN, *Therapeut. Monatsh.*, 1897, 95.
33. C. L. SCHLEICH, *ibid.*, 1897, 97.
34. C. LONGARD, *ibid.*, 1897, 34.
35. W. SYNIEWSKI, *Liebig's Ann. der Chemie*, 1902, **324**, 201.
36. A. REICHARD, *Zeit. ges. Brauw.*, 1908, 161; via *J. Soc. Chem. Ind.*, 1908, **27**, 461.
37. W. VON KAUFMAN, *Biochem. Zeit.*, 1917, **78**, 371.
38. — *Ber.*, 1917, **50**, 198.
39. M. JACOBY, *ibid.*, 1919, **52**, 558.
40. W. VON KAUFMAN and A. LEWITE, *ibid.*, 1919, **52**, 616.
41. J. WOHLGEMUTH, *Biochem. Zeit.*, 1919, **94**, 213.
42. G. WOKER, *Ber.*, 1916, **49**, 2311.
43. G. WOKER and H. MAGGI, *ibid.*, 1919, **52**, 1594.
44. H. MAGGI, *Helv. Chim. Acta*, 1918, **1**, 433.
45. — *Fermentforschung*, 1919, **2**, 304.
46. G. WOKER, *Biochem. Zeit.*, 1919, **99**, 307.
47. H. SALLINGER, *Ber.*, 1919, **52**, 651.
48. M. SAMEC and A. MAYER, *Kolloidchem. Beih.*, 1921, **13**, 165.
49. E. BLUMER, G.P. 179,590, 1904. (Lapsed.)

50. F. BELTZER, *9th Int. Congr. Appl. Chem.*, 1912, 7, 7.
51. WULKAN, G.P. 403,183, 1921. (Lapsed.)
52. W. PLATT, *Cereal Chem.*, 1930, 7, 1-34.
53. C. L. ALSBERG, *Wheat Studies*, 1936, 12, No. 6, 221.
54. C. H. F. FULLER, *J. Soc. Chem. and Ind.*, 1938, 57, 562.
55. KUHLMANN and GOLOSOWA, *Cereal Chem.*, 1936, 202.
56. S. WOODRUFF, *Ind. Eng. Chem.*, 1938, 30, 1409.
57. W. OSTWALD, *Kolloid-Zeit.*, 1919, 25, 26.
58. R. HUTCHINSON, 'Food and the Principles of Dietetics,' Arnold, London, 1937.
59. D. W. KENT-JONES, 'Modern Cereal Chemistry,' Northern Publ. Co., Liverpool, 3rd edit., p. 241, 1939.
60. CORN PRODS. REFIN. CO., F.P. 844,509, 844,510, 26/7/1939 : E.P. 552,732, 10/2/1938 ; U.S.P. 2,222,874, 26/11/1940.
61. T. J. SCHOCH, *Cereal Chem.*, 1941, 18, 121.
62. M. SAMEC, 'Kolloidchemie der Stärke,' Dresden and Leipzig, 1927, p. 393.
63. PRINGSHEIM, 'Chemistry of the Saccharides,' McGraw-Hill, N.Y., 1932, p. 216.
64. O. STAMBERG and BAILEY, *Cereal Chem.*, 1939, 16, 330.
65. M. SAMEC and J. R. KATZ, *Kolloid.-Beih.*, 1939, 49, 455.
66. C. S. HANES and M. CATTLE, *Proc. Roy. Soc.*, 1938, 125B, 387.
67. FREEMAN and HOPKINS, *Biochem. J.*, 1936, 30, 442.
68. K. H. MEYER, P. BERNFELD and E. WOLFF, *Helv. Chim. Acta*, 1940, 23, 854.
69. L. H. LAMPITT, C. H. F. FULLER and N. GOLDENBERG, *J. Soc. Chem. Ind.*, 1941, 60, 175.
70. S. WOODRUFF, *Ind. Eng. Chem.*, 1938, 30, 1409.
71. STAUDINGER and HUSEMANN, *Ann.*, 1937, 527, 195.
72. M. SAMEC and BENKOVIC, *Kolloid. Beih.*, 1936, 43, 272.
73. SAMUEL, *Chem. and Ind.*, 1936, 672.
74. M. SAMEC, *Kolloid. Beih.*, 1912, 4, 132.
75. J. R. KATZ and WEIDINGER, *Zeit. physik. Chem.*, 1937, 180A, 423.
76. C. H. F. FULLER, *J. Soc. Chem. Ind.*, 1938, 562.
77. J. R. KATZ *et al.*, *Zeit. physik. Chem.*, 1930, 150A, 37, 60 ; 155A, 199 ; 169A, 339 ; 170A, 421.
78. K. H. MEYER and BRENTANO, *Arch. Sci. phys. nat.*, 1936 [V], 18, Suppl. III.
79. — *et al.*, *Helv. Chim. Acta*, 1937, 20, 1331 ; 1940, 23, 845 and 854.
80. M. SAMEC and J. R. KATZ, *Zeit. physik. Chem.*, 1932, 158A, 321.
81. E. L. HIRST, M. M. T. PLANT and WILKINSON, *J. Chem. Soc.*, 1932, 2375.
82. S. WOODRUFF and HAYDEN, *J. Agric. Res.*, 1936, 52, 233.
83. — and McMASTERS, *Ill. Agric. Exp. Stat. Bull.* No. 445, 1938.
84. — *Ann. Rept. Ill. Agric. Exp. Stat.*, 1934, 252.
85. TANNER and INGLIS, *Food Res.*, 1940, 5, 503.
86. ZSIGMONDY, 'Colloids and the Ultramicroscope,' 1909.
87. M. SAMEC, *Biochem. Zeit.*, 1928, 195, 49.
88. RICHARDSON, HIGGINBOTHAM and FARROW, *I. Text. Indust.*, 1936, 27, T. 131.
89. FARGHER and PROBERT, *ibid.*, 1927, 18, T. 559.
90. JACKSON and HUDSON, *J. Amer. Chem. Soc.*, 1937, 59, 2049.

91. KRUYT, 'Colloids,' Wiley, N.Y., 1927.
92. ——— and KOERS, *Kolloid. Beih.*, 1937, **47**, 1191.
93. G. T. LEUCK (to Corn Prod. Ref. Co.), U.S.P. 2,222,872 ;  
2,222,874 ; 2,222,785.

ADDITIONAL REFERENCE

- H. L. VAN DE SANDE-BAKHUYZEN, *Proc. Soc. Exp. Biol. Med.*, 1926, **23**,  
506. (Spherocrystals appear after three weeks when amylose  
solution is added to alcohol.)

## CHAPTER 8

## STARCH AND THE HYDROGEN BOND

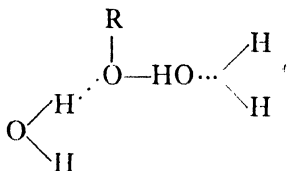
Contributed by G. V. CAESAR, *Research Laboratory, Stein Hall & Co., New York, N.Y.*

IN recent years the conception of a new form of valence linkage through hydrogen (the 'hydrogen bond') has been exhaustively investigated and experimentally established. Its literature is growing very rapidly, and the scope of this chapter does not permit of an adequate review. A few references only<sup>1-8</sup> must suffice. At present the nature of the bond is regarded as largely ionic, in effect a hydrogen bridge between two electronegative atoms, viz.,  $O-H \cdots O$ ,  $N-H \cdots O$ ,  $N-H \cdots N$ ,  $N-H \cdots F$ , etc., using Pauling's designation ( $-H \cdots$ ). The discovery of this form of secondary valence promises to be one of the most important in the history of physical chemistry, and can no longer be ignored in the study of many types of compounds. Hydroxylated compounds, especially, have been found to associate through this form of linkage, and expression has been given to the thought that it might play an important role in starch and cellulose structure and behaviour.<sup>5, 6, 9-12</sup> Unfortunately, these suggestions appear to have been more or less overlooked by the majority of starch investigators (and of cellulose also). I have particularly welcomed this opportunity to attempt very briefly and, I fear, inadequately, a portrayal of the  $O-H \cdots O$  linkage in starch, and its influence on the behaviour of starch and some of its derivatives.

When an aqueous dispersion of a starch is hydrated, let us say, by cooking, the initial physical reaction is a swelling or gelatinisation of the granular packages. This effect is largely a function of temperature of the water, although starch granules may become more or less swollen at room temperatures if allowed sufficient time. But temperature is the primary factor, and for a given botanical variety of starch there will exist a temperature range (usually narrow) within which the bulk of the granules swell or hydrate. This gelatinisation range may also be affected to some extent by the mode of manufacture of the starch. What is the very interesting mechanism of this hydration? Why is hot water, above a certain temperature, effective, and cold water relatively ineffective? And why do sufficiently high aqueous concentrations of certain salts, such as NaI, NaSCN, etc., gelatinise starch

in the cold? Incidentally, how and why is starch gelatinised in the cold by strong aqueous alkalis?

The hydrogen bond suggests a very plausible and probable answer to these problems. That hydration in general is some form of association with water molecules through hydrogen bonding is now accepted by many physical chemists. In the case of starch hydration, the phenomenon may be represented as follows:



water penetrating the highly organised granule, and swelling it as the water becomes available to the clustering hydroxyl groups. In order to be available to the starch hydroxyls, the penetrative power of the water must be high. Now water itself is known to be 4-co-ordinated, or associated,<sup>13</sup> its hydrogen bonds, in the liquid phase, being continuously destroyed and reformed, the destruction rate being highest at elevated temperatures—as would be anticipated from bond energy considerations. At lower temperatures in the liquid phase the relatively large size of the water molecule aggregates would impede penetration; at higher temperatures these aggregates would be progressively dissociated until at some temperature dependent upon the closeness of the 'packing' of the particular starch aggregates, considerable penetration would ensue and the granules would swell or gelatinise.

This theory of starch hydration is supported by the well-known fact that starch is gelatinised in high aqueous concentrations of certain salts (see p. 51) and by the light that has very recently been thrown upon the effect of these salts upon the co-ordination of liquid water. Buswell, Gore, and Rodebush<sup>14</sup> have found through infra-red absorption studies that the 4-co-ordinated structure of water appeared to be at least in part destroyed by 4M concentrations of the lyotropic series NaI, NaSCN, NaBr, etc., the effect of NaI being > NaSCN > NaBr. Since NaI and NaSCN are effective at high aqueous concentrations in gelatinising starch in the cold, the above postulation of the mechanism of the hydrating action would appear to be singularly strengthened. It seems not improbable, also, that water should be powerfully dissociated by strong alkalis.

As gelatinisation proceeds—through cooking or other means—

the larger swollen sacs rupture more or less and a heterogeneous jelly-like mass is formed, gradually becoming more dispersed and lower in viscosity or consistency. Dispersion is greatly promoted by mechanical agitation or by other physical means (see p. 253), but becomes progressively more difficult owing to the elasticity of the hydrated masses. Cooling, or even cessation of agitation, promotes an increase in consistency—in some instances, as in the cereal starches, amounting to a gel or a plastic paste.<sup>15</sup> These marked changes in consistency with change in temperature, or with quiescence or ageing, may be rationalised by the assumption, well warranted, of a network of hydrogen bonds acting through water aggregates and more or less immobilising them. If this network has a relatively fine structure, an irreversible gel may result; if relatively coarse, enough of the hydrogen bonds may be destroyed by reheating and agitation to permit of a renewed mobility of starch and water aggregates. By suitable physical processing the aqueous pastes of certain botanical varieties of starch may remain in a relatively mobile or fluid state over a wide temperature range and for long periods of time; other varieties will persist in reverting to a gel or to a plastic or semi-plastic state. The reasons are not yet known with any reasonable degree of certainty, although these queer effects are possibly due to spatial configurations<sup>18</sup> of the associated primary valence chains in part, at least, assisted by the effect of non-carbohydrates. There is no good reason to assume that 'chain-length' or chain configuration are the same in all starches—except perhaps in a very broad sense—or that the 'chain-length' is anything but a mean value.

The extraordinary variations in the viscous properties of an aqueous starch paste are partly illustrated by the curves of Fig. 29. Ten per cent. concentrations of a high grade tapioca or cassava were prepared by mild cooking, and two methods of physical dissociation (A and B). Method A involved thorough mechanical agitation by an ordinary stirring mechanism; method B involved special means of drastic physical disorganisation. The time for both treatments was approximately the same, also the temperature; and hydrolytic degeneration—as indicated by chemical tests\*—was slight in both cases. Method B produced a clear fluid dispersion of exceptional stability and homogeneity. The paste produced by method A had to be diluted to 5 per cent. in order to permit kinematic viscosity measurements between 10° and 80° C. Curves 1 and 2 represent the plots of the log of kinematic viscosity (as measured by a 'modified' Ostwald pipette)

\* Taylor's alkali labile method.



against the reciprocal of the absolute temperature between the limits 10.0 and 80.0 degrees C., curve 1 at 5 per cent. concentration, curve 2 at 10 per cent.

There is an astonishing difference between these curves, not only in their values but in their form. Being plotted on semi-log paper, the slope of the curves is representative of the percentage rate of change of viscosity as a function of  $1/K$ . Curve 1 shows a relatively high percentage rate of change at both extremes of temperature; curve 2 is almost exponential, particularly at the

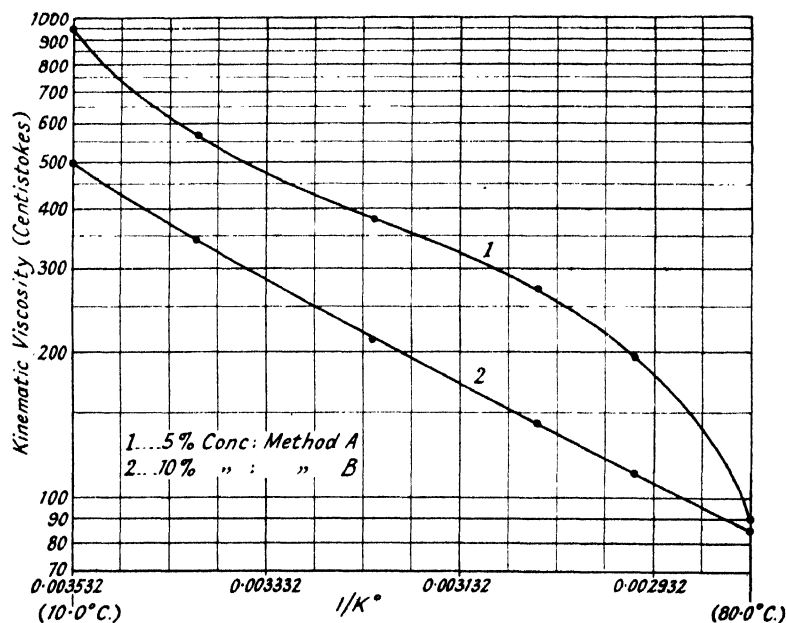


FIG. 29.

higher temperatures. The latter is, in fact, remarkably similar to the form of the curve for pure water. It is obvious that curve 2 is characteristic of a fluid of a high degree of homogeneity, and that curve 1 is characteristic of considerable heterogeneity.

What is the cause of these profound differences in the viscous properties of the same starch? Method B has not degenerated it into a thin-boiling type. The form of curve 2 is not that of a thin-boiling starch or 'white' dextrin, nor do chemical tests indicate a thin-boiling type. The only probable and satisfactory explanation appears to lie in phenomena of association through hydrogen bonding. The aqueous dispersion represented by

curve 1 is a heterogeneous mass of micellar aggregates, many of them relatively large complexes of starch polymers associated through and with co-ordinated water molecules. We would expect the mobility of such complexes to be sensitive to temperature changes, especially at low and at high temperatures where the rates of formation and destruction, respectively, of hydrogen bonds would be greatest. The extended S-form of curve 1 is precisely what might be anticipated from these considerations.

But how about curve 2? Why is it so flat; and considering that its solid concentration is double that of curve 1, why is it so extremely fluid, relatively?

The profound disorganising treatment of method B has torn apart the clotted aggregates left by method A. The micellar organisation has been sieved out,\* as it were, into much smaller associated fragments, so small indeed that the water has in a very real sense become unbound, a result to which changes in the spatial configuration of the starch molecule itself may not too improbably contribute.<sup>18</sup> This is admittedly speculative. But in any event the form of curve 2 is, as has been stated above, remarkably similar to that of water or of other pure and more or less associated liquids. It might be added, parenthetically, that the plot of log. of viscosity against the reciprocal of the absolute temperature is linear for an 'ideal' liquid. According to the theory of viscous flow postulated by Eyring,<sup>16</sup> the viscous behaviour of associated liquids must be abnormal and be expressed by a form of curve more or less similar to curve 2 . . . 'the energy of activation for viscous flow is not independent of temperature . . . and in addition to the normal work required to make a "hole," it is necessary for the hydrogen bonds to be broken before the activated state for flow can be attained.'<sup>16</sup> It is most interesting and suggestive that the same starch can be made to yield, through different physical processes, such a variation in viscous flow behaviour as shown by curves 1 and 2. Too few investigators of starch have had a thorough appreciation of the remarkable mutations to which it is subject in aqueous dispersion.

The case for the O—H . . . O linkage in starch is further supported by the viscous behaviour of 'chlorinated' starches. There is reason to believe that some of the secondary alcohol groups in the glucopyranose units are oxidised to ketose groups by hypochlorite treatment. Oxidation of this sort would obviously inhibit hydrogen bonding. So-called 'chlorinated' or oxidised starches might therefore be expected to show reduced associative

\* Confirmed by the R.C.A. Electron Microscope.

tendencies, dependent upon the severity of hypochlorite treatment. This is confirmed by the viscous behaviour of aqueous dispersions of 'chlorinated' starches: they are phenomenally fluid, clear, and stable, as compared to the aqueous pastes from 'thin-boiling' starches modified by hydrolytic scission of main valence chains.

The case for the high-soluble 'canary' dextrans is similar. This type may be made very stable in viscosity, even at extremely high concentrations, exhibiting little tendency to associate upon ageing. A conception of the structure of these dextrans<sup>17</sup> for which there is considerable support postulates that they probably are, essentially, more or less etherised fragments of the original amylose, hydroxyl hydrogen being largely eliminated through both oxidation and dehydration. Here again, if this picture be approximately correct, association through hydrogen bonding should be minimised. On the other hand, a high-soluble 'white' dextrin, manufactured with a higher acid content and at lower temperatures for shorter times, would be expected to contain a much higher proportion of OH groups and to show very different viscous properties. This is in agreement with the facts, the plot of viscosity versus temperature showing a characteristically steep slope, merging at low temperatures into the plastic state, at high concentrations. This is strong presumptive evidence of the liberal presence of OH groups forming an O—H . . . O network.

In conclusion, the postulation of the hydrogen bond in starch and its derivatives makes for a much clearer understanding of the many puzzling anomalies of chemical and physical behaviour. It is greatly to be desired that some method be developed, such as a modification of present infra-red technique, to put this postulation to direct experimental proof.

#### REFERENCES

1. BUSWELL, RODEBUSH and ROY, *J. Am. Chem. Soc.*, 1938, **60**, 2239, 2444.
2. GILLETTE and SHERMAN, *ibid.*, 1936, **58**, 1135.
3. GORDY and STANFORD, *ibid.*, 1940, **62**, 497.
4. PAULING, 'Nature of the Chemical Bond,' Cornell Univ. Press, Ithaca, N.Y., 1939.
5. RODEBUSH and BUSWELL, *J. Phys. Chem.*, 1939, **43**, 219.
6. SIDGWICK, 'The Covalent Link in Chemistry,' Cornell Univ. Press, 1933.
7. ——— *Chem. Soc. Ann. Repts.*, 1935, **31**, 34.
8. STANFORD and GORDY, *J. Am. Chem. Soc.*, 1940, **62**, 1247.
9. HIRST, PLANT and WILKINSON, *J. Chem. Soc.*, 1932, 279.
10. MARK, *J. Phys. Chem.*, 1940, **44**, 764.

11. MEYER and MARK, 'Der Aufbau der hochpolymeren organische Naturstoffe,' Akademische Verlagsgesellschaft, Leipzig, 1930.
12. TAYLOR and KERESZTESY, *Ind. Eng. Chem.*, 1936, **28**, 502.
13. GLASSTONE, 'Textbook of Physical Chemistry,' D. Van Nostrand, N.Y.C., p. 498.
14. BUSWELL, GORE and RODEBUSH, *J. Phys. Chem.*, 1941, **45**, 543.
15. CAESAR and MOORE, *Ind. Eng. Chem.*, 1935, **27**, 1447.
16. GLASSTONE, LAIDLER and EYRING, 'Theory of Rate Processes,' McGraw-Hill Co., N.Y.C., ch. IX.
17. TAYLOR, Private Communications.
18. CAESAR and CUSHING, *J. Phys. Chem.*, 1941, **45**, 776.

## CHAPTER 9

## THE REACTION OF STARCH WITH IODINE

THE well-known action of iodine on starch to form the blue so-called 'starch iodide' has been extensively studied, and has given rise to much controversy since its discovery by G. de Claubry<sup>1</sup> in 1814; and the question does not seem to have been settled satisfactorily even yet. The fact that, as yet, we cannot explain fully the mechanism of the formation of the iodine coloration does not prevent its use as an empirical index of the degradation of starch. Degradation of starch by acid or enzymic hydrolysis, oxidising agents or thermal decomposition, produces a progressive change in the colour given with iodine and it is possible to separate dextrins of decreasing complexity which show colours varying from blue-violet, through violet and reddish-brown to pale red when iodine is added to them (see p. 422). From a consideration of the colour developed by the  $\alpha$ -amylodextrin, which is of the retrograding type, or of the 12-unit dextrin, which exemplifies the non-retrograding type, it does not appear that chain-length is the *sole factor* governing the particular colour with iodine.<sup>74</sup> At the same time there appears to be a lower limit to the chain-length below which no iodine coloration is produced. Dextrins of 6 units chain-length give no coloration<sup>75</sup> and those of 8 or 12 units a red coloration. At the point of disappearance of the iodine reaction, i.e. the achroic point, therefore, it is unlikely that any dextrins of chain-length longer than 6 to 7 units remain in the solution. The work of C. S. Hanes and M. Cattle<sup>57</sup> is of importance (see p. 486).

G. de Claubry found that the addition of iodine solution to dilute solutions of starches obtained from many sources gave an intense blue coloration, and he also studied the effect of certain acids, alkalis and other reagents upon it. Leroy<sup>2</sup> found that water was necessary for the formation of the blue colour, an observation confirmed by H. B. Stocks,<sup>3</sup> who studied the action of iodine vapour on starch.

C. F. Roberts,<sup>4</sup> F. E. Hale,<sup>5</sup> and H. B. Stocks,<sup>3</sup> and a number of other workers<sup>3</sup> have all suggested that the presence of hydriodic acid or an iodide in addition to water is essential for the development of the blue colour, a conclusion supported by F. Mylius,<sup>6</sup> and it has been suggested by M. Samec that the iodide actually forms part of the complex formed, possibly as

the polyiodide ion  $I'_3$ . Also, it would appear from the work of W. Harrison<sup>9</sup> that certain salts other than iodides enable the reaction to take place, and intensify the coloration produced.

Meineke<sup>10</sup> added aqueous iodine solution to a starch solution without any blue colour being developed, although the resultant solution was quite yellow; after adding a little potassium iodide, the blue colour appeared immediately. C. F. Roberts<sup>4</sup> added a chloroform solution of iodine, which had been carefully freed from hydriodic acid, to a starch solution and no blue colour was obtained; on treating this mixture in such a way as to produce hydriodic acid, e.g. by heating, or by adding acid or potassium iodide, the blue colour appeared. A. Vogel<sup>11</sup> records that iodine in absolute alcohol gives no reaction with dried starch.

**The Effect of Heat.**—On heating to the boiling-point the blue solution obtained by the action of iodine on starch solution, the colour disappears, but returns on cooling, and, according to S. Pickering,<sup>12</sup> the temperature at which this colour disappears varies with the intensity it possessed before heating. A. Vogel<sup>13</sup> has also mentioned that higher temperatures are required to decolorise the solution as the concentration of the starch iodide is increased (see also ref. 33), and this the author has confirmed. Jacquelain<sup>14</sup> carried out microscopical examinations on starch granules that were heated up to 200° C., and from his results concluded that the combination between the starch and iodine was not a chemical one. Since the colour intensity of the reaction increases as the temperature is decreased, J. J. Pohl<sup>15, 16</sup> concludes that hot water has a greater affinity for starch than has iodine, whereas with cold water the reverse is true.

J. Personne<sup>17-18</sup> considers that the decoloration of the blue starch iodide is due to the partial volatilisation of iodine with the concurrent formation of a colourless compound between the starch and the residual iodine. On the other hand, A. Payen<sup>19-20</sup> thinks that the expansion of aggregates of starch is the cause of the blue colour disappearing on heating, and that on cooling re-aggregation takes place and thus the blue colour reappears. E. Sonstadt<sup>21</sup> heated strongly dried starch iodide to a red heat: it charred, and upon analysis one-fifth of the iodine was found in the residual carbon.

**Sensitivity of the Reaction.**—The starch-iodide reaction forms a valuable means of following the course of the conversion of starch to dextrins either by the torrification method or by means of the action of diastatic or hydrolytic agents; and its use in volumetric work is well known. As the degradation of the starch increases in the course of any process for producing

dextrin, so the colour given by iodine solution with a sample withdrawn from the batch changes from a deep blue through violet and red to brown, and finally no colour at all is produced (see p. 470). The sensitivity of the reaction between starch and iodine has been studied by E. Chretien and H. Vandenberghe<sup>22</sup> in their work on another problem. They find that at room-temperature (19° C.) 0.15 ml. of N/100 I will give a distinct blue colour to a solution of as little as 1 pt. of starch in 50,000 pts. of distilled water containing 50 pts. of potassium iodide.

The first mention of strips impregnated with starch for use in detecting iodine appears to have been made by L. Jonas,<sup>23</sup> whose strips were made of cotton. The sensitivity of the reaction has also been studied by Stromeyer,<sup>24</sup> C. Meineke,<sup>10</sup> J. F. Norris and H. Fay,<sup>25</sup> J. Pinnow,<sup>26</sup> G. Rivat,<sup>27</sup> and V. Greismayer.<sup>28</sup> The last two workers deduce that dextrans have a greater affinity for iodine than has starch, and that the intensity of the blue coloration obtained when soluble starch is used, is inversely proportional to the amount of dextrans present. The presence of non-electrolytes does not appreciably affect the sensitivity of the reaction,<sup>29</sup> although in presence of albumin an excess of iodine is required for the appearance of the blue colour.<sup>30</sup> The reaction is inhibited by the presence of chloral hydrate,<sup>31</sup> tannin, and certain phenolic compounds, such as pyrogallol and resorcinol, but not in the presence of phenol itself.<sup>32</sup> Electrolytes, such as acids and salts, tend to increase the sensitivity of the reaction,<sup>9, 34</sup> being maximum for potassium iodide solution,<sup>10, 34</sup> although, according to J. Pinnow,<sup>26</sup> sodium sulphate appears to act best. The presence of electrolytes also affects the colour under certain conditions<sup>77</sup> (*vide infra* Simerl and Browning). Excess of alkali inhibits the reaction,<sup>35</sup> and when testing prepared dextrans for matching it is necessary to ascertain if they have a strongly alkaline reaction; if they have, the dextrin solution must be neutralised with dilute sulphuric acid before carrying out the iodine test. The alkaline type of product is met with in the so-called 'arable gums,' which are dextrans containing a small percentage of soda ash and borax (see p. 286). The precipitation of the starch-iodide complex brought about by the addition of salts can also be brought about if iodine and iodide are added in excess.<sup>78, 79</sup>

**An Abnormal Starch-Iodide Reaction.**—F. Dafert,<sup>36, 37, 38</sup> U. Kreuzler,<sup>37</sup> and Y. Shimoyama<sup>39</sup> discovered that starch from the glutinous rice occurring in China gave a red or reddish-brown coloration with iodine, although aqueous extractions gave no indication that dextrin was present. Y. Tanaka<sup>40</sup> has studied this

starch, and confirms the findings of the earlier workers. Under the microscope the starch does not differ from common rice starch, and on hydrolysis by amylase the reaction appeared to follow a similar course to that of ordinary starch, except that the ratio of dextrin to maltose was higher. He was unable to detect the presence of albuminoids or dextrans, and so far no explanation of this peculiar reaction has been advanced.

The percentage of iodine found in the starch-iodide complex varies with the conditions of preparation between 3 and 23 per cent.<sup>33, 76</sup>

**The Composition of Starch Iodide.**—The exact composition of starch iodide has given rise to much speculation, and many workers consider that definite compounds of starch and iodine are formed. C. F. Schoenbein<sup>41</sup> was of this opinion, but doubted the existence of a colourless starch iodide, as suggested by Personne to explain the disappearance of the blue colour on heating.

G. Rouvier,<sup>42</sup> in a series of papers, suggests that the compounds  $(C_6H_{10}O_5)_nI_n$ , where  $n$  equals 2, 3, 4 or 5, are formed as the amount of iodine added to a fixed amount of starch is increased. These compounds, it is suggested, have the power of dissolving more iodine. The amount of iodine found in these compounds varies from 3 to 19.6 per cent. By dissolving starch in hot glycerine, and precipitating with a potassium-iodide-iodine solution, J. Toth<sup>7</sup> obtained starch iodides from wheat, rice and potato starches containing 22.6-22.8 per cent. of iodine. Using wheat or rice starch, the largest iodine-content Rouvier could obtain was 19.6 per cent., and for potato starch the maximum was 18.6 per cent. In the presence of insufficient iodine a potato-starch derivative was obtained containing 13.5 per cent. iodine, whilst derivatives of wheat and rice starches contained about 9 per cent. It would appear that potato starch has the ability to bind more iodine than wheat starch under certain conditions.<sup>47, 77, 82, 83</sup> Under similar conditions, C. O. Harz<sup>43</sup> obtained figures of about 7 and 19 per cent. for the amount of iodine in the compounds formed in the presence of insufficient and excess iodine, respectively. M. Padoa and B. Savarè,<sup>44</sup> using a starch iodide containing about 19 per cent. iodine, carried out conductivity measurements, the results of which tend to show that starch iodide is an additive product of iodine, starch, and potassium iodide or hydrogen iodide. A constant relation between the iodine and the hydrogen iodide was, however, not found.

L. W. Andrews and H. M. Goettsch<sup>46</sup> have proposed formulæ of  $(C_6H_{10}O_5)_{12}I$  and  $(C_6H_{10}O_5)_{12}I_2$  for the compounds they



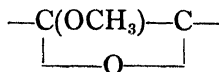
obtained, whilst the formulæ  $(C_6H_{10}O_5)_{18}I_2$  and  $(C_6H_{10}O_5)_{18}I_4$  have been proposed by H. von Euler and K. Myrbäck<sup>47</sup> from their work on the distribution of iodine between starch solution and benzene, using starches which had been pre-treated in various ways.

Opposed to those who ascribe formulæ to the product or products formed are others who hold that the process is one of adsorption.<sup>46, 69</sup> Thus A. Lottermoser<sup>48-49</sup> obtained typical adsorption curves, his measurements being based on the determination of electric potential in the system free iodine/iodide ion as formulated in the Nernst equation, and also on the distribution ratio of free iodine between 0.1 N potassium iodide solution and carbon tetrachloride. L. Berczeller<sup>50</sup> found that no iodine was adsorbed by the starch from iodine solutions in carbon tetrachloride or benzene, whilst the amount adsorbed from alcohol was less than that from water. E. Angelescu and J. Mirescu<sup>51</sup> have derived an exponential formula for the adsorption of iodine by maize starch. Conductivity measurements by N. R. Dhar<sup>45</sup> also point to the formation of an adsorption compound.

Other physico-chemical methods used in the study of the starch-iodine reaction include osmotic-pressure measurements,<sup>52</sup> measurement of the depression of freezing-point,<sup>53</sup> and colorimetric methods.<sup>54</sup>

According to M. Bergmann and S. Ludewig<sup>55-56</sup> the bridge oxygen atoms in starch are centres of adsorption, and this may be a partial explanation of the progressive movement of colour towards the red end of the spectrum as starch is progressively degraded through dextrin to glucose.

It would appear that the iodine coloration is purely a question of adsorption depending ultimately on certain residual affinities of the starch molecule. Bergmann found that components containing the internal oxygen bridge of the type



such as the cyclo-acetals of acetoin and acetol, give blue-black compounds with iodine. Bluish compounds were also obtained by G. Barger and W. W. Starling<sup>71</sup> by the action of iodine on certain derivatives of  $\alpha$ - and  $\gamma$ -pyrones, whilst colloidal or semi-colloidal substances such as saponarin and cholalic acid were found by Barger and E. Field<sup>72</sup> to give the 'iodine reaction' (see also Mylius<sup>70</sup>). W. Biltz<sup>73</sup> had previously observed the phenomenon with lanthanum subacetate.

From their work on amylases C. S. Hanes and M. Cattle<sup>57</sup> conclude that starch contains groups along the molecular chain which can adsorb iodine to give the characteristic coloration shown by starch and its various degradation products. As hydrolysis with  $\beta$ -amylase continues there is a progressive decrease in the number of adsorbing groups following the cleavage of successive maltose residues from the end of the chain. With  $\alpha$ -amylase they consider that there is a preliminary disruption of the associated molecules, followed by a progressive destruction of the adsorbing groups as the chains are split into achroo-dextrins. By means of their technique they find it possible to follow quantitatively the alteration in the iodine coloration under the action of different amylases, and so to delineate throughout the various degradation processes the relation between the iodine reaction and the reducing power. This provides a highly selective method for the recognition of points of similarity and difference in the action of various starch-splitting enzymes. They deduce that, in the visible region the absorption spectrum of an aqueous solution of the iodine-iodide mixture is not appreciably altered by its participation in the starch-iodide complex.

Jackson and Hudson<sup>80</sup> have broken open the pyranose rings, giving aldehydic groups in positions 2 and 3 by oxidising starch with periodic acid. The product is highly colloidal but shows no iodine reaction, thus showing that the presence of pyranose rings is at least a partial condition for the starch-iodide reaction to take place. That the many OH groups present in the starch molecule play no part in the reaction is shown by the fact that fully methylated starch is still able to develop the blue colour.<sup>79, 81</sup>

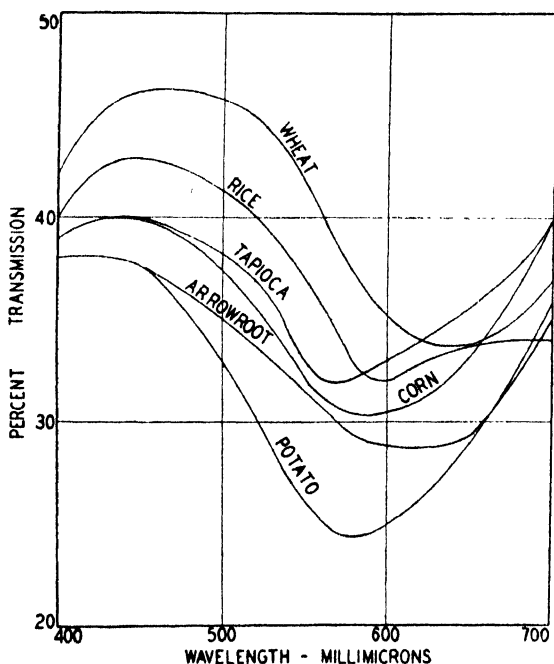
An admirable and comprehensive review of the action of amylases in relation to the structure of starch is given by C. S. Hanes,<sup>59</sup> and readers are referred to the original paper and also to the work discussed on pages 480 and 486. R. H. Muller and H. M. McKenna<sup>58</sup> have examined the starch-iodide system by a photo-electrical method and find that beyond a preliminary 'dissociation stage' Beer's law is followed, and the use of Duboscq type of instrument in analysis is indicated.

The fact that starch iodide can be precipitated in the presence of certain salts has been used as the basis of several methods for the estimation of starch (see p. 397), compounds of constant composition being obtained, providing the conditions of precipitation are rigidly standardised.

**Use of Starch Iodide.**—Starch iodide is a strong disinfectant, and is comparatively stable. In contact with tissue it continues active for much longer than iodine itself, and if the iodine-content

is around 1 per cent. it is devoid of irritant action. The use of starch-iodide solution has also been suggested for the irrigation method of treating wounds.

**The Starch-Iodide Reaction in the Spectro-photometric Determination of Starch.**—Johnson<sup>64</sup> followed the course of enzymatic hydrolysis of starch by the iodine coloration and Blake<sup>65</sup> used the Duboscq colorimeter for this type of work. The degree of hydrolysis of modified starches has been followed by H. P.



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FIG. 30.

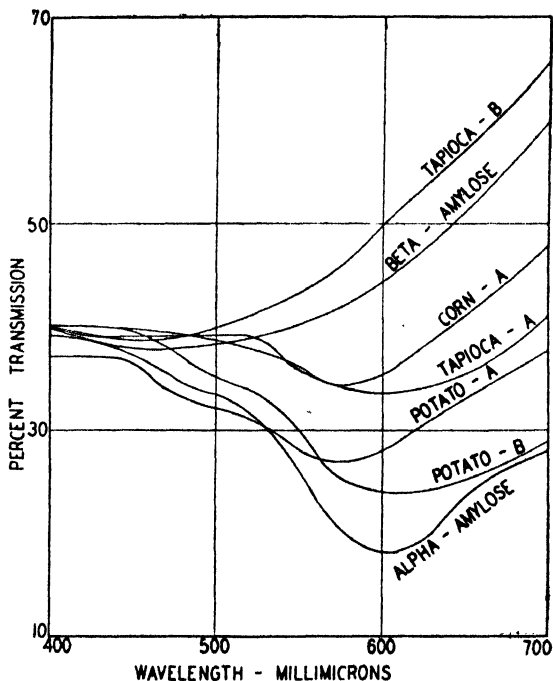
Das Gupta<sup>66</sup> and by G. B. Jambuserwala,<sup>67</sup> using the Lovibond Tintometer. Reference should also be made to the work of J. J. Chinoy.<sup>68</sup>

Several workers have made careful photometric studies of a few starches, and L. E. Simerl and B. L. Browning<sup>63</sup> have studied a number of starches and dextrans, using a General Electric Hardy recording spectrophotometer, with special reference to starches used in paper-making and the influence of extraneous material.

With an iodine concentration of 0.1 gram per litre, and a potassium-iodide ratio of 1.5 to 1 by weight, the starch concentration

does not affect the position of the minimum in the absorption curve; neither small changes in the potassium iodide concentration nor the presence of aluminium sulphate affect the minimum, although both affect the colour and the total absorption.

Fig. 30 shows that the maximum absorption for the six starches examined occurs over the range 5600 to 640 $\mu\mu$ . It will be seen that the curves have the same general shape but considerable differences exist in the wave-length of maximum absorption and in the transmission at any given point in the spectral range.

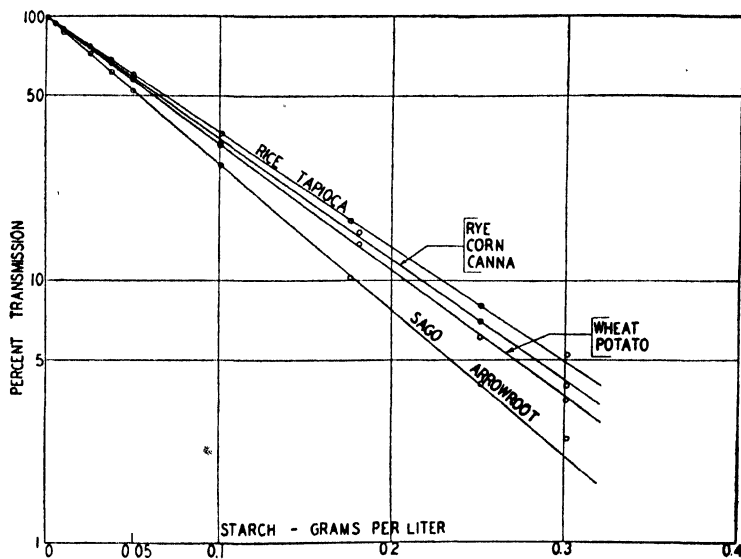


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FIG. 31.

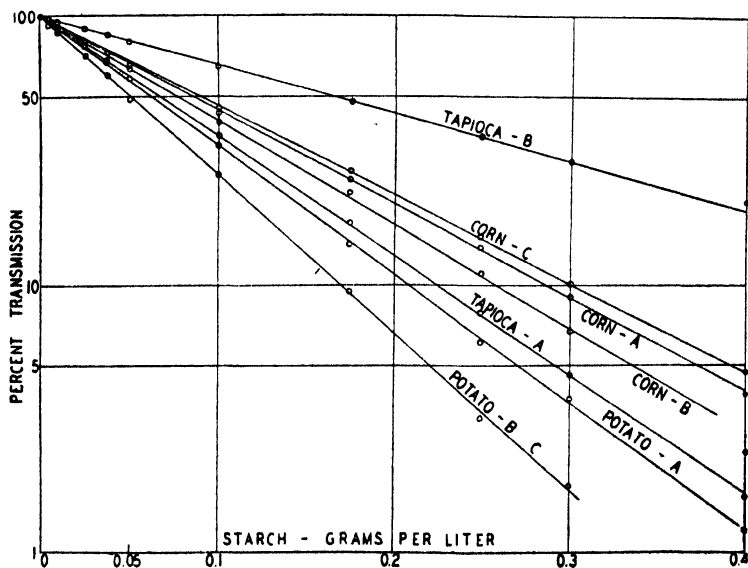
Fig. 31 shows that an oxidised or hydrolysed starch has a different colour reaction with iodine, but that the transmission in the band of maximum absorption may not be uniformly effected. The maximum absorption, except for  $\beta$ -amylose and tapioca dextrin, occurs in the range 570 to 610 $\mu\mu$ . Modification to dextrins eliminates the region of maximum absorption and the transmission rises rapidly and uniformly in the red.

At certain concentrations of iodine, deviation from Beer's law may be expected, but at higher concentrations, and by the use



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FIG. 32.



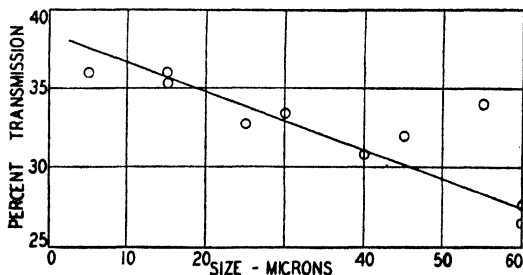
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FIG. 33.

of suitable filters, the logarithm of the fractional transmission does become proportional to the starch concentration. In Figs. 32-33 the transmission of the iodine solution with no starch present is assumed to be 100 per cent., and straight-line transmission curves are obtained over the range of 5 to 95 per cent. transmission. It will be seen that each starch has its own transmission curve having a slope which is characteristic of the starch type.

According to L. Paloheims and I. Antila,<sup>61</sup> the particle size of the starch influences the intensity of colour of the starch-iodine reaction, starches characterised by large particle size showing a lower transmission than those of small particle size.

Fig. 34 shows this relation under the conditions of Simerl and Browning's test,<sup>63</sup> but it should be noted that the relationship is inapplicable to modified starches.



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FIG. 34.

Providing the kind of starch and its history are known, these workers consider that the photo-colorimetric procedure is capable of high accuracy, the nature of the starch itself being the limiting factor, and if raw starches are used, the deviation from the mean of the highest and lowest transmission curves of the starches examined amounts to  $\pm 12$  per cent. Fig. 34 illustrates that the particle size may be very important in determining differences in transmission of various starches, whilst for modified starches the possible error due to starch may be as much as  $\pm 25$  per cent. In the presence of dextrans, which may be present in paper together with raw or modified starch, the photo-colorimetric method becomes totally unreliable, whilst reducing agents and alkalis should be absent to obtain accurate results. Considerable errors may be introduced if the paper contains acid-soluble fillers and the starch is removed by acid extraction. Other applications of the starch-iodide reaction will be found throughout these pages. L. H. Lampitt, C. H. F. Fuller and N. Goldenberg<sup>66</sup>

have examined the absorption spectra of the starch-iodide complexes formed from the different fractions obtained by ball-milling wheat starch (see p. 72).

They find that the absorption spectra given by the H.W.S. fractions (see p. 72) are unchanged by grinding except for a decrease in absorption at longer wave-lengths, which becomes apparent simultaneously with the first significant decrease in the mean molecular weight. The absorption by the C.W.S. fractions (see p. 72) complexes are considerably lower than those given by the corresponding H.W.S. fractions, the greatest difference being in the visible part of the spectrum.

The C.W.S. fractions obtained by milling for 50 to 470 hours show a progressive increase in absorption but between 1000 and 7000 hours' milling the absorption at the longer wave-lengths decrease. When milled from 470-1000 hours the two processes overlap. The mean molecular weight begins to decrease between 300 and 470 hours grinding. These workers conclude that the depolymerisation of wheat starch brought about by 7000 hours milling is due to fission of the weaker, lateral links between the repeating units of 24-30 anhydroglucose units. The increasingly larger absorption of the C.W.S. fraction-iodine complex cannot at present be explained but it appears to be unconnected with the depolymerisation of this fraction by milling.

The change in molecular weight has a much less effect on the absorption at shorter wave-lengths of the starch-iodide complex, and changes in the starch are not easily followed by examination of this spectral region. The most sensitive region for the following of depolymerisation is from 4300 to 7500 Å. Samec<sup>87</sup> found no connection between the reducing power and the iodine reaction of various starch fractions, but the work of Lampitt *et al.* agrees very well with that of Hanes,<sup>57, 59</sup> Muller and McKenna<sup>58</sup> and Simerl and Browning.<sup>63</sup> Lampitt and co-workers conclude that the absorption of the starch-iodide complexes appears to depend on both the relative and actual proportions of starch, iodine and iodide in solution, and the characteristics of the curves given by the various fractions depend both on molecular weight and on the chain-length of the repeating units, the effect of the chain-length predominating.

It should be noted that other workers<sup>82, 84, 85</sup> have noticed that starch depolymerised to a considerable extent still gives the blue colour with iodine, but they did not examine the phenomena quantitatively.

The oxidative effect of iodine on starch is fully discussed by F. F. Farley in Chapter 3, Section 2.

## REFERENCES

1. G. DE CLAUBRY, *Gilbert's Ann. Phys.*, 1814, **48**, 297.
2. LEROY, *Phil. Mag.*, 1834, **4**, 313.
3. H. B. STOCKS, *Chem. News*, 1887, **56**, 212; 1888, **57**, 183.
4. C. F. ROBERTS, *Amer. J. Sci.*, 1894, **47**, 422.
5. F. E. HALE, *J. Chem. Soc.*, 1902, **82**, i, 533; 1903, **84**, i, 151.
6. F. SEYFERT, *Zeit. angew. Chem.*, 1881, **1**, 15.
7. J. TOTH, *Chem.-Ztg.*, 1891, **15**, 1523, 1583.
8. F. MYLIUS, *Zeit. physiol. Chem.*, 1887, **11**, 306.
9. W. HARRISON, *Kolloid-Zeit.*, 1911, **9**, 5.
10. MEINEKE, *Chem.-Ztg.*, 1894, **18**, 157.
11. A. VOGEL, *Jahrb. Chem.*, 1873, 829.
12. S. PICKERING, *Chem. News*, 1880, **42**, 311.
13. A. VOGEL, *Neues Repert. Pharm.*, 1876, **25**, 565.
14. V. A. JACQUELAIN, *Ann. Chim. Phys.*, 1840, **73**, 167.
15. J. J. POHL, *J. prakt. Chem.*, 1861, **83**, 35.
16. R. FRESENIUS, *Ann. Chem. Pharm.*, 1857, **102**, 184.
17. J. PERSONNE, *Compt. rend.*, 1865, **61**, 993.
18. — *J. pharm. chim.*, 1861, **39**, 49.
19. A. PAYEN, *Compt. rend.*, 1865, **61**, 466.
20. — *ibid.*, 1865, **61**, 1021.
21. E. SONSTADT, *Chem. News*, 1873, **28**, 248.
22. E. CHRETIEN and H. VANDENBERGHE, *Ann. chim. anal.*, 1921, **26**, 19.
23. L. JONAS, *Ann. der Pharm.*, 1836, **20**, 40.
24. STROMEYER, *Ann. Phys.*, 1815, **49**, 146.
25. J. F. NORRIS and H. FAY, *Amer. Chem. J.*, 1900, **23**, 119.
26. J. PINNOW, *Zeit. anal. Chem.*, 1902, **41**, 485.
27. G. RIVAT, *Chem.-Ztg.*, 1910, **34**, 1141.
28. V. GREISMAYER, *Ann. Chem. u. Pharm.*, 1871, **160**, 40.
29. J. PINNOW, *Zeit. anal. Chem.*, 1902, **41**, 485.
30. E. PUCHOT, *Compt. rend.*, 1876, **83**, 225.
31. E. SCHAER, *Pharm. Centralhalle*, 1896, **37**, 540.
32. E. HEINTZ, *Jahrb. Agric. Chem.*, 1879, 499.
33. M. SAMEC, 'Kolloidchemie der Stärke,' Dresden and Leipzig, 1927, pp. 314-362.
34. J. M. KOLTHOFF, *Chem. Zentbl.*, 1919, **2**, 717.
35. C. M. VAN DEVENTER, *Chem. Centbl.*, 1888, 424.
36. F. W. DAFERT, *Landwirtsch. Jahrb.*, 1885, **14**, 837.
37. F. W. DAFERT and U. KREUSLER, *Centr. Agrik.*, 1885, **14**, 259.
38. F. W. DAFERT, *Ber. Deuts. botan. Gesell.*, 1887, **5**, 108.
39. Y. SHIMOYAMA, *Botan. Jahresh.*, 1889, **14**, ii, 315.
40. Y. TANAKA, *J. Ind. Eng. Chem.*, 1912, **4**, 578.
41. C. F. SCHOENBEIN, *J. prakt. Chem.*, 1861, **84**, 402.
42. G. ROUVIER, *Compt. rend.*, 1892, **114**, 128, 749, 1366; 1893, **117**, 281, 461; 1894, **118**, 743; 1895, **120**, 1179; 1897, **124**, 565.
43. C. O. HARZ, *Chem. Centbl.*, 1898, **1**, 1018.
44. M. PADOA and B. SAVARÈ, *Reale Accad. Lincei*, 1905, **14**, 467.
45. N. R. DHAR, *J. Phys. Chem. Ithaca*, 1924, **28**, 125.
46. L. W. ANDREWS and H. M. GOETTSCHE, *J. Amer. Chem. Soc.*, 1902, **24**, 865.
47. H. VON EULER and K. MYRBÄCK, *Arkiv for kemi, mineralogi och geologi*, 1921, **8**, 29; *Ann.*, 1922, **427**, 1.



48. A. LOTTERMOSER, *Zeit. angew. Chem.*, 1921, **34**, 427.
49. — *Kolloid-Zeit.*, 1923, **33**, 271.
50. L. BERCEZELLER, *Biochem. Zeit.*, 1922, **133**, 502.
51. E. ANGELESCU and J. MIRESCU, via *Chem. Abstracts*, Easton, 1926, **20**, 686.
52. H. RODEWALD and A. KATTEIN, *Sitzber. preuss. Akad. Wiss.*, 1899, 628.
53. H. FRIEDENTHAL, *Centr. Physiol.*, 1899, **13**, 54.
54. M. KATAYAMA, *Zeit. anorg. Chem.*, 1908, **56**, 209.
55. M. BERGMAN and S. LUDEWIG, *Ber.*, 1924, **57**, 753.
56. — *ibid.*, 1924, **57**, 961.
57. C. S. HANES and M. CATTLE, *Proc. Roy. Soc.*, 1938, **125B**, 387 and 414; *Rep. Food Invest. Board for 1937*, H.M. Stationery Office, 1938.
58. R. H. MÜLLER and M. H. MCKENNA, *J. Amer. Chem. Soc.*, 1936, **58**, 1017.
59. C. S. HANES, *New Phytol.*, 1937, **36**, 101, 189.
60. H. VON EULER and S. BERGMAN, *Kolloid-Zeit.*, 1922, **31**, 81.
61. L. PALOHEIMS and I. ANTILA, *Biochem. Zeit.*, 1931, **238**, 401.
62. G. W. PUCHER and H. B. VICKERY, *Ind. Eng. Chem. (Anal. Ed.)*, 1936, **8**, 92.
63. L. E. SIMERL and B. L. BROWNING, *ibid.*, 1939, **11**, 125.
64. W. A. JOHNSON, *J. Amer. Chem. Soc.*, 1908, **30**, 798.
65. J. C. BLAKE, *ibid.*, 1918, **40**, 623.
66. H. P. DAS GUPTA, *J. Indian Inst. Sci.*, 1936, **19A**, 37.
67. G. B. JAMBUSERWALA, *J. Text. Inst.*, 1938, 149T.
68. J. J. CHINOY, *Mikrochemie*, 1939, **26**, 132.
69. A. LOTTERMOSER, *J. prakt. Chem.*, 1905, **71**, 296.
70. F. MYLIUS, *Ber.*, 1895, **28**, 385. (Discusses analogy of starch iodide and iodochohic acid.)
71. G. BARGER and W. W. STARLING, *J. Chem. Soc.*, 1915, **107**, 411.
72. — and E. FIELD, *ibid.*, 1912, **101**, 1394.
73. W. BILTZ, *Ber.*, 1904, **37**, 1719.
74. W. N. HAWORTH, E. L. HIRST and M. M. T. PLANT, *J. Chem. Soc.*, 1935, 1214.
75. WALDSCHMIDT-LEITZ, M. REICHEL and A. PURR, *Naturwiss.*, 1932, **20**, 254; *Z. physiol. Chem.*, 1934, **223**, 76.
76. J. J. CHINOY, F. W. EDWARDS and H. R. NANJI, *Analyst*, 1934, **59**, 673.
77. M. SAMEC and MAYER, *Kolloid. Beih.*, 1921, **13**, 272.
78. — and KLEMEN, *ibid.*, 1925, **21**, 55.
79. — and WALDSCHMIDT-LEITZ, *Z. physiol. Chem.*, 1931, **203**, 16.
80. JACKSON and HUDSON, *J. Amer. Chem. Soc.*, 1937, **59**, 2049; 1938, **60**, 989.
81. MEYER, WERTHEIM and BERNFELD, *Helv. Chim. Acta*, 1940, **23**, 865.
82. WALDSCHMIDT-LEITZ, M. SAMEC and MAYER, *Z. physiol. Chem.*, 1937, **250**, 192.
83. M. SAMEC, *ibid.*, 1935, **236**, 103.
84. BAIRD, W. N. HAWORTH and E. L. HIRST, *J. Chem. Soc.*, 1935, 1201.
85. WALDSCHMIDT-LEITZ, M. SAMEC and MAYER, *Z. physiol. Chem.*, 1936, **242**, 165; 1935, **236**, 168.
86. L. H. LAMPITT, C. H. F. FULLER and N. GOLDENBERG, *J. Soc. Chem. Ind.*, 1941, 99T.
87. M. SAMEC, *Kolloid. Beih.*, 1937, **47**, 91 (see also ref. 78).

## ADDITIONAL REFERENCES

- W. P. WILLIAMS, E.P. 501,246, 9/6/1939. (Starch iodide for medicinal purposes.)
- F. W. KUESTER, *Liebig's Ann. der Chem.*, 1894, **283**, 360. (Considers starch iodide is well-defined solid solution of iodine in starch.)
- C. GAUTIER and T. NOGIER, *Compt. rend. Soc. biol.*, 1910, **69**, 156. (Starch iodide decolorised by rays from mercury vapour lamp.)
- W. HARRISON, *J. Soc. Chem. Ind.*, 1910, **29**, 1335. (Starch iodide solution is colloidal dispersion of iodine protected by the starch.)
- M. SAMEC, *Congr. Chim. Ind., Compt. Rend.*, 18th Congr., 1938, 472. (Considers various kinds of starch exist differing more particularly in iodine reaction; the behaviour of these 'amyloses' and 'erythroamyloses' is discussed.)

## CHAPTER 10

## ETHERS AND ESTERS OF STARCH

As might be expected from its constitution, starch forms a series of ethers and esters, often by a fairly simple reaction. A great amount of work has been done on these compounds, and those who require an exhaustive treatment of the subject should consult the works of E. C. Worden.<sup>1</sup>

**Treatment with Sulphuric Acid.**—C. Blondeau<sup>2</sup> treated starch with concentrated sulphuric acid, and from the reaction mixture separated two distinct compounds by means of the lead salts. The reaction was also studied by other workers, who isolated the calcium and barium salts as well as the lead compounds. These substances, however, have not attained any industrial importance.

R. Tamba<sup>7</sup> has prepared amylodisulphuric acid by the action of chlorosulphonic acid and chloroform on starch suspended in pyridine. The potassium salt was separated by treatment with alcoholic potash. Another method, patented by W. Traube,<sup>8-10</sup> is to treat starch suspended in carbon bisulphide or sulphuric acid with sulphur trioxide, the products obtained being used as protective colloids.

**Treatment with Nitric Acid.**—Braconnot<sup>11</sup> appears to have been the first to examine the action of nitric acid on starch, and to the product obtained he gave the name 'xyloidine'. He noted that when textiles are treated with this substance and dried, they retain their stiffness and become impervious even to boiling water. His work was soon followed by that of B. Ballot,<sup>3</sup> and J. Pelouze,<sup>12</sup> who was the first worker to suggest the use of nitro-starch as an explosive. From his work he concluded that 'xyloidine' and pyroxlin are not identical. Later, other workers suggested the use of the nitro-compounds for explosives. The subject has been fully examined by F. Ritter,<sup>16</sup> and recently the U.S. Government stated that such explosives had been perfected. Berthelot measured the heats of formation and decomposition of 'xyloidine'.<sup>17-18</sup>

O. Muehlhaeuser<sup>19</sup> obtained various nitro-bodies from starch containing 10.5-13.5 per cent. of nitrogen, and showed them to be true esters, whilst W. Will<sup>20</sup> obtained products containing 14.04 and 18.9 per cent. nitrogen, which he regarded as the hexanitrate. These compounds are soluble in ethyl alcohol and ethyl acetate, and are stable at 50° C., but decompose with explosive

violence at  $194^{\circ}$  C. H. T. Brown and J. H. Millar<sup>21</sup> prepared compounds containing 7.8 and 11.5 per cent. of nitrogen, and from measurements of the freezing-point of the solution in acetic acid concluded that the molecular weight was 987. Samples of nitro-starch obtained by A. V. Sapozhnikov<sup>22</sup> contained 13.4 per cent. nitrogen and gave a molecular weight of 1845, as determined by the boiling-point method, using acetone as solvent. By the action of ammonium sulphide on the nitro-bodies, soluble starch is obtained.

E. Berl and R. Büttler,<sup>23</sup> using a mixture of fuming sulphuric acid and nitric acid on four different varieties of starch, obtained nitrates containing 12.86-13.85 per cent. of nitrogen, and noted that a 5 per cent. solution of cellulose nitrate in acetone has a viscosity nine thousand times greater than the corresponding starch nitrate (13 per cent. nitrogen). As a general rule cellulose derivatives show a greater viscosity in solution than the corresponding starch derivatives. A. Béchamp<sup>24-25</sup> found that starch can be recovered from the nitrate by treatment with certain ferrous salts. Other work on nitro-compounds is indicated in the references.<sup>26-29</sup> It should be noted that before starch is nitrated or acetylated, it should be freed from oil by solvent extraction, and stabilisation of the nitrate is readily effected by washing the starch nitrate with hot, strong aqueous alcohol to which may be added a small quantity of acetone.<sup>29</sup>

K. H. Meyer, P. Bernfeld and W. Hohenemser<sup>114</sup> have prepared both nitrates and acetates of amylose and amylopectin from maize starch and studied the concentration viscosity relationships. The slope of the curve for amylopectin acetate is much greater than that of the amylose acetate.

**Action of Acetic Acid.**—P. Schuelzenberger<sup>30-31</sup> early noted the formation of two starch acetates, one soluble in water and alcohol, and the other soluble in acetic acid and alcohol, but not in water. By the saponification of either compound dextrin was obtained. Later<sup>32</sup> he prepared the triacetate, using starch and soluble starch, and regenerated the starch from each product by hydrolysis. Using acetic anhydride, F. Pregl<sup>33</sup> obtained the triacetate, insoluble in alcohol, and then regenerated the starch by hydrolysis. Using more sulphuric acid as a catalyst, he obtained a triacetate melting at  $150^{\circ}$  C., which was soluble in alcohol and gave a dextrin on hydrolysis.

C. F. Cross, E. J. Bevan and J. Traquair<sup>34-35</sup> examined the action of glacial acetic acid on starch at  $100-105^{\circ}$  C. and produced a number of products of commercial interest. The action of the acid on the starch at this temperature is proportional to the time

of heating, and to the ratio of starch to acid. J. Boeseken <sup>36</sup> and co-workers found that the rate of acetylation of wheat starch is less than that of cellulose, and that hydriodic acid appears to be the best catalyst. If sulphuric acid is used as the catalyst, the reaction is accelerated by increasing the amount of acid, but not proportionally.

The lowest products of the starch-acetate series show many of the properties of gelatine, and are marketed under the trade name of 'Feculose'. It forms transparent and elastic films which are used industrially for making transparent foils and for purposes for which gelatine is normally employed.

J. R. Whinfield and G. G. Ritchie <sup>115</sup> claim a process giving fully acetylated derivatives which give solutions of high viscosity at low concentrations. They treat the dried starch prior to acetylation with a limited amount of formic acid, alone or with a minor proportion of acetic acid, containing not more than 2 per cent. of water. Within a short time the starch swells up to a fluffy mass. This is left to stand until swelling is complete (about 24 hours).

The material is next acetylated with acetic anhydride containing about 0.1 per cent. of sulphuric acid, the temperature being kept below 50° C. to prevent hydrolysis. After 3 hours the clear viscous mass is precipitated with a large volume of water and the resulting acetate dried and ground.

J. D. Law <sup>37</sup> made the interesting observation that, in the presence of a large amount of zinc chloride, a mixture of acetic acid and acetic anhydride does not acetylate or even hydrolyse starch, whereas cellulose under the same conditions forms the triacetate. Y. Tsuzuki <sup>38</sup> has successfully acetylated starch in a non-aqueous medium in the presence of zinc chloride, for example, by heating 20 gm. starch, 30 gm. glycerol, and 2 gm. of zinc chloride for 15 hours at 170° C., cooling to 70° C., adding 140 c.c. of acetic anhydride, then heating for 30 minutes at 80° C. It is probable that the first stage of the reaction is the formation of a glycerol derivative.

If the reaction with acetic anhydride is carried out in pyridine, omitting the sulphuric acid catalyst, products are obtained different in nature from those given by the usual reaction using the acid catalyst.<sup>39</sup> Tsuzuki <sup>40</sup> also carried out acetylations, using sodium or potassium thiocyanate instead of sodium acetate, and obtained the same yields of identical products; furthermore, the salts did not require to be dehydrated before the reaction.

An I.G. Farbenindustrie <sup>41</sup> patent claims the preparation of starch acetate, acetopropionate, etc., by carrying out the reaction

in liquid sulphur dioxide under a pressure exceeding 12 atmospheres.

H. Stein<sup>42</sup> claims the use of an inert liquid swelling agent, e.g. fused chloroacetic acid, at a temperature below 100° C. To the fused solution the anhydride of an aliphatic acid (or if mixed esters are required, a mixed anhydride) is added together with an esterification catalyst such as perchloric acid or thionyl chloride. To obtain products partially or wholly soluble in acetone and benzene this worker treats alkali starch with acetyl chloride.<sup>105</sup> For further work on these esters the reader should consult the references.<sup>44-50, 72</sup>

The catalytic effect of sulpho-fatty acids of the Twitchell type on the reaction has been studied by R. Escales and H. Levy,<sup>51</sup> who find that they may be used at temperatures up to 80° C. without causing depolymerisation of the starch. They confirm the observations that the starch acetate solutions have a lower viscosity than the corresponding cellulose acetate solutions, and that the former are hygroscopic.

The diethers of starch have been prepared by J. K. Chowdhury<sup>52</sup> by the action of monochloroacetic acid in the presence of caustic soda; the remaining hydroxyl-groups were afterwards partially methylated with dimethyl sulphate and alkali. A number of chloroacetylated products were obtained by Z. H. Skraup and co-workers<sup>53</sup> by treating starch with acetic anhydride saturated with hydrogen chloride. Other papers on the acetylation of starch are given in the references.<sup>43, 107, 108</sup> Similar compounds, and also the formate, were obtained by A. G. Kldiashvili, using chloroacetic acids or formic acid.<sup>54</sup> The formate is soluble in water.<sup>96</sup> D. Gottlieb, C. G. Caldwell and R. M. Hixon<sup>109</sup> obtained the monoformate whatever the reaction conditions and could not obtain the di- and tri-formates reported by Traquair.<sup>35</sup> They report that the mono-formate gives a red iodine coloration. Spurlin,<sup>110</sup> summarising the general experience with starch, states that 'the substituents are distributed among the OH groups according to the laws of chance, and the three sorts of hydroxyls are substituted to an extent relative to one another that is determined by the relative reactivities of the hydroxyls . . . and the nature of the reaction. . . .' The above workers, however, find that the formyl group is attached to the 6-carbon atom. Partial derivatives of starch have been reported by Gomberg and Buchler<sup>111</sup> and by Tomecko and Adams.<sup>112</sup>

**Other Esters of Starch.**—Carbohydrate esters of the higher fatty acids<sup>59-60</sup> or cyclic carboxylic acids are made, according to an I.G. Farbenindustrie patent,<sup>55</sup> by swelling the starch to give an

aqueous paste of at least 30 per cent. strength and, after converting to the alkali derivative, treating with the acid chloride or a mixture of acid chlorides. The examples in the patent describe the preparation of the laurate-benzoate and the phenacetate of starch. The palmitate, stearate, and undecylate are made<sup>56</sup> by treating the unmodified starch with the acid chloride in the presence of a tertiary base and a diluent, such as chloroform or benzene, at the boiling-point of the diluent. The linoleate and esters of tung-oil acids are viscous liquids, becoming insoluble owing to oxidation in the air, and are prepared by treating the starch with the halide of the acid in the presence of the tertiary organic base.<sup>57</sup> P. Berthon<sup>58</sup> moistens starch with a mixture of benzene and pyridine, treats the mass with palmityl chloride in benzene, and after heating, adds alcohol to precipitate the ester which is used for the making of films. H. Gault<sup>61</sup> obtains the lauryl ester by treatment with the acid chloride in pyridine, and the products in this case are soluble in water. The preparation of the hexapalmitate and hexastearate is also described by P. Karrer.<sup>62</sup> By the action of chlorotriphenylmethane in pyridine, B. Helferich and H. Koester<sup>63</sup> obtained the triphenylmethane ester, which swells in organic solvents, but is decomposed by water and acids with extreme rapidity, giving starch and hydroxytriphenylmethane. W. S. Reich and A. F. Damansky<sup>64</sup> have prepared the di- and tricinnamates from soluble starch and the acid chloride, using pyridine as the reaction medium, and by suitably adjusting the conditions of the reaction they obtain good yields of the desired product.

G. Genin<sup>97</sup> swells the starch and then treats with the anhydride of a fatty acid. The stearic esters so prepared are soluble in hydrocarbons and their chlorinated derivatives, and they give soft transparent, slightly yellow waxy films; the mixed ester obtained from a mixture of acetic and stearic anhydrides has better film-forming properties which improve progressively with increasing acetate-content; the solubility also changes progressively, and it is possible to produce solutions in some esters and ketones. The esterification of starch is also discussed by A. F. Damansky.<sup>98</sup>

**Starch Ethers.**—A large number of starch ethers have been prepared in the last 10 years by different workers, and some have found their way, to some extent, into industrial use. The I.G. Farbenindustrie<sup>65</sup> products, Colloresin DK and D, are well known in the textile-printing trade; they are methyl ethers of cellulose containing 23-24 per cent. methoxy-groups. The methyl ethers of starch are of no interest commercially, as their solutions have a very low viscosity. Those desiring a complete

survey of this large field are referred to the extensive literature available elsewhere on the subject; only a very short summary can be offered here.

The ethers are generally prepared by the action of the requisite halide<sup>66</sup> or other alkylating (e.g. alkyl sulphate), or arylating, agent on the starch alkali in the presence of, for example, caustic alkali,<sup>67-77</sup> tertiary bases,<sup>78</sup> or dehydrating agents like calcium chloride<sup>79-81</sup> under a variety of conditions. Diazomethane has been used for methylation<sup>82</sup> of starch, and another patent claims the action of hydrogen chloride on a suspension of alkali starch in methyl alcohol. G. Kasiwaya<sup>113</sup> notes, incidentally, that rice starch is more easily methylated than millet or kaoliang starches. The action of ethylene oxide and its analogues or homologues,<sup>83-85</sup> under a variety of conditions, on starch or alkali starch is covered by several patents, the products being claimed as thickening agents for a variety of purposes, e.g. textile-printing, whilst in another patent<sup>86</sup> ethylene chloride, chlorhydrin, epichlorhydrin and similar compounds are used. If the sodium salt of the alkyl sulphuric acid is used, e.g. sodium methyl sulphate, more of the reagent is required and the reaction takes longer to complete.<sup>95</sup> By altering the conditions with these reagents different products may be obtained. To obtain products giving a thick viscous paste suitable as an adhesive for plywood, wood veneer or spiral tubing, the starch is first gelatinised, then methylated and heated to 80° C. To obtain substances more suitable as textile-finishing agents, the starch is methylated, diluted, and then gelatinised. Du Pont<sup>106</sup> claims that derivatives soluble in aqueous and/or organic media to give highly viscous solutions are obtained by treating starch, or a soluble derivative of it, with less than 1 mol. of di- $\beta$ -chloroethyl ether per  $C_6H_{10}O_5$  unit under such conditions that less than  $\frac{1}{4}$  mol. enters into the reaction.

Benzylated starches have been examined by B. V. Maxorov and K. A. Andrianov,<sup>99-100</sup> who find that pure starch gives products different from those obtained by the use of potato-flour and bran starches. The crude starches give resinous products having gloss, brittleness, low viscosity, and solubility in acetone, alcohols and aromatic hydrocarbons. Heating these products with formaldehyde or glyoxal raises the melting-point and lowers the solubility, and if sufficient of these reagents be used it is possible to obtain insoluble and infusible resins which the above workers think may be of interest in electrical work. F. Pancirolli<sup>104</sup> treats alkali starch with *p*-nitrobenzyl chloride and obtains the *p*-aminobenzyl starch by reduction. This compound can be diazotised and coupled with naphthols.



**Starch Xanthogenates.**—When dry starch is saturated with carbon disulphide and caustic soda stirred in, the product dissolves in water; on adding iodine solution the starch dixanthogenate-iodine complex is precipitated. The amount of iodine in this complex corresponds very closely with the theoretical amount calculated from the amount of starch originally taken.<sup>87</sup> If the starch be treated with a 5-20 per cent. caustic soda solution followed by carbon disulphide, allowed to stand for 24 hours, and then poured into alcohol, a mass of starch viscose containing two atoms each of sodium and sulphur separates. The compound undergoes progressive decomposition with a corresponding decrease in viscosity, but the decomposition is slower than in the case of cellulose viscose.

To attain completion, the ripening of starch viscose should be allowed to continue for a longer time than with cellulose viscose, the reaction sometimes taking one or two months. With some metals, starch xanthate gives characteristic compounds, which are colloidal and protected by the sodium starch xanthate.<sup>92</sup> Starch xanthate can be used as an adhesive, giving very strong bonds. On acetylation, according to Knoevenagel,<sup>93</sup> no water appears to be eliminated. The preparation of starch xanthogenate is also described by R. Wolfenstein and E. Oeser.<sup>89</sup> Stern<sup>94</sup> describes the preparation of a starch xanthate to be used as an adhesive for wood veneers: 200 kg. of starch are agitated with an equal weight of water at 60° C. and 15 kg. of caustic soda dissolved in 75 kg. water are slowly added; then to the alkali starch solution 7.5 kg. of carbon disulphide are added, the mass being well stirred and kept cool. The odour of the carbon disulphide decreases after several hours, and finally disappears.

Stable derivatives of starch are claimed to be formed<sup>90</sup> when a xanthogenate is oxidised in the presence of ammonia or ammonium derivatives, such as hydroxylamine, containing no organic radicals. The products so obtained are claimed for use in making films or filaments.

By treatment of the xanthate with a monohalogen fatty acid, ester, or other derivative, xantho-fatty acids are produced,<sup>91</sup> those from cellulose being of use as thickening agents, but the starch derivatives appear to have found no commercial use as yet. According to the conditions of treatment, the xantho-fatty acids produced may be soluble or insoluble in water. It is interesting to note that the derivatives of cellulose, having a higher viscosity in solution than the corresponding starch derivatives, are often of industrial value, but the starch compounds, being as a rule of low viscosity, are unsuitable.

Friese, Smith and Hess<sup>101</sup> report that the triacetates of amylose and amylopectin from wheat differ in their solubilities in chloroform, acetone, benzol, and ethyl acetate, the former being fairly soluble and the latter insoluble. These results are, however, not confirmed by the later work of Brigl and Schinle,<sup>102</sup> or that of Taylor and Walton.<sup>103</sup>

## REFERENCES

1. E. C. WORDEN, 'Technology of the Cellulose Esters,' Vol. 8. E. & F. N. Spon, London, 1921.
2. C. BLONDEAU, *Rév. sci. ind. Paris*, 1843, **15**, 69.
3. B. BALLOT, *Rapport annuel sur les progrès de la chimie, Berzelius, Paris*, 1844, p. 222.
4. H. FEHLING, *ibid.*, 1847, p. 342.
5. M. HOENIG and S. STANILAU, *Monatsh. f. Chemie, Wien*, 1886, **6**, 708.
6. — *ibid.*, 1886, **7**, 455.
7. R. TAMBA, *Biochem. Zeit.*, 1923, **141**, 274.
8. W. TRAUBE, F.P. 657,204, 1928.
9. — *Ber.*, 1928, **61B**, 754; E.P. 294,572, 1928. (Lapsed.)
10. — E.P. 322,003, 1928. (Lapsed.)
11. H. BRACONNOT, *Ann. chim. et phys. Paris*, 1833, **52**, 290.
12. J. PELOUZE, *Compt. rend.*, 1846, **23**, 809,892.
13. A. PAYEN, *ibid.*, 1847, **24**, 85.
14. A. BÉCHAMP, *Reportorium für die Pharmacie, Buchner's*, 1860, **51**, 255.
15. H. REINSCH, *ibid.*, 1849, **3**, 6.
16. F. RITTER, *Dingler's Polytechn. J.*, 1861, **161**, 146.
17. BERTHELOT, *Ann. chim. et phys.*, 1876, **9**, 316.
18. — *Compt. rend.*, 1885, **100**, 314.
19. O. MUEHLHAUSER, *Dingler's Polytechn. J.*, 1892, **284**, 137.
20. W. WILL, *Ber.*, 1898, **31**, 68; via *J. Chem. Soc.*, 1898, **74**, 227.
21. H. T. BROWN and J. H. MILLAR, *ibid.*, 1899, **75**, 308.
22. A. V. SAPOZHNIKOV, *J. Russ. Phys. Chem. Soc.*, 1903, **35**, 126; via *Bull. Soc. chim.*, 1905, **34**, 1173.
23. E. BERL and R. BÜTLER, *Zeit. für ges. Schiess- u. Sprengstoffwesen*, 1910, 82; via *J. Soc. Chem. Ind.*, 1910, **29**, 373.
24. A. BÉCHAMP, *Compt. rend.*, 1853, **37**, 134.
25. — *Ann. Chim. Phys.*, 1862, **64**, 311.
26. S. S. SADTLER, *Met. and Chem. Eng.*, 1917, **16**, 361.
27. K. KESSELER and R. ROHM, *Zeit. angew. Chem.*, 1922, 125.
28. G. R. ANCHORS, U.S.P. 1,329,353, 1920. (Lapsed.)
29. HAJIMA OKADA, *Cellulose Ind. Tokyo*, 1927, **3**, 3; via *Chem. Abst.* 1928, **22**, 686.
30. P. SCHUELZENBERGER, *Compt. rend.*, 1865, **61**, 485.
31. — *ibid.*, 1869, **68**, 814.
32. — *Ann. Chim. Phys.*, 1870, **21**, 235.
33. F. PREGL, *Monatsh. Chem.*, 1901, **22**, 1049.
34. C. F. CROSS, E. J. BEVAN, and J. TRAQUAIR, *Chem.-Ztg.*, 1905, **29**, 527.
35. J. TRAQUAIR, *J. Soc. Chem. Ind.*, 1909, **28**, 288.
36. J. BOESEKEN, J. VAN DEN BERGH, and A. KERSTJENS, *Rec. trav. chim. Pays-Bas*, 1916, **35**, 320.
37. J. D. LAW, *Chem.-Ztg.*, 1908, 365.
38. Y. TSUZUKI, *Bull. Chem. Soc. Japan*, 1929, **4**, 153.

39. P. BRIGL and R. SCHINLE, *Ber.*, 1929, **62B**, 99.
40. Y. TSUZUKI, *Bull. Chem. Soc. Japan*, 1929, **4**, 21 ; via *Chem. Abstr.*, 1929, **23**, 21.
41. I.G. FARBENIND., U.S.P. 1,928,269, 1930.
42. H. STEIN, G.P. 573,191.
43. H. PRINGSHEIM and M. LASSMANN, *Ber.*, 1922, **55**, 1409.  
I.C.I. Ltd., E.P. 493,513 (lapsed), U.S.P. 2,206,354. (Preparation of starch amines.)
44. I.G. FARBENIND., E.P. 293,757, 1927. (Lapsed.)
45. ——— E.P. 293,316, 1927. (Lapsed.)
46. H. GAULT and P. EHRMANN, *Chim. et Ind., Special No.* 574, May, 1924.
47. EASTMAN KODAK Co., U.S.P. 1,928,652, 1931.
48. C. DORÉE, 'The Methods of Cellulose Chemistry,' pp. 295.
49. I.G. FARBENIND., E.P. 279,864, 1927.
50. W. A. SCHOLTEN'S Chemische Fabr., F.P. 732,306, 1932.
51. R. ESCALES and H. LEVY, *Kunststoffe*, 1923, **25**, 52 and 64.
52. J. K. CHOWDHURY, *Biochem. Zeit.*, 1924, **148**, 76.
53. Z. H. SKRAUP, *Monatsh. f. Chemie*, 1905, **26**, 1415.
54. A. G. KLDIASHVILI, *J. Russ. Phys. Chem. Soc.*, 1905, **36**, 905 ;  
*ibid.*, 1905, **37**, 421.
55. I.G. FARBENIND., F.P. 668,686, 1929.
56. ——— G.P. 484,242, 1923.
57. ——— G.P. 478,127, 1924.
58. P. BERTHON, U.S.P. 1,651,366, 1923. (Lapsed.)
59. I.G. FARBENIND., E.P. 297,766, 1927. (Lapsed.)
60. ——— E.P. 283,181, 1927 (lapsed) ; U.S.P. 1,940,589.
61. H. GAULT, *Compt. rend.*, 1923, **177**, 592.
62. P. KARRER and Z. ZEGA, *Helv. Chim. Acta*, 1923, **6**, 822 ; via  
*Chem. Abstr.*, 1924, **18**, 80.
63. B. HELFERICH and H. KOESTER, *Ber.*, 1924, **57B**, 587.
64. W. S. REICH and A. F. DAMANSKY, *Compt. rend.*, 1933, **197**, 275.
65. ANON, *S.N.F. Bull. Tech.*, J.H. 354, *Technical Note*, No. 9847.
66. L. LILIENFELD, G.P. 475,884, 1921. (Lapsed.)
67. ——— G.P. 360,415, 1914. (Lapsed.)
68. ——— Austrian P. 82,866.
69. M. GOMBERG, *J. Amer. Chem. Soc.*, 1921, **43**, 1904.
70. C. G. TOMECKO and R. ADAMS, *ibid.*, 1923, **45**, 2698.
71. G. FRANK and K. MIENES, G.P. 575,349, addition to G.P. 555,930.
72. I. FUKUSHIMA and Y. TAKAMATSU, *J. Soc. Chem. Ind. Japan*, 1929,  
**32**, 130 ; *Chem. Abstr.*, 1929, **23**, 5058.
73. I.G. FARBENIND., F.P. 656,861, 1928.
74. ——— F.P. 640,174, 1927.
75. ——— E.P. 326,865, 1928. (Lapsed.)
76. L. LILIENFELD, E.Ps. 12,854/12, 6,035/13, 6,387/13, 163,016,  
163,017, 200,815 (all lapsed) ; Can. P. 222,377 ; U.S.P.  
1,350,820, 1920.
77. ——— E.P. 181,393, 1921. (Lapsed.)
78. ——— F.P. 699,217, 1930.
79. ——— F.P. 676,344, 1929.
80. W. HARRISON, G.P. 497,240, 1927.
81. I.G. FARBENIND., G.P. 492,319, 1926.
82. L. SCHMID and M. ZENTNER, *Monatsh.*, 1928, **49**, 111.
83. BAYER, G.P. 363,192, 1920. (Lapsed.)

84. I. G. FARBENIND., E.P. 359,618, 1930.
85. BAYER, G.P. 368,413, 1920. (Lapsed.)
86. H. DREYFUS, E.P. 166,767, 1920. (Lapsed.)
87. C. F. CROSS, E. J. BEVAN, and J. F. BRIGGS, *J. Chem. Soc.*, 1907, **91**, 612.
88. H. OST, F. WESTHOFF, and L. GESSNER, *Liebig's Ann. der Chemie*, 1911, **382**, 340.
89. R. WOLFFENSTEIN and E. OESER, *Chem. Zentr.*, 1925, **2**, 366.
90. W. HARRISON, E.P. 286,332; E.P. 286,331, 1926. (Both lapsed.)
91. L. LILIENFELD, E.P. 231,800; E.P. 231,805, 1924. (Both lapsed.)
92. P. WENGRAF, *J. Soc. Dyers and Col.*, 1930, **46**, 245.
93. KNOEVENAGEL, *Ann.*, 1914, **402**, 118.
94. STERN, U.S.P. 1,412,020, 1922. (Lapsed.)
95. DU PONT and NEMOURS, E.P. 454,963, 1935; U.S.P. 2,116,867, 1934.
96. J. TRAQUAIR, *J. Soc. Chem. Ind.*, 1909, **28**, 290.
97. G. GENIN, *Rev. gén. Mat. Plast.*, 1936, **12**, 5.
98. A. F. DAMANSKY, *Ann. Chim.*, 1934, **11**, 491.
99. B. V. MAXOROV and K. A. ANDRIANOV, *Plast. Massi*, 1933, **6**, 1.
100. B. V. MAXOROV, *Rev. gén. Mat. Plast.*, 1935, **11**, 336, 373, 375.
101. FRIESE, SMITH and HESS, *Ber.*, 1928, **61**, 1975.
102. BRIGL and SCHINLE, *ibid.*, 1929, **62**, 99.
103. T. C. TAYLOR and R. P. WALTON, *J. Amer. Chem. Soc.*, 1929, **51**, 3437.
104. PANCIROLI, *Boll. R. Staz. Sperim. Ind. Carta*, 1937, **32**, 314.
105. H. STEIN, G.P. 411,156, 1921. (Lapsed.)
106. DU PONT NEMOURS, E.P. 520,625.
107. A. FRANK, *Rev. brasil. Chim.* (Sao Paulo), 1940, **10**, 33.
108. TOKUZO NISIDA, Japan Pat. 130,827, 29/6/1939.
109. D. GOTTLIEB, C. G. CALDWELL and R. M. HIXON, *J. Amer. Chem. Soc.*, 1940, **62**, 3342.
110. SPURLIN, *ibid.*, 1939, **61**, 2222.
111. GOMBERG and BUCHLER, *ibid.*, 1921, **43**, 1904.
112. C. G. TOMECKO and R. ADAMS, *ibid.*, 1923, **45**, 2698.
113. G. KASIWAYA, *J. Chem. Soc. Japan*, 1938, **59**, 1261.
114. K. H. MEYER, P. BERNFELD and W. HOHENEMSER, *Helv. Chim. Acta*, 1940, **23**, 885.
115. J. R. WHINFIELD and G. G. RITCHIE, E.P. 535,949, 1940.

## ADDITIONAL REFERENCES

- HENKEL ET CIE, E.P. 529,993, 31/5/1939. (Alkali starch added to cellulose-ethers while latter are being alkylated, to give mixture of ethers.)
- W. P. M. MATLA, *Chem. Weekbl.*, **33**, 120. (Starch nitrates. A review.)
- T. URLANSKI and Z. JANISZEWSKI, *Rocz. Chem.*, 1937, **17**, 349. (Nitration with  $N_2O_5$ .)
- P. WENGRAF, *Textilber. (Eng. Ed.)*, 1930, 94. (Parallel reactions of starch and cellulose esters.)
- P. P. SHORYGIN *et al.*, *J. Gen. Chem. (U.S.S.R.)*, (In English), 1938, **8**, 1917. (Some new glycol ethers of starch.)
- W. PHILOPPOFF and K. HESS, *Ber.*, 1938, **71**, 841. (Viscosity of trimethyl starch in  $CH_2Cl$  measured over a wide range.)
- R. SUTRA, *Compt. rend.*, 1932, **195**, 1079. (Acetolysis of starch.)

## PART II

## THE MANUFACTURE OF STARCH AND STARCH PRODUCTS

## CHAPTER I

## ROOT STARCHES

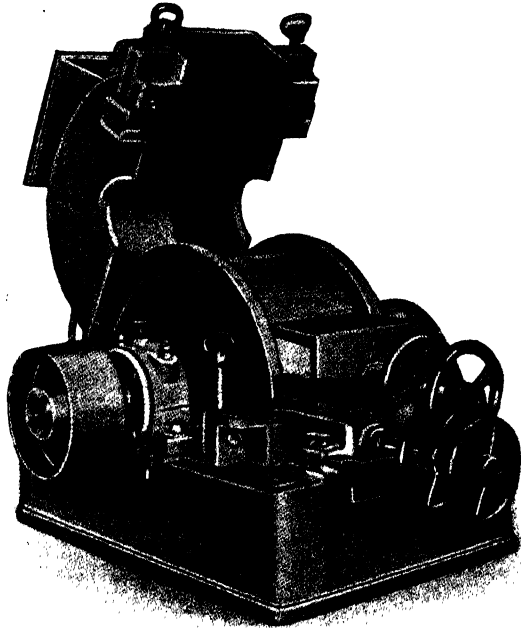
**Manufacture of Potato Starch.**—Potato starch is manufactured in Germany, Holland, Poland, Russia, and France (formerly also in Czechoslovakia), the inception of the industry dating from the middle of the eighteenth century. It was not until 1830 that potato starch was produced on a large scale in certain German factories. The industry became firmly established with the development of the paper and textile industries, and later when glucose was used in the fermentation industry.

A feature of the potato-starch industry is the number of small rural factories which supply the raw material, in the form of wet starch containing about 40-50 per cent. water, to factories producing glucose; the latter can thus cut down their overhead expenses by 20-30 shillings per ton, and as the crops are grown on the spot, transport costs are minimised, and the waste pulp being sold to neighbouring farms for fodder, competition with the larger town factories can be maintained.

Both the amount and the quality of starch present in potatoes are subject to wide variations; climate, soil conditions and variety of potato playing a large part in determining these values. According to L. Raab,<sup>1</sup> who examined 61 varieties of potatoes, the amount of starch varies from 10-30 per cent., a normal crop yielding anything from 16-22 per cent., one yielding about 13 per cent. being regarded as poor. In Germany, by means of cross-fertilisation and careful selection, potatoes have been improved from the point of view of starch manufacture, and the average starch-content has been raised to 25-40 per cent.

Potatoes, especially those raised on heavy soil, invariably have dirt adhering to them so tenaciously that simple washing does not remove it. They are therefore soaked for several hours and then tumbled into a revolving cylinder of heavy wire which is partly immersed in a trough of running water, or they are agitated in concrete troughs. It is most important that all dirt and impurities be thoroughly removed at this point, in order that the best possible product may be obtained at the final stage.

After washing, the potatoes are thoroughly rasped or mashed in such a way as to rupture the maximum number of cells containing the starch, in order to obtain the highest yield. In one machine for this purpose a toothed cylinder revolves in an outer casing at a very high speed (Fig. 35). The resulting watery pulp is washed practically free from loose starch, and the washed pulp is passed for a further mashing to a mill, generally of the Excelsior type, in which the product passes between two discs,

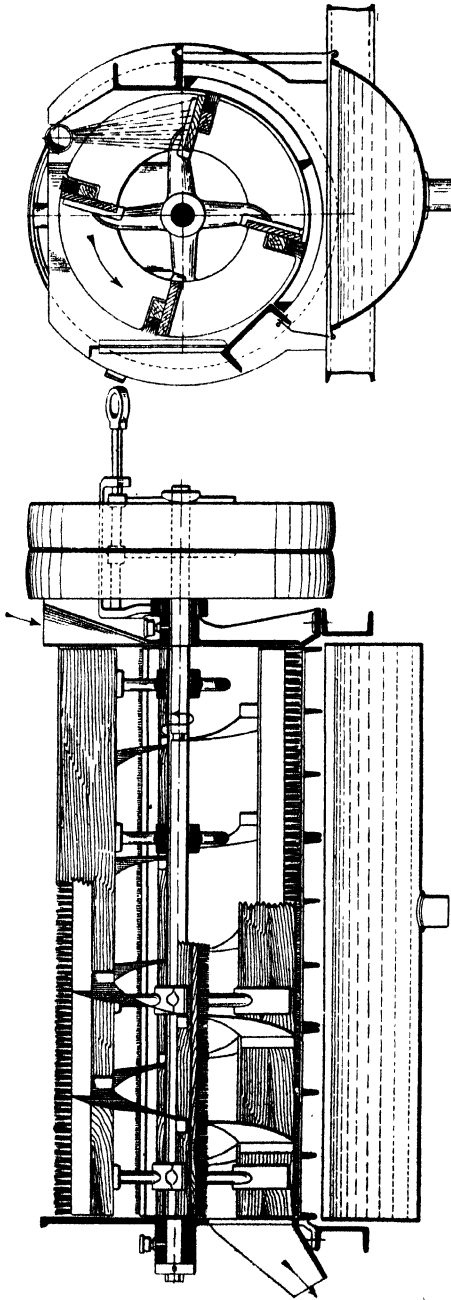


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FIG. 35.—Potato rasping machine.

one of which is rotated while the other is stationary. Heavy milling by means of rollers or Buhr stones, besides costing more, tends to loss of starch by the rupture of the larger granules.

In one process,<sup>2</sup> after the raw material has been pulped, air is excluded until it is centrifuged. In the liquor discharged from the centrifugal machine is the enzyme tyrosinase, which converts the tyrosine present into the dark bluish-black compound, melanine. In the usual processes, however, sulphurous acid is added to the pulped mass to prevent the oxidation of the tyrosine and to decolorise any melanine already formed.



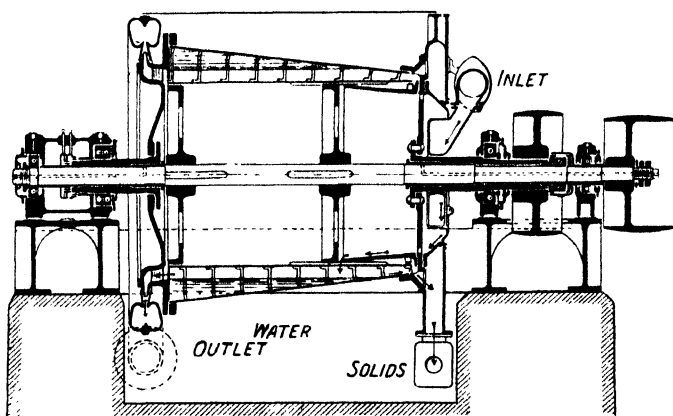
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FIG. 36.—Brushing sieve.

The next process is washing the pulp free from starch, which is carried out on a shaking sieve. The first sieve is generally a coarse one, and a considerable amount of finely divided fibre passes through with the starch. Further passage through finer sieves removes much of this fibre. Shaking sieves are noisy when running, are more expensive to run than those of the brush type, are apt to become clogged, and, although not so well adapted to remove starch completely from the pulp, are useful as refining sieves. They can be used to extract the first run of starch from the mash before it is returned to the mill to be re-mashed. In the case of brush sieves, which may be rectangular, circular or cylindrical, the brushes are moved so as to obtain the maximum sweeping effect, and to cause the pulp to travel slowly towards the discharge-outlet. Water is supplied continuously to the

mass by means of overhead sprinklers when open sieves are used, and for cylindrical sieves water is sometimes emitted through jets in the central shaft itself (Fig. 36). The brushing movement is responsible for a large amount of fibre being forced through the mesh, which does not happen to such an extent with sieves.

The raw milk-starch from the sieves contains soluble matter, fibre, and little nitrogenous matter in addition to the starch. To obtain starch of a good colour the iron-content of the water used should be low,<sup>8</sup> and the starch should be separated from the wash water as soon as possible, which is done by tabling the starch, whereby a lot of fibre is carried off in the liquid leaving



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FIG. 37.—Cross-section of a starch separator.

the tables. In some factories the liquid is removed by a centrifugal method, the liquid either passing out through the perforated circumference of the drum, which is lined with filter cloth, or if the periphery of the drum is not perforated, it is drawn off by an axial discharge-pipe. The starch is deposited against the outer wall as a compact mass, and when it half fills the drum the centrifuge is stopped and the starch removed.

Fig. 37 shows the cross-section of a continuous separator used in a German starch factory. The starch-milk of S.G. 1.020 enters the rotating chamber at the narrow end, the separated water runs to the wide end, whereas the starch is deposited on the walls of the narrow chamber and moves slowly but continuously to the narrow end, impelled by an endless screw rotating against the wall of the chamber at a slightly different rate. The



discharged starch is in the form of a paste containing about 55 per cent. of moisture.

When the tabling method is employed, the starch-milk is diluted and flows over inclined tables, some 20-30 yards long, about  $1\frac{1}{2}$  yards wide, and 10-13 inches deep, which have a weir at the lower end. Most of the largest granules fall to the bottom in the first 8 or 10 yards, the granule-size of the starch deposited becoming progressively smaller as the weir is approached. The liquor leaving the tables carries with it, besides much fibre in suspension, some starch of very small granule-size and soluble salts. The discharged liquor is led to a battery of settlement tanks arranged in series, where the remainder of the starch contaminated with fibre is deposited.

After the first sedimentation, the starch on the tables is again mixed with water and subjected to a second tabling, which may be preceded by sieving through shaking sieves to remove fibre. At this stage the starch liquor may be fed into centrifuges of the type in which the water escapes through perforations around the outside of the drum. In this way a starch with a lower moisture-content (35-40 per cent.) is obtained than by tabling alone (45-50 per cent. moisture). By using centrifuges after tabling, difficulties arising through excessive foaming of the liquor do not occur, as they do when raw starch is centrifuged in this type of machine without previous tabling.

**Refining the Starch.**—Refining is carried out by further washing and tabling; the wooden tables are arranged in parallel from a common supply channel and are narrower than those mentioned above. The fall of the tables is smaller, in order to reduce the rate of flow of the starch-milk. In some factories it is customary to treat the starch prior to this tabling with potassium permanganate solution in order to oxidise any impurities present, and to follow this with a sulphurous-acid treatment, but care must be taken to prevent excessive action, otherwise the starch may be so changed as to give a product which, on solution, possesses a reduced viscosity.

E. Wiegler<sup>3</sup> has studied the use of sulphurous acid in the manufacture of potato starch, and finds that the velocity of sedimentation of the starch from starch-milk increases with increase in the acidity of the milk above the normal. At normal  $pH$  the volume of the sediment gradually increases with time to the final value. With acidities greater than normal, however, the volume of the sediment rises rapidly to a value greater than the final value, and then gradually diminishes until the final value is obtained.

After the supernatant liquid has been run off, the starch on the

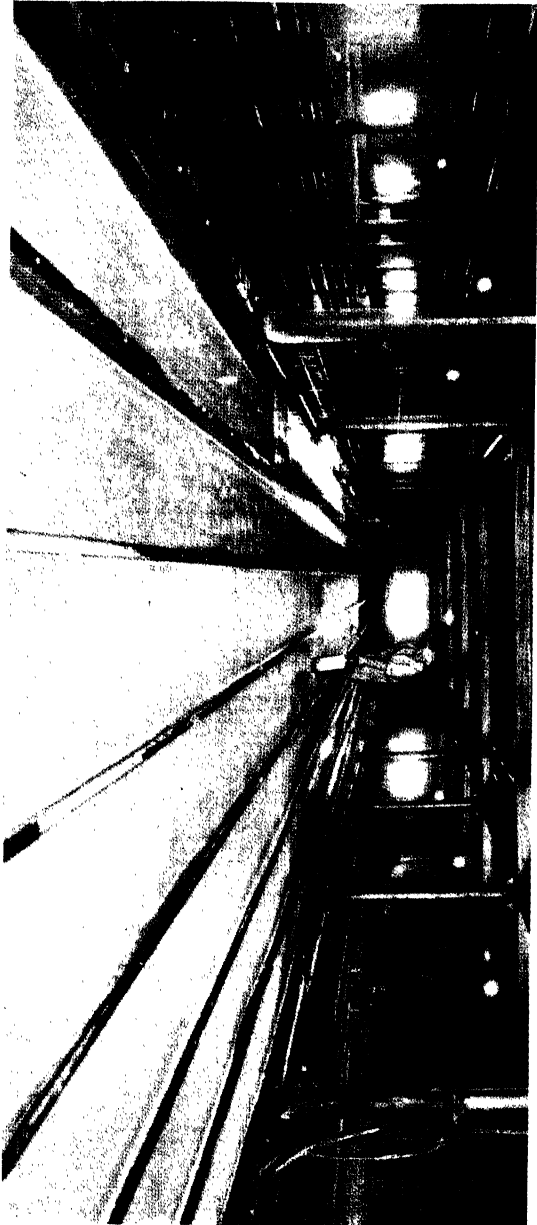


FIG. 38.—Flushing starch from the concrete ' tables '.

[Facing p. 156.]



refining tables is coated on the surface with a coloured layer containing impurities, which is scraped off with wooden squeegees and flushed away with clean water, the liquid from the flushing being returned to a starch-liquor undergoing one of the earlier steps of the process. The starch from the tables may be centrifuged to lower the moisture-content, and if washed into the centrifuge the residual impurities form the inside layer of a centrifuge cake. This inside layer is scraped off, the starch removed from the centrifuge and conveyed to the drier.

**Drying the Starch.**—Starch is generally dried in heated-air chambers of various designs, worked on the counter-current principle to avoid the formation of pellets of gelatinised starch. In one process the starch containing 35-40 per cent. of moisture enters the chamber and meets a current of air at a temperature of about 30° C. It is passed by means of endless belts to the bottom of the chamber, where the air is about 45° C. The heat is supplied by means of steam pipes between the layers of belting. In another process the starch is laid on a coarse grid at the top of a heated chamber, where some drying taking place; it crumbles and falls on to another finer grid below, and so on until at the bottom it consists of very small dry lumps and much powder. From time to time a man with a rake moves the starch on the various grids. This process is very effective, but slow, and has the disadvantage that some time is taken for the charge to accumulate during which bacterial action may operate.

Another form of dryer which gives powdered starch is the drum-dryer, in which revolving arms keep the starch continually in motion towards the discharge-vent of the drum. The axis of the drum is slightly inclined to resist the passage of the starch, and a current of warm air passes over the starch, carrying off the moisture. As an extension to this process the drum may be of the closed, vacuum type; the drum is jacketed and hot air or steam is circulated in the jacket so that the internal temperature ranges between 35° and 40° C. Grid dryers and those of either of the drum types are less apt to give a product containing pellets of gelatinised starch, which are sometimes present in starch dried in a belt-dryer through local overheating of the wet starch on the belt near the hot pipe. After drying, the starch is powdered, bolted, and bagged.

I. D. Buromskii and A. A. Matyushenko<sup>4</sup> carried out some interesting work on the preparation of potato starch by fermentation. They found that frozen or thawed potatoes stored in piles, or potatoes kept under water, may undergo a peculiar anærobic fermentation of the lactic-acid type which leaves the starch intact.

They also separated and described several bacteria responsible for this fermentation.

**Some Difficulties Occurring in the Manufacture of Potato Starch.**—The production of starch being a seasonable occupation necessitates the storing of some of the potato crop, as the whole cannot be processed at once. Temperature-control during storage is very important, and not easily realised in practice. If the potatoes are stored at  $-3^{\circ}$  C. they freeze, and on thawing rot so quickly that they have to be processed immediately. The yield of starch from stored potatoes is affected by three factors: evaporation, formation of sugar from the starch by the action of enzymes, and destruction of this sugar by respiration. The enzymes and bacteria present have full play at ordinary temperatures, and the metabolism of the tubers follows its normal course whereby the starch is destroyed. Respiration can take place freely, leading to a concomitant rise in temperature which, if not counteracted, accelerates the respiration still further, and the potatoes may even sprout. Both evaporation and respiration are almost arrested at  $0^{\circ}$  C. but the enzymatic production of sugar from the starch can still take place. In practice, the best temperature for storage of potatoes is considered to be between  $6^{\circ}$  and  $10^{\circ}$  C. (see also p. 296).

The reader may have seen potatoes being stored in the countryside. Long mounds, triangular in cross-section,  $1\frac{1}{2}$  to 2 yds. wide and 1 to  $1\frac{1}{2}$  yds. high, lightly covered with straw and a layer of earth, may be seen at the side or in a corner of a field. These mounds are known as 'clamps,' and constitute the best method of storing potatoes until the approach of warm weather. When a severe winter is expected, the amount of straw and earth covering the clamp is increased.

Frozen or diseased potatoes give rise to difficulties in manufacture, as they become soft and disperse in the wash-water. The colloidal, non-starchy matter present in suspension appears to protect the starch and prevent satisfactory sedimentation, besides frequently carrying coloured matter, which gives a poor appearance to the final product. Sedimented starch drained on the tables retains approximately its own weight of water, whereas a mass of fine potato fibres is able to retain five to seven times its weight of water. Hence fibre not only retards the settling of the starch but increases the water-retention of the 'green' starch.

H. Ducomet and A. Girard<sup>5</sup> have utilised rotten potatoes for the manufacture of starch, providing that the decomposition has not been carried too far. The potatoes awaiting treatment are kept under water, which is periodically renewed, so that further

decomposition is prevented. The disposal of large amounts of wash-water used in the manufacture of starch has been dealt with by J. Haline.<sup>6</sup>

Sprockhoff<sup>31</sup> puts the loss of potato starch, in the form of starch refuse or slime, as high as 10 to 15 per cent. The slime consists of about 50 per cent. of very minute starch granules mixed with traces of proteins, fibre, iron, bacteria, etc. This form of waste is generally experienced in plants using settling vats and is due to the action of bacteria and enzymes on the slow-settling starch granules. The protein matter present is converted to a gelatinous material which protects the granules and delays their settling. The amount of waste may be reduced to 2 per cent. by using centrifuges instead of settling tanks and by treatment of the starch-milk in the final stages with sodium hypochlorite which destroys the protective, gelatinous, protein coating on the granules, thus accelerating separation.

Potato starch has a characteristic odour, and several methods have been suggested to destroy or lessen its intensity. Thus C. Hellfrisch<sup>7</sup> treats the crude starch with chlorine. Sulphurous acid,<sup>11</sup> ozone, sulphites,<sup>10</sup> hypochlorites, sodium carbonate or caustic alkali<sup>9</sup> is often employed at some stage of the process to give a better-coloured product and to assist the settling of the starch. With almost all these treatments, however, care must be taken to see that the starch itself is in no way affected. Throughout the whole process of making potato starch a good-grade water, as free from iron as possible, should be used in order to get a good, lustrous starch. This is especially important in the manufacture of tapioca starch, in which the presence of tannin leads to the formation of iron tannate, which cannot be removed, and gives the starch a dull grey appearance, but lime and alumina have little or no effect on the appearance of the starch.

E. Peschke and F. Tobler<sup>33</sup> have noted that a higher yield of better starch can be rapidly and smoothly obtained by treating the starch-bearing material with *Bacillus felsineus* or the retting preparation 'felsinozima'. The cellulosic material is digested but the starch is unattacked and is obtained in a more finely granular form than by mechanical processes. In view of the interest in biological methods of production in recent years it is surprising that this observation has received so little commercial exploitation.

**Cassava Starch or Brazilian Arrowroot.**—This starch is obtained from the tuberous roots of the manioc or cassava plant, which was originally a native of Central America, but is now grown in Brazil, Madagascar, and the East Indies. Two chief

varieties of root are cultivated, the bitter variety, *Jatropha Manihot* or *Manihot utilissima*, and the sweet variety, *Jatropha dulcis* or *Manihot palmata*. The roots usually contain a small amount of hydrocyanic acid, which, however, disappears when they are processed for extracting the starch. The bitter variety yields more starch and contains more hydrocyanic acid than the sweet variety. The acid, formed by the action of an enzyme on a glucoside,<sup>12</sup> phaseolunatin, is present to the extent of 0.01-0.035 per cent. in the tubers of the bitter manioc, and the cortical layers of the sweet variety contain a like percentage. The interior portions of the latter, however, contain only 0.004-0.015 per cent. of the acid.<sup>13, 30</sup> On drying in the sun the content of hydrocyanic acid falls to 0.0017 per cent. and, if oven dried, to 0.0006 per cent. Y. Nemoto<sup>34</sup> considers all trace of hydrocyanic acid is removed when the tapioca flour is used for bread-making, and has studied the hydrogen-cyanide content, at various stages, in the preparation of flour, starch, etc., from different varieties of manioc.

On the average the tubers of the manioc plant contain 50-70 per cent. of water and 20-30 per cent. of starch, but the bitter manioc may contain more starch.

Methods of preparation vary somewhat in different countries, but in general the pulped roots are washed on sieves, and the starch so obtained is purified by more or less well-known methods. The operations of separating and purifying the starch are very similar to those used for potato starch. In the West Indies the roots are washed, peeled and pulped, either by hand or by a simple wheel-grater, and the pulp squeezed in coarse bags to expel the juice from which a fairly pure starch is deposited. The pulp remaining in the bags is rubbed through coarse sieves, and after drying, is known as manioc flour or farine.

In the lowlands of Java the roots are grown for about 11 to 13 months, or, for better starch, 13 to 16 months. The roots are collected, cleaned, sliced or ground into meal, and dried in the sun or in kilns. The dried meal is then shipped to Europe for starch separation. A large amount of starch is, however, made on the island by washing the starch from the pulped roots in a revolving cylindrical sieve of fine brass-gauze about 5 ft. in diameter and 15 ft. long. The waste matter is discharged from the end and the starch separated into two grades in a second cylindrical sieve covered with coarse cotton-cloth. The first-grade starch is deposited in about 4 hours; the second grade, which takes longer, is then agitated with water in another vat and resettled. After drying at 42° C. it is sieved through fine silk screens and bagged.

The success of the operations depends chiefly on the careful control of the rate of flow of the water in the sieves.

**The Manufacture of Sweet-Potato Starch.**—In the last few years efforts have been made in America to supply part of the domestic demand for root starches by the establishment of an industry for extracting starch from the tubers of the sweet potato (*Ipomea batata*) (see Fig. 39). C. H. Boehringer<sup>14</sup> reports that this source has been used by the Japanese on a small scale for a hundred years, and that in 1933 over 30 per cent. of Japanese starch produced came from this raw material. The colour and



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FIG. 39.—Sweet potatoes weighing 6 to 10 lb. each.

quality of this starch is variable. The average starch-content of the sweet potato is 23.8 per cent. (maximum 26.4 per cent., minimum 21.7 per cent.) and the final starch contains about 12 per cent. of moisture.

Experimental work in America was started so long ago as 1895 by M. B. Hardin,<sup>15</sup> and continued for several years.<sup>16-18</sup> More recent attempts by R. T. Balch and H. S. Paine,<sup>19</sup> and by F. H. Thurber<sup>20</sup> have led to the production of a starch suitable for commercial requirements. As a very full account containing all technical details has been given by H. S. Paine and his co-workers,<sup>21</sup> a brief summary here must suffice.



The potatoes are thoroughly washed, crushed in a hammer-mill (this replaces the saw-toothed drum-rasp used in European

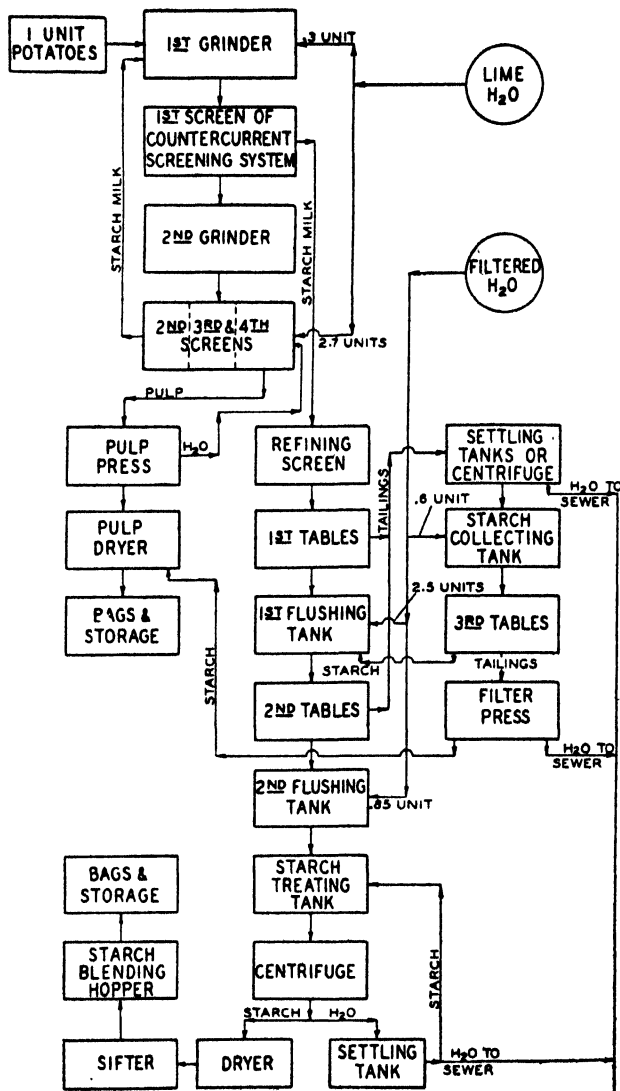
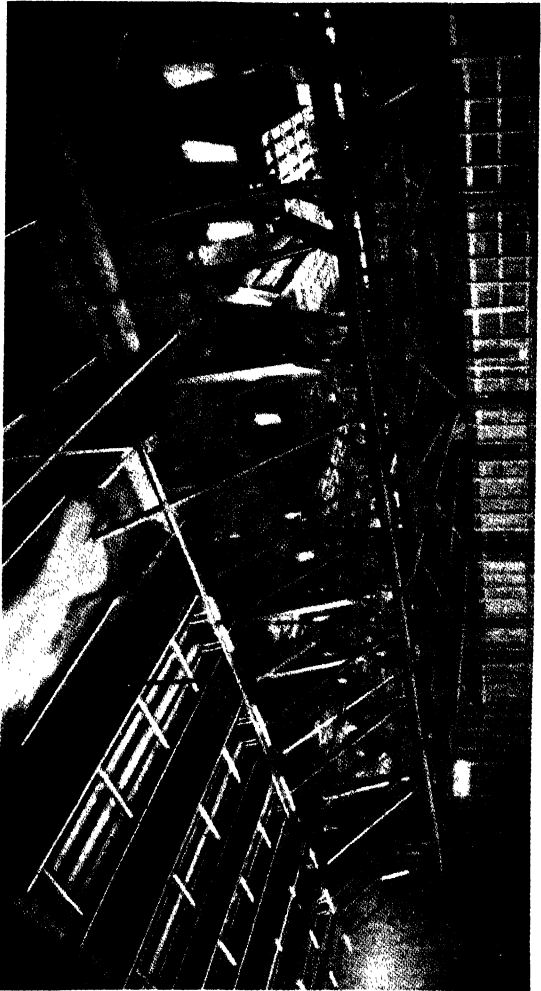


FIG. 40.—Flow diagram of starch process.

potato-starch factories), mixed with a clear saturated solution of calcium hydroxide, and the mass passed over a series of electrical





*[Reproduced by courtesy of 'Industrial and Engineering Chemistry',*  
*FIG. 41.—A shaker screen installation.*

*[Facing p. 163.*

shaker-screens to remove the starch set free (see Fig. 41). The screened mash is again re-ground, washed with calcium hydroxide solution, and re-sieved, giving a suspension with a  $pH$  of 8.6-9.2, which is the optimum range for all the tabling operations; in addition, the calcium hydroxide flocculates certain impurities and dissolves the pigment present which would otherwise discolour the starch. The starch liquor is again sieved to remove fibres, etc., and tabled at about  $5^{\circ}$  Bé., the tables sloping  $1/32$ -in. per ft. The tables are made of concrete, and are 19 in. wide by 110 ft. long. After sedimentation the starch is re-tabled with fresh water and the deposit from this flushed off at  $10-15^{\circ}$  Bé. and re-screened. To obtain a good-coloured product, the starch from the second tabling is bleached for 2 hours with a slight excess of sodium hypochlorite at a  $pH$  value just above 8.3, the residual chlorine eliminated by the use of sulphur dioxide, and a final adjustment of  $pH$  value made. The liquor is then centrifuged in the perforate-basket type of centrifuge at 1200 r.p.m. which delivers starch with a water-content of 35 per cent. to the dryer, which is of the batch-vacuum type, 4 ft. in diameter and 20 ft. long. After drying, the starch is pulverised and screened. Fig. 40 shows the flow diagram of the process.

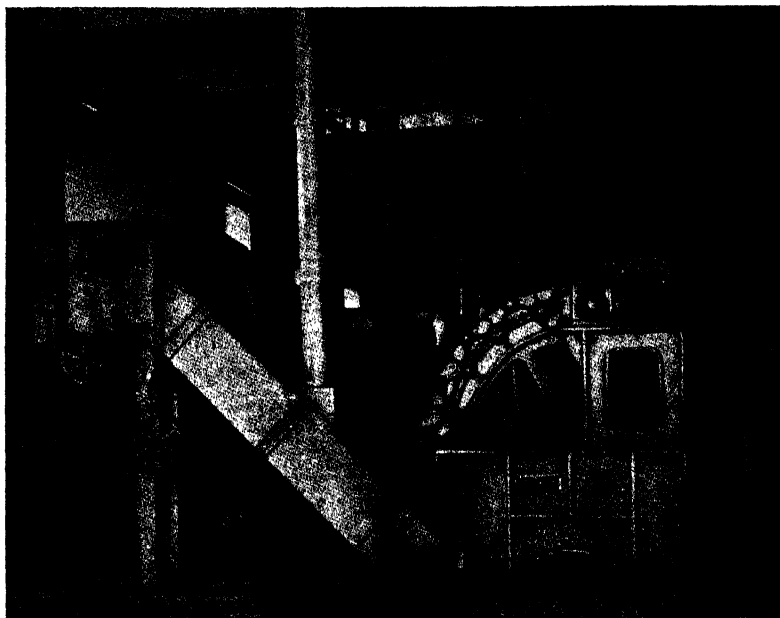
Any starch in the overflow water from the centrifuge and in the table-tailings is recovered by cone-bottom settlers and imperforated centrifuges. It is diluted to  $4.5^{\circ}$  Bé., and after adjusting the  $pH$  value to 9.2, with saturated lime water, it is then returned to the starch going to the first tabling process, and thus the factory produces only one grade of product.

This starch has been found to be of value<sup>22</sup> in warp sizing, and gives good clear colours and soft 'handle' when used for finishing cotton goods. The viscosity of the starch made by the above process increases slightly during the first 3 or 4 hours of heating, which is an advantage in the above type of work (see p. 327). W. T. Scriber<sup>23</sup> finds that the starch gives clearness of colour, greater smoothness, and stiffness to fabrics treated in laundry work compared with other starches. It has also been found valuable for paper-making<sup>24</sup> and for the manufacture<sup>21</sup> of adhesives and dextrin, where it can replace cassava starch.

Sweet potatoes do not store well, and an interesting observation made by E. F. Hopkins<sup>26</sup> is that when the sliced tubers were treated with certain vapour or liquid reagents, such as carbon tetrachloride or carbon bisulphide, the cell wall becomes very permeable to liquid, so that 60-70 per cent. of the juice present can be removed by applying pressure (see Fig. 42). Without this treatment only about 6-7 per cent. of the juice was removed.

The remaining moisture can be reduced to 12 per cent. by using hot waste gases, and the product may then be stored until required for processing.

By finely grinding the dried material and sieving, a product is obtained having a starch-content (dry basis) of 80-90 per cent. H. S. Paine and K. Ward <sup>25</sup> find that adhesives suitable for cartons,



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FIG. 42.—Pulp press and dryer.

fibre containers, etc., can be readily made from this flour, but they suffer from the drawback of possessing some colour, although for a number of purposes this is not objectionable. This flour may find some outlet in the baking <sup>27-28</sup> and brewing <sup>29</sup> industries, and it is also eminently suitable for alcohol production.

#### REFERENCES

1. L. RAAB, *J. Chem. Soc.*, 1872, **25**, 1111.
2. JAHN & CO., E.P. 439,262, 3/12/1935.
3. E. WIEGL, *Zeit. Spiritusind.*, 1935, **58**, 205.
4. I. D. BUROMSKII and A. A. MATYUSHENKO, *Microbiol., U.S.S.R.*, 1936, **5**, 40; via *Chem. Abst.*, 1936, **38**, 110, 6030.

5. H. DUCOMET and A. GIRARD, *Compt. rend. Acad. Agric. France*, 1917, **3**, 61.
6. J. HALINE, *J. Soc. Chem. Ind.*, 1916, **35**, 1126.
7. C. HELLEFRISCH, E.P. 24,456, 1895.
8. O. SAARE, *Zeit. Spiritusind.*, 1886, **9**, 511.
9. — *ibid.*, 1892, **15**, 319, 335 and 344.
10. ANON, *ibid.*, 1918, **41**, 399.
11. — *ibid.*, 1918, **41**, 407.
12. CARMODY, *Lancet*, 1900, No. 4018, 736.
13. DUNSTAN, HENRY, and AULD, *Proc. Roy. Soc.*, 1906, **78B**, 152.
14. C. H. BOEHRINGER, *Assist. Trade Commiss. Tokyo, Spec. Rept.*, 1936, **55**, Jan. 7th.
15. M. B. HARDIN, *S. Carolina Agric. Exp. Station, Ann. Rept.*, 1895.
16. F. H. SHIVER, *ibid.*, *Bulletin*, 1898, **28**; *ibid.*, 1901, 63.
17. C. C. McDONNELL, *ibid.*, 1908, 136.
18. T. C. KEITT, *ibid.*, 1912, 165.
19. R. T. BALCH and H. S. PAINE, *Ind. Eng. Chem.*, 1931, **23**, 1205.
20. F. H. THURBER, *ibid.*, 1933, **25**, 565 and 919.
21. H. S. PAINE, F. H. THURBER, R. T. BALCH, and W. R. RICHEL, *ibid.*, 1938, **30**, 1331.
22. F. H. THURBER, *ibid.*, 1933, **25**, 565.
23. W. T. SCREIBER, M. N. GEIB, and C. C. MOORE, *Bur. Stds., J. Res.*, 1933, **11**, 765.
24. C. G. WEBER, M. D. SHAW, and M. J. O'LEARY, *Nat. Bur. Stds. Misc. Publ., M.* 150, 1935.
25. H. S. PAINE and K. WARD, U.S.P. 2,124,994, 1935.
26. E. F. HOPKINS, *Science*, 1938, **87**, 71.
27. C. E. MANGELS and S. C. PRESCOTT, *Chem. Age (N. Y.)*, 1921, **29**, 132.
28. H. C. GORE, *Ind. Eng. Chem.*, 1923, **15**, 1238.
29. H. S. PAINE and E. YANOVSKY, U.S.P. 2,126,133, 1938.
30. C. C. MOORE, *U.S. Bur. Chem., Bull.* 106, 1907.
31. SPROCKHOFF, *Zeit. Spiritusind.*, 1930, **58**, 78.
32. R. D. DE G. PAULA and J. L. RANGEL, *Ministerio trabalko, ind. comm., Inst. nac. tec. (Rio de Janeiro)*, 1940, 66; via *Chem. Abstr.*, 1940, **34**, 7031.
33. E. PESCHKE and F. TOBLER, *Faserforsch.*, 1925, **4**, 252; *Chem. Zentr.*, 1925, **96**, II, 1233.
34. Y. NEMOTO, *Rev. Aliment. Chim. Ind.*, 1940, **4**, No. 33, 5.

## ADDITIONAL REFERENCES

- ANON, *Amer. Dyestuff Rep.*, 1936, **25**, 313; *Bull. Assoc. Chim.*, 1936, **23**, 553. (Recent equipment for separating tuber starches.)
- L. W. JIRAK, *Zeit. Spiritusind.*, 1935, **58**, 165. (Methods and calculations for plant control.)
- W. KRONER, *ibid.*, 1937, **60**, 39 and 45. (Black substance deposited on potato-starch machinery due to proteins.)
- H. G. KNIGHT, *Mfrs. Record*, 1937, **106**, 40. (Sweet-potato starch manufacture.)
- W. KRONER and G. STEINHOFF, *Zeit. Spiritusind.*, 1937, **60**, 143. (Estimation of starch in frozen potatoes.)
- W. TARGENER, *Deut. Zuckerind.*, 1937, **62**, 69. (Estimation of starch in potatoes.)

- C. P. McCORD, *Mfg. Conf.*, 1927, 621. (Starch-drying precautions.)  
P. NETTIN, *Rev. Sci.*, **65**, 363. (Technology of starch-making.)  
J. A. MACLACHLAN, *J. S. Afric. Chem. Inst.*, **12**, 3. (Manufacture of starch.)  
M. SPROCKHOFF, *Zeit. Spiritusind.*, 1929, **52**, 306. (Sources of waste in starch manufacture.)  
A. H. CROWN, *Proc. Amer. Assoc. Text. Chem. Col.*, 1930, **59**; *Amer. Dyestuff Rep.*, 1930, **19**, 83. (Manufacture of starch, dextrin, and British gums.)  
G. J. MULLER, *Chem. Met. Eng.*, 1941, **48**, No. 3, 78 and 83. (Modern technology in potato starch manufacture.)  
G. E. GOVIER, *Rubber Age*, 1930, **10**, 490. (Manufacture of starch for the rubber industry.)  
C. H. PETERS, *Zeit. Spiritusind.*, 1930, **53**, 120. (Starch industry in Russia.)  
T. MINAGAWA, *J. Agric. Chem. Soc. Japan*, 1933, **9**, 342; via *Chem. Abst.*, **27**, 3959. (Amylosynthase and synthetic starch.)  
J. T. GIBBONS, *Amer. Dyestuff Rep.*, 1934, **23**, 286. (Manufacture.)  
H. P. DAS GUPTA and V. SUBRAHMANYAN, *Agric. Livestock, India*, 1934, **4**, 645. (Starch from indigenous tubers and grains.)  
W. D. HANSEN and H. VOGEL, *Mitt. deut. Landw. Ges.*, 1926, **41**, 12. (Potato starch.)  
MARILLER, *Bull. Assoc. chim. suc. dist.*, 1927, **44**, 299. (Purification and drying of potato starch.)  
M. SPROCKHOFF, *Zeit. Spiritusind.*, 1928, **51**, 180. (Grating machines used in potato-starch manufacture.)  
— *ibid.*, 1929, **52**, 191. (Yield tables for potato starch.)  
O. WOLFF, *ibid.*, 1929, **52**, 110, 221, 231. (Control operations in potato-starch manufacture.)  
W. BUDBERG, *ibid.*, 1930, **53**, 113. (The 'ter Meer' centrifuge in potato-starch manufacture.)  
M. SPROCKHOFF, *ibid.*, 1930, **53**, 119. (Potato starch.)  
— *Chem.-Ztg.*, 1930, **54**, 299. (Tables showing starch-content of starch-milk in relation to density.)  
W. BIELECKI, *Przemysl Chem.*, 1930, **14**, 145. (Potato starch.)  
J. EBEL, *Chem.-Ztg.*, 1933, **56**, 829. (Advances in potato-starch manufacture.)  
B. BLEYER, *Zeit. Spiritusind.*, 1933, **56**, 45. (Potato starch.)  
H. TRYLLER, *ibid.*, 1933, **56**, 60. (Effect of weather on potato starch.)  
B. HOSPES, *ibid.*, 1935, **58**, 237. (Yield tables in potato-starch manufacture.)  
J. JIRAK, *ibid.*, 1935, **58**, 81. (Control of washing-out process in potato-starch manufacture.)  
M. PLATZMANN, *ibid.*, 1936, **59**, 361. (Starch-content of potato flakes.)  
P. A. SINGER, U.S.P. 1,528,995, 1925. (Drying moist starch in hot air cyclone separator.)  
N. E. GOLDTHWAITE, *Colorado Agric. Exp. Stat. Bull.*, 1925, [296], 3. (Composition of Colorado potatoes.)  
B. LAMPE, *Zeit. Spiritusind.*, 1931, **54**, 36. (Determination of starch-content of potatoes with Reimann balance.)  
G. FOTH, *ibid.*, 1924, **47**, 352. (Determination of starch in potatoes of very low starch-content.)  
MASCHINEBAU-ANSTALT HUMBOLDT, G.P. 441,911, 1925. (Potatoes treated in disc mills open to the whole circumference of the discs.)

- C. J. DE WOLFF, *Chem. Weekblad*, 1927, **24**, 18. (Formation of sugar in potatoes during drying.)
- W. KRÖNER, *Zeit. ges. Getreidew.*, 1939, **26**, 162. (Flow sheet and details of potato-starch manufacture with analytical details.)
- *Zeit. Spiritusind.*, 1939, **62**, 163. (Furnace gases which contain SO<sub>2</sub> should not be used for drying starch.)
- C. A. BRAUTLECHT, *Ind. Eng. Chem.*, 1940, **32**, 893. (Processes and production costs for manufacture of white potato starch in U.S.A. discussed.)
- W. R. RICHEL, *Proc. Oklahoma Farm. Chemurgie Conf.*, 1937, **1**, 4. (Plant operation for sweet-potato manufacture discussed.)
- C. C. MOORE, *Potato Mag.*, 1920, **2**, 10, 20, 22 ; **3**, 8, 20, 22. (Detailed description of potato-starch manufacture.)
- W. EKHard, *Zeit. Spiritusind.*, 1924, **47**, 183. (Potato-starch manufacture.)
- H. S. PAINE, F. H. THURBER, R. T. BALCH and W. R. RICHEL, *Chem. Met. Eng.*, 1939, **46**, 69. (Sweet-potato starch.)
- E. SZEGO, *Bull. assoc. Chim.*, 1939, **56**, 158. (Plant control in potato-starch factories discussed.)
- W. KRÖNER, Fr.P. 823,308, 18/1/1938. (Starch separated from finely divided potatoes by upward stream of water in inclined tube.)
- A. FRANK, *Rev. brasil. chim.* (Sao Paulo.), 1940, **10**, 120. (Preparation of cassava starch.)
- H. G. KNIGHT, *Kansas State Hort. Soc. Biennial Rept.*, 1940, **45**, 131. (Sweet potato starch. General.)



## CHAPTER 2

## CEREAL STARCHES

**The Manufacture of Wheat Starch.**—In the manufacture of wheat starch the chief problem lies in the separation of the starch from gluten. Gluten consists of two protein substances, glutenin and gliadin, and in water swells to a sticky mass which retains the starch. A review of the state of our present knowledge of wheat proteins has been made by D. B. Jones,<sup>18</sup> to which readers are referred for further information. A. Schhukin<sup>20</sup> has examined the chemical composition of hard and soft wheats (see also Table II, p. 413). To separate the starch, the gluten may be destroyed by fermentation, as in the older processes, or mechanically kneaded in flowing water, by which the starch is liberated and carried away. Processes based on the mechanical separation are in general use to-day, as the gluten can be recovered and is a valuable by-product.

In the old fermentation method (*v.s.*), often known as Hale's method, the grain is steeped until it is soft enough to be coarsely crushed in a mill, the temperature of the steeping water playing a large part in determining the time of steeping. This preliminary steeping may, however, be omitted. A mash is made of the crushed grain and left to ferment. The period of induction of fermentation can be shortened if liquor from a previous fermentation is introduced into the mass. The period of fermentation required to bring the mass to the required state varies with climatic conditions. In the summer about 7 to 10 days are required, and in the winter the process may take as long as 30 days. Proteins and other nitrogenous matter decompose during the fermentation, and acids are produced which in turn help to dissolve the gluten, the latter becoming more soluble as its degradation proceeds. Gases, chiefly carbon dioxide, ammonia, and hydrogen sulphide, are evolved in the early stages of the process, and the whole mass has an offensive, putrefying odour, which constitutes a drawback to the process. As the fermentation proceeds, the mass becomes acid from the formation of acetic, butyric and lactic acids, and clots of moulds appear on the surface. The fermentation should not be carried to the stage at which the liquid becomes viscous, as it has then proceeded too far and difficulties will be encountered. At the desired stage, supernatant liquor is run off and the remaining sludge transferred to

washing drums. The suspension of starch flows out through the wall of the drum, which may be made of cloth or fine metal-gauze. By revolving the drum horizontally and passing in water through the central shaft the mass may be washed until very little starch remains. The starch-milk is run off by means of troughs to settling tanks, where it is washed several times by agitation, settling, and decantation. The mass of starch is then removed to draining-boxes lined with flannel, or it is spread upon porous draining-floors, after which it is sent to the drying room.

The fermentation process is rarely, if ever, used at the present time. Not only is the valuable gluten lost, but the process is a lengthy one, offensive in operation, and gives rise to effluents, the disposal of which offers difficulties.

**The Alsatian Process** dispenses with fermentation, and is occasionally employed on the Continent. The grain is steeped until soft and the steeping water is changed frequently to avoid acid-formation and fermentation. The grain is then roughly crushed and the mass kneaded in a continuous stream of water supplied from sprinklers over the trough. The process is slow, as about 10 hours are required for the treatment of 1 ton of wheat. It has the advantage, however, that good-grade gluten is recoverable if the steeping is not too prolonged. A temperature of 30° C. is recommended by Rehwald to hasten this step of the process. One ton of wheat yields about 750-1000 lb. of first-quality starch and about 220-450 lb. of starch of second quality.

**Martin's Process** is the one most generally used now, partly owing to the low initial outlay for plant, and partly to the good yields of first-quality starch obtained. About 45-55 per cent. of good starch on the weight of wheat, 10-20 per cent. of inferior starch, and 10-15 per cent. of good-grade gluten free from husks may be obtained. The disadvantages of the process are the higher-priced starting materials and the relatively large yield of second-grade starch, contaminated with gluten, that is produced. This, however, can be utilised as a good basic material for sizes and adhesives. It is a fairly rapid process. Wheat flour, the starting material, is made into a dough with about 40 per cent. its weight of water and allowed to stand for 1 hour. The gluten swells, and the mass is transferred in lumps to the washing-machine, in which the dough is kneaded by means of a grooved roller on a grooved bed flanked by sieves, while a constant stream of water is supplied by means of sprinklers. The starch-milk is collected in troughs beneath the machine and is led into settling-tanks. The remaining gluten is removed after each operation.

**Fesca's Process** also uses flour as the raw material, and separates the starch from the gluten by means of a centrifuge. A suspension of flour in water is made and centrifuged, using a non-perforated drum. In this way a layer of starch is obtained on the wall of the drum, whilst the gluten forms a layer on the inside, a certain amount of gluten also remaining in suspension. Knives or scrapers, that may be set at a required distance from the centre of the centrifuge drum, are used for scraping off the gluten layer. The liquid from this process is rich in nitrogenous material and mineral salts and is incorporated in feeding-stuffs, which are then dried. To obtain a sharper separation of the starch from the gluten, Klopfer has suggested several modifications of this process, namely, the use of a 1 per cent. solution of sodium bicarbonate,<sup>14</sup> or of sodium chloride,<sup>15</sup> or of green malt extract<sup>16</sup> in the making of the flour suspension. The addition of these substances is claimed to improve the separation without destroying the nutritive value of the gluten.

This process is economical; it requires very little plant and water, and has low overhead charges. About 50 per cent. of the flour solids appears in the gluten layer, which contains about 22 per cent. protein, 67 per cent. starch, and other solids; this by-product finds a valuable outlet in the manufacture of feeding-stuffs, and is used in the preparation of macaroni, semolina, and similar products.

In all the above processes the starch obtained requires purification before being used for a number of purposes, and this is carried out by washing with water, sieving, and sedimenting. The percentage of small starch grains present is determined by the rate of flow of the starch liquor over the tables or through the vats. When the centrifuge method is employed in the purification process it is usually carried out after the primary purification in order to avoid trouble due to the gluten particles choking the filter cloth; or, again, the centrifuge may be of the non-perforated type. After successive washings and sedimentation the starch cakes are filter-pressed or centrifuged in a perforated drum, and dried at 30° C., this temperature being increased to 60-70° C. later, when most of the moisture has been driven off. The cakes so obtained have a brownish discoloured layer on the outside, which is scraped off and may be used for low-grade work and for adhesive-making.

In some cases the treatment of glutenous starch to obtain pure starch is carried out with water containing a small amount of acid, usually acetic acid, which exerts a dispersing action on the gluten. Sulphuric acid is sometimes used, but suffers from the disadvantage

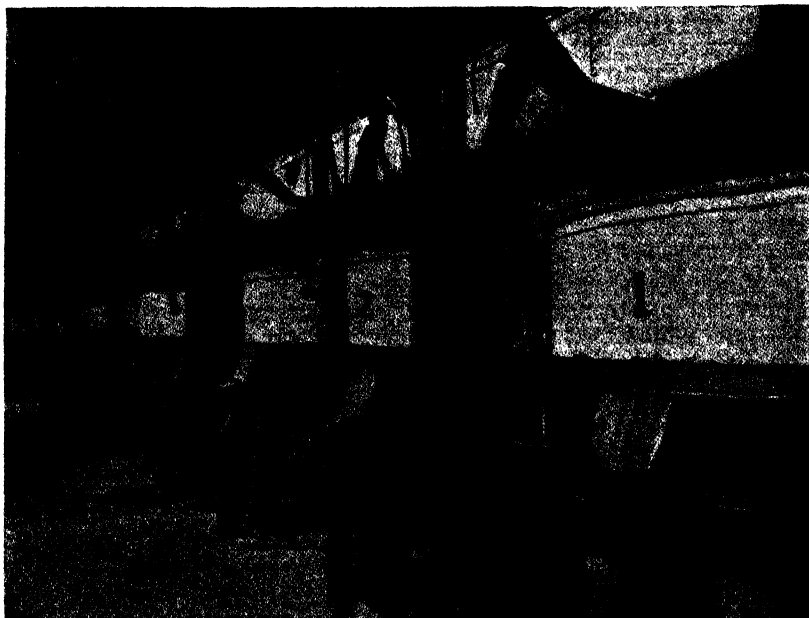
that the acid is non-volatile, whereas if acetic acid is used, any remaining traces are driven off in the drying process. Caustic soda is another excellent but non-volatile dispersing agent for gluten. If crystal starch is required, the acid treatment is often omitted, as the presence of small amounts of gluten improves the coherence of the product, whereas with very white starches containing practically no gluten it is sometimes necessary to add a small amount of white dextrin to obtain the necessary binding effect. Good crystals of maize starch (*v.s.*) may, however, be prepared even when all the gluten has been removed and no dextrin added.

**Manufacture of Maize Starch.**—The largest producer of maize starch is the United States of America, where it is generally known as 'corn starch,' and is widely employed.

The amount and quality of the starch produced from maize depend upon the conditions of cultivation in a similar manner to the variations in potato starch, but between narrower limits.

In manufacture the starch has to be separated from the associated nitrogenous substances quickly to prevent deterioration being brought about by fermentation or acid formation. Centrifugal methods have been employed for this separation.<sup>9</sup> The protein matter, or gluten, which is present to the extent of about 8-10 per cent., consists of water-soluble albumins, alkali-soluble glutelins, the globulins that are soluble in dilute salt solutions, and prolamines, which are soluble in aqueous alcohol and occur only in the grains. Most of the nitrogenous substances are separated as the substantially water-insoluble gluten, but some of the more soluble globulins, together with the water-soluble albumins, are removed from the corn when it is steeped at the beginning of the process. This steeping-water also contains soluble salts, which form some 33 per cent. of the solid matter removed at this stage. The gluten consists of about 50 per cent. protein matter, 5 per cent. oil, and 35 per cent. starch, together with fibre and a little mineral matter. The starch, which constitutes 55-65 per cent. of the dry weight of the grain, is contained in the endosperm, from which it is removed by crushing and maceration. In the old process the whole grain was treated, but present-day procedure is to separate the embryos and treat them to recover the oil they contain. F. Baines<sup>10</sup> has protected a process whereby the grain is heated with five to fifteen times its weight of water in a closed vessel at 85-95° C., and a somewhat similar method is employed by J. Wildsmith.<sup>11</sup> The by-products from the manufacture of maize starch are of commercial value, and are dealt with on page 360.

**Early Process. Extracting the Starch.**—A. E. Williams<sup>1</sup> and G. Archbold<sup>2</sup> have described the earlier processes. In these the whole grain was steeped in water for several days at about 60° C., the water containing 0.25 per cent. sulphur dioxide or 0.3 per cent. calcium bisulphite to retard excessive fermentation. It was then lightly ground between Buhr-stones (see Fig. 43) to disintegrate the grains, and the mass well washed on sieves. After re-grinding and washing the mass a second time, to extract any



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FIG. 43.—Buhr mills in which two large stones revolve over each other.

residual starch, the two lots of starch-milk were mixed and concentrated by tabling, the gluten and fibre being carried off by the water and collected by sedimentation in large tanks.

**Treating the Starch.**—The starch was next washed from the tables into a tank where it was treated with about 1 per cent. caustic soda, calculated on the weight of starch in suspension. This treatment caused the residual gluten to swell and at the same time facilitated the removal of the fatty material. The starch-milk, after further dilution, was again tabled, and the raw starch, which then contained about 0.1 per cent. alkali, again mixed with water.

After sieving to remove impurities, this starch-milk was passed to washing vats where, during the sedimentation, some fermentation took place and the acid formed neutralised the residual alkali. After several washings, followed by sedimentation, the starch was passed to draining boxes, as in the present-day process. The draining was followed by drying, which is carried out to-day on similar lines and which may conveniently be considered at this point.

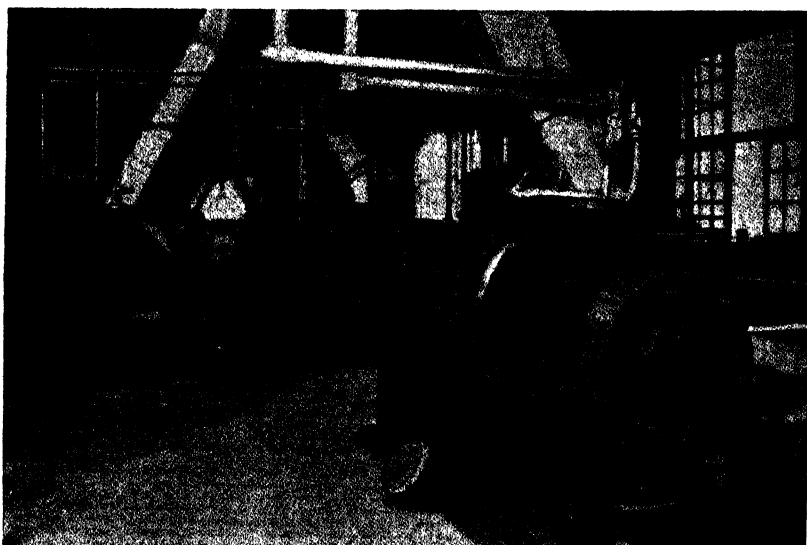
**Drying.**—In the draining boxes the moisture-content is reduced in a few hours to 45-55 per cent., approximately. The blocks are then transferred to a warm chamber, where more moisture is lost, and the surface of the block forms a crust which is discoloured by soluble impurities coming to the surface. This discoloured starch is scraped off and returned to another batch being processed. The blocks of scraped starch are treated according to the type of product required; for lump starch they are broken and air-dried, for crystal starch they are wrapped in paper and dried slowly at 30-40° C. It is preferable to allow the blocks to remain at room-temperature for several days before heating, and then to raise the temperature to about 35° C. over two to three weeks. Pearl starch is obtained by drying at 71-80° C. for 18-20 hours.

The starch produced by the older methods has not, in general, such a good colour as that obtained in the present-day sulphurous acid process. The starch from the latter is largely used in glucose-syrup manufacture, and tends to give more fluid solutions; starch from the alkali process finds a big outlet in laundry work.

It will have been noted that in the preparation of wheat and maize starch the chief problem is to separate the gluten. The differences in manufacturing procedure for wheat and maize starch arise from the differences in the properties of the gluten in the two materials. Gluten from wheat can be kneaded into a tough, elastic, coherent mass, whereas that from maize remains in a finely dispersed state, necessitating special treatment to separate it effectively from the starch.

**Modern Process.**—The sulphur-dioxide process was probably introduced by L. von Wagner<sup>12</sup> in 1886; it is superior to the alkali process in that the oil from the embryos is recovered and the starch more easily purified. W. P. Kaufman<sup>13</sup> has described the modern process, the first stage of which, after any necessary pre-washing, consists in steeping the corn in water for some 30-40 hours at 40-60° C. to soften the grains. The water contains 0.3 per cent. of sulphur dioxide. The grain is steeped in a battery of vats on the counter-current principle, and then crushed with

rollers or in a Fuss mill (see Fig. 44) with sufficient water to maintain the proper gravity; the embryos are set free, together with a small amount of starch, and the germs are separated by flotation in a V-shaped tank having a screw-conveyor at the bottom. After grinding the germs, the oil is expelled by hydraulic presses or removed by solvent extraction. The coarse solid matter removed from the V-shaped tank by the screw and conveyor, and freed from the germs, is ground with water in a Buhr-stone mill, the liquid being delivered into inclined shaking sieves (No. 9 gauze or 90 meshes to the linear in.), and then passes



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FIG. 44.—Attrition or Fuss mills.

through a series of finer sieves (160 linear in., No. 17) or bolting cloth of 200 mesh, in which the fibrous and cellulosic matter is largely retained. The matter retained in the first sieve is generally re-ground and passed through the sieves again to obtain all the starch possible from it.

The starch is then concentrated by deposition on wooden tables which are 100-130 ft. long, 1-2 ft. wide, and 6-10 in. deep, with a fall of about 4 in. between the ends, the fall being greatest in the first 50 ft. The liquor is adjusted to 6-7° Bé. and fed by a pipe, 3-6 in. in diameter, or by a perforated box, to the tables, which are arranged in parallel. Here more fibrous and glutenous

matter is removed in suspension, the starch sinking to the bed of the tables owing to its greater specific gravity. The 'green' starch on the tables contains approximately 50 per cent. of water, together with 0.5 per cent. of protein matter, and is removed when a depth of 8-9 in. is attained at the head of the table. Any slight surface deposition of gluten is first squeegeed off the starch, which is then flushed with water from the table over rotary filters. In this state the starch may be used directly for the manufacture of glucose syrup, but to obtain the starch for marketing it must be further purified by deposition, either with or without treatment with alkali. If the alkali treatment is omitted the protein-content remains around 0.5 per cent., but by treatment with alkali the figure is lowered to 0.3 per cent., as the alkali causes the gluten to swell and facilitates its removal. The fat is saponified and more easily carried away in the washings.

Recent patents<sup>4, 13</sup> make use of the fact that gluten and impurities are more strongly absorbed at an air/water interface than starch, so that air blown through raw starch liquor causes the formation of a froth which contains only about 10 per cent. by weight of starch but very appreciable quantities of gluten. By this process it is claimed that, after sweeping away the foam, the protein-content of the starch has been lowered from 0.5 to 0.3 per cent. The paste is next made up to 22° Bé. with water, and pumped into cloth-lined boxes, where it is dried as described above in the account of the old process.

**Rice Starch.**—The production of rice starch offers similar problems to the production of maize starch because the gluten in the rice grain does not form a coherent mass as does wheat gluten. One of the earliest methods was due to O. Jones<sup>19</sup> and this, with unimportant modifications, is used to-day. Rice grains are rich in starch, and each granule is coated with a very compact layer of gluten that has to be softened before it can be removed. Granules of rice starch are very small, and take a long time to settle, hence many factories use centrifugal methods of separating the partially purified and the purified starch. Where settling methods are used, as much as six weeks may elapse from the time the grain enters the factory to the time the starch is ready for marketing, so that the use of centrifugal methods, although more expensive, economises both space and time.

Rice starch is generally made from the broken white grains which have been rejected for use in the foodstuffs industry, or from 'cargo rice,' which is the grain still enclosed in the outer brown cuticle. The by-product from the latter type of raw material is of value for cattle food, as it contains phosphorus compounds and rice oil.



The grain is fed by gravity into cement or iron vats, provided with air-agitation and fitted with perforated false bottoms. Caustic soda solution of sp. gr. 1.005 is run in until the level of the liquid is 12 to 24 in. above the grain. After standing for 24 hours, with periodic air-agitation, the liquid is withdrawn, the grains washed with water, and fresh liquor added. The steeping is continued for a further 36 to 48 hours, and then the grains are soft enough to squash by gentle pressure between thumb and finger, or the mass may have started to disintegrate. The mass of soft grains is ground with caustic soda solution of such a strength that the outflow liquor from the disintegrator has sp. gr. 1.240. Ammonia solution may be used instead of caustic soda solution; it saves half the time but is more costly.

The mass is now passed to the settling tanks, or in some factories to the centrifuges, in which case the amount of solids in suspension is kept higher than if it is to be settled. The imperforate-drum type of centrifuge is used, and after the operation has been completed any heavy fibrous matter is found against the outer wall, followed by a layer of starch containing some fine fibrous matter, whilst the last and inner layer consists of gluten admixed with some starch. The inner layer is scraped off before the main bulk of the starch is discharged.

The wet starch is agitated with water, to which is often added 0.25 per cent. of formaldehyde solution (35 per cent. strength) to inhibit fermentation, and either settled or centrifuged, and after removing the gluten layer the washing and separation processes are repeated. In some factories a bleaching process is introduced at this stage, and in others a blueing agent (see p. 338) is added in order to improve the colour of the final starch.

There is no set rule for the order in which operations for purifying the starch are carried out. In some factories screening precedes, and in others it follows the centrifugal separation. Centrifuging the starch before screening saves wear on the silk screens, as the material passing through is much less alkaline than if the order of operations is reversed. With centrifugal separation the amount of alkali present is double that used when settling is employed, and the concentration of starch is also greatly increased.

The starch liquor is adjusted to sp. gr. 1.210 and passed into draining-boxes, which have perforated bottoms and are lined with cotton fabric. In many modern plants, suction can be applied to the bottom of the boxes and, to hasten draining, air-pressure may be applied through covers fitted on the top. As it is important that no air bubbles should form in the mass, the sides of the boxes

are tapped while the liquor is still free enough to liberate entrapped bubbles. When the starch has drained sufficiently to set to a solid block the boxes are discharged, and the blocks of moist starch are cut into four pieces and placed on porous plates in a crusting-stove.

In the crusting-stove the starch blocks are dried for about 48 hours at 50-60° C., then removed, and the brownish discoloured outer layer of starch scraped off and returned to one of the washing tanks. The starch-block is now ready for the final drying operation.

The drying process is very important, and must be strictly controlled, as the characteristic appearance of crystal or lump rice starch depends upon the attainment of correct drying conditions. If crystal starch is to be prepared, the blocks, after crusting and scraping, are wrapped in paper. For the first 48 hours the temperature is kept at 35° C., and the humidity of the air circulated in the drying-stove is not allowed to fall below 14 grains per cubic foot. At the end of 48 hours the temperature is raised by 3° C. in each succeeding 24 hours, and the humidity of the circulated air is allowed to fall until, when the temperature of 50° C. has been reached, practically dry air is being used. The temperature is then raised to 57-60° C. for 36-48 hours, after which the starch is removed from the stove and the paper removed. For ordinary purposes, where crystal starch is not required, a shorter drying-time and higher initial temperature may be used, as the careful drying described above is carried out solely with the object of obtaining the starch in compact lumps.

## REFERENCES

1. A. E. WILLIAMS, *Ind. Chem.*, 1923, 9, 129.
2. G. ARCHBOLD, *J. Soc. Chem. Ind.*, 1902, 4.
3. W. P. KAUFMANN, *ibid.*, 1910, 527.
4. E.P. 159,838, 1921. (Lapsed.)
5. BANER, U.S.P. 1,061,720, 1913. (Drying of starch.) (Lapsed.)
6. — U.S.P. 1,035,302, 1912. (Drying of starch.) (Lapsed.)
7. — U.S.P. 1,099,276, 1914. (Drying of starch.) (Lapsed.)
8. JEFFRIES, U.S.P. 1,007,782, 1,007,783, 1911. (Lapsed.)
9. A. PELTZER, U.S.P. 2,013,668, 1935.
10. F. BAINES, E.P. 18,258, 1891. (Lapsed.)
11. J. WILDSMITH, E.P. 4,146, 1883. (Lapsed.)
12. L. VON WAGNER, E.P. 4,758, 1886 : (Lapsed.) *J. Soc. Chem. Ind.*, 1886, 5, 330.
13. H. HOLDEN, U.S.P. 1,221,990, 1917. (Lapsed.)
14. KLOPPER, G.P. 102,465. (Lapsed.)
15. — G.P. 201,969. (Lapsed.)
16. — E.P. 11,159, 1907 ; E.P. 19,726, 1908. (Both lapsed.)
17. A. E. WILLIAMS, *Ind. Chem.*, 1930, 6, 387.

18. D. B. JONES, *Cereal Chem.*, 1937, **14**, 771.
19. O. JONES, E.P. 8,488, 1840. (Lapsed.)
20. A. SCHHUKIN, *Naukh-Agron. Zhurnal [Russia]*, 1926, **3**, 379.

## ADDITIONAL REFERENCES

- A. RICHE, *J. pharm. chim.*, 1880, **1**, 137. (Describes fermentation process for making maize starch.)
- L. BONDONNEAU, *Bull. Soc. encour. ind. nat.*, 1893, **92**, 849. (Trough-settling superior to tank-settling.)
- E. LECONTE, *Elektrochem. Zeit.*, 1904, **11**, 113. (Preparation and purification of rice starch using electric current.)
- K. HEMBD, *Zeit. Spiritusind.*, 1919, **42**, 395. (Good starch deposits more quickly than poor starch.)
- F. B. WISE, *Assoc. Rice Millers*, 1921, **1**, 18. (Detailed description of rice-starch manufacture.)
- O. K. A. KRIZKOVSKY, *Zeit. Spiritusind.*, 1923, **46**, 123. (Detailed description of Martin's method.)
- E. PAROW, *ibid.*, 1923, **46**, 1, 6, 12, 17, 23. (Describes machinery for starch manufacture.)
- A. P. WEST and O. A. CRUZ, *Philippine J. Sci.*, 1933, **52**, 1. (Rice-starch manufacture in the Philippines.)
- H. BADER, *Ann. brass. distill.*, 1934, **32**, 113. (Rice starch.)
- O. K. A. KRIZKOVSKY, *Chem.-Ztg.*, 1928, **52**, 425, 466, 486, 526. (Maize starch.)
- A. E. WILLIAMS, *Ind. Chem.*, 1933, **9**, 129. (Maize starch.)
- ANON, *Chem. Trade J.*, 1935, 409; *Gel. Leim u. Klebst.*, 1935, 168. (Rice starch, Continental methods.)
- S. MIZAKA and K. HAYASKI, *J. Soc. Trop. Agric.*, 1936, **8**, 185. (Amylo-synthase from Formosa rice.)
- W. KRÖNER, *Zeit. Spiritusind.*, 1938, **61**, 235, 243, 253. (Significance of the starch-industry from viewpoint of food supply.)
- M. SOBORNOV, *Spirto-Voctohnaya Prom.*, 1938, **15**, 29. (The starch-content of cereals cannot be estimated from the density.)
- F. B. DEHN, E.P. 277,400, 1926. (Counter-current principle in maize starch production.)
- E. C. R. MARKS, E.P. 277,572, 1927. (As above, using SO<sub>2</sub> in the water.)
- E. SCHLÜTER, E.P. 225,101, 4/3/1924. (Lapsed.) (Production of rice starch.)
- H. KAUTZ, Swiss P. 115,305, 1925. (Manufacture of rice starch.)
- F. L. JEFFRIES, U.S.P. 2,065,313, 22/12/1936. (Details of manufacture of maize starch.)
- A. PELTZER, U.S.P. 2,097,531. (Centrifugal separation of starch and gluten.)
- INTERNATIONAL PAT. DEVEL. Co., Fr.P. 813,220, 28/5/1937; Fr.P. 813,221. (Maize starch and gluten separated by aeration of partly purified starch suspension.)
- A. E. STALEY MFG. Co., U.S.P. 2,058,683. (Manufacture of maize starch.)
- A. H. KELLING, U.S.P. 2,100,744. (Manufacture of maize starch in which substantially all the waters, except steep waters, are re-used.)

- K. SUBBA RAO, *Current Science*, 1939, **8**, 250. (The sorption-desorption cycle of polished rice followed. Absorption maximum increased from one cycle to the next.)
- F. J. JEFFERIES (to Int. Pats. Desel. Co.), U.S.P. 2,098,293. (Preparation of starch as dustless flakes for malting purposes.)
- CORN PRODUCTS REFIN. CO., F.P. 844,308, 24/7/1939. (Plant described for manufacture of maize starch.)
- E.P. 530,226; Conv. 11/7/1938; 6/12/1940. (Starch dried by disintegration in stream of hot gas at a temperature above the gel point.)
- U.S.P. 2,228,717. (Corn steeped in water containing 0.05-0.1 per cent. chlorobenzene or chlorotoluene and a mixture of 2 parts NaHSO<sub>3</sub> and 1 part NH<sub>4</sub>HSO<sub>3</sub> to give SO<sub>2</sub> content of 0.12 per cent.)
- P. CHATSKII, *Spirto-Voctochnaya. Prom.*, 1939, **16**, No. 2, 36. (Nomo-grams for starch-content of barley and rye.)

## CHAPTER 3

## THE OXIDATION OF STARCH

*Contributed by F. F. FARLEY,\**

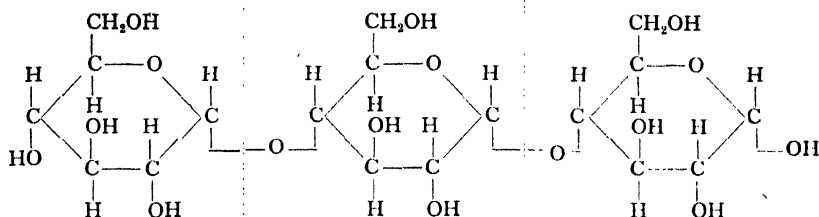
OXIDATION has been employed commercially for at least forty years as a means of producing modified starch. The various oxidised starches are used chiefly in the textile, paper, laundry and food industries because of the particular properties which can be conferred on starch by oxidation. Oxidised starches yield pastes which are more fluid and more transparent than pastes from raw starches. Sufficient oxidation furnishes products which are soluble in hot water to a clear solution. Furthermore, soluble starches made with oxidising agents are in general whiter than those made with acid.

For the surface sizing of paper the more fluid pastes from oxidised starches have preference over the viscous pastes formed from ordinary starch.<sup>207</sup> Use is also made of oxidised starches in the mill sizing of textiles. The limited 'setting up' (see p. 236) which high grade tapioca starch shows on standing may be made a property of other starches by oxidation.

Oxidation of starch decreases the amount of starch which can retrograde or precipitate on ageing. The textile industry is interested in soluble starches which give a permanently clear solution; starches whose solutions become cloudy during storage are undesirable. The starches used in textile work whose solutions are claimed to be permanently clear are generally produced by oxidation and may contain added substances to inhibit retrogradation (see p. 112). Likewise, in the manufacture of 'clear gum' confectionery a starch is demanded which will not become opaque on storage (see p. 303). Cheap, rapid drying and efficient adhesives are produced by oxidation beyond the thin-boiling stage.<sup>47</sup>

Although many aspects of the structure of starch are still in dispute, it is generally agreed that starch consists largely of chains of  $\alpha$ -glucopyranose units joined by 1-4 linkages :

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Starch.

It is apparent from the above formulation that the site of attack of an oxidising agent is not very limited. There are available for oxidation terminal aldehydic groups, primary alcoholic groups and secondary alcoholic groups. The priority which one or the other of these groups may enjoy in the initial stage of starch oxidation has been the subject of much speculation.

A survey of the literature on the oxidation of starch disclosed that among the large number of papers only a few contributed substantially to the study of the mechanism of the oxidation. This lack of data on the oxidative mechanism is due in part to the fact that the oxidations were, on the one hand, too mild and, on the other hand, too extensive to throw light on the intermediate stages. Many investigations have been concerned with the physical properties of the slightly oxidised starches and with the end products of extensive oxidation to sugar fragments, but relatively little work has been done on the hydrolysis and investigation of intermediates. However, the mechanism of one type of oxidation, periodic acid oxidation, has been greatly clarified by Jackson and Hudson,<sup>123, 124</sup> and Caldwell and Hixon.<sup>28</sup>

In order that satisfactory comparisons may be made among the oxidising agents used on starch, the oxidants have been compared on an equivalent basis rather than on a weight basis. The reason is obvious when it is recalled that one pound of chlorine may be equivalent to several pounds of a chemical which liberates chlorine. The weight of the oxidants has been converted to equivalents and reported as the number of equivalents for each glucose anhydride ( $C_6H_{10}O_5$ ) unit. For example, two equivalents of chlorine per glucose unit would mean 70 grams of active chlorine per 162 grams of starch. This system shows the error in many comparisons which have been made in the literature on a weight basis and makes possible a fundamental comparison of all oxidising agents.

Although the fact that starch can be hydrolysed by acid has been far more important to commerce and research than has the oxidation of starch, the hydrolysis of starch was not discovered until twenty years after starch had been oxidised. Fourcroy,<sup>66</sup> in 1792, had oxidised starch by means of concentrated nitric acid, later by chlorine gas, both before Kirchoff,<sup>137</sup> in 1811, discovered the important conversion of starch to glucose by means of acid.

The course of the oxidation of starch depends to a large extent on the *pH* of the medium. Starch has been oxidised in acid, alkaline and neutral solutions.

### I. OXIDATION OF STARCH IN ACID MEDIA

In an acid medium hydrolysis of starch occurs along with oxidation. Oxidation in acid solution gives, therefore, oxidised forms of the hydrolytic products of starch.

(a) **Oxidation by Nitric Acid.**—Doroschewski and Rakowski<sup>44</sup> observed during quantitative studies of starch hydrolysis by very dilute nitric acid that concentrations over 0.6 N nitric acid resulted in oxidation. Stronger nitric acid solutions oxidised starch to saccharic, tartaric and oxalic acids and carbon dioxide.<sup>72, 88, 96, 135, 143, 212</sup> Hachihama and co-workers<sup>72, 96</sup> reported a 49 per cent. yield of saccharic acid and a 10 per cent. yield of oxalic acid.

By using concentrated nitric acid Bechamp<sup>6</sup> produced oxidised starches of varying solubility in cold water. Oxidised starch containing nitrogen was obtained by many of the following when the oxidation was carried out by concentrated nitric acid. Petit's<sup>180</sup> product contained 6 per cent. nitric acid. The nitric acid, however, was liberated by treatment with water. The purified resulting product had acidic and reducing properties. Further oxidation produced the same acids as obtained with dilute nitric acid, viz. saccharic, tartaric and oxalic. This last acid, oxalic, was also obtained by Fourcroy<sup>66</sup> and Pelouze<sup>176</sup> when they used concentrated nitric acid on starch. Braconnot<sup>17</sup> made a product soluble in cold water. By using a large excess (117 equivalents) of concentrated nitric acid, Will and Lenze<sup>239</sup> produced a trinitrate (14 per cent. N<sub>2</sub>) of starch which exploded at 194° C. Brown and Millar<sup>23</sup> used 4.6 equivalents of strong nitric acid to produce a sesquinitrate (9.1 per cent. N<sub>2</sub>) from soluble starch, limit dextrin, maltodextrin and Nægeli's amylo-dextrin. Although the regenerated starch was little altered by nitration and still had a blue iodine colour, the distinct acid

character of the nitrated dextrins together with their loss of reducing power was a proof that oxidation had occurred. The starch nitrates of Berl and Bütler<sup>11</sup> containing 12.86 to 13.44 per cent. nitrogen were prepared from corn, potato, rice and soluble starches by eight equivalents of nitric acid mixed with sulphuric acid.

Lieben<sup>143</sup> obtained a mononitro-starch (6.76 per cent. N<sub>2</sub>) by the action of fuming nitric acid. The nitro-starch of Berl and Smith<sup>12</sup> contained 14.08 per cent. N<sub>2</sub> and gave a strongly reducing acid when the nitrogen was removed by alcoholic sodium hydroxide. Pelouze<sup>177</sup> suggested, because of its combustibility and its resemblance to guncotton, that nitro-starch should be used as an explosive (see p. 142).

Some of the patents on nitric acid oxidation of starch are listed.<sup>52, 115, 121, 153, 158, 162, 166, 187, 216</sup>

(b) **Oxidation by Ammonium Nitrate.**—The decomposition of ammonium nitrate was accelerated by the presence of starch.<sup>59</sup> Evidence of oxidation was the carbon dioxide and carbon monoxide evolution. Ammonium nitrate was also used in a patented process.<sup>190</sup>

(c) **Oxidation by Chromic Acid.**—Several investigators allowed chromic acid to act on starch and dextrin under mild conditions to produce a small amount of oxidation. Starch thus treated was converted to soluble starch,<sup>43, 105, 192, 201, 229</sup> or to dextrins.<sup>49, 112, 143</sup> The amount of chromic acid employed by Harz<sup>112</sup> was one equivalent per glucose unit. Reychler's<sup>192</sup> oxidised starch, though insoluble in hot water, dissolved readily in very dilute alkali. It was produced in the cold by 2.5 equivalents of chromate. Jambuserwala<sup>125</sup> used dilute chromic acid for the oxidative hydrolysis of starch. He presented data on the reducing value and viscosity of the products. In a later publication Jambuserwala and Kanitkar<sup>127</sup> reported the gradual increase in acidity during oxidation. Several earlier workers had observed the acidic properties of these chromic acid products.<sup>192, 240</sup>

Cross, Bevan and Beadle<sup>38</sup> reported a yield of about 10 to 11 per cent. furfural when starch was oxidised in the cold by six equivalents of chromic acid, the furfural being distilled from a solution of the oxidised starch in 12 per cent. hydrochloric acid and coming, presumably, from polyglucuronic acids. Mann, Krüger and Tollens<sup>151</sup> repeated the experiment of Cross, Bevan and Beadle but heated the oxidation mixture, but obtained no test for uronic acids. Alkaline permanganate, however, gave an oxidised starch which showed the presence of uronic acid



by furfural evolution and by a colour test. It seems probable that heating of the chromic acid solution caused destruction of the polyglucuronic acids.

Semichon and Flanzky<sup>206</sup> obtained large amounts of formaldehyde. When starch was heated with Hehner's mixture containing 7.4 per cent. potassium dichromate and 75 per cent. sulphuric acid, Lieben and Molnar<sup>144</sup> were able to oxidise starch completely. By that time chromic acid had already been used to determine cellulose quantitatively by complete oxidation. Richardson, Higginbotham and Farrow<sup>193</sup> applied the method to starch successfully. They oxidised the starch completely by boiling in a mixture of sulphuric acid and potassium dichromate, then titrated the excess dichromate with ferrous ammonium sulphate. The amount of starch was calculated from the equivalents of dichromate used for oxidation.

The chromic acid treatment of starch has also been patented.<sup>161, 191</sup>

(d) **Oxidation by Permanganates.**—Langbein<sup>141</sup> found that one gram of starch would reduce 1.5 grams of potassium permanganate in acid solution. By less drastic treatment with permanganate, Lieben<sup>143</sup> obtained a brown product. Very mild oxidation, such as that obtained by Wolff<sup>240</sup> on using 1 per cent. potassium permanganate in the cold, or by Reychler<sup>192</sup> or Nakamura,<sup>160</sup> yielded soluble starches.

Patents have been registered for the use of acidic permanganates on starch.<sup>128, 191</sup>

(e) **Oxidation by Hydrogen Peroxide.**—The action of hydrogen peroxide on starch has attracted more workers than that of any other oxidising agent.<sup>4, 14, 21, 30, 48, 58, 77, 80, 85, 87, 105, 107, 165, 167, 168, 195, 196, 213, 220, 242</sup> Hydrogen peroxide alone, according to Wurster,<sup>242</sup> hardly attacks starch at ordinary temperatures but in boiling acid solution produces dextrins and glucose. Gruzewska<sup>77, 87</sup> identified dextrins, maltose and oxalic acid but no glucose among the oxidation products when the reaction was carried out at 37° C. As the oxidation proceeded a precipitate of retrograded amylose formed, the iodine colour and material precipitable by alcohol disappeared, the reducing power showed a slight decrease, and the acidity increased to a constant value in twice the time required for the iodine colour to reach the achroic-point.<sup>78, 85, 86</sup> Soluble starch behaved like a mixture of amylopectin and amylose, on each of which control oxidations had been run. Neuberg and Miura,<sup>165</sup> contrary to Gruzewska's findings, claimed that 1.3 to 2.7 per cent. glucose was among the products when two equivalents of peroxide were used in the presence of ferrous sulphate. Their

claim was based on the rotation and reducing power of the resulting solution and on the melting-point and nitrogen analysis of the glucosazone. Gerber<sup>79, 80</sup> claimed to have simulated an amyolytic hydrolysis of starch by using very dilute hydrogen peroxide (0.1 per cent. and less). Dextrins and maltose were the products; the latter was oxidised when large amounts of peroxide were used. Omori<sup>167</sup> stated that the action of hydrogen peroxide on starch is governed by a mechanism different from that of diastasis.

Fernbach and Wolff<sup>58</sup> sought accelerators for solubilising starch by means of hydrogen peroxide. They, too, measured the production of acid during the oxidation. Durieux<sup>48</sup> used ferric chloride as an accelerator of the oxidation after finding that colloidal iron had no effect on the rate. Biedermann and Jernakoff<sup>14</sup> obtained considerable catalysis in the peroxide oxidation by using iron or copper salts. Omori<sup>167</sup> claimed that hydroxyphenol was a better catalyst than the heavy metal ions. According to Omori,<sup>168</sup> the combination of iron salt and hydrogen peroxide on starch was made more effective in its modifying power by the addition of cystine, tyrosine, glutathione or thioglycollic acid.

With increasing amounts of hydrogen peroxide for a definite time of oxidation, Durieux<sup>48</sup> found that the reducing power soon reached a maximum value, then fell to zero. The maximum in acidity was passed while the reducing power was decreasing. When this catalysed oxidation was carried out with a constant amount of hydrogen peroxide the acidity reached a constant value after a certain time. Samec<sup>195, 196</sup> noted the large increase in acidity and stated that there were a considerable number of carboxylic acid groups produced in a sample of starch oxidised by hydrogen peroxide. The distillates from peroxide-oxidised starch contained substances which absorbed iodine.<sup>30</sup> Brown<sup>21</sup> obtained formic acid in the distillate. He stated, however, that neither glucose nor maltose was found in the residue, although aldehyde and reducing tests were positive.

Palit and Dhar<sup>174</sup> found that 69.6 per cent. of the original starch was oxidised to carbon dioxide in two hours at 50° C. by hydrogen peroxide and ferric sulphate. In the oxidation of starch at 100° C.<sup>106, 107</sup> hydrogen peroxide was more effective than Aktivin or sodium perborate; at 137° C. Aktivin was the most effective. Patents<sup>31, 35, 104, 114, 222</sup> have been registered for the peroxide oxidation.

(f) **Oxidation by Halogens.**—(i) *Fluorine.*—Carbon was deposited when Moissan<sup>156</sup> allowed fluorine to react with starch.

The carbon burned in the fluorine and produced enough heat to make the decomposition very rapid.

(ii) *Chlorine*.—Many early investigators used chlorine to modify starch. De Gassicourt<sup>37</sup> gives Fourcroy and Parmentier credit for converting starch to sugar by chlorine treatment before Kirchoff<sup>137</sup> discovered the conversion of starch to sugar by means of acid. The product obtained by Fourcroy and Parmentier had both a bitter and a sweet taste. Some time later, however, Liebig<sup>145</sup> reported that starch was very difficultly decomposed by the prolonged action of chlorine, only  $\frac{1}{10}$ th of the starch in solution being decomposed by chlorine gas in eight hours. When Städeler<sup>215</sup> distilled starch with manganese dioxide and hydrochloric acid, carbon dioxide was evolved, chloral, formic acid and an oil were formed. Lieben's<sup>143</sup> product was coloured.

Wolff<sup>240</sup> obtained a slightly modified starch with acid properties. Gerber<sup>81</sup> reported that a very small amount of chlorine added to starch paste before the addition of an amylase accelerated hydrolysis by the enzyme. A larger amount of chlorine inhibited the digestion by amylase. Similarly, in the bleaching of wheat flour by chlorine, enzymatic activity was increased.<sup>3</sup> The amount of chlorine used was very small, 300 to 500 parts per million, but it nevertheless increased acidity and diastatic activity and improved baking qualities. These changes were not encountered to any noticeable extent when nitrogen peroxide, nitrogen trichloride or benzoyl peroxide were used for flour bleaching.

Samec<sup>195, 196</sup> oxidised moist starch with chlorine gas according to Kindscher's patent,<sup>136</sup> but at room-temperature rather than at 100° C. Samec's chlorinated starch was oxidised to a greater extent than the starches oxidised by any of the other agents or methods which he used. The gel-phase of this chlorinated starch had the lowest molecular weight, the highest titratable acidity, the highest hydrogen-ion concentration and the highest conductivity of all his oxidised products.

When chlorine water was allowed to act on amylose ( $\beta$ -amylose), Fletcher and Taylor<sup>61</sup> noted that the *pH* value dropped rapidly from 7 to 1, while the reducing value underwent a sharp rise and the viscosity a simultaneous drop a few hours after the inception of oxidation. The reducing value and viscosity soon reached a constant value. This was not true of the reducing value when a phosphate buffer was used to keep the *pH* value of the medium above 2. In the latter instance the sharp changes of viscosity and reducing value came in two minutes.

Numerous patents have been registered for the modification of starch by chlorine.<sup>8-10, 13, 75, 76, 109, 122, 132, 136, 149, 158, 209, 214, 237</sup>

It is interesting to note in connection with modification of starch by chlorine that Hirst, Plant and Wilkinson<sup>117</sup> used chlorine as a catalyst in acetylation. When amylopectin ( $\alpha$ -amylose) is acetylated by this procedure the acetate obtained varies with the method of preparation, being more soluble and less viscous the more chlorine is used; amylose ( $\beta$ -amylose), however, always yields the same acetylated product.<sup>108</sup> It is possible that the catalyst, chlorine, is oxidising or modifying in some other way the amylopectin before it is acetylated.

(iii) *Bromine*.—Habermann<sup>94, 95</sup> found that treatment of dextrin with five equivalents of bromine per glucose anhydride unit evolved carbon dioxide and bromoform and gave gluconic acid as a product. He prepared the calcium, barium, lead and cadmium salts and ethyl ester of the gluconic acid, the first two of which were crystalline. Further oxidation converted the gluconic acid to bromoform, bromoacetic acid and oxalic acid. Lieben<sup>143</sup> also obtained gluconic acid. Franchimont,<sup>67</sup> in order to exclude oxidation, treated dried potato starch with dry bromine and with bromine in chloroform. He reported the starch to be absolutely unchanged if all moisture was excluded. If moist air was present, hydrobromic acid was formed and acted on the starch. When dry hydrobromic acid and dry bromine in chloroform were added to dry starch, an orange product resulted. Lintner<sup>146</sup> and Syniewski<sup>225</sup> prepared a soluble starch by using bromine. Gerber<sup>81</sup> found that bromine, like chlorine, made starch more susceptible to amylytic hydrolysis if small amounts of bromine were added to starch paste before digestion.

Bergmann and Ludewig<sup>7</sup> shook iodine-potassium iodide solution and bromine-potassium bromide solution with starch and with acetylated starch of 46 to 48 per cent. acetyl-content to determine the affinity of the starch for these halogens. The presence of acetyl groups had very little effect on the adsorption of the halogens. The authors stated that this agreed with the view that bridge oxygen atoms were responsible for halogen adsorption of starch (see p. 132). Whilst starch and starch triacetate absorbed both iodine and potassium iodide from I—KI solution, only the bromine was absorbed from bromine-potassium bromide solution. Because of the short time that the starch was in contact with the bromine (four minutes), Bergmann and Ludewig regarded it as improbable that the bromine adsorption (0.08 equivalents Br/glucose) could be due only to oxidation and substitution, and considered some of the bromine held by adsorption.

Everett and Sheppard<sup>51</sup> measured the optical rotations, various colour reactions, titratable acidities, Sumner reducing values, Folin-Wu reducing values, and the ratio between the last two, of solutions of polysaccharides treated with bromine water. Rotations showed that maltose and maltobionic acid were not intermediates in the hydrolysis and oxidation of starch, soluble starch, dextrin and glycogen. The oxidations resembled those of  $\alpha$ -glycosides. Colour tests and Sumner/Folin-Wu ratios gave evidence of the formation of keturonic acid derivatives of the anhydroglucose polymers. These higher keturonic acids reduced analytical sugar reagents at room-temperature. Only one patent appears to cover the use of bromine.<sup>182</sup>

(iv) *Iodine*.—When the coloured starch-iodine complex was decolorised by heating to 100° C., Personne<sup>179</sup> claimed that some of the iodine was lost by volatilisation and some of the iodine converted the coloured complex partly to a colourless starch-iodine compound and partly to sugar (see p. 129). These last two processes undoubtedly involved hydrolysis and oxidation. Rodewald and Kattein,<sup>184</sup> however, solubilised starch by heating with iodine at 130° C., recovered the starch by precipitation and iodine removal and reported that the product resembled pure starch grains. Andrews and Goettsch<sup>1</sup> were able to restore the original intensity of the starch-iodine colour if the heating to 100° C. and subsequent cooling were carried out quickly. Otherwise the returning colour became progressively weaker, depending on the length of the heating period. The iodine colour vanished after heating for 65 hours with 2.3 equivalents of iodine. Hale<sup>97</sup> claimed that loss of iodine in starch indicator was due to an oxidation of the impurities in the starch. In the titration of arsenite with iodine more iodine was used in the presence of starch than in its absence. The impurities causing this loss of iodine, were soluble dextrans which gave a blue colour with iodine.

The degradation of dextrin and glycogen by heating with iodine at 100° C. until decolorised was investigated by Vintilescu and Faltis.<sup>231</sup> Hydrolysis and oxidation took place, the acidity being greater than that corresponding to the hydriodic acid. Formaldehyde and formic acid could be distilled from the liquid. The residue contained a non-reducing humic oxidation product which contained no combined iodine.

According to Angelescu and Mirescu<sup>2</sup> the decolorisation of the starch-iodine complex on heating (see p. 129) is due to diminution of adsorption, alteration of the degree of dispersion of the starch, and to irreversible changes of the starch or the

iodine. This last would include oxidation of the starch. They further state that the temperature of decolorisation does not correspond to the temperature of recoloration nor is it reproducible a second time with the same solution.

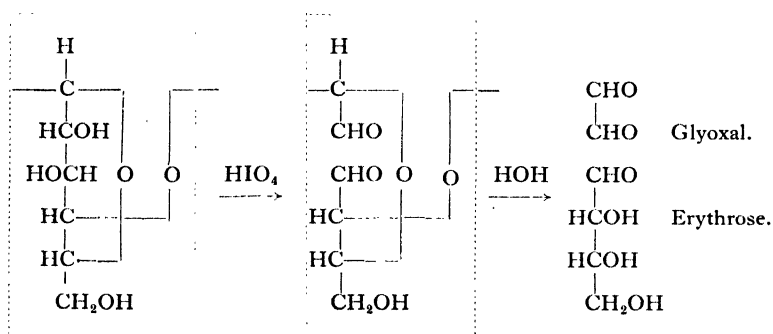
(g) **Oxidation by Oxy-halogen Acids.**—(i) *Hypochlorous Acid.*—Brautigam<sup>18</sup> found that hydrolysis of starch to glucose preceded oxidation by hypochlorous acid. The final products were oxalic acid and carbon dioxide. Craik<sup>32</sup> obtained an inconclusive test for maltose among the soluble products and reported only slight acidity at the end of the reaction. The rotation of the solution increased. On the basis of his experiments with  $\beta$ -glucosan and disaccharides, he postulated an addition of the hypochlorous acid to the ring or bridge oxygen atoms. Fletcher and Taylor's<sup>61</sup> experiments with chlorine water on amylose ( $\beta$ -amylose) have been discussed in the section on oxidation by chlorine. Jambuserwala and Kanitkar<sup>126</sup> used 0.25, 1, 2 and 3 per cent. acidified hypochlorite on corn, wheat, sago and farina starches. They measured the  $R_{Cu}$  reducing value (mg. Cu per gram of starch), the carboxyl-content, the alkali-labile value, viscosity, and a few properties of value in the textile industry, e.g. the adhesive value. With an increasing amount of oxidant the  $R_{Cu}$  curves showed a slight decrease then a rapid increase along a straight line. The carboxyl-content of each starch gradually increased during oxidation, the viscosity and adhesive value showed a sudden, initial drop to a low value with only a small amount of oxidant. Thereafter the decrease of these last two properties was very slight upon further oxidation. Seck<sup>205</sup> patented the oxidation by hypochlorous acid.

(ii) *Chloric Acid.*—Schmerber<sup>201</sup> prepared a soluble starch by using chloric acid prepared from a chlorate and sulphuric acid. Haake and Haake<sup>90</sup> as well as Mirow<sup>154</sup> covered the process in patents.

(iii) *Periodic Acid.*—Periodic acid reacted quantitatively with corn starch, according to Jackson and Hudson,<sup>123</sup> and oxidised the glycol grouping of the non-terminal glucose units to a dialdehyde structure. One mole of oxidant reacted per  $C_6H_{10}O_5$  unit to split each anhydrohexose unit between the second and third carbon atoms into a two- and a four-carbon fragment. Hydrolysis of the oxidised starch would be expected to yield glyoxal from the two-carbon fragments and *d*-erythrose from the four-carbon fragment (see p. 190).

After hydrolysis Jackson and Hudson observed that the rotation was near the  $[\alpha]_D$  of  $-14.5^\circ$  reported for *d*-erythrose. Caldwell and Hixon<sup>28</sup> isolated the glyoxal as the phenylosazone and as

the benzylphenylosazone. Further studies of this reaction, Malaprade's reaction,<sup>150</sup> on a series of dextrin fractions were employed by Caldwell and Hixon to determine the molecular size of the dextrans. The quantity of formaldehyde liberated from the aldehydic end of the dextrin chain gave values for the chain-lengths which correlated with the values determined by reducing value. These dextrans had chain-lengths between 25 and 134 glucose units, indicating a much higher value for starch than the 25 to 30 units proposed by Haworth, Hirst and Woolgar.<sup>113</sup>



Jackson and Hudson<sup>124</sup> later isolated the same two derivatives of glyoxal from the hydrolysis products of oxidised starch. They were further successful in identifying *d*-erythrose in the hydrolysate by bromine oxidation of the solution to give *d*-erythronic acid. The erythronic acid was characterised as the brucine salt and as the *d*-erythronic lactone. Jackson and Hudson stated that the presence of other types of units in the oxidised starch in minor quantities was not excluded. Another product was, indeed, soon discovered. Grangaard, Michell and Purves<sup>84</sup> isolated a crystalline compound in yields of 0.7 to 0.9 per cent. after they had hydrolysed the oxidised starch in methyl alcohol containing hydrogen chloride. This crystalline substance had the formula, C<sub>13</sub>H<sub>16</sub>O<sub>8</sub>(OCH<sub>3</sub>)<sub>4</sub>, was stable to further oxidation by periodic acid or Fehling's solution. However, after mild acid hydrolysis, it reduced Fehling's solution, pointing to a methyl acetal of an aldehyde or ketone. Corn, wheat, potato, arrowroot, and soluble potato starches all yielded the same product.

(h) **Oxidation by other Per-compounds.**—In his studies on soluble starches produced by oxidation Samec<sup>195, 196</sup> included sodium perborate and ammonium persulphate among the oxidants. The same measurements were made on these oxidised starches as were reported above in discussing Samec's work on

starches oxidised by hydrogen peroxide or chlorine. Neither ammonium persulphate nor sodium perborate gave an extensively oxidised product. There was a slight increase in acidity over that calculated from the phosphoric-acid content, but, in general, these two oxidants gave less modification than the other five oxidants, excluding air, which Samec used. Nakamura<sup>160</sup> likewise found about the same order of oxidising power, since the phosphorus-content was decreased more by ammonium persulphate than by sodium perborate and more by potassium permanganate than by either of the other two agents. Sodium perborate and ammonium persulphate have been used to produce slight physical modification of starch granules.<sup>105</sup>

Various patents describe the use of persulphates and perborates.<sup>20, 29, 62, 63, 70, 71, 99, 104, 114, 118, 128, 133, 140, 203, 211, 217-219</sup>

(i) **Oxidation by Oxides in Acid Solution.**—Döbereiner<sup>42</sup> distilled a solution of starch in sulphuric acid and manganese dioxide with the result that carbon dioxide was evolved and formic acid was found in the distillate.

According to Schmidt and Graumann<sup>202</sup> chlorine dioxide, an agent for removing incrustations from plant skeletal substances, did not attack starch in 24 hours. Practically none of the chlorine dioxide was used in this time. Samec and Ulm<sup>199</sup> concurred in the above opinion to the extent that the microscopic appearance of potato and wheat starches was unchanged, but found chemical changes in the treated starches. Chlorine dioxide removed amounts of phosphorus from wheat and potato starches which varied with the length of treatment, 0.2 N chlorine dioxide on wheat starch for eight days removing 84 per cent. of the phosphorus. When the starches were treated with chlorine dioxide and pyridine instead of chlorine dioxide alone, noticeable acidity was developed beyond that demanded by the phosphoric-acid content, and from this Samec and Ulm concluded that oxidation had occurred.

(j) **Oxidation by Irradiation**—Massol<sup>152</sup> and Bielecki and Wurmser<sup>15</sup> stated that irradiation of soluble starch solutions for ten days at 45° C. gave splitting and oxidation. The products were dextrans, pentoses, glucose, formaldehyde and acidic substances. Both the conductivity and hydrogen-ion concentration rose to constant values in 100 hours. Ono<sup>169</sup> disintegrated starch paste by irradiation with ultrasonic waves and reported that oxidation may have been responsible for a small amount of depolymerisation.

(k) **Oxidation by Air in Acid Solution.**—This method was patented.<sup>26</sup>



(1) **Oxidation by Ozone.**—Gorup-Besanez<sup>83</sup> reported that potato starch in water was indifferent to ozone, showing no change in several days. König<sup>138, 139</sup> bleached and deodorised amylaceous materials by passing ozone through solutions of starch or dextrin until the desired effect was obtained. Siemens and Halske<sup>208</sup> patented the preparation of an 'ozone starch' by double treatment with chlorine and ozone. According to Schaeffer and Scheurer<sup>200</sup> the Siemens and Halske products varied in iodine colour from blue to violet and from insolubility in cold water to complete solubility, the latter giving strong reduction of Fehling's solution. Pieper's patent<sup>181</sup> used ozone to prepare clear dextrans and gums free from odour and repulsive taste. The ozone was passed over the starch during roasting. Friedenthal<sup>69</sup> mentioned that a commercial soluble starch, known as 'ozone starch,' had a molecular weight of 9450. Löb<sup>147</sup> passed a silent electric discharge through 1 per cent. solutions of starch, noted the formation of ozone, the gradual disappearance of the starch-iodine colour, the reduction of Fehling's solution, the formation of osazones, but no true criterion of oxidation.

## 2. OXIDATION OF STARCH IN ALKALINE MEDIA

In alkaline media the various enolisation reactions of Nef<sup>164</sup> can occur previous and concurrent to oxidation. Evans<sup>27</sup> and co-workers have studied the mechanism of the oxidation of sugars in alkali and have found evidence for enolisation. The action of alkaline oxidising solutions would, therefore, be expected to produce oxidised forms of hydrolysed and enolised starch. Theoretically, no hydrolysis should occur, but there exists evidence to the contrary, viz. the increase in reducing power of starch upon heating in alkali.

(a) **Oxidation by Hypochalites.**—(i) *Alkaline Hypochlorite*—Schmerber<sup>201</sup> and Dollfus and Scheurer<sup>43</sup> solubilised starch with bleaching powder. Haller<sup>103</sup> noted that the temperature rise depended on the amount of sodium hypochlorite he used on potato starch. In the manufacture of oxidised starches by the wet process the rise in temperature must be strictly controlled, otherwise, as pointed out by Radley, the final properties of the product will differ from those desired. Trotman<sup>229</sup> reported an oxidation procedure for the modification of starches for sizing of cotton which used hypochlorite. Rassow<sup>188</sup> also measured the temperature rise as well as the decrease in viscosity and the increase in reducing power. Lobenstein's<sup>148</sup> thesis

concerned the preparation of soluble starch by means of alkaline sodium hypochlorite.

The last two authors published a comprehensive treatise in collaboration,<sup>189</sup> in which they traced the temperature-rise during oxidation partly to heat of adsorption, partly to heat of decomposition of the sodium hypochlorite, and partly to heat of oxidation. The extent of the temperature-rise depended on the amount of the hypochlorite solution. Completely dry starch gave an even more vigorous reaction, probably due to heat of hydration or heat of swelling. R. and W. Haake<sup>93</sup> made use of this fact to treat starch with 10 per cent. sodium hypochlorite (20-28° Bé.), or other highly concentrated oxidising liquid, when the heat of reaction served to evaporate the moisture and left a nearly dry product. When large amounts of hypochlorite were used<sup>189</sup> in 0.19 N alkali, the solutions soon became acidic and reduced Fehling's solution in the cold. The reduction increased with the amount of hypochlorite used, while the viscosity decreased. The viscosities of all oxidised starches were considerably less than that of native starch. Barium starches were prepared by adding saturated barium hydroxide to the oxidised starches, and precipitating the salt with alcohol. Barium analyses of three fractions showed one barium atom per two, four and six glucose units, respectively. No sharp differentiations between the abilities of oxidised and native starch to be stained by dyes were observed, although many acidic, basic and direct dyes were tried. When a 5 per cent. paste of one of these oxidised starches was aged, the viscosity dropped from 184 to 40 seconds in less than ten days. On long standing the oxidised starch powders increased their titratable acidity considerably during the first three months and reached a constant acidity only after twelve to eighteen months.

Samec and Blinc<sup>197</sup> allowed alkaline hypochlorite to act until a test sample of the starch went into solution at 95° C. The microscopic appearance, including the shape, and birefringence was very little changed although some granules showed cracks and stratification (see Photomicrograph 14, p. 377). The specific rotation was 198°, the iodine colour blue to violet, the molecular weight by osmotic-pressure method somewhat lower than that of native starch, but the viscosity at 55° C. was greatly increased (by 64 per cent.). Although there was a decrease in conductivity in the solution and in the dialysed sol of the oxidised starch from that of native corn starch, the purified gel (by dialysis) showed an eight-fold increase in conductivity on oxidation. Oxidation had increased the hydrogen-ion concentration of the sol from  $0.8 \times 10^{-5}$

to  $51 \times 10^{-5}$ . The reducing power of the sol was high, the rotation lower than that from native starch. Samec and Blinc explained the increase in viscosity in spite of the decrease in particle size as being due to the production of hydrophilic groups which augmented hydration. Because a decrease in molecular size accompanies oxidation of starch, the authors tended to believe that glucosidic bonds were broken which liberated aldehydic groups for oxidation to carboxylic groups.

Fletcher and Taylor<sup>61</sup> treated corn  $\beta$ -amylose with alkaline hypochlorite at pH 10 and 13. The viscosity remained practically constant for thirty days but the alkali-labile value and initial reducing value fell practically to zero. Fletcher and Taylor likewise surmised that the reaction was primarily an oxidation of aldehydic groups at the end of the chains, but that some disintegration of the carbohydrate accompanied this change.

Ammonium hypochlorite was used by Jambuserwala and Kanitkar<sup>127</sup> to oxidise corn starch. The milliequivalents of COOH, the  $R_{Cu}$  values and the alkali-labile values were directly proportional to the amount of available chlorine used (1, 2 and 3 per cent.). The copper numbers of these alkaline hypochlorite treated starches were smaller than those of the starches oxidised in neutral solutions (actually acidic: pH 5.2 to 6.8) by the same authors.<sup>126</sup> This confirmed an earlier conclusion that the copper numbers of cellulose increased with decreasing alkalinity of the hypochlorite solution.

Some of the patents on the modification of starch by alkaline hypochlorite are included in the bibliography.<sup>16, 25, 38, 40, 46, 73, 74, 82, 89-93, 114, 116, 120, 133, 157, 163, 190, 204, 214, 222, 230, 233</sup> This is one of the most common agents used in the commercial oxidation of starch,<sup>129</sup> producing a wide variety of products depending upon the concentration of the hypochlorite, the temperature of the mixture and the length of time the oxidation is allowed to proceed.<sup>207</sup> According to Radley one of the commercial processes consists in 'suspending the starch in water and adding either sodium or calcium hypochlorite solution from time to time. When, by previous experience, the reaction is judged to be approaching completion, a sample of the suspension is withdrawn, rapidly filtered, and washed on a vacuum filter; it is then slurred with a given amount of water, the suspension neutralised, heated to 80° C., and the viscosity determined. When the desired degree of fluidity has been reached, the process is stopped and the mass thoroughly washed.'

In the surface sizing of paper ordinary starch forms gels which are too viscous. The gels of 'chlorinated' starches, i.e. those

produced by chlorine treatment, have a greater fluidity. The property of high grade tapioca starch paste of 'setting up' to only a limited degree on standing is transferred to other kinds of starch by hypochlorite oxidation. Hypochlorite starches are also finding use in the mill sizing of textile fibres.

Oxidation beyond the thin-boiling stage produces cheap, rapid drying and efficient adhesives.<sup>47</sup> These adhesives may contain as little as 8 per cent. or as much as 50 per cent. starch (see p. 278).

(ii) *Alkaline Hypobromite*.—When De Chalmot<sup>36</sup> oxidised starch with two equivalents of bromine per glucose unit in the presence of sodium carbonate, part of the starch was liquefied. The starch mixture reduced Fehling's solution in the cold and gave a small amount of a phenylosazone. De Chalmot concluded that oxidation had taken place because neither bromine nor sodium carbonate alone would act on starch perceptibly in a week. His proposed mechanism for the oxidation included conversion of the primary alcohol groups (6th carbon atom) through aldehydic to carboxyl groups. The carboxyl groups were then eliminated as carbon dioxide without affecting the rest of the hexose unit.

Hönig and Ruziczka<sup>119</sup> used  $\frac{1}{3}$  and  $\frac{1}{2}$  equivalent of bromine per glucose unit and obtained maltobionic acid as the calcium and brucine salts. The solution was kept alkaline with barium hydroxide.

One patent<sup>180</sup> was secured on the bromine oxidation process.

(iii) *Alkaline Hypoiodite*.—Myrbäck<sup>218</sup> oxidised the reducing end-groups of starch with 0.054 equivalent of iodine per glucose unit. The oxidised starch gave the same yield of maltose by  $\alpha$ - or  $\beta$ -amylase as raw starch.

(b) **Oxidation by Alkaline Chlorite**.—Liebig<sup>145</sup> noted that starch was attacked only with difficulty by salts of chlorous acid. The textile industry<sup>46</sup> has found an application for sodium chlorite in the kierung operation to remove starch in strong alkali and at a high temperature.

(c) **Oxidation by Alkaline Aktivin**.—Feibelmann,<sup>53-56</sup> Frère,<sup>68</sup> Haller,<sup>98, 102, 106, 107</sup> Ekhard,<sup>50</sup> Trotman<sup>229</sup> and Walker<sup>234</sup> reported on the production of soluble starch by means of 'Aktivin,' sodium-*p*-toluenesulphochloroamide,  $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_2\text{N}(\text{Cl})\text{Na}$ . This compound liberates chlorine at a definite rate when heated with water. By heating with Aktivin and water the starch is gelatinised and thinned. About 1 per cent of Aktivin, which amounts to 0.006 equivalent of chlorine, is used to produce soluble starch. Copper salts catalyse the

oxidation and bleaching. The efficiency of Aktivin<sup>56</sup> in comparison with calcium hypochlorite and perborates is claimed to be in the ratio of 2.4 : 1.3 : 1.0. Haller, Hackl and Frankfurt<sup>106, 107</sup> titrated several oxidised starches with iodine and alkali to determine the extent of oxidation. The starch from Aktivin oxidation at 137° C. consumed more iodine than that from hydrogen peroxide or sodium perborate. Hydrogen peroxide was the most effective of the three at 100° C.

Several patents were registered for the Aktivin oxidation,<sup>99-101, 114, 190, 204</sup>

(d) **Oxidation by Alkaline Permanganates.**—Lieben<sup>143</sup> obtained a brown product by permanganate oxidation. Lintner<sup>146</sup> used increasing amounts (1.5 to 6 equivalents) of potassium permanganate on soluble starch at 60-70° C. to obtain gummy products whose iodine colours ranged from blue through red to brown, or colourless. The optical rotatory power decreased in parallel fashion with the iodine colour; a violet iodine colour corresponded to  $[\alpha]_D$  of 182.4° to 170°, a red-brown colour to 153.1°, while rotations of the achroic solutions were 154.3° to 128.4°. The respective amounts of permanganate at each of these three stages were 1.5, 4 and 6 equivalents. Only a small reduction of Fehling's solution and negative colour reactions with phloroglucinol or orcinol were given. All of the gums were acidic, which led Lintner to call them 'dextrinic acids.' With lead acetate or barium hydroxide, solutions of these dextrinic acids gave a precipitate. Schmerber<sup>201</sup> reported a decrease in viscosity and increase in transparency upon oxidation of starch with 0.015 equivalent of potassium permanganate. Mann, Krüger and Tollens,<sup>151</sup> on distillation of an oxidised starch with 12 per cent. hydrochloric acid, reported that considerable furfural was collected. This oxidised starch had been prepared with ten equivalents of permanganate on starch paste for one half hour. The product also gave the naphthoresorcinol test for uronic acid. Syniewski<sup>225</sup> isolated a crystalline osazone melting at 195° from starch oxidised by permanganate.

Dextrin treated with a large amount of calcium permanganate in the presence of ammonia yielded 0.57 per cent. urea at room-temperature and 2.85 per cent. at 95° C., according to Fosse.<sup>64</sup> The addition of cupric carbonate increased the yield to 2.57 per cent. urea from dextrin and gave 2.0 per cent. urea from starch. Heating the last two mixtures at 95° C. increased the yields to 21.85 and 24.28 per cent. respectively. The urea was isolated as the xanthidrol derivative. Later experiments by Fosse<sup>65</sup> measured the yield of hydrocyanic acid from carbohydrates treated with

a solution of calcium permanganate, ammonia and silver nitrate. Sugars, dextrin and starch gave about 1 per cent. hydrocyanic acid. The addition of cupric carbonate increased the yield from dextrin and starch only to 1.6 per cent.

Samec<sup>195, 196</sup> observed an increased conductivity and an increased acidity of the dialysed gel-phase of a permanganate oxidised starch. The equivalents of titratable acidity of this gel-phase were greatly in excess of the gram atoms of phosphorus. Samec interpreted this to mean that carboxyl groups had been added by oxidation. The acidity was due in part to phosphoric acid groups and in part to carboxyl groups. For the dialysed sol-phase of this product, however, the gram atoms of phosphorus and the equivalents of titratable acidity were practically identical.

Nakamura<sup>160</sup> found that potassium permanganate decreased the phosphorus-content of starch to a greater extent than did ammonium persulphate or sodium perborate. The viscosity was decreased over that of the original starch and the products were more readily acetylated. Permanganate was also used by Haller<sup>105</sup> for very mild modification.

Randall, Benger and Grocock<sup>186</sup> oxidised numerous organic substances with alkaline permanganate. The ease of oxidation of some carbohydrates diminished in the order: glucose, maltose, starch, methyl glucoside, cellulose. These authors measured the time required for complete oxidation at reflux temperatures and the percentage of the carbon appearing as carbon dioxide, oxalic acid and acetic acid. For starch the time was three hours and the respective percentages were 66.0, 24.1 and 7.5. Symons and Buswell<sup>223</sup> found that the biochemical oxygen demand (BOD) of starch under the conditions of the common test was 79 to 88 per cent. of the theoretical.

The patents on permanganate oxidation have been listed.<sup>19, 34, 131, 161, 181, 203, 210</sup>

(e) **Oxidation by Alkaline Peroxides.**—At ordinary temperatures alkaline peroxide hardly attacks starch, according to Wurster,<sup>242</sup> but does modify starch in boiling solution to dextrin and glucose. Von Asboth<sup>232</sup> added hydrogen peroxide, made alkaline with ammonia, to a boiling starch paste. On concentration of the solution a precipitate of 'starch cellulose' formed. The filtrate, on treatment with several volumes of alcohol, gave as a precipitate a tough substance which constituted 80 per cent. of the original starch. This precipitate gave a blue-violet iodine colour, was non-reducing, had an optical rotation of 178°. On standing, the alcoholic filtrate gave a further precipitate constituting 15 per cent. yield which was non-reducing,

gave a violet-red iodine colour and an optical rotatory power of  $170^\circ$ . Hot concentrated barium hydroxide gave a fourth precipitate, not coloured by iodine, reducing silver solutions but not Fehling's solution and analysing to be  $C_5H_8O_4 \cdot H_2O$ . This loss of one carbon atom from the hexose units agreed with the observed evolution of carbon dioxide. The filtrate from these four precipitates still contained some sugars.

A cold solution of sodium peroxide gave in one hour none of the above drastic changes, when Syniewski<sup>224</sup> used it for oxidation. Although the product was soluble to 12.5 per cent. in cold water and completely soluble in hot water, it nevertheless gave a blue iodine colour, no reduction of Fehling's solution, showed retrogradation on standing, and had a rotation of  $183^\circ$  to  $189^\circ$ , depending on the concentration of the starch in solution. The precipitate from barium hydroxide treatment contained one barium atom per three glucose units.

Another preparation<sup>225</sup> gave the same barium analysis but higher rotatory power,  $195^\circ$ . Acetyl and benzoyl derivatives corresponded to seven free hydroxyl groups for every three glucose units. Molecular weights, according to Raoult's method, were 773 and 880 for the acetyl and benzoyl derivatives respectively, somewhat lower than the 798 and 1148 calculated for hepta-derivatives of a trisaccharide. Syniewski's third paper<sup>226</sup> stated that sodium peroxide did not oxidise the starch but merely solubilised it. A molecular weight determination of the soluble starch corresponded to a chain of nine glucose units.

Samec and Jencic<sup>198</sup> oxidised starch according to Syniewski's method, using a time of two hours instead of one. The product was easily soluble in water, showed some reducing power and a lowered viscosity. Later, Samec<sup>195, 196</sup> extended the time of oxidation to six hours but still obtained only mild oxidation as evidenced by the acidity, iodine colour, viscosity and osmometric molecular weight.

Radley<sup>186</sup> mentions that adhesives may be made with hydrogen peroxide (see p. 278). Two patents use barium peroxide for oxidation;<sup>111, 182</sup> other peroxides are also used in patents.<sup>39, 82, 90, 178</sup>

Corn Products Refining Co.<sup>316</sup> treat aqueous starch suspensions with calcium peroxide at  $46-52^\circ C$ . for 24-48 hours, neutralise with hydrochloric acid, filter and dry. The product has a Scott test index of 65-35 and a Stormer test index of 560-60 and forms a gel having more body than acid-treated thin-boiling starch of equivalent hot paste viscosity.

(f) **Oxidation by Air in Alkaline Solution.**—Dhar and co-workers<sup>41, 155, 171-175</sup> published a series of papers on the

induced oxidation of carbohydrates by air. Air was passed through alkaline solutions containing carbohydrates and an oxidisable substance such as sodium sulphite, ferrous hydroxide or cerous hydroxide. The last-named substance underwent oxidation at room-temperature and induced the oxidation of the carbohydrate. The carbon dioxide produced from the carbohydrate was liberated after oxidation and absorbed in calcium hydroxide solution for measurement. The amount of oxidation in a given time increased with the alkaline concentration of the solution except when sodium sulphite was the inductor. One-tenth of a gram of starch in 100 ml. of solution containing cerous hydroxide or ferrous hydroxide was completely oxidised by 36.5 litres of air in 5.5 hours when the concentration of sodium hydroxide was 0.5 to 0.7 per cent. Starch was found to be more easily oxidised than any of the several sugars used. This order does not agree with that determined by permanganate.<sup>186</sup> Sodium sulphite induced the oxidation of 26 per cent. of the starch even when no alkali had been added to the solution, but did not give complete oxidation at any alkaline concentration up to 0.7 per cent. When air was passed through an alkaline starch solution containing no inductor, 23 to 35 per cent. of the starch was oxidised at different concentrations of sodium hydroxide. When the concentration of sodium hydroxide was kept constant 31.7 per cent. of the starch was oxidised in 5.5 hours, while 45.3 per cent. was oxidised in 9 hours. But when sodium bicarbonate was substituted for the sodium hydroxide, the values were 35.9 and 31.9 per cent. respectively, showing a decrease on longer oxidation.

In the presence of sodium bicarbonate or various inductors<sup>174</sup> glycogen showed the following percentage oxidation: sodium bicarbonate present, 12.0 per cent.; sodium sulphite present, 21.8 per cent.; ferrous hydroxide, 64.5 per cent.; cerous hydroxide, 87.5 per cent. However, in the presence of ferric hydroxide, which is not a reducing agent, there was 67.9 per cent. oxidation, more than in the presence of ferrous hydroxide. Also, surprising is the fact that cupric hydroxide gave a large amount of oxidation, 69.8 per cent.

Further experiments by Dhar and associates<sup>173, 175</sup> studied the effect of sunlight on the oxidation of carbohydrates by air in neutral solution. 36.5 litres of air passed through starch solution in 5.5 hours oxidised 35.6 per cent. of the starch in tropical sunlight, 38.8 per cent. in sunlight of greater intensity, 66.9 per cent. in the latter case if zinc oxide (a photosensitiser) was present, 92.9 per cent. if the photocatalyst was ferric nitrate, and 100 per cent. if the photocatalyst was uranium nitrate. The



respective values for glycogen oxidation were 15.9, 19.7, 38.7, 82.3 and 71.0 per cent. In the photochemical oxidations, as in the induced oxidations, starch was oxidised more easily than any of the sugars or glycogen.

Samec<sup>195, 196</sup> passed air for three days through a 15 per cent. starch suspension which was 0.05 N in sodium hydroxide and obtained very little evidence of oxidation. The osmometric molecular weight was high, the iodine colour blue to lilac, the titratable acidity and the percentage of dialysable substance very low.

In the production of dextrans by dry heating in air at 200° C., Katz and Weidinger<sup>130</sup> found a measurable acidity due to weak oxidation. The amount of acidity developed was always less than 0.01 equivalent per glucose unit. In an atmosphere of nitrogen no acidity was developed.

Starch was oxidised by Baudisch and Deuel<sup>5</sup> for 48 hours with sodium pentacyanoaquaferroate and oxygen but failed to give any acetol.

The oxidation of starch by air has likewise been patented.<sup>170</sup>

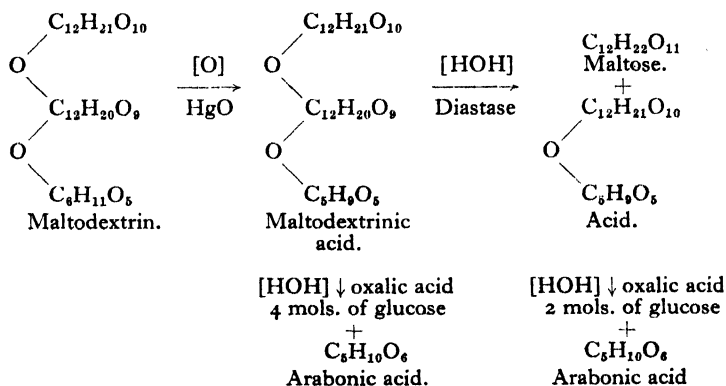
(g) **Electrolytic Oxidation.**—Leconte<sup>142</sup> used electrolysis as one step in the preparation of a very white grade of rice starch. An alkaline suspension of the starch was electrolysed between aluminium, zinc or other metallic electrodes. Harvey<sup>110</sup> and White<sup>238</sup> patented a process for the Perkins Glue Company which was designed to convert starch to a glue. An aqueous suspension of the starch was made, conducting by the addition of hydrochloric or sulphuric acid, a salt or alkali. Iron or carbon electrodes were used with a direct current of 3.5 amperes at 110 volts. The electrolysis was stopped before the starch reached the soluble stage. It is apparent that some oxidation occurred, due to the products of electrolysis of the conducting substances. Siemens and Halske<sup>209a</sup> and F. Hermite<sup>115a</sup> used similar methods with sodium or magnesium chloride as the electrolyte.

Fink and Summers<sup>60</sup> attempted to electrolyse starch between graphite electrodes in 2 per cent. potassium bromide solution at 40° C. kept neutral by means of calcium carbonate. They reported no reaction, stating that electrolytic oxidation in the bromide bath seemed specific for aldoses.

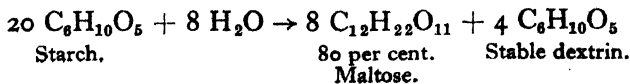
(h) **Oxidation by Alkaline Mercuric Oxide.**—Brown and Millar<sup>22</sup> oxidised a maltodextrin (of about five or six glucose units) by heating at 100° C. with mercuric oxide in barium hydroxide solution. Under these conditions oxidation proceeds

further than the conversion of the aldehydic group of the terminal glucose unit to a carboxyl group. Malt amylase ( $\alpha$ - and  $\beta$ -amylase) hydrolysed the oxidised maltodextrinic acid to maltose and a smaller acid. The original maltodextrin was only slowly attacked by  $\alpha$ -amylase, indicating that  $\beta$ -amylase splits maltose units off the non-aldehydic end of the starch chain.

During the oxidation, after three atoms of oxygen were consumed per mol. of maltodextrin, the reduction of Fehling's solution ceased. The barium salt at this point gave chiefly an acidic fraction of  $[\alpha]_D$  189-192°. Analysis of the calcium salt corresponded to the formula  $(C_{25}H_{44}O_{26})_2Ca$ . Diastatic hydrolysis of the maltodextrinic acid gave maltose and a smaller acid whose calcium salt analysed as  $(C_{17}H_{29}O_{16})_2Ca$ . Hydrolysis of these two calcium salts by oxalic acid gave 85.55 and 67.7 per cent. glucose, respectively, and an acid whose calcium salt analysed as  $(C_5H_9O_6)_2Ca$ , presumably calcium arabonate. These data fit the following scheme of oxidation and hydrolysis :



Brown and Millar <sup>24</sup> prepared another dextrin from hydrolysis of starch by malt extract to 80 per cent. maltose, and also oxidised this dextrin with mercuric oxide in barium hydroxide solution. The resulting acid was converted to the calcium salt, whose analysis and hydrolysis to glucose and arabonic acid suggested this formula for the acid:  $(C_6H_{10}O_5)_{39}-C_5H_9O_5$ . This meant that the original dextrin contained 40 glucose units, a value which agreed exactly with the reducing power of the dextrin. The usual equation for malt digestion at that time (1899) was :



On the basis of their findings Brown and Millar proposed a new equation :



They stated further that the molecular weight of starch could not be less than five times that of the stable dextrin. Since the molecular weight of the dextrin was 6498, starch would have a minimum molecular weight of 32,400, or possess 200 glucose units. The molecular weight of this same dextrin, determined cryoscopically, was 6221 and the molecular weight of a soluble starch by the same procedure was between 20,000 and 30,000. Brown and Millar then proposed a ring structure for starch.

(i) **Oxidation by Alkaline Persulphates.**—Persulphate made alkaline by ammonia modifies starch for cotton sizing according to Trotman.<sup>229</sup> 0.036 equivalent was used. Supf<sup>221</sup> has a patent on alkaline persulphate oxidation.

### 3. OXIDATION OF STARCH IN NEUTRAL MEDIA

In a neutral solution acid or alkaline hydrolysis and enolisation would be expected to be eliminated. Oxidation in neutral solution, then, should not be complicated by these other reactions, and the study of its mechanism should be simplified.

(a) **Oxidation by Bromine.**—Franchimont<sup>67</sup> found no reaction when dry bromine and dry starch were brought together in chloroform. Syniewski<sup>227, 228</sup> oxidised amylo-dextrin by means of bromine in the presence of barium carbonate. The amylo-dextrin had been prepared by autoclaving starch paste for 12 hours at 125-138° C. When the iodine colour disappeared, the oxidation was stopped by removal of the excess bromine. The barium salt was precipitated with alcohol, had  $[\alpha]_D = 191^\circ$ , reduced Fehling's solution in the cold to the equivalent of 23 per cent. maltose, gave a red solution with hydroxylamine and an orange-red precipitate with phenylhydrazine. A naphtho-resorcinol test indicated glucuronic or malturonic acid units. Distillation of the amylo-dextrinic acid in 12 per cent. hydrochloric acid gave furfural equivalent to 33.9 and 34.5 per cent. glucuronic anhydride. This was in agreement with the assumed formula for amylo-dextrinic acid which contained 24 glucose units and 12 carboxyl groups. Syniewski assumed that the carboxyl groups were formed from the sixth carbon atom of the glucose units by the oxidation of primary alcohol groups. His naphtho-resorcinol test lends support to this mechanism but might also indicate keturonic acids or aldehydic acids, as he

stated. Syniewski concluded that no keto or aldehydic acids were present in this case. It is, indeed, improbable that any aldehydic groups would remain unoxidised in the presence of bromine. On the other hand, there is some evidence that Syniewski's amyloextrinic acid contained keto groups, viz. the reduction of Fehling's solution in the cold, the coloured compound with hydroxylamine, the precipitate with phenylhydrazine, and the reducing power of 23 per cent. maltose equivalent.

Felton, Farley and Hixon<sup>57</sup> oxidised starch paste with increasing amounts of bromine in the presence of calcium carbonate and recovered the calcium salts of the oxidised starch. Uronic acid determinations (by carbon dioxide evolution) showed that the oxidation of primary alcohol groups to uronic carboxyl groups passed through a maximum of 50.7 per cent. glucuronic anhydride when six equivalents of bromine were used per glucose anhydride unit. The calcium content of the oxidised starch underwent a steady increase proportional to the amount of bromine used. By means of the curve for uronic-acid content the authors were able to calculate the amount of calcium necessary for the uronic carboxyl groups. When these values of uronic acid calcium were subtracted from the values for total calcium, the difference would indicate the calcium values of the non-uronic carboxyl groups. These latter could result from the oxidation of terminal aldehydic groups or from the oxidative splitting of hexose units at the site of a ketone group. Some indication that oxidative splitting of ketone groups was the chief source of non-uronic acids came from the reducing power of the products, a measure of the production of ketone groups from secondary alcohol groups. The reducing power of the oxidised starches passed through a maximum at two equivalents of bromine, then decreased proportional to the amount of bromine used. Just beyond this maximum, at three equivalents of bromine, the non-uronic acid groups began to appear and were produced in proportion to the amount of bromine used. The logical assumption was that most of the non-uronic carboxyl groups resulted from oxidative decomposition of the reducing units.

Felton, Farley and Hixon also oxidised, by the same procedure, amylose ( $\beta$ -amylose) and amylopectin ( $\alpha$ -amylose) prepared by electro-dialysis from corn-starch paste. Practically the same product was obtained from each fraction, although the oxidation of the amylose required one-fourth as much time as that of the amylopectin.

Fink and Summers<sup>60</sup> electrolysed starch in a solution containing potassium bromide and calcium carbonate but observed no reaction.

Kihara<sup>134</sup> also oxidised starch paste with bromine and calcium carbonate. His observations were that the oxidised starch was precipitated by calcium or barium hydroxides but not by copper sulphate or Fehling's solution, and that takadiastase scarcely hydrolysed the product. Kihara prepared an acetyl derivative which melted at 145° C.

(b) **Oxidation by Iodine.**—When Andrews and Goettsch<sup>1</sup> heated starch at 100° C. with 2.3 equivalents of iodine in the presence of calcium carbonate, 110 hours were required to reach the achroic-point. This same stage was reached in 65 hours in the absence of calcium carbonate.

The action of acids on starch can be regulated to give products which give mobile solutions with water (see pp. 271-273). Both oxidising agents and acids yield products which give films of lower tensile strength than the untreated starch, but these products possess certain advantages, which are described in the chapters on Paper and Textiles, which more than offset this defect. The oxidised starches are invariably whiter than those produced by the action of acid, maize starch being preferred by most manufacturers for the production of oxidised starches, whilst potato starch is generally used for the production of soluble starches by the acid process.

TABLE 5  
SUMMARY OF THE PRODUCTS FROM THE OXIDATION OF STARCH

<i>Product.</i>	<i>Reference No.</i>
Acetic acid . . .	186.
Arabonic acid (?) . . .	22.
Bromoacetic acid . . .	94.
Bromoform . . .	94, 95.
Chloral . . .	215.
<i>d</i> -Erythronic acid . . .	124.
<i>d</i> -Erythrose . . .	123.
Formaldehyde . . .	15, 28, 206, 231.
Formic acid . . .	21, 28, 42, 58, 215, 231.
Furfural (by distillation)	33, 228.
Gluconic acid . . .	31, 94, 95, 119, 143.
Glyoxal . . .	28, 124.
Hydrocyanic acid . . .	65.
Hydroxypyruvic acid . . .	12.
Maltobionic acid . . .	119.
Nitrostarch . . .	11, 12, 23, 121, 143, 177, 180, 225, 239.
Oxalic acid . . .	18, 31, 66, 72, 77, 78, 94, 96, 143, 176, 180, 186, 187.
Polyglucuronic acid . . .	57, 134, 227, 228.
Polyketuronic acid . . .	51, 57.
Saccharic acid . . .	31, 72, 96, 135, 180, 212.
Tartaric acid . . .	88, 115, 162, 166, 180, 187, 216.
Urea . . .	64.

## REFERENCES

1. L. W. ANDREWS and H. M. GOETTSCH, *J. Am. Chem. Soc.*, 1902, **24**, 865.
2. E. ANGELESCU and J. MIRESCU, *Bul. soc. chim. România*, 1930, **11**, 81; *Chem. Abstr.*, 1930, **24**, 2913.
3. C. H. BAILEY, 'Chemistry of Wheat Flour,' pp. 212, 220 *et seq.* Chemical Catalog Co., New York, 1925.
4. M. BAMBERGER and J. NUSSBAUM, *Monatsh. Chem.*, 1919, **40**, 411.
5. O. BAUDISCH and H. J. DEUEL, *J. Am. Chem. Soc.*, 1922, **44**, 1585.
6. A. BÉCHAMP, *Compt. rend.*, 1854, **39**, 653.
7. M. BERGMANN and S. LUDEWIG, *Ber.*, 1924, **57**, 691.
8. C. BERGQUIST, E.P. 294,979, 1928; *Chem. Abstr.*, 1929, **23**, 2065.
9. — U.S.P. 1,851,749, 1932.
10. — U.S.P. 1,871,027, 1932.
11. E. BERL and R. BÜTLER, *J. Soc. Chem. Ind.*, 1910, **29**, 373T.
12. BERL and SMITH, *ibid.*, 1908, **27**, 534T.
13. BERNARDI, Ital. P. 126,803, 1913; *Chem. Abstr.*, 1915, **9**, 2160.
14. BIEDERMANN and JERNAKOFF, *Biochem. Zeit.*, 1924, **149**, 309.
15. J. BIELECKI and R. WURMSER, *ibid.*, 1912, **43**, 154.
16. BOCHSKANDL, U.S.P. 2,070,576, 1937.
17. H. BRACONNOT, *Ann. chim. phys.*, 1833, (2) **52**, 290.
18. BRÄUTIGAM, *Pharm. Ztg.*, 1901, **46**, 636.
19. O. BREDT, G.P. 156,148, 1904; *Chem. Zentr.*, 1905, I, 643; E.P. 22,370, 1903.
20. BROOKS, U.S.P. 779,583, 1905.
21. BROWN, *J. Biol. Chem.*, 1936, **113**, 417.
22. H. T. BROWN and J. H. MILLAR, *J. Chem. Soc.*, 1899, **75**, 286.
23. — *ibid.*, 1899, **75**, 308.
24. — *ibid.*, 1899, **75**, 315.
25. BRYANT, U.S.P. 1,937,543, 1933.
26. BUCHSWEILER AKT.-GES., ADMINISTRATION DER MINEN, G.P. 227,606, 1910; *Chem. Abstr.*, 1911, **5**, 2200.
27. BUSCH, CLARK, GENUNG, SCHROEDER and EVANS, *J. Org. Chem.*, 1936, **1**, 1.
28. C. G. CALDWELL and R. M. HIXON, *J. Biol. Chem.*, 1938, **123**, 595.
29. L. CERF, U.S.P. 698,632, 1902.
30. CHAKRABARTI and DHAR, *J. Indian Chem. Soc.*, 1929, **6**, 617.
31. CHEMISCHE FABRIK GEDDON RICHTER AKT.-GES., G.P. 544,693, 1932; *Chem. Abstr.*, 1932, **26**, 3518.
- 31a. CORN PROD. REF. CO., E.P. 530, 344.
32. J. CRAIK, *J. Soc. Chem. Ind.*, 1924, **43**, 171T.
33. C. F. CROSS, E. J. BEVAN and C. BEADLE, *Ber.*, 1893, **26**, 2520.
34. DAVID, U.S.P. 769,061, 1904.
35. DEBUIGNE, F.P. 777,722, 1935; *Chem. Zentr.*, 1935, II, 140.
36. DE CHALMOT, *Am. Chem. J.*, 1895, **17**, 535.
37. DE GASSICOURT, *Beitr. Chem. Physik*, 1812, **1**, 1; after Walton<sup>28</sup>.
38. DEUTSCHE MAIZENA G.M.B.H., F.P. 771,029, 1934; *Chem. Abstr.*, 1935, **29**, 952.
39. — F.P. 844,571, 1939; *Chem. Abstr.*, 1940, **34**, 7648.
40. — F.P. 844,572, 1939; *Chem. Abstr.*, 1940, **34**, 7648.
41. N. R. DHAR, *J. Phys. Chem.*, 1924, **28**, 943.
42. DÖBEREINER, *Ann. Physik Chem.*, 1829, **15**, 307.

43. DOLLFUS and SCHEURER, *Bull. soc. ind. Mulhouse*, 1896, **66**, 241.
44. DOROSCHIEWSKI and RAKOWSKI, *J. Russ. Phys. Chem. Soc.*, 1907, **39**, 427; *Chem. Zentr.*, 1907, II, 1325.
45. DUBEAU, MACMAHON and VINCENT, *Am. Dyestuff Reptr.*, 1939, **28**, 590.
46. DUINTJER WILKENS MEIHUIZEN & Co., F.P. 772,836, 1934; *Chem. Abstr.*, 1935, **29**, 1676; E.P. 420,275, 1935; *Chem. Abstr.*, 1935, **29**, 3546.
47. DULAC and ROSENBAUM, 'Industrial Cold Adhesives,' Griffin & Co., London, 1937.
48. O. DURIEUX, *Bull. soc. chim. Belg.*, 1913, **27**, 90.
49. J. M. EDER, *J. prakt. Chem.*, 1879, **19**, 294.
50. M. EKHard, *Z. Spiritusind.*, 1926, **49**, 196.
51. EVERETT and SHEPPARD, 'Oxidation of Carbohydrates in Acid Solution,' p. 43, University of Oklahoma School of Medicine, Oklahoma City, 1936.
52. FARBENFABRIKEN, F.P. 383,902, 1907; *Chem. Abstr.*, 1909, **3**, 1105.
53. R. FEIBELMANN, *Z. ges. Textil-Ind.*, 1924, **27**, 410; *Chem. Zentr.*, 1924, II, 2702.
54. — *Melliand Textilber.*, 1926, **7**, 144.
55. — *Am. Dyestuff Reptr.*, 1928, **17**, 436.
56. — *Indian Textile J.*, 1931, **41**, 317; *Chem. Abstr.*, 1931, **25**, 5774.
57. FELTON, F. F. FARLEY and R. M. HIXON, *Cereal Chem.*, 1938, **15**, 678.
58. A. FERNBACH and J. WOLFF, *Proc. Seventh Intern. Congr. Applied Chem.*, 1909, **6B**, 124.
59. A. FINDLAY and C. ROSEBOURNE, *J. Soc. Chem. Ind.*, 1922, **41**, 58T.
60. FINK and SUMMERS, *Trans. Electrochem. Soc.*, 1938, **74**, 625.
61. FLETCHER and T. C. TAYLOR, *J. Am. Chem. Soc.*, 1938, **60**, 3018.
62. FLICK, G.P. 217,336, 1909; *Chem. Abstr.*, 1910, **4**, 1557.
63. — E.P. 25,121, 1909, incomplete; *Chem. Abstr.*, 1911, **5**, 224.
64. R. FOSSE, *Bull. soc. chim. France*, 1921, (4) **29**, 158.
65. — *Compt. rend. soc. biol.*, 1922, **86**, 175.
66. FOURCROY, 'Encyclopédie Méthodique de Chymie,' 5, p. 81, Paris, 1792; after Walton <sup>285</sup>.
67. A. P. N. FRANCHIMONT, *Rec. trav. chim.*, 1883, **2**, 91.
68. J. FRÈRE, *Rev. prod. chim.*, 1924, **27**, 721; *Chem. Zentr.*, 1925, I, 722.
69. H. FRIEDENTHAL, *Centr. Physiol.*, 1898, **12**, 849.
70. F. FRITSCHÉ, E.P. 1,351, 1908; *Chem. Abstr.*, 1909, **3**, 600.
71. F. FRITSCHÉ, U.S.P. 910,524, 1909.
72. FUJITA, MAESHIMA and HACHIHAMA, *J. Soc. Chem. Ind., Japan*, 1938, **41**, *Suppl. binding*, 63.
73. FULLER, E.P. 383,778, 1933; *Chem. Abstr.*, 1933, **27**, 6006.
74. FULLER, U.S.P. 1,937,752, 1933.
75. — U.S.P. 1,942,544, 1934.
76. — U.S.P. 2,014,799, 1935.
77. Z. GATIN-GRUZEWSKA, *Compt. rend. soc. biol.*, 1907, **63**, 224.
78. — *Compt. rend.*, 1909, **148**, 578.
79. C. GERBER, *ibid.*, 1912, **154**, 1543.
80. — *Compt. rend. soc. biol.*, 1912, **72**, 1002.
81. — *ibid.*, 1912, **73**, 356 and 358.
82. GERSON and SACHSE, G.P. 167,275, 1906; *Chem. Zentr.*, 1906, I, 1128.
83. VON GÖRUP-BESANEZ, *Ann.*, 1859, **110**, 86.

84. GRANGAARD, MICHELL and PURVES, *J. Am. Chem. Soc.*, 1939, **61**, 1290.
85. GRUZEWSKA, *Compt. rend. soc. biol.*, 1910, **68**, 274.
86. — *Bull. soc. chim.*, 1910, (4) **7**, 744.
87. — *Compt. rend. soc. biol.*, 1910, **68**, 1084.
88. GUÉRIN, *Ann. Chem.*, 1933, **8**, 24.
89. W. HAAKE, G.P. 547,421, 1932; *Chem. Zentr.*, 1932, I, 2907.
90. R. HAAKE and W. HAAKE, G.P. 164,385, 1905; E.P. 19,720, 1901; *Chem. Zentr.*, 1905, II, 1566.
91. — — U.S.P. 813,647, 1906.
92. — — U.S.P. 1,792,088, 1931.
93. — — G.P. 543,457, 1932; *Chem. Abstr.*, 1932, **26**, 2613.
94. J. HABERMANN, *Ann.*, 1872, **162**, 297.
95. — *ibid.*, 1874, **172**, 11.
96. HACHIYAMA and FUJITA, *J. Soc. Chem. Ind., Japan*, 1935, **38**, *Suppl. binding*, 744.
97. F. E. HALE, *Am. J. Sci.*, 1902, **13**, (4), 379.
98. R. HALLER, *Melliand Textilber.*, 1924, **5**, 389.
99. — — E.P. 229,623, 1925; *Chem. Abstr.*, 1925, **19**, 3169.
100. — — U.S.P. 1,564,955, 1925.
101. — — G.P. 438,119, 1926; *Chem. Zentr.*, 1927, I, 1240.
102. — — *Zeit. ges. Textil-Ind.*, 1927, **30**, 416; *Chem. Zentr.*, 1927, II, 1902.
103. — — *Kolloid-Zeit.*, 1927, **41**, 81.
104. — — G.P. 665,268, 1938; *Chem. Abstr.*, 1939, **33**, 1988.
105. — — *Helv. Chim. Acta*, 1940, **23**, 596.
106. R. HALLER, HACKL and FRANKFURT, *Melliand Textilber.*, 1928, **9**, 757.
107. — — *The Melliand*, 1930, **2**, 86.
108. C. S. HANES, *New Phytologist*, 1937, **36**, 101.
109. HARTWIG, U.S.P. 798,509, 1905.
110. HARVEY, U.S.P. 1,366,653, 1921.
111. — — U.S.P. 2,006,164, 1935.
112. E. HARZ, *Wochschr. Brau.*, 1905, **22**, 721.
113. W. N. HAWORTH, E. L. HIRST and WOOLGAR, *J. Chem. Soc.*, 1935, 177.
114. HENKEL AND CIE, E.P. 289,053, 1928; *Chem. Zentr.*, 1928, II, 393.
115. HENZERLING, U.S.P. 1,834,057, 1931; *Chem. Zentr.*, 1932, I, 1439.
- 115a. F. HERMITE, G.P. 70,275; E.P. 1061, 1892.
116. HILDEBRANDT, G.P. 545,081, 1932; *Chem. Abstr.*, 1932, **26**, 3135.
117. E. L. HIRST, M. M. T. PLANT and WILKINSON, *J. Chem. Soc.*, 1932, 2375.
118. HOLSTE, E.P. 30,390, 1909; *Chem. Abstr.*, 1911, **5**, 2444.
119. HÖNIG and RUZICZKA, *Biochem. Zeit.*, 1930, **218**, 397.
120. F. HÖPFLER, G.P. 558,145, 1932; *Chem. Abstr.*, 1933, **27**, 1229.
121. HOUGH, G.P. 172,549, 1906; *Chem. Zentr.*, 1906, II, 938.
122. INT. PAT. DEVEL. Co., F.P. 657,605, 1928; *Chem. Abstr.*, 1929, **23**, 4591.
123. JACKSON and HUDSON, *J. Am. Chem. Soc.*, 1937, **59**, 2049.
124. — — *ibid.*, 1938, **60**, 989.
125. G. B. JAMBUSERWALA, *J. Textile Inst.*, 1938, **29**, 149T.
126. G. B. JAMBUSERWALA and K. R. KANITKAR, *ibid.*, 1939, **30**, 85T.
127. — — *ibid.*, 1940, **31**, 1T.



128. KANTOROWICZ, U.S.P. 1,207,177, 1916.
129. J. R. KATZ, *Textile Research*, 1939, **9**, 146. (Extensive patent lists given.)
130. — and WEIDINGER, *Z. physik. Chem.*, 1939, **184A**, 100.
131. KERR, U.S.P. 2,052,308, 1936.
132. — U.S.P. 2,108,862, 1938.
133. KESSELER and DÖRING, G.P. 535,839, 1931; *Chem. Abstr.*, 1932, **26**, 1150.
134. KIHARA, *J. Agr. Chem. Soc., Japan*, 1939, **15**, 107; *Chem. Abstr.*, 1939, **33**, 6253.
135. KILIANI, *Ber.*, 1925, **58**, 2345.
136. H. KINDSCHER, G.P. 149,588, 1904; *Chem. Zentr.*, 1904, **I**, 976; 168,980, 1906; *Chem. Zentr.*, 1906, **I**, 1514.
137. G. S. C. KIRCHHOFF, *Tekhnologicheskii Zhurnal*, **9**, pt. 1, 3-26, 1812; abstracted in *Acad. Imp. Sci., St. Petersburg, Mém.*, 1811, (5) **4**, 27.
138. L. KÖNIG, *J. Soc. Chem. Ind.*, 1894, **13**, 824A.
139. — E.P. 9,674, 1894.
140. KÜHL and SOLTAN, G.P. 522,555; *Chem. Zentr.*, 1931, **I**, 3525.
141. G. LANGBEIN, *Pharm. Z. Russ.*, 1868, **7**, 573; *Chem. Zentr.*, 1869, 847.
142. E. LÉCONTE, *Zeit. Elektrochem.*, 1904, **11**, 113; *Chem. Zentr.*, 1904, **II**, 1076.
143. A. LIEBEN, *Ber.*, 1875, **8**, 1017; — and REICHARDT, *ibid.*, 1020.
144. LIEBEN and MOLNAR, *Monatsh. Chem.*, 1929, **53**, 1.
145. J. LIEBIG, *Ann. Physik Chem.*, 1829, **15**, 541.
146. C. J. LINTNER, *Zeit. angew. Chem.*, 1890, **3**, 546.
147. LÖB, *Biochem. Zeit.*, 1912, **46**, 121.
148. LOBENSTEIN, *Thesis*, Leipzig; *Chem. Abstr.*, 1931, **25**, 3867.
149. J. R. MACMILLAN, U.S.P. 1,567,609, 1925.
150. MALAPRADE, *Bull. soc. chim., Mém.*, 1934, **1**, 833.
151. MANN, KRÜGER and TOLLENS, *Zeit. angew. Chem.*, 1896, **9**, 33.
152. MASSOL, *Compt. rend.*, 1911, **152**, 902.
153. MILITZ, U.S.P. 941,159, 1909; *Chem. Abstr.*, 1910, **4**, 525.
154. MIROW, G.P. 273,235, 1914; *Chem. Abstr.*, 1914, **8**, 2825.
155. R. MITTRA and N. H. DHAR, *Zeit. anorg. Chem.*, 1922, **122**, 146.
156. MOISSAN, *Ann. chim. phys.*, 1891, (6) **24**, 224.
157. MONTGOMERIE, E.P. 490,070, 1938.
158. MORI, Japanese Pat. 100,061, 1933; *Chem. Abstr.*, 1934, **28**, 2564.
159. K. MYRBÄCK, *Biochem. Zeit.*, 1936, **285**, 290.
160. NAKAMURA, *J. Faculty Agr. Hokkaido Imp. Univ.*, 1935, **38**, Pt. 1, 1.
161. D. R. NANJI, E.P. 273,481, 1927; *Chem. Abstr.*, 1928, **22**, 2076.
162. NAQUET, *Ber.*, 1892, **25**, 884R; G.P. 64,401, 1892.
163. NATIONAL ADHESIVES CORP., F.P. 729,259, 1932; *Chem. Abstr.*, 1932, **26**, 6176.
164. NEF, *Ann.*, 1904, **335**, 191; 1907, **357**, 214; 1910, **376**, 1; 1914, **403**, 204.
165. C. NEUBERG and S. MIURA, *Biochem. Zeit.*, 1911, **36**, 37.
166. ODELL, U.S.P. 1,425,605, 1922.
167. OMORI, *J. Biochem., Japan*, 1931, **14**, 339.
168. — *ibid.*, 1932, **16**, 483.
169. ONO, *Rev. Phys. Chem., Japan*, 1940, **14**, 25; *Chem. Abstr.*, 1940, **34**, 5721.
170. PAIRA, E.P. 9,370, 1909; *Chem. Abstr.*, 1909, **3**, 3018.

171. C. C. PALIT and N. H. DHAR, *J. Phys. Chem.*, 1925, **29**, 799.  
172. — *ibid.*, 1926, **30**, 939.  
173. — *ibid.*, 1928, **32**, 1263.  
174. — *ibid.*, 1930, **34**, 711.  
175. — *ibid.*, 1930, **34**, 993.  
176. J. PELOUZE, *Ann.*, 1839, **29**, 38 ; *J. prakt. Chem.*, 1839, **18**, 168.  
177. — *Compt. rend.*, 1846, **23**, 809 and 892.  
178. F. G. PERKINS, U.S.P. 1,020,656, 1912.  
179. J. PERSONNE, *J. pharm. chim.*, 1861, (3) **39**, 49.  
180. P. PETIT, *Compt. rend.*, 1892, **114**, 1375.  
181. PIEPER, G.P. 79,326, 1894 ; *Ber.*, 1895, **28**, 575R.  
182. PIERSON, U.S.P. 2,023,973, 1935.  
183. J. A. RADLEY, see pp. 110 and 115.  
184. See p. 127, 1st Edition.  
185. See p. 187, 1st Edition.  
186. RANDALL, BENDER and GROOCCOCK, *Proc. Roy. Soc. (London)*, 1938, **A165**, 432.  
187. RANKIN, U.S.P. 1,520,885, 1924.  
188. RASSOW, *Melliand Textilber.*, 1931, **12**, 468.  
189. RASSOW and LOBENSTEIN, *Kolloidchem. Beih.*, 1931, **33**, 179.  
190. REISZ, Hungarian Pat. 104,972, 1933 ; *Chem. Abstr.*, 1933, **27**, 4123.  
191. A. REYCHLER, G.P. 360,128, 1922 ; *Chem. Zentr.*, 1923, II, 40 ;  
F.P. 544,580, 1922 ; *Chem. Zentr.*, 1923, II, 1004.  
192. — *Bull. soc. chim. Belg.*, 1923, **32**, 221.  
193. W. A. RICHARDSON, R. S. HIGGINBOTHAM and F. D. FARROW,  
*J. Text. Inst.*, 1936, **27**, 131T.  
194. H. RODEWALD and A. KATTEIN, *Z. physik. chem.*, 1900, **33**, 579.  
195. M. SAMEC, *Kolloidchem. Beih.*, 1929, **28**, 155.  
196. — *Kolloid-Zeit.*, 1933, **64**, 321.  
197. M. SAMEC and M. BLINC, *Kolloidchem. Beih.*, 1933, **38**, 48.  
198. — and JENCIC, *ibid.*, 1915, **7**, 137.  
199. — and ULM, *ibid.*, 1936, **43**, 287.  
200. G. SCHAEFFER and O. SCHEURER, *Bull. soc. ind. Mulhouse*, 1893,  
**63**, 363.  
201. O. SCHMERBER, *ibid.*, 1896, **66**, 238.  
202. E. SCHMIDT and E. GRAUMANN, *Ber.*, 1921, **54**, 1860.  
203. SCHOLTEN'S CHEM. FABR., E.P. 392,178, 1933 ; *Chem. Abstr.*,  
1933, **27**, 4951.  
204. SCHOTZ, E.P., 345,985, 1931 ; *Chem. Abstr.*, 1931, **25**, 5477.  
205. W. SECK, E.P. 437,890, 1935 ; *Chem. Abstr.*, 1936, **30**, 3270.  
206. SEMICHON and FLANZY, *Compt. rend.*, 1932, **195**, 254.  
207. R. S. SHANE, *J. Chem. Educ.*, 1937, **14**, 460.  
208. SIEMENS and HALSKE, *Ber.*, 1893, **26**, 959R ; G.P. 70,012, 1893.  
209. — G.P. 103,399 and 103,400, 1899 ; *Chem. Zentr.*, 1899, II, 892.  
209a. — G.P. 88,447 ; U.S.P. 798,509, 1905.  
210. O. A. SJOSTROM, Canadian Pat. 356,463, 1936 ; U.S.P. 2,052,320,  
1936 ; *Chem. Abstr.*, 1936, **30**, 7378.  
211. SOCIÉTÉ ANONYME TRUST CHIMIQUE, G.P. 134,301, 1902 ; *Chem.*  
*Zentr.*, 1902, II, 836.  
212. SOHST and B. TOLLENS, *Ann.*, 1888, **245**, 1.  
213. SOLLAZZO, *Boll. chim. farm.*, 1934, **73**, 917 ; *Chem. Abstr.*, 1935,  
**29**, 2924.  
214. SPROCKHOFF, G.P. 618,265, 1935 ; *Chem. Abstr.*, 1936, **30**, 319.

215. STÄDELER, *Ann.*, 1847, **61**, 101.  
 216. STOKES and PETER, U.S.P. 1,870,472, 1932.  
 217. STOLLE and KOPKE, F.P. 384,764, 1907; *Chem. Abstr.*, 1909, **3**, 1105.  
 218. — G.P. 199,753, 1908; *Chem. Abstr.*, 1908, **2**, 3167; Austrian Pat., 37,835, 37,836 and 40,449.  
 219. — G.P. 202,229, 1908; *Chem. Zentr.*, 1908, II, 55.  
 220. C. SUNDER, *Bull. soc. ind. Mulhouse*, 1924, **90**, 558.  
 221. SUPF, G.P. 364,314, 1922; *Chem. Zentr.*, 1923, II, 257.  
 222. SUTHERLAND, E.P. 121,943, 1919; *Chem. Abstr.*, 1919, **13**, 1109.  
 223. SYMONS and BUSWELL, *Ind. Eng. Chem., Anal. Ed.*, 1929, **1**, 161.  
 224. W. SYNIEWSKI, *Ber.*, 1897, **30**, 2415.  
 225. — *ibid.*, 1898, **31**, 1791.  
 226. — *Ann.*, 1899, **309**, 282.  
 227. — *Roczniki Chem.*, 1922, **2**, 83; *Chem. Abstr.*, 1923, **17**, 1627.  
 228. — *Ann.*, 1925, **441**, 277.  
 229. TROTSMAN, *Dyer*, 1928, **60**, 160.  
 230. VAN DER MEULEN, G.P. 624,988, 1936; U.S.P. 2,053,012, 1936; *Chem. Abstr.*, 1936, **30**, 7378.  
 231. VINTILESCU and FALTIS, *Bul. soc. chim. România*, 1923, **5**, 59; *Chem. Abstr.*, 1924, **18**, 1475.  
 232. VON ASBOTH, *Chem. Ztg.*, 1892, **16**, 1517 and 1560.  
 233. VON BRUEDER, U.S.P. 646,724, 1900.  
 234. WALKER, *Am. Dyestuff Repr.*, 1935, **24**, 374.  
 235. R. WALTON, 'Comprehensive Survey of Starch Chemistry,' p. 239. Chemical Catalog Co., New York, 1928.  
 236. — *ibid.*, p. 2, Bibliography.  
 237. W. WATSON and D. W. KENT-JONES, U.S.P. 1,519,014, 1924.  
 238. WHITE, E.P. 172,145, 1922; F.P. 525,462, 1921; G.P. 369,971, 1923; *Chem. Abstr.*, 1922, **16**, 1049; *Chem. Zentr.*, 1923, II, 1004.  
 239. WILL and LENZE, *Ber.*, 1898, **31**, 68.  
 240. WOLFF, *Compt. rend.*, 1905, **141**, 1046.  
 241. WROE, G.P. 231,960, 1900; *Chem. Abstr.*, 1912, **6**, 942.  
 242. C. WURSTER, *Ber.*, 1889, **22**, 145R.

## ADDITIONAL REFERENCES

- J. A. RADLEY, *Manuf. Chem.*, 1936, **7**, 246. (Soluble starches.)  
 G. LAQUEUILLE, *T.I.B.A.*, 1938, **16**, 677. (General.)  
 W. B. NEWKIRK, *Ind. Eng. Chem.*, 1939, **31**, 153. (Industrial uses of soluble starches described.)  
 J. E. POLLAK, E.P. 483,773, 1938. (Thick-boiling starch by action of heat on starch-halogen mixture.)  
 ANON, *Bull. Shirley Inst.*, 1939, **12**, 124. (Use of soluble starch in sizes discussed.)  
 H. INUKAI, *Bull. Kyoto Ind. Res. Inst.*, 1927, **1**, 1; 1928, **3**, 13. (Properties of soluble starches made by various methods described.)  
 W. A. NIVLING, U.S.P. 2,204,615. (Soluble starch prepared by carrying out reaction in gaseous medium.)  
 J. PELOUSE, *Compt. rend.*, 1838, **7**, 713. (Oxalic acid obtained by action of nitric acid.)  
 P. BERG and H. NEUBERGER, U.S.P. 1,133,914, 1915.

- A. VON ASBOTH, *Chem. Ztg.*, 1892, 1527, 1560. (Action of ammoniacal hydrogen peroxide on starch.)
- ANON, *T.I.B.A.*, 1935, 18, 633. (General. Halogen compounds used to oxidise starch.)
- I. G. FARBEININD, G.P. 538,839, 1924. (Air-dry starch ground with 3 per cent. its weight of calcium hypochlorite for several hours.)
- C. BRENDER, E.P. 17,650, 1898. (Uses chlorates to oxidise starch.)
- A. ASHWORTH, E.P. 19,720, 1901. (Chlorates and oxidation catalysts, e.g. copper sulphate or vanadium chloride used.)
- SIEMENS and HALSKE, U.S.P. 798,509, 1905. (Chlorine gas used for oxidation.)
- W. VON SIEMENS and C. WITT, E.P. 24,455, 1895. (Permanganates followed by hydrochloric acid to solubilise, then sulphur dioxide to decolorise.)
- J. MULLER, U.S.P. 2,173,040; U.S.P. 2,173,041, 12/9/1939. (Per-sulphates at 50-100° C. Amount of agent used is such that product is neutral, due to basic substances commonly present in starch.)
- CORN PRODUCTS REF. CO., E.P. 530,344, 16/9/1938. (Calcium peroxide used to obtain thin-boiling, thick-setting product.)
- E.P. 534,503, 16/11/1939. (Starch chlorinated in dry state with hypochlorite.)
- M. D. ROZENBROEK and N. V. CHEMISCHE FABR. SERVO, E.P. 534,112, 27/12/1938. (Powdered starch and alkaline reagent mixed and chlorine passed in.)
- J. S. REICHERT and S. A. MCNEIGHT, U.S. Applic. 343,455, 1/7/1940. (Starch treated with aqueous organic mono-per acids. Citations against by U.S. Pat. Office: Debuigne, Ref. 35; Flick, Ref. 63; Stoddard, U.S.P. 1,718,609, 1,687,805 and 1,687,804; McKee, U.S.P. 1,767,543.)
- V. I. NAZAROV, *Colloid J. (U.S.S.R.)*, 1940, 6, 254. (Oxidation with permanganate takes place throughout granule and follows closely the equation for a unimolecular reaction.)
- F. F. FARLEY and R. M. HIXON, *Ind. Eng. Chem.*, 1942, 34, 677. (Electrolysis in alkaline sodium chloride solution. Oxidation localised between radical starch crystallites.)

## CHAPTER 4

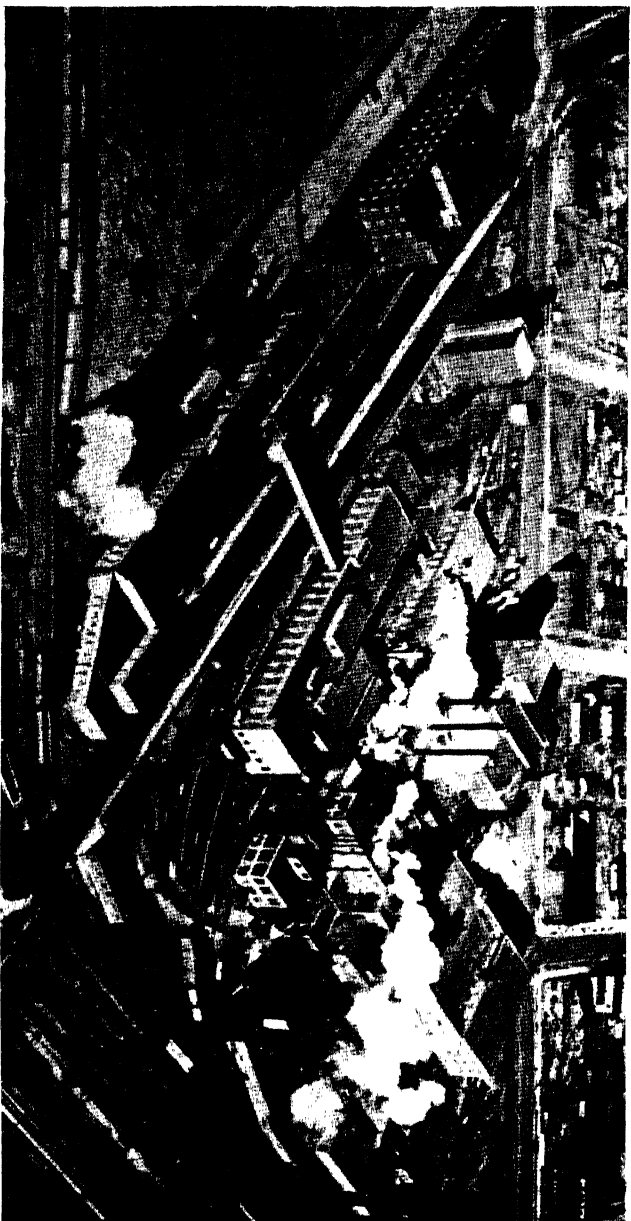
## GLUCOSE AND MALTOSE

**The Manufacture of Glucose.**—Kirchhoff discovered, in 1811, that heating starch with dilute sulphuric acid transformed it into sugar, although his object had been to prepare a light-coloured substitute for gum arabic. As the English were blockading France at that time, the importance of the discovery was quickly realised and manufacturing operations began at once. It was thought at first that the sugar obtained was identical with cane sugar, and could be substituted for it in any product, but later it was found to be less soluble and less sweet, and, as it could not be used in every case as a substitute for sugar, it fell into disrepute for a long time. When the blockade was lifted, production of starch sugar fell off greatly, owing to the heavy fall in price consequent to the market being flooded with colonial products, and to the strong competition of beet sugar. It had, however, gained a foothold in the brewing industry and for the manufacture of spirituous liquors. That starch sugar is identical with grape sugar was established by Saussure<sup>1</sup> in 1814, and he correctly explained the mechanism of the process as one of hydrolysis.

Commercial dextrose is marketed in three forms: (a) starch syrup, glucose syrup or corn syrup, (b) glucose, (c) commercial dextrose. The first is a thick, pale or colourless syrup containing from 12-20 per cent. moisture and colloidal matter like dextrin, which prevents crystallisation taking place. The solid glucose appears as pale to brown-coloured amorphous masses, although in reality it is made up of minute crystals. The colour and amount of dextrose are determined by the method of manufacture, and the terms '70' or '80' sugar are used to denote the percentage content of dextrose. Commercial dextrose is a fine crystalline powder containing from 80 to 99.5 per cent. of pure sugar, according to the conditions of crystallisation. One refined brand, known as 'Cerelose,'<sup>2</sup> contains 1 molecule of water of crystallisation, has an average sugar-content of 91.4 per cent., and ash-content 0.05 per cent.

W. B. Newkirk<sup>36-37</sup> has described in some detail the preparation of anhydrous glucose and dextrose hydrate and his work is discussed below.

Starch syrup for confectionery should contain as little acid as possible, otherwise during 'boiling' in confectionery manufacture



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Fig. 45.—Illinois plant of Union Starch and Refining Company. Aerial view showing layout.

*[Facing p. 212.]*

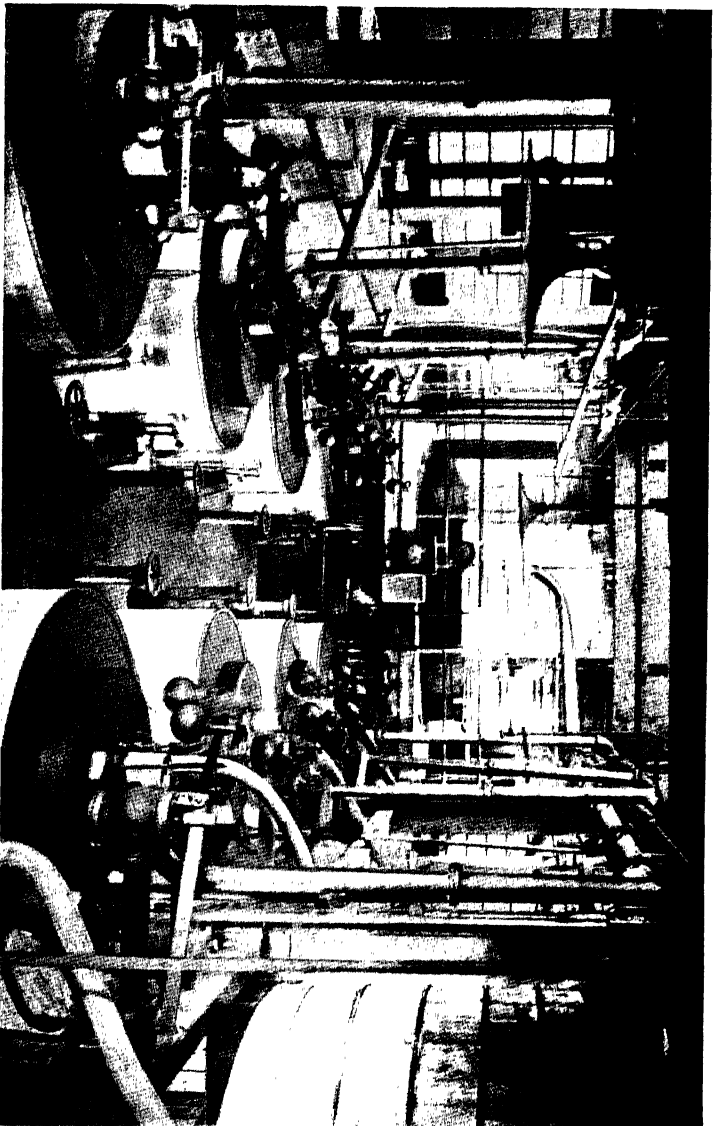


FIG. 46.—Converter floor where starch is converted to sugar liquor.

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*[Facing p. 213.]*

the sucrose used in conjunction with it will be inverted. On the other hand, W. Ekhard<sup>3</sup> points out that some acidity is required, otherwise a yellow colour develops during the 'boiling.' This acidity, which is due to acid phosphates, should not fall below 0.3 per cent. if the yellowing is to be avoided. For these reasons Preuss<sup>4</sup> considers that sodium carbonate should be used to neutralise the hydrochloric acid used in the conversion, as the salt formed is without hydrolytic action on sucrose and, in the concentration as left in the syrup, does not affect the flavour; whereas if chalk is used, the resultant calcium chloride would have a strong inverting action when used in confectionery. If sulphuric acid has been used to hydrolyse the starch, chalk may be used, as the resulting calcium sulphate can be readily removed.

The amounts of oil and protein in the syrup are other factors determining its value for confectionery processes; when these exceed 0.14 and 0.19 per cent. respectively,<sup>5</sup> they cause darkening when the syrup is heated to 145° C. Potato starch, being freer from protein matter than maize starch, gives syrups that are superior in this respect.

**Raw Materials.**—The amylaceous matter used is generally 'green' starch, from maize, wheat, rice, or potatoes, and is often produced at the factory, especially maize starch. The grains of the cereal may be used, but then a less pure product may result, although rice with its high starch-content lends itself perhaps more readily to this treatment than the other cereals. L. E. Stout and C. G. Ryberg<sup>30</sup> have suggested the use of sweet-potato starch as a starting material and have determined the effect of pressure and of acid concentration on the rate of hydrolysis. The isoelectric point of the undesirable humus was found to lie at pH 5.1 and the conditions for decolorising the syrup were determined. The results show that sweet-potato starch does not differ very much from maize starch in its ease of conversion or the conditions it requires and that a product comparable in flavour and appearance can be produced. Highly modified soluble starch has been used by C. B. Duryea.<sup>6</sup>

The acids generally favoured are sulphuric acid in Europe and hydrochloric acid in America. Other acids have been proposed from time to time, including nitric,<sup>7</sup> hydrofluoric<sup>8</sup> and phosphoric<sup>9</sup> acids, but these have not found much favour. Of these acids it will be noticed that the last two could be readily removed by chalk, but this advantage does not appear to have outweighed the disadvantage of higher cost.

**Earlier Process.**—In making glucose by the earlier processes sulphuric acid is employed, to the extent of about 0.05-1.25 per



cent., to hydrolyse the starch, which is suspended in 2-2½ times its weight of water contained in wooden vats provided with a steam coil. Water and acid are first heated to boiling-point and the starch-milk run in. If 'green' starch is used, allowance is made for the moisture in it (45-55 per cent.); for the production of solid sugar the higher figure for acid is employed. During the boiling which follows the addition of the starch-milk, volatile odoriferous substances are driven off, especially when potato starch is used (see p. 246), and, in that case, it is preferable to boil for a longer time, so that the characteristic odour may be completely expelled.

The course of the conversion is followed in the first stages by means of the iodine reaction, which indicates the point at which the transformation of the starch into glucose and lower dextrans is complete. From this point the hydrolysis of the residual dextrans is followed by withdrawing samples of the syrup and adding double the volume of alcohol, when these compounds are precipitated. The liquor is neutralised when this test gives no precipitate.

As lime would react with the glucose, chalk is used as the neutralising agent, and when nearly neutral the liquid is again boiled for a short time before the final addition of chalk. Although at this point no precipitate is obtained in the alcohol test, yet some substance other than glucose is present which is not precipitated by alcohol at that concentration, and it appears in the final glucose, unless boiling is continued for a further short time.

The calcium sulphate is allowed to settle, or filtered through a filter press, and the liquor is evaporated until more calcium sulphate separates, when it is again filtered and evaporated in vacuum pans to a thick syrup. If a good coloured, solid sugar is required, the syrup is filtered through animal charcoal before the last evaporation, and the final syrup soon begins to crystallise.

The above process may be carried out under pressure if desired, and with the proportions mentioned above, the temperature should not exceed 130° C.; also, the time of heating should be carefully controlled, otherwise the sugar decomposes and gives a lower yield of an inferior product.<sup>11</sup> If the process is carefully carried out glucose of 92-97 per cent. purity is obtained. E. Parow<sup>5</sup> has shown that with potato starch increase of pressure leads to a rapid increase in the rate of hydrolysis, but, as previously indicated, it is preferable to operate at the moderate pressure for a longer time in order to expel the characteristic smell of potatoes from this starch. The more recent process uses pressure.

**The More Recent Process.**—In this process, which in the

United States is used almost exclusively, water containing 0.12 per cent. of hydrochloric acid on the weight of starch to be processed is heated to the conversion temperature in cylindrical copper vessels, which may be as much as 20 ft. high and 6 ft. in diameter, and the starch suspended in cold water is pumped in. The process of charging may take from 15 to 20 minutes, and the continual influx of new material prevents the formation of gelatinous lumps; after the addition is finished, a few more minutes suffice to complete the conversion. The temperature throughout the entire operation is maintained between  $132^{\circ}$  and  $137^{\circ}$  C. If solid glucose is being prepared, the acid may be increased to 0.2 per cent. on the weight of starch present, to give a liquid of  $10^{\circ}$  Bé., and higher temperatures and pressures may be used. When some dextrin is still present, as shown by the iodine and alcohol tests, which may occur after some 5-7 minutes' heating following the final addition of starch-milk, the conversion is stopped by releasing the pressure. The process is interrupted at this point when liquid glucose is being produced. For the preparation of solid glucose, the operation is continued until no further matter is precipitated in the alcohol test; a further 10 minutes' heating is then given to complete the conversion of the matter which is not precipitated by the alcohol. The most economic time-pressure values for maximum dextrose production from maize have been worked out by R. C. Ernst, C. E. Brown and J. B. Tepe.<sup>34</sup>

The mass is neutralised with soda ash, insoluble matter is removed by filtration, and the liquor is concentrated to a density of about  $30^{\circ}$  Bé. The resulting syrup is passed through a battery of three decolorising tanks connected in series, and filled with animal charcoal. The colourless liquid is further evaporated under vacuum to a syrup of  $42-45^{\circ}$  Bé. If it has been arranged for the necessary amount of dextrin to be left in, no crystallisation takes place, otherwise on cooling white or pale-coloured masses of sugar separate; or if anhydrous dextrose is desired, the warm syrup is thoroughly seeded with a small quantity of anhydrous glucose, and the mass allowed to crystallise. The crystals are separated from the mother-liquor by centrifuging. To prepare the monohydrate the liquor is 'seeded' with monohydrate crystals.

M. S. Badollet and H. S. Paine<sup>14</sup> point out that neutralisation of the acid with sodium carbonate brings about clarification, as the isoelectric point of the bulk of the colloidal matter present is reached at neutrality. Some colloidal matter, however, still remains dispersed, and the above workers, finding that this has a positive charge, have added such materials as bentonite, colloidal

aluminates, aluminium silicate, and other colloidal clays and earths, which have a negative charge at the  $pH$  value of the acid starch-conversion liquors. They find that the bentonite flocculates the colloidal matter present in the converter liquor, and that settling out is more rapid and efficient than when sodium carbonate is used. Bentonite also eliminates the defect experienced with sodium carbonate, viz. that an excessive amount tends to re-disperse the colloidal material. This treatment prepares the liquor better for the bone-char filtration, and tends to reduce the rate of exhaustion of the bone-char. Sodium aluminate used in



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FIG. 47.—Two rows of dextrose crystallizers.

this way appears to be commercial practice, the results being reported satisfactory in the production of corn syrup and corn sugar.

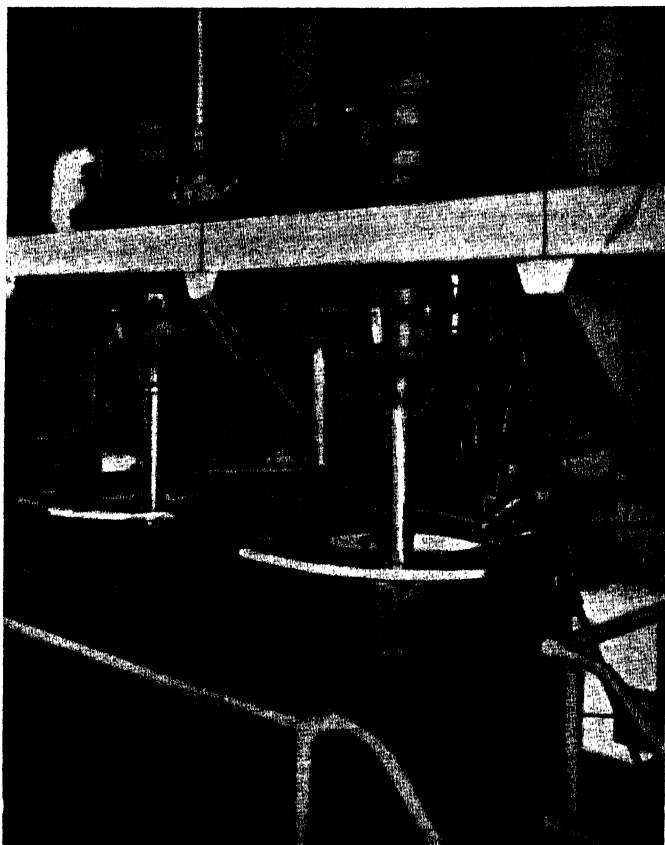
Fellars, Millar, and Onsdorff<sup>15</sup> found that dextrose syrups heated to high temperatures tended to darken, the colour effect being largely dependent on the  $pH$  value of the solution. W. Kröner and H. Kothe<sup>16-17</sup> find that at temperatures over  $100^{\circ}$  C. this darkening is a function of the temperature, and deviations from proportionality between the glucose concentration and discoloration are due to the effect of the sugar on the  $pH$  value of the solution. A sharp minimum occurs at  $pH$  2.3-3.0. Unbuffered

alkaline sugar solutions attain, after heating, a constant  $pH$  of 3.3 (in air) or 3.8 (air excluded). Under the conditions they employed, heavy metal salts had little effect on the discoloration, which was, however, increased in the presence of amino-acids, possibly owing to their effect on the  $pH$  of the solution. The discoloration due to the presence of proteins appears to be purely additive.

In further work<sup>28</sup> the starch was hydrolysed with hydrochloric acid at a given  $pH$  value at temperatures between 102-170° C. and a definite degree of discoloration of the solutions was produced for a given production of reducing sugar, irrespective of the temperature; the colour production was low in the early stages of the hydrolysis but increased rapidly later. Again, at  $pH$  0.6-1.75 a similar relation exists between the amount of discoloration, measured as the extinction coefficient and sugar production so long as complete hydrolysis is not reached. Increase of colour is very rapid after the hydrolysis has been completed. Proportionality between the starch concentration and the colour developed could not be established, but it was noted that inferior starches gave more colour than good grade starches under similar conditions. The colour of the sugar solution is deepened on neutralisation and this appears to be due to the colouring matter acting as an indicator with a transition point between  $pH$  values of 4.5 and 6.5.<sup>29</sup> The technical implications of the above results are fully discussed by these workers with especial reference to the choice of acid concentration, temperature and time of heating and the starch concentration, and for these the reader is referred to the original papers.

One process of purification, introduced by Duryea,<sup>10</sup> is to treat the liquor from the converters with 1 part tannin to every 4800 parts liquor, heat to 90° C., filter, treat with animal charcoal, and concentrate. The corn syrup contains about 40 per cent. of dextrin, and, according to H. Berlin,<sup>13</sup> the final mother-liquor from solid glucose manufacture contains about 70 per cent. glucose, 18.3 per cent. gentiobiose, which has probably been formed by reversion, and 12 per cent. of some other non-fermentable matter. In order to repress, to a great extent, the formation of gentiobiose and other condensation products, which appear to be formed during the later stages of the saccharification, and to remove traces of heavy metal salts, E. C. R. Marks<sup>27</sup> interrupts the conversion when about 40-42 per cent. of sugar has been formed, adjusts the  $pH$  value to between 4.5 and 4.8, filters, readjusts to  $pH$  5.5-6.5, concentrates to  $d-1.2-1.26$  and again filters through bone-char. After dilution and re-acidification the conversion is completed.

**The Crystalline Forms of Anhydrous Dextrose and Dextrose Hydrate.**—W. B. Newkirk<sup>35, 36</sup> set out to improve the method of Porst and Mumford<sup>38</sup> for producing chemically pure dextrose in which the crystallisation is carried out in the quiescent

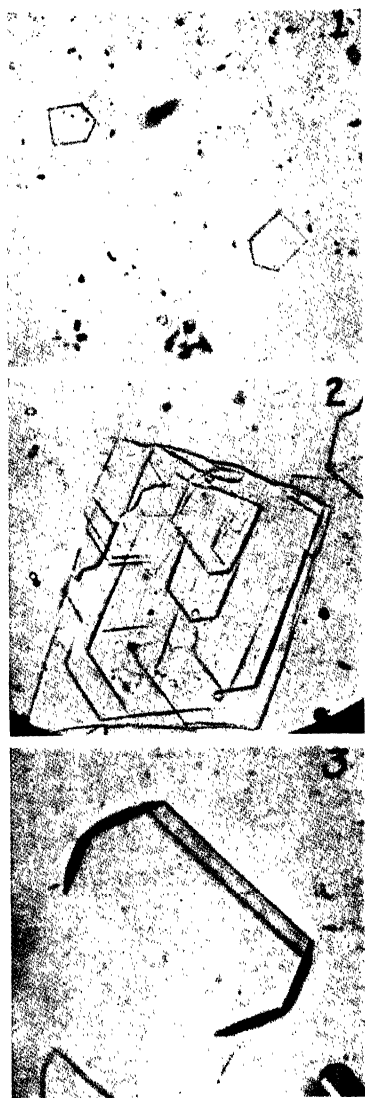


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FIG. 48.—Electric high-speed centrifugals. Bronze and monel metal construction.

state to form a semi-solid mass which is paddled and then centrifuged. The difficulties with this method is the long time of centrifuging, the retention of mother-liquor, washing difficulties leading to the retention of 10-20 per cent. water.

Becke was the first to record<sup>39</sup> the extremely thin, fragile, leaf-like pentagonal crystal of dextrose hydrate shown in the



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FIG. 49.

[Facing p. 218.



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FIG. 50.

[See p. 219.

Photomicrographs Nos. 1 and 2, Fig. 49, taken by Sjostrom and published by W. B. Newkirk,<sup>35</sup> the first being from hydrol, the final liquor of the process, the second from honey. In order to get good quick washing the type of crystal shown in Photomicrograph No. 3 is ideal and is produced by the twinning of two of the hemimorph forms after a rotation of  $180^\circ$ . The shape of the crystal is affected to a great extent by the conditions of crystallisation. *Still crystallisation* of normal factory liquors produces 'wartlike aggregations'<sup>39</sup> or aggregates resembling a sea-urchin<sup>35</sup> shown in Photomicrograph No. 4. These masses readily break down on centrifuging, forming a compact mass difficult to wash without waste. Photomicrograph No. 5 shows a typical hexagon crystal which gives a readily centrifuged and washable product, and Photomicrograph No. 6 shows the striking effect of the presence of impurities on crystal growth. The crystals here are almost perfect hexagons, thicker than those in Photomicrograph No. 5, and the top and bottom faces are lenticular instead of flat, thus preventing aggregation.

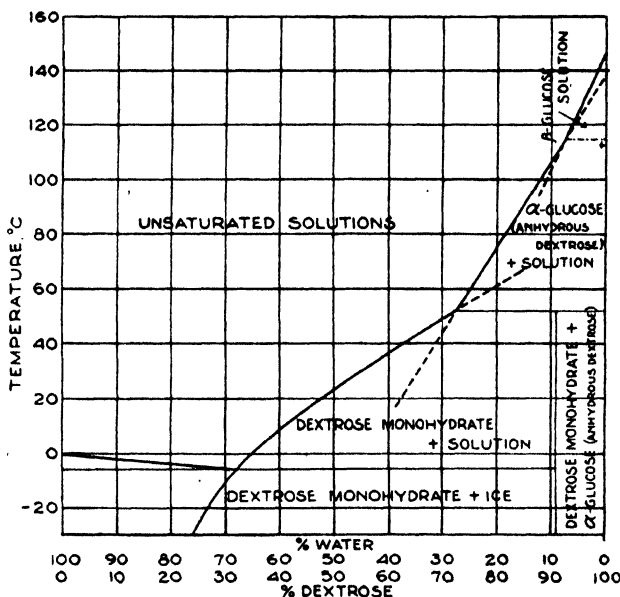
By *crystallisation in motion* long, thin, mechanically weak crystals are obtained (Photomicrograph No. 7, Fig. 50), and Behr<sup>40</sup> had suggested that *absolutely* quiescent conditions were required to obtain anhydrous crystallisation from convertor liquors. As Newkirk has pointed out, both the anhydrous and the hydrate forms can be produced simultaneously in the same liquor under these conditions. Photomicrograph No. 8 shows Behr's anhydrous dextrose, and No. 9 shows the dual crystallisation, whilst No. 10 shows anhydrous but weak dextrose crystals from strongly-agitated high gravity liquors. The true anhydrous crystal from water solution is shown in No. 11, and with proper gravities for slow motion Newkirk finds there are conditions between the anhydrous form shown in No. 8 and those in No. 11 which allow of easy washing, but another difficulty was then met.

In Fig. 51 the anhydrous  $\alpha$ -dextrose solubility curve is quite steep, showing that either heavy gravities must be used, with the danger of forming crystals as in Photomicrograph No. 10, which is undesirable, or lighter gravities may be used and the final temperature lowered with the danger of producing dextrose hydrate. As anhydrous dextrose deposits the liquor becomes lighter in gravity and the forced cooling of the crystalliser reduces the temperature. If hydrate is made in the same building spontaneous seeding will find the right conditions present at this point to induce rapid hydrate formation, preventing adequate washing and causing solidification of the mass in the plant. The



best conditions for obtaining easily washed, anhydrous, hydrate-free crystals (Photomicrograph No. 12) are obtained by the use of vacuum pans as crystallisers, where light-gravity liquors of low viscosity can be crystallised by some withdrawal of water without finishing in low temperature conditions.

**Producing Anhydrous Dextrose.**—Besides the two  $\alpha$ -dextrose crystals which give trouble, anhydrous  $\beta$ -dextrose is present and can be crystallised out, for the solution contains an equilibrium between the  $\alpha$ - and  $\beta$ -isomerides. In this process it is necessary

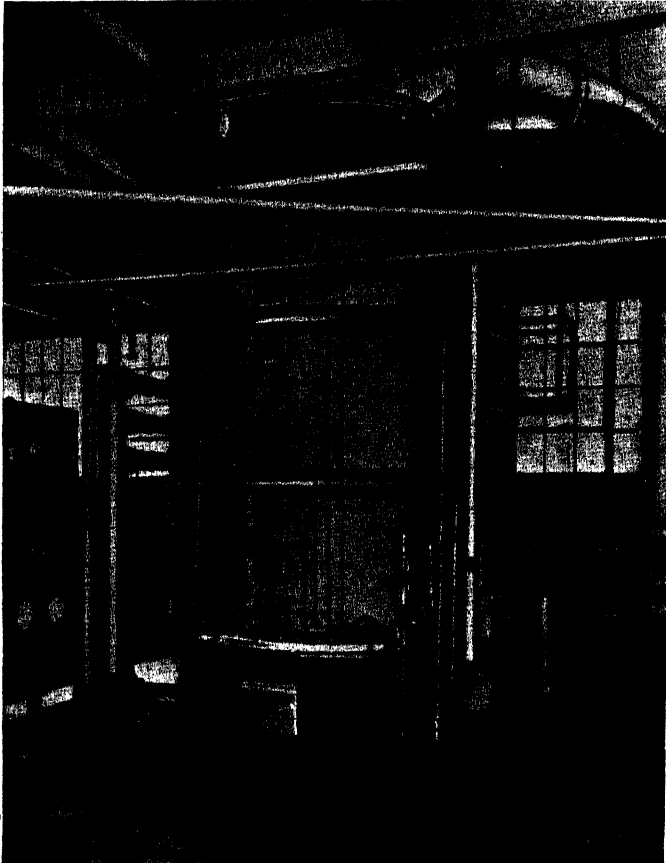


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FIG. 51.—Phase diagram of dextrose solubility.

to have supersaturation of 5-8 per cent. to obtain crystallisation, and it must be supersaturation of the required isomer. The speed of mutarotation is also very important as anhydrous  $\alpha$ -dextrose cannot be deposited quicker than it is formed. Strict control of the gravity, temperature and amount and distribution of the solid nuclei induced at the graining state is important. W. B. Newkirk<sup>35, 36</sup> has described the process used at the Corn Products Refining Co. at Argo, U.S.A. Centrifugally washed hydrate (Cerelese) instead of being dried is dissolved to give a solution of 28-30° Bé. and treated for 30 minutes at 160° F.

with 0.8 per cent of Darco (carbon) on the weight of solids. The liquor is then filter-pressed, any cloudy liquor being re-circulated through the press until delivered clear. The liquor, after further filtering through streamline filters, is delivered to



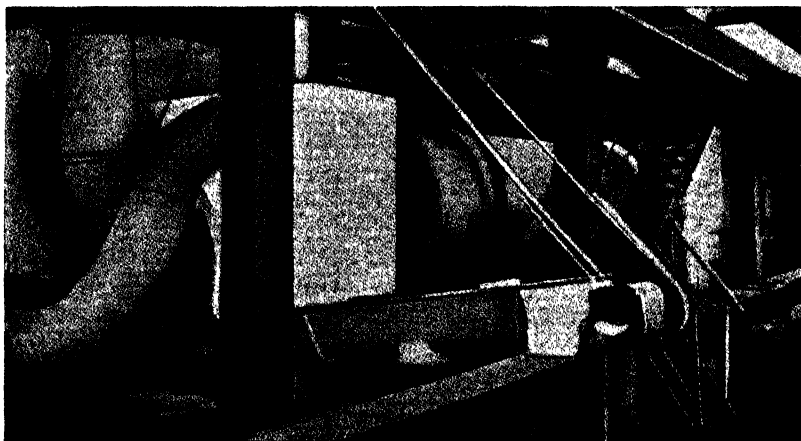
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FIG. 52.—Brass crystallising pan used in anhydrous dextrose manufacture.

a copper or brass strike pan, sufficient liquor being led in so that by the time the charge has reached graining conditions some 15-20 per cent. of the volume of the finished strike is present. The grain is taken in by gravity alone and without seeding, and

after introduction of the grain a relatively large charge of 30° Bé. liquor is run in until the pan is approximately full and the strike is boiled on water. The finished strike is then fed into the brass centrifuges and the cake washed with hot water to prevent the mother-liquor becoming cooled and therefore supersaturated with respect to hydrate dextrose. The crystals then go to the dryer-granulator from which they are discharged as pure anhydrous dextrose.

W. B. Newkirk<sup>12</sup> hydrolyses the starch as far as possible, filters the liquor through bone-char, and after concentrating, allows it to crystallise at 38° C. with stirring. The mother-



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FIG. 53.—Dextrose dryer.

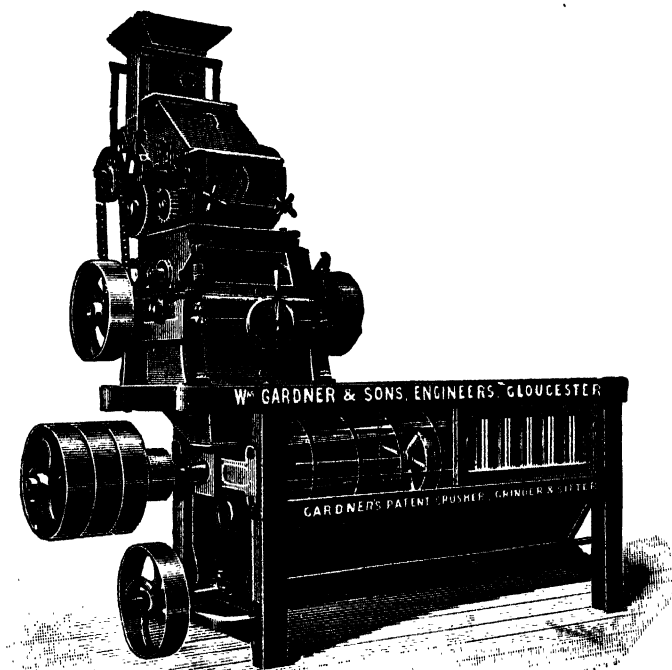
liquor is separated by centrifuging, and the crystals, after washing with water, should have a purity of 99-100 per cent.

**Uses of Glucose.**—Most of the sales of refined dextrose made from corn sugar in the U.S.A. is absorbed by the food industry. The free use of dextrose in the U.S.A., without declaration on the label, was authorised by the Secretary of Agriculture in 1930.

Many canned goods are improved by using pure glucose<sup>2</sup> instead of sucrose. The optimum amounts used vary from 20-50 per cent. of the total sugar present; it cannot be used in concentrations over 50 per cent. because it crystallises out. Canned prunes, plums, rhubarb and apple sauces, tomato juice, sweet pickles, sweet and sour cherries (excluding black, sweet varieties), apricots, and some varieties of peaches are improved by

20-40 per cent. of 'Cerelose.' With peas, beets, apple butter, strawberry and pineapple preserves, citrus marmalade, cranberry sauce, currant, apple and quince jellies, some of the sucrose may be replaced by pure glucose without impairing the flavour or quality.

The addition of 1 per cent. pure glucose to cucumber pickle brines accelerates fermentation, produces a higher final acidity, and aids in maintaining an active flora of lactic bacilli.



[Reproduced by courtesy of Messrs. Wm. Gardner & Sons, Ltd.]

FIG. 54.—Sugar grinding and sifting machine.

Glucose is also widely used in the manufacture of confectionery, mixtures of glucose and sucrose being 'boiled' together until the correct properties have been imparted to the mass, which is then coloured with pure dyestuffs and formed into 'sweets.' According to the type of boiling, and the process to which the boiled sugar is subjected, boiled 'sweets' and those of the fondant type are produced. Recently, beet sugar has been widely employed instead of corn glucose, with excellent results when care has been taken to make slight adjustments in the process to allow for the greater content of sulphur dioxide in the beet sugar.

Further uses of glucose are as a conditioning agent in dressings and sizes, and in adhesives.

Glucose is also used as the starting-point for the manufacture of certain industrial chemicals. Gluconic acid is obtained by fermenting glucose solutions with the culture of *Penicillium leteum purpurogenium*. The conditions of the action, e.g. pH value of the medium, are carefully regulated, and a 60 per cent. yield of gluconic acid is obtained in a manufacturing cycle of 11 days. P. A. Wells and G. E. Ward<sup>31</sup> have given an excellent résumé of the uses of glucose in the fermentation field in which they deal with bacterial fermentation to yield lactic acid and the production of gluconic acid by submerged and surface fermentations. It is of interest that by submerged fermentation, using rotary fermenters, with an air pressure of two atmospheres they found *Aspergillus niger* gave a practically quantitative conversion of 20 per cent. glucose solutions to gluconic acid in 24 hours instead of the more usual 60 per cent. yield obtained in 10 to 12 days by the usual methods. The electrolytic method of producing gluconic acid has now been displaced in America by the fermentation process, and in 1939 some 500,000 lb. of calcium gluconate was produced in the U.S.A. A. J. Moyer and co-workers<sup>32, 33</sup> has dealt at some length with the production of gluconic acid by the method of submerged growth under increased air pressure; and according to some recent work by the U.S. Dept. of Agriculture the addition of a small amount of boric acid or borax promotes the biochemical reactions partly by keeping the calcium gluconate in solution. Kojic acid is produced in 50-60 per cent. yields by the action of *Aspergillus flavus* on glucose solutions.<sup>31</sup> Recently,<sup>18, 25</sup> sorbitol and mannitol have been manufactured from glucose by an electrolytic method, and the former is already finding an outlet as a humidifying agent, softener and plasticiser. Citric acid has also been manufactured from glucose.<sup>19</sup>

**The Manufacture of Maltose.**—By treating starch paste with malt diastase, maltose may be obtained in a yield of about 80 per cent., 20 per cent. of dextrans being produced simultaneously. This enzyme action was first recorded by Irvine in 1785, but it was not until 1819 that de Saussure<sup>20</sup> isolated maltose from the products of this reaction and obtained the sugar in a crystalline form. Later, it was re-examined by Dubrunfaut,<sup>21</sup> who named it 'maltose.' These observations passed unnoticed until 1872, when C. O'Sullivan<sup>22</sup> rediscovered it and carried out further work.

Dubrunfaut, after his initial examination of the reaction, worked with Cuisinier and together they patented a process for preparing

maltose in a crystalline state or as a syrup, these products being intended for use in distilleries and breweries for sweetening wines, making liqueurs, or for brewing. In their method a starch paste was prepared by heating starch and water together, and this was treated at 70-80° C. with 5-10 per cent. of a 'green' malt infusion in order to liquefy it; the mass was slightly acidified with hydrochloric acid and converted to a maltose-dextrin mixture at 50° C. with from 5-20 per cent. of a diastase preparation. The solution was filtered and allowed to stand for 15 hours, concentrated, refiltered and then further concentrated. After a treatment with animal charcoal, the liquid was crystallised.

As these workers used crude raw materials, such as ground maize, the pastes obtained in their process were too thick to handle easily and, owing to the difficulty of obtaining a homogeneous distribution of the diastase preparation and of the 'green' malt, excessive amounts of these had to be employed to carry the reaction to the required stage in an economic time. The presence of extraneous substances in the flours used also contributed largely to difficulties in refining the syrups obtained. In order to get thin fluid pastes in the first instance, weaker starch pastes had to be employed, which led to excess costs for evaporation. These factors, in addition to the competition from glucose, which by then was being produced by the well-established and economic saccharification of starch by acids, contributed to the commercial failure of the process.

A better process is that introduced by Duryea<sup>20</sup> in which a thin-boiling starch solution of 16° Bé. is treated with malt extract at 59° C.

Ptyalin, an enzyme present in saliva, also rapidly effects the conversion of starch into maltose; pancreatin has a similar action, but the reaction is carried further to produce glucose.

To prepare pure maltose, barley diastase may be used instead of malt diastase, with the consequent avoidance of the formation of alcohol-soluble dextrans, which would cause trouble in the purification processes.<sup>23</sup> Pure maltose may be obtained<sup>24</sup> by digesting crushed barley with 2-4 parts of 20 per cent. alcohol for 24 hours, filtering off the liquid and precipitating the diastase by the addition of twice its volume of absolute alcohol. The precipitate is washed with absolute alcohol, then with ether, and finally dried *in vacuo* over sulphuric acid. Fifty grams of starch are heated with 1600 ml. of water, and the paste treated with 1 gram of the diastatic preparation for 5-6 hours at 50° C., the temperature being allowed to fall to room-temperature at the end of this time, and the action allowed to continue for a few more

hours. After evaporating the product to a syrup, it is poured into industrial spirit until the strength of the latter has fallen to about 80 per cent. The precipitated dextrans, etc., are filtered off and the liquid evaporated to a thick syrup, which is seeded with a crystal or two of pure maltose and then set aside to crystallise. The crystals so obtained may be further purified by washing with absolute alcohol, redissolving in a little water, retreating with industrial spirit, filtering, and evaporating. The resulting maltose readily crystallises from strong aqueous solutions. Maltose has no extensive industrial uses.

## REFERENCES

1. T. DE SAUSSURE, *Bull. Pharm.*, 1814, **6**, 499.
2. C. R. FELLERS, *Div. Agric. Food Chem. Symposium, Amer. Chem. Soc.*, 1937.
3. W. EKHard, *Zeit. Spiritusind.*, 1923, **46**, 228.
4. E. PREUSS, *ibid.*, 1904, **27**, 478.
5. E. PAROW, *ibid.*, 1922, **45**, 229.
6. C. B. DURYEY, E.P. 11,800 and 11,801, 1907.
7. A. SEYBERLICH and A. TRAMPEDACH, E.P. 8,000, 1885.
8. F. MALINSKY, *Zeit. Spiritusind.*, 1899, **22**, 240.
9. H. J. HADDON, E.P. 6,176, 1882.
10. C. B. DURYEY, E.P. 280,680, 1927.
11. E. PAROW, *Zeit. Spiritusind.*, 1905, **28**, 121.
12. W. B. NEWKIRK, U.S.P. 1,471,347, 1923.
13. H. BERLIN, *J. Amer. Chem. Soc.*, 1926, **48**, 2627.
14. M. S. BADOLLET and H. S. PAINE, *Ind. Eng. Chem.*, 1927, **19**, 1245.
15. C. R. FELLERS, J. MILLAR, and T. ONSDORFF, *ibid.*, 1937, **29**, 946.
16. W. KRÖNER and H. KOTHE, *Zeit. Spiritusind.*, 1937, **60**, 191, 199 and 207; *ibid.*, 1938, **61**, 209, 217 and 227.
17. — *Ind. Eng. Chem.*, 1939, **31**, 248.
18. R. L. TAYLOR, *Chem. Met. Eng.*, 1937, **44**, 588.
19. CHAUNCEY CHEM. CO., F.P. 819,279.
20. T. DE SAUSSURE, *Phil. Trans. Roy. Soc. Lond.*, 1819, **109**, 29.
21. A. P. DUBRUNFAUT, *Ann. chim. d. phys.*, 1847, **21**, 178.
22. C. O'SULLIVAN, *J. Chem. Soc.*, 1872, **25**, 579.
23. J. L. BAKER and F. DAY, *Brit. Assoc. Rep.*, 1908, 671.
24. C. J. LINTNER, *J. prakt. Chem.*, 1886, **34**, 386.
25. L. LIGHT, *Chem. Age*, 1936, **34**, 531.
26. C. B. DURYEY, *J. Ind. Eng. Chem.*, 1914, 419.
27. E. C. R. MARKS, E.P. 291,991, 19/8/1927.
28. H. KOTHE and W. KRÖNER, *Zeit. Spiritusind.*, 1939, **62**, 191, 197, 205, 213.
29. — *ibid.*, 1939, **62**, 245, 253.
30. L. E. STOUT and C. G. RYBERG, *Ind. Eng. Chem.*, 1939, **31**, 1451.
31. P. A. WELLS and G. E. WARD, *ibid.*, 1939, **31**, 172.
32. A. J. MOYER *et al.*, *Ind. Eng. Chem.*, 1937, **29**, 777.
33. — *ibid.*, 1937, **29**, 653.
34. R. C. ERNST, C. E. BROWN and J. B. TEPE, *ibid.*, 1939, **31**, 1247.

35. W. B. NEWKIRK, *Ind. Eng. Chem.*, 1936, **28**, 760.
36. — *ibid.*, 1939, **31**, 18.
37. — *ibid.*, 1924, **16**, 1173.
38. C. E. G. PORST and N. V. S. MUMFORD, *ibid.*, 1922, **14**, 217.
39. F. SOXHLET, *J. prakt. Chem.*, 1880, **21**, 242.
40. A. BEHR, *Ber.*, 1882, **15**, 1104.

## ADDITIONAL REFERENCES

- H. F. BAUER, *Eighth Int. Congr. Appl. Chem.*, 1912, **13**, 21. (Preparation of chemically pure glucose from commercial product.)
- E. PAROW, *Zeit. Spiritusind.*, 1921, **44**, 177, 187. (Discusses American technique for making glucose.)
- J. K. DALE, *Chem. Age*, 1923, **31**, 295. (Manufacture of glucose.)
- A. E. WILLIAMS, *Ind. Chemist*, 1930, **6**, 495. (Manufacture of glucose.)
- W. B. NEWKIRK, E.P. 246,098, 11/8/1925. (Production of crystalline dextrose.)
- C. B. DAVIS, U.S.P. 1,618,148, 1927. (Colloidal tannate of iron added to precipitate electropositive colloids.)
- W. R. FETZER (to Union Starch and Refining Co.), U.S.P. 2,210,659, 6/8/1940. (Some of the glucose returned to next batch to reduce reversion products formed.)
- CORN PRODUCTS Co. LTD., E.P. 536,020, 1941. (Glucose produced from potatoes without first separating the starch.)

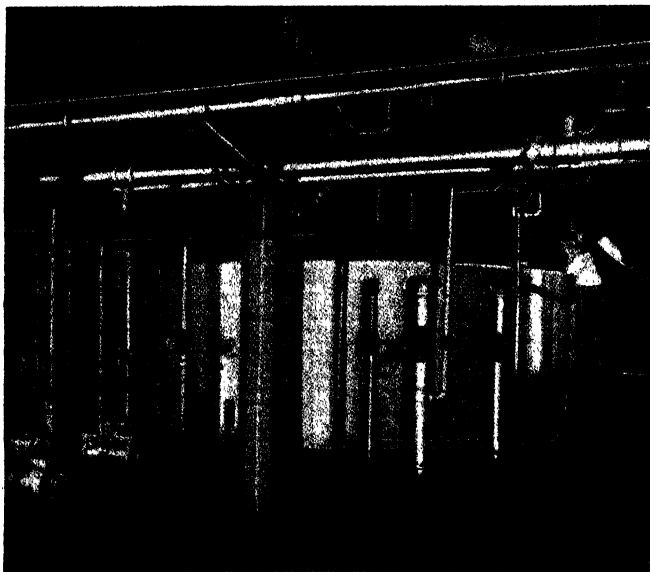


## CHAPTER 5

## ETHYL ALCOHOL AND ACETONE

**The Manufacture of Ethyl Alcohol.**—For the preparation of ethyl alcohol on a commercial scale the cereals, barley, maize, rye, oats, wheat or rice are treated to transform the starch into sugar, which is then fermented to yield alcohol.

A 30 per cent. suspension of one of the cereals is steamed, under a pressure ranging from 30-60 lb. per square in., or even



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FIG. 55.—A mash tub.

higher, to gelatinise the starch, and on cooling to 50-55° C., about 4 per cent. of malt is added to saccharify it. After 1 to 3 hours the conversion is complete, as shown by the absence of the blue coloration when iodine is added to a test portion, and at this point the temperature is raised to 80° C. by the introduction of live steam. This has the disadvantage of destroying the diastase of the malt and of raising heating costs, but its object is to destroy air- or water-borne organisms which would introduce side reactions tending to lower the yield of alcohol and bring extraneous

substances into the wash, so necessitating extra care in the separation of the pure alcohol. The greatest precautions against infection by such organisms and absolute cleanliness should be observed throughout the process.

To destroy these micro-organisms and yet retain the diastase in an active condition for the further work it has to do, ammonium bifluoride or hydrofluoric acid is added to the wash. The wort is separated from the solid matter, and to every 100 gallons of liquor at  $17-21^{\circ}\text{C}$ . in the fermentation vat, 4-5 lb. of a freshly prepared yeast culture is added and the temperature allowed to rise to within the optimum limits, i.e.  $26-30^{\circ}\text{C}$ . The reaction



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FIG. 56.—Fermenters in a modern distillery.

is completed within 2 days, although in practice an extra day at about  $26^{\circ}\text{C}$ . is added. The wash is then ready for distillation.

In the older system the starch was hydrolysed and saccharified by the action of sulphuric or hydrochloric acid, and after adjusting the hydrogen-ion concentration, was submitted to fermentation. Owing to the formation of stable dextrans, however, this process does not give high yields, and it is now more usual to hydrolyse and saccharify by the use of 'green' malt. A. Boidin<sup>1</sup> pointed out the difficulties of handling stiff starch pastes and of obtaining homogeneous incorporation of the converting agent, and he overcame them by adding to the mash sufficient acid to convert the secondary acid phosphates present into primary acid phosphates. This procedure is based on the work of A. Fernbach.<sup>2-3</sup>

Fernbach and his co-workers<sup>2-5</sup> found that the viscosity of

starch paste was influenced by other compounds co-existing in the cereal, among which secondary phosphates are especially potent.<sup>5</sup> Fernbach<sup>2-4</sup> also found that the optimum  $pH$  value for the saccharification of starch with malt extract lies between 4.4-4.5, which corresponds to the conversion of the secondary to primary phosphates. The mash is therefore brought to a  $pH$  value between 4.4 and 4.5 by the addition of acid until it is neutral to methyl orange. Boidin found that at this  $pH$  value a much paler mash is obtained; hence adjusting the  $pH$  value of the mash to 4.4-4.5 with hydrochloric acid gives greater fluidity, optimum conversion, and a paler mash.

It has been suggested that in the fermentation of the mixture of stable dextrin and maltose with yeast, the maltose is converted to alcohol, and as the conversion proceeds the malt enzymes attack the stable dextrin, forming more maltose which is, in turn, fermented. This point has not yet been satisfactorily established. H. Pringsheim and W. Fuchs<sup>6-7</sup> consider that the yeast contains an amylase which itself converts the stable dextrin to maltose, or acts as a co-enzyme to the malt enzyme in this conversion. This explains why the general practice is to add ammonium bifluoride instead of heating the wort to 80° C., which would destroy the diastase. Side reactions can also be reduced by the addition of lactic or other organic acids, but the presence of a buffer is necessary, because any free acid would inhibit the conversion of the stable dextrin to maltose.

**The Amylo Process.**—A. Collette and A. Boidin patented a process in 1898 whereby the conversion could be carried out under aseptic conditions, thus ensuring ease of working and purity of product. In this process, known as the 'Amylo' process, ground maize is mixed with water and about 17.5 lb. of concentrated hydrochloric acid added per ton of maize flour, i.e. sufficient to adjust the  $pH$  value of the suspension to between 4.4-4.5, which is then gelatinised under a pressure of 30-60 lb. The gelatinised mass is then transferred to a covered vat containing hot water in an amount sufficient to raise the final temperature and sterilise the whole; it is then cooled to 30° C. A culture of one of the moulds, *Mucor*  $\beta$ , *Mucor*  $\gamma$ , *Rhizopus Delmar* or *Amylomyces Rouxii* on rice is next added to the sterile mass, then after 24 hours a pure yeast culture, and the fermentation allowed to proceed as in the above processes. The amounts of mould and yeast used are very small, being 1 and 2 parts, or even much less, to 100,000 parts of mash, respectively. This process is now widely used throughout the world, but not in Great Britain, as it precludes compliance with the statutory requirement that the specific

gravity of brewer's wort, which determines the strength of the alcohol formed, shall be determined by a saccharometer before fermentation. The preparation of *samsu* by the Chinese in Malaya is described by R. O. Bishop and G. L. Teik,<sup>23</sup> who note that *Amylomyces Rouxii*, grown on a mixture of starch, soya beans, clay and vegetable tissue, is added to a boiled rice in the proportion of 1 part of the mixture to 3 parts of boiled rice. The yield of alcohol obtained in this manner is 54 per cent. of the theoretical, but this could be improved but at the expense of the flavour and aroma.

The fermented liquor is distilled in a patent, or Coffey, still and finally 'rectified,' the first and final fractions being rejected from stills working a 'batch' process. Wood charcoal is used either before or after rectification to assist in the purification.

**The Production of Acetone.**—Before<sup>8</sup> the war (1914-1918) the United States and Austria were the main sources of supply of acetone, which was produced from calcium acetate, but as the large supplies required for aeroplane dopes and cordite manufacture were far in excess of the amounts available at that time, recourse was made to other sources. Alcohol was employed as the starting material in one process and calcium carbide in another. As these processes involved the intermediate production of acetic acid, which itself was in urgent demand, they were used for the manufacture of the latter, and not for acetone.

Dr. C. Weizmann, in 1915, suggested to the Admiralty the use of bacterial fermentation of maize and other substances containing starch.<sup>10</sup> Fermentation of potatoes with Professor Fernbach's bacteria had previously been used, but the low yields obtained were unsatisfactory; however, with damaged rice as the source of starch, the new process worked very well.

The use of horse-chestnuts was also suggested, and they were found fairly satisfactory if they were of good quality, dried to a moisture-content of less than 1 per cent., and ground to a meal as fine and as free from husks as possible.

In the Weizmann process 1 part of acetone and 2 parts of butyl alcohol are produced simultaneously; the latter, having only a restricted industrial use, was converted by a catalytic process to methylethyl ketone, which in the pure state is as good as acetone for cordite manufacture.

The Weizmann culture is used to inoculate a sterilised medium made up of 1000 ml. of wort, sp. gr. 1.008, containing 1 per cent. gelatine, 1 per cent. calcium carbonate, and 2 per cent. agar. Full details of preparing other convenient culture media for factory use are given by A. Gill.<sup>10</sup> One hundred millilitres of

culture media is used to inoculate the contents of a 3-gallon pail, care being taken to avoid infection during this process, and after 24 hours this culture is used to inoculate a 'seed' tank. The seed tanks are of the enclosed type capable of withstanding either vacuum or steam pressure; they are made of iron, and are of 800-3000 gallons capacity. The operation is carried out as follows: 784 lb. of finely-ground maize are mashed, made up to 1300 gallons, run into the cooker, and boiled with live steam for 1 hour. The pressure is then raised to 30 lb. for 2 hours, and the mass blown over into the sterilised seed tanks, having been cooled to 37° C. on the way. The volume of the mash is now about 1600 gallons. The tanks are then inoculated with the 'pail culture,' 3 gallons of this being added to every 800 gallons of mash. The 'seed' is generally ready 16 hours later.

The fermentation mash is prepared in the same way as the seed mash, but 6 per cent. maize is a preferable concentration, and up to 10 per cent. has been used successfully. The mass is blown over to the fermentation vats, which have been previously sterilised, neutralised with caustic soda or milk of lime, 200 gallons of seed added to 3000 gallons of fermentation media (6.6 per cent.), and the fermentation allowed to proceed. It is generally complete in 30-36 hours, when carbon dioxide and hydrogen cease to be evolved; in a good fermentation this gas evolution falls suddenly. The fermented mash is then distilled as soon as possible, because on standing, even for a short time, the acidity of the liquid rises, and there is a loss of acetone and butyl alcohol.

In maize-mash fermentation there is a residue of 13 per cent. of solid matter, which is high in oil and protein-content and can be used as cattle food in admixture with carbohydrate-containing materials. Owing to the price of raw materials, the success of the process appears to be more assured abroad than at home, as the chief sources of raw materials here are potatoes and horse-chestnuts.

J. H. Northrop, L. H. Ashe, and R. R. Morgan,<sup>9</sup> in an attempt to eliminate the production of butyl alcohol in Weizmann's method, have elaborated a process for producing acetone and ethyl alcohol by the use of an organism *B. acetoehtylicum* to ferment beet molasses containing 1.05 gram of sugar per ml. molasses. Before fermentation, the molasses is diluted with fifteen times its volume of water and heated under 15 lb. pressure for 4 hours. The mass is cooled to 40° C. and inoculated with 12½ per cent. of fermented mash, prepared 24 hours previously. As the bacteria tend to form a slime at the bottom of the vat during the fermentation, the tank is filled first with inert material,

such as brush or limestone chips. The fermented mash is run over into the still by displacing it with fresh mash flowing into the bottom of the tank, and a continuous process less liable to contamination by air-borne bacteria is thus assured. The pH of the mash is brought to between 8.5 and 9.5 by the addition of lime, and as the fermentation proceeds this figure falls to about 6.0.

In this method about three times as much ethyl alcohol as acetone is produced, the yield of the mixed liquid being about 33 per cent. on the volume of the molasses. Small amounts of propyl and butyl alcohol are also produced.

Papers dealing with the preparation of other alcohols and related compounds will be found in the references.<sup>11-22</sup>

## REFERENCES

1. A. BOIDIN, *Compt. rend.*, 1906, **143**, 511.
2. A. FERNBACH, *Ann. Brasserie*, 1899, **2**, 400, 433, 457.
3. — *ibid.*, 1900, **3**, 324.
4. A. FERNBACH and L. HUBERT, *Compt. rend.*, 1900, **131**, 293.
5. A. FERNBACH and J. WOLFF, *ibid.*, 1906, **143**, 38.
6. H. PRINGSHEIM and W. FUCHS, *ibid.*, 1906, **143**, 380.
7. — *Ber.*, 1923, **56**, 1762.
8. F. NATHAN, *J. Soc. Chem. Ind.*, 1919, **38**, 271T.
9. J. H. NORTHROP, L. H. ASHE, and R. R. MORGAN, *J. Ind. Eng. Chem.*, 1919, **11**, 723.
10. A. GILL, *J. Soc. Chem. Ind.*, 1919, **38**, 271T.
11. H. A. LEVY, *Ind. Eng. Chem.* (News Ed.), 1938, **16**, 326. (Glycerol.)
12. E. SIMON and C. WEIZMANN, *Enzymologia*, 1937, **4**, 169. (Acetone-butanol.)
13. H. R. STILES, U.S.P. 2,098,199. (Acetone-butanol fermentation.)
14. K. BERNHAUER, "Gährungschemisches Praktikum," J. Springer, Berlin, 1936. (Fermentation analyses and technique.)
15. A. M. SIMSKAYA, *Biokhimiya*, 1936, **1**, 603. (Acetone-butanol fermentation.)
16. E. SOTNIKOV, Russ. P. 43,649, 1935. (Citric acid from barley.)
17. J. C. WOODRUFF, H. R. STILES, and D. A. LEGG, U.S.P. 2,089,522. (Acetone-butanol fermentation.)
18. D. A. LEGG and H. R. STILES, U.S.P. 2,089,562. (Acetone-butanol fermentation.)
19. R. NAKAZAWA and others, *J. Agric. Chem. Soc. Japan*, 1937, **13**, 815. (Alcohol from sweet potatoes.)
20. W. SCHWARTZ, *Zeit. angew. Chem.*, 1937, **50**, 294. (Formation of fats from starch.)
21. L. A. UNDERKOFER, E. L. FULMER, and M. M. RAYMAN, *J. Ind. Eng. Chem.*, 1937, **29**, 1290. (Acetone-butanol fermentation.)
22. L. DEMIDCHUK and E. BELINSKAYA, *Brodilnaya Prom.*, 1936, **13**, 9; via *Chem. Zentr.*, 1937, **1**, 1043. (Spirit vinegar from starch.)
23. R. O. BISHOP and G. L. TEIK, *Malay. Agric. J.*, 1928, **16**, 14.

## ADDITIONAL REFERENCES

- M. A. TUBANGUI *et al.*, *Philippine J. Sci.*, 1939, **70**, 123. (Fermentation of cassava for production of acetone and *n*-butyl alcohol.)
- KILP, *Zeit. Spiritusind.*, 1930, **53**, 71. (Discusses use of potato flakes in alcohol manufacture.)
- W. J. EDMONDS, E.P. 268,749, 14/3/1927. (Acetone-butanol anaerobic fermentation.)
- I. G. FARBENIND. E.P. 282,347, 1927. Addition to E.P. 269,950. (Dihydroxyacetone fermentation in which extractive substances from spent brewers grains used in nutrient medium.)
- G. W. FREIBURG, E.P. 237,228, 1925. (Acetone-butanol fermentation.)
- J. RAUX, *Brasseur franc.*, 1940, **4**, 7. (Discusses use of cassava starch to replace rice and maize starches in brewing industry.)
- E. LÜHDER, *Z. Spiritusind.*, 1931, **54**, 7. (Discusses manufacture of alcohol from potato flakes.)
- S. SUGIZAKI, *J. Agr. Chem. Soc. Japan*, 1940, **18**, 281. (Discusses alcoholic fermentation of acorns with *Rhizopus javanicus*.)
- ANON, *Chem. Age*, 1940, June 1st, 301. (Alcohol from potatoes.)

## CHAPTER 6

## DEXTRIN AND BRITISH GUMS

DEXTRIN, as understood commercially, consists of the degradation products obtained by treating starch in a variety of ways. It is liable to contain soluble starch and sugar, according to its degree of conversion, some dextrans approaching soluble starch in their composition, others being not far removed from glucose. Dextrin appears on the market in several forms: as powders varying in colour from white, through yellow, to brown; as granulated particles resembling gum senegal in appearance; as thick, viscous, coloured liquids or as a white paste. All of these contain a mixture of true dextrans.

In 1811 Kirchhoff<sup>15</sup> obtained a gummy substance by heating starch in the presence of an acid, but the production of dextrin by roasting starch appears to be due to B. Lagrange, who published his method in the same year that Kirchhoff reported his work on the production of starch sugar and dextrin by the action of acid on starch. Later, the treatment of starch with sulphuric acid was further investigated by Biot and Persoz,<sup>16</sup> who examined the products obtained and gave to the gummy material they separated the name 'dextrin,' because of the direction of its optical rotation. The product known to the trade as 'British gum' had been discovered previously, according to authentic sources, owing to a fire breaking out in a Dublin textile mill in which starch was stored. The brown-coloured powder which was left after the fire had played on the sacks was found to be soluble in water and to give a sticky solution. It was then discovered that the same result could be obtained by heating the starch in an iron pan.

Scientifically classified, dextrans are the degradation products of starch having the same empirical formulæ as the original starch, i.e.  $(C_6H_{10}O_5)_n$ . In starch the value of  $n$  is fairly large, but in dextrans it progressively decreases as the degradation of the starch continues. The pure dextrans are soluble in water, insoluble in alcohol, and with iodine solution give either a red or a brown coloration, or no colour at all.

The physical properties of dextrans vary greatly with the method of treatment.<sup>1</sup> Some, when dissolved in water, give thick, viscous pastes, others thin flowing liquids; some dry quickly when exposed to the air, whilst with others the process takes place slowly; with some the viscosity of their solutions does not alter



appreciably on storage, whereas solutions of others show a decided increase in viscosity with age, and also the phenomena of 'retrogradation' or reversion, becoming cloudy or pasty according to the degree of reversion. Generally, the further the conversion is carried the more stable is the resulting dextrin and the less it tends to show reversion, although this applies less to mixtures containing caustic soda and borax.

It will be readily seen that there is no absolute method for referring to an industrial dextrin. Users are accustomed to order the dextrin they require according to the trade- or code-number of their supplier, and if they wish to change their supplier, they have to submit a sample of the required dextrin to the proposed new supplier for matching purposes. A more definite classification has long been wanted, and the author has found that if the starch from which the dextrin is made is specified, as well as the viscosity at 50° C. of a 1-1 solution of the dextrin in water, a better and more exact description is obtained. Thus, a yellow potato dextrin of good solubility, sugar-content of 2.5 per cent., moisture-content of 11 per cent., which yields a fairly adhesive solution, would, under this classification, be termed yellow potato dextrin, 57. The dextrin is dissolved in the water at 80° C., making allowance for its moisture-content, and the solution allowed to cool to 50° C., when its viscosity at that temperature is determined. This method was found to work quite well in one factory,<sup>1</sup> but is by no means claimed to be the best that could be evolved, and is open to some objections.

It is sometimes said that the process of making dextrin is highly complicated, and that secret processes are the rule, but such statements are quite unfounded. The process is well known and simple, being more of a craft than an exact science, and experience is required to get the best results. It is this fact, together with the general lack of enthusiasm on the part of makers to give any indication of how they obtain their products, which has probably given rise to the secret-process myth.

**Methods of Manufacture.**—In the manufacture of dextrin the processes used fall naturally under two main headings, the Torrification or Dry Method, and the Wet Method. In processes embraced by the first title the starch is heated, either alone or in the presence of small amounts of a catalyst, generally an inorganic acid; in the second, the starch is suspended in water and heated with a catalyst or, after forming a paste, it is treated with enzymes. The wet method of making dextrans will be dealt with later.

J. R. Katz<sup>34</sup> considers that dextrans and British gums made by

heating dry starch at about 180° C. are closely analogous to those obtained by boiling starch with dilute acids or treating it with amylolytic enzymes, but, as we shall see, their properties may differ widely.

If starch is heated to a relatively high temperature, say about 160-190° C., it gives up its moisture, turns first a yellow and then a brown colour, and is then found to be soluble in water. The product known as 'British gum' has a strong smell, poor flavour, and gives deeply coloured solutions. The addition of a little nitric or hydrochloric acid to the starch before heating allows these reactions to take place at a lower temperature, so that the flavour, odour and colour of the product are markedly improved. Bloede<sup>14</sup> appears to have been the first worker to exploit the acid-roasting process on a commercial scale in America, and in his original patent the starch was sprinkled with nitro-hydrochloric acid and then heated on iron plates in an oven.

In the method involving the use of an acid, the first product formed is soluble starch, and after this three stages, merging into one another, may be distinguished, viz. the formation of amylo-dextrin, erythro-dextrin, and achroo-dextrin, in that order. By continued action of the acid on the last substance dextrose is produced, and by further heating at a high temperature the sugar appears to revert partly to dextrin, but this point has not yet been fully cleared up.

The various stages described above are readily recognised by the colours which their solutions give with a few drops of iodine solution, and this test is one of those used in factory control. When treated with iodine solution, a solution of the soluble starch first formed gives a blue coloration, a preponderance of amylo-dextrin gives a violet, erythro-dextrin a red-brown coloration, and achroo-dextrin a pale brown or colourless solution.

**Raw Materials.**—Starch is generally used for making dextrin, but dried and ground tubers of potato or cassava plants may be employed, in which case a higher temperature, more catalyst, and a longer period of heating are needed in order to obtain the same degree of conversion as with starch; and an additional process of solution in water and filtration is necessary to obtain a usable product.

Maize, potato, and tapioca starches are most used for dextrin-making, and although rice and wheat starches can be used, they offer greater difficulties to conversion without any particular gain in the way of special properties. The starch should be of the finest quality if a good lustrous dextrin is required, especially if the product is to be used in paper-making, where black specks or

discoloration would condemn it at once. The choice of starch depends on the type of product required and the market price of both commodities.

Maize dextrin has quite a distinctive odour and flavour, but for cheap work it is extensively used, especially in the United States. For high-class work potato and tapioca dextrins are well established, and the latter is especially valuable, as it has a slightly greater strength than potato dextrin and at the same time is relatively odourless and tasteless, so that it can be used in adhesives for postage stamps, envelope-flaps, labels, etc., where the bitter taste of potato dextrin would be a disadvantage. Maize dextrins, made by the roasting process, are often used in conjunction with inorganic salts (see Adhesives) for adhesives without the addition of any other type of dextrin; maize dextrins made by enzyme-conversion are used as the basis of certain adhesives to which are added potato or tapioca dextrins to impart desirable adhesive properties; such a composition forms the basis of some of the best photographic mounting pastes on the market (see p. 251).

The mode of preparation of the starch during manufacture sometimes affects the ease of conversion, and it has been asserted that starch free from any traces of sulphur compounds is less liable to give rise to fire hazards than starch containing them. This may or may not be true, but in regard to fire hazards, it should be mentioned that the provision of ample window space in the factory is one of the best methods of minimising damage should an explosion take place.

The rate of conversion to dextrin of a particular starch can be judged with some accuracy from its average grain size—the larger the granules the more readily is the batch converted to dextrin. The starches with the smaller granules, such as tapioca or maize starch, require a higher temperature than potato starch, and this rule appears to hold for individual deliveries of starch. It has been stated that the presence of small amounts of hydrocyanic acid slows down the conversion, especially that of tapioca starch, but as other starches which are known to contain no hydrocyanic acid show variations in ease of conversion from delivery to delivery, this explanation does not seem tenable. The presence of alkali may explain the greater usage of the acid catalyst required in some cases, but this does not explain why some starches cannot be fully converted even in the presence of great excess over that normally used of the hydrolytic agent. It is significant, however, that the lower-grade starches show this effect much more frequently than the high-grade starches, but a good-grade starch

which has been separated by a centrifuge method is likely to contain all the small granules present in the liquor and to show this resistance to conversion into dextrin to a small degree. We have not seen this explanation put forward before and, if the difficulty of gelatinising the smallest starch granules with water is taken into consideration, it appears to fit most of the facts. Another interesting point which probably has some bearing on the above observation is, that batches of starch having a smaller average particle size than usual are more liable to give the unstable type of dextrin which, although its solutions have the correct viscosity when first made, show an increased viscosity on standing and also the phenomenon of retrogradation to a marked degree. Such dextrans will give perfectly clear solutions when dissolved in water, but these solutions on standing for 24-48 hours appear quite pasty or opaque. To overcome this drawback, the time of maturing, the amount of acid catalyst used, or the conditions of roasting employed to produce the type of dextrin required from a normal starch, may have to be adjusted so as to obtain the desired product. As alteration in any of these values is liable to give rise to corresponding changes in the amount of sugar formed, the statement that dextrin manufacture is an art and not a science will be appreciated.

Starch containing sulphites gives a paste which has a lower viscosity than that which has been freed from these compounds, and the first stages of the conversion of such a starch may proceed rapidly.

**The Choice of Acid.**—As already stated, hydrochloric and nitric acids are both suitable for use in dextrin-making, but sulphuric, nitro-hydrochloric, oxalic and phosphoric acids have all been employed, and especially the first two of these. For use in the conversion process, an acid should not leave any residue on evaporation, which in the presence of iron salts is detrimental to the final colour of the dextrin. There is, however, a patent<sup>2</sup> in which certain metallic chlorides and nitrates are added to the acid to check its evaporation during the roasting process, thus shortening the time required. It is claimed that this addition decreases the acid consumption, and that a practically pure white product of very good solubility is readily obtained. In one example in this patent, 100 kg. potato starch are heated for 40 minutes at 120-130° C. with 5 litres of water in which 150 ml. concentrated hydrochloric acid and 20 grams manganous chloride are dissolved. A. E. Williams<sup>3</sup> states that he has tried this process, but that no marked advantage could be obtained. A slight shortening of the time of conversion was noted, but it

would appear that the process is of no special interest, especially as such dextrans cannot be used in food manufacture.

As will be seen later, an indication of the acid used in its manufacture is given by the colour of the dextrin to be matched. The conditions of roasting have to be carefully watched when sulphuric or phosphoric acid is used, but the volatile acids allow some little latitude, and should be given preference as during the heating process they vaporise and penetrate the mass of starch, giving a more uniform distribution of catalyst throughout the mass. Phosphoric and sulphuric acids give products which are inclined to be more than usually hygroscopic. A further point in favour of the volatile acids is that they evaporate during the roasting, and hence the end-point of the roasting can be more readily controlled. The treatment of starch by means of the hot vapours of acids, such as hydrochloric or nitric acid, is sometimes used on a commercial scale. Spraying the hot dextrin with 3-5 per cent. formaldehyde after the acid treatment is finished gives a product which is more soluble but unaffected with regard to viscosity and colour.

The hygroscopicity of a dextrin and a tendency for its solution to be stable or otherwise depend largely on the presence of dextrose, and the amount of dextrose formed during conversion is largely dependent on the acid used and on the time and the temperature of processing. O. Philipp<sup>17</sup> appears to have been the first worker to note that the amount of acid influences the amount of sugar formed in a conversion. The volatile acids give lower values, generally speaking, than the non-volatile acids in this respect. S. P. Aiger<sup>35</sup> mentions that rice starch requires five to six times the amount of acid for conversion as does potato starch.

**Pre-treatment of Starch before Torrification.**—Various processes have been patented and are in use by which the starch is treated in some way before the actual roasting process is carried out. The National Adhesives Co.<sup>4</sup> sensitises the starch to the action of acids by treating with hypochlorite solutions, adjusted to the pH value necessary to give any desired characteristic to the finished product. This process also destroys certain impurities that may be present in the starch and allows of the preparation of finer coloured products than would otherwise be possible. The treated starch appears to contain some combined chlorine, and the presence of calcium chloride acts as a catalyst in the subsequent conversion to dextrin. In certain cases, where a particular type of glue containing calcium chloride is to be made, the presence of this salt due to the pretreatment is immaterial.

The catalyst may also be incorporated in the magma of starch after the chlorination is complete, thus giving a more uniform distribution. The drying and dextrin-formation in this instance may be effected at the same time. In Böhme's patent<sup>5</sup> the main bulk of the starch is swollen before it is mixed with a small amount of powdered starch containing all the catalyst necessary to convert the whole mass to dextrin, and then heated to 105-150° C.

Another process that has considerable vogue is the drying of the acidified starch before torrification; this will be discussed later.

**Main Steps in Dextrin Manufacture.**<sup>6</sup>—The following are the main operations carried out in dextrin manufacture. It will be seen that some of the steps may be omitted, according to the design of the plant or the preference of the operator:—

1. Addition of the catalyst.
2. Maturing (optional).
3. Drying (optional).
4. Roasting or torrification.
5. Cooling.
6. Re-moistening.
7. Grinding and bagging operations.

In some processes the starch is acidified and then roasted immediately, in others the starch is actually acidified in the roasters.

**Addition of Catalyst.**—A small batch of starch may be mixed with the requisite amount of acid, and this acidified powder is incorporated with the rest of the starch.<sup>5</sup> A much more satisfactory method, however, is to spray the acid, by means of an atomiser-jet,<sup>18-21</sup> on to the starch, which is suitably agitated during the addition. Air under pressure—about 20 lb. per square in. suffices—is used to force the acid through the fine jet so that it meets the starch in the form of a very fine mist. The acid is generally first diluted with two to five times its volume of water, and additional catalysts such as inorganic salts may be added to the solution or sprayed on separately, according to their compatibility with the acid.

The amount of acid used is generally smaller for potato starch than for maize or tapioca starches. To obtain a white potato dextrin, about 80 ml. of hydrochloric or nitric acid can be diluted to 300 ml. with water and sprayed on to 100 kg. starch. This amount may have to be increased or decreased according to the particular delivery of starch. For a tapioca dextrin, 200 ml. of either of the above acids is diluted to four times its volume with water and added to the starch; this figure, again, is only an

approximation. Some tapioca starches give a pink shade when nitric acid is added, but others remain white. This affects the final shade of dextrin, and the cause of this colour change has, so far, remained unexplained.

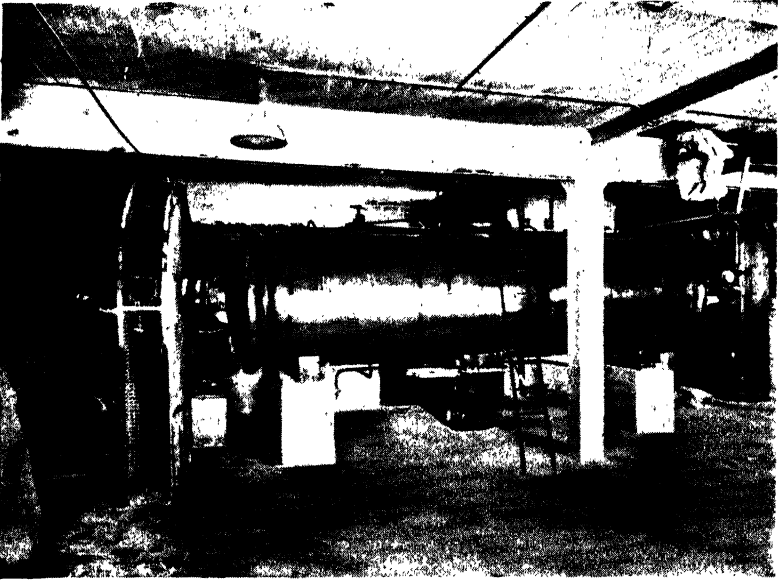
**Maturing the Starch.**—By the term 'maturing' is meant storing the starch so as to allow the catalyst to diffuse thoroughly throughout the mass, and the slow conversion of the treated starch into the soluble modification. When the starch is ready for processing it will rapidly dissolve in water at 80° C., and the longer the maturing, the more quickly will the formation of dextrin take place and the lower will be the temperature required for the conversion. The maturing may take anything from 12 hours up to 5 days to reach the required stage, the starch generally being stored in hardwood bins during this time. A well-matured starch gives a better coloured dextrin, and shows better stability for the same degree of conversion than one which has been inadequately matured. When ready for processing, the starch is withdrawn from the bottom of the bin by a conveyor. This process is by no means always used, but is employed in a number of factories on the Continent and by a few in England.

**Drying the Starch before Roasting.**—This step is quite optional, and many factories omit it entirely. Bloede,<sup>25</sup> by heating and pre-drying starches before roasting, obtained them in a very dry state, and from such dried starches he obtained dextrins that differed from those obtained by roasting the same starches without this special pre-treatment. The moisture in the starch, including that added with the catalyst, is often expelled in the roasters by careful adjustment of the temperature. In the process in which the starch is matured, however, removal of the moisture before the starch reaches the roasters constitutes an important step. If the starch is insufficiently dried, sugar is formed during the roasting in an amount depending on the percentage of residual water. Owing to the rapid conversion which takes place in the roaster, the temperature and time of roasting cannot exceed certain limits, so that the re-conversion of sugar to lower dextrins cannot take place. In the generally used older processes, however, a longer time of roasting is required, viz. some 3 hours, together with a somewhat higher temperature, and thus some of the sugar formed initially appears to be reconverted into dextrin or dextrin-like substances.

Drying may be carried out in the roaster by cautious heating and agitation, or in a vacuum dryer. In the latter method, care must be taken that the temperature of the jacket is not raised too quickly, as when this happens the starch forms lumps coated with

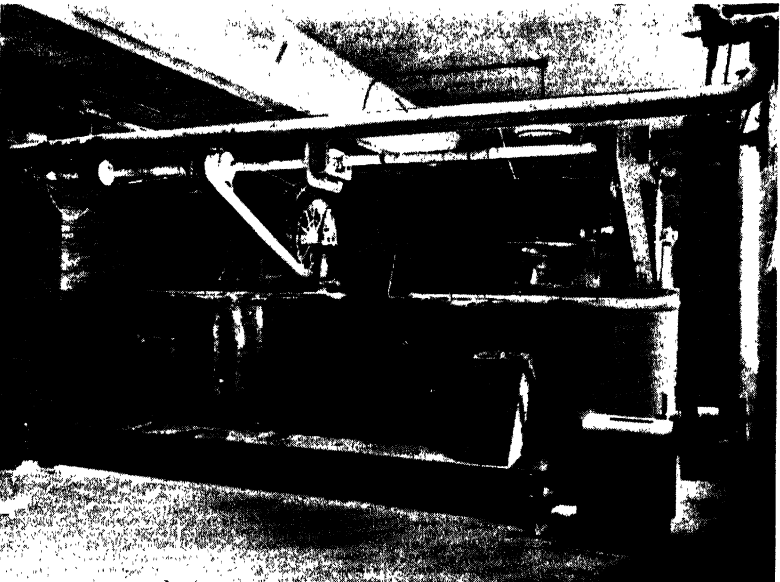






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FIG. 57.—Rotary vacuum dryer, of the batch type, for drying starch.



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FIG. 58.—Hot-plate dryer, consisting of mechanically agitated gas-heated pans for the production of dextrin. This type of dryer or roaster can also be arranged for steam or oil heating.

*[Facing p. 243.]*

a gelatinised starch, thus preventing thorough expulsion of the water. Potato starch seems more liable to this defect than other starches, probably owing to the greater amount of water present in the first instance, and partly to the greater ease with which it gelatinises. It is a good plan to start the vacuum pump about 5 or 10 minutes before the steam is admitted to the outer jacket. The degree of vacuum is generally about 25-26 inches. The temperature in the vacuum chamber is allowed to rise to about 70° C. in about 30 minutes, and then the starch is generally ready to discharge. Some batches of starch are much more easily dried than others, and are ready in 10 to 15 minutes, but the reason for this is unknown to the author. The progress of drying can be watched by means of a gauge in the water-trap fitted to the drier. Some starch coming over as dust also collects in the water-trap; it is removed with the water at the end of each run and collected by sedimentation.

A certain amount of acid, especially when a volatile acid is used as catalyst, is found in this water, and amounts to approximately two-thirds of the quantity of acid added to the starch in the first place. The starch from the dryer is often halfway to the stage of conversion to which it is to be taken, depending on the batch of starch; the content of reducing sugar varies from 1.5 to 3.5 per cent., or even higher, and the moisture-content is about 3 per cent. when it is passed to the roasters.

**The Roasting Process.**—At one time the starch was moistened with the catalyst solution, shaped into bricks, and heated on trays in a heating-room at about 95-150° C. Now, heated pans or vessels are used that are fitted with an efficient stirring device, for one of the main essentials of the process is that the starch should be uniformly heated: at no point should the starch be stationary or the temperature exceed that of the rest of the vessel.

Many factories employ steam-jacketed pans in which to roast the starch, the jackets withstanding a pressure of 100 lb. steam, which gives a temperature sufficiently high for the production of most, if not all, dextrins, but not for British gums. The highest temperature required for a dextrin is about 180° C., whereas for British gums over 200° C. is necessary. Tapioca dextrin requires the higher temperature-range of 140-175° C., whilst maize is often roasted in the region of 125-140° C., and potato starch is converted at temperatures between 105 and 135° C.

Uhland's apparatus contains heated oil in the outer jacket of the dextrinising vessel, and is very suitable for continuous working. Lehmann's dextrinising vessel is air-jacketed, the air-space below it being heated by conduction from a fire, or burners, along

tubes containing water. The pressure within these tubes, which are sealed, is indicated by a manometer, and regulated to about 150 atmospheres. Many factories, however, still employ gas-fired roasters with every satisfaction.

In one process, due to H. Wulkan,<sup>7</sup> about 5 per cent. of the starch is mixed with the total amount of catalyst (0.2 to 0.4 per cent.) to be used, with little or no previous dilution, and then mixed with the remainder of the starch. This mixture is fed through rollers into a steam-jacketed vessel, where it is agitated and moved continuously towards the discharging device through which it is withdrawn as dextrin. As the operation is carried out in a more or less confined space, practically no moisture is lost.

Heating by steam appears to give most satisfaction from the points of view of ease of control and even distribution of heat, and superheated steam has sometimes been employed. In vessels using superheated steam an early trouble was experienced, in that the portion of the roaster near the inlet absorbed more of the heat than the rest of the vessel. Some roasters are now on the market in which this defect is largely overcome by having a number of inlets feeding into small compartments in the outside jacket. These small sections are each fitted with a water-condensate trap, which automatically discharges any water formed.

Another type of roaster is heated by circulating hot oil in the outside jacket, and by this means temperatures high enough to obtain British gums can be obtained. Electrically-heated converters have been suggested, and should prove of value where electricity is cheap. The whole question of design of the roaster is purely one of engineering, in which a sufficiently high and yet evenly distributed temperature is the chief consideration to be kept in mind.

Some makers claim that under a partial vacuum the torrifaction process is speeded up, whilst others claim that the same object is achieved by roasting in closed vessels owing to the pressure set up by the liberated moisture. Krause<sup>8</sup> considers that by removing the vapours from the surface of the material in the roaster the water is more rapidly eliminated and a better conversion is obtained, and he has designed a simple mechanism to this end. Roasting in an open vessel appears to be conducive to a lower sugar-content, owing to the moisture being able to pass away freely as it is given off. The author has not observed any speeding up of the process by the use of partial vacuum, but has found that the final sugar-content is lower than when the roasting is carried out at normal pressures.

By roasting under pressure, the conversion can be carried out

at a lower temperature than at atmospheric pressure, and in the absence of a catalyst, but it is possible that some of the compounds liberated in the conversion of the starch to dextrin play a part. Under pressure dextrin forms rapidly, but the final product contains too high a proportion of sugar for some purposes, e.g. for the making of certain adhesives. Well-defined derivatives, the so-called achroodextrins, which are good adhesives, are obtained, according to Haake,<sup>9</sup> by mixing 100 kg. potato starch with 0.2 per cent. hydrochloric acid, and exposing the mixture for 2 or 3 seconds to a pressure of about 2500 kg./cm.<sup>2</sup> without cooling. In this manner it is claimed that a very light-coloured product is obtained.

H. E. Bode<sup>36</sup> dries the starch under vacuum, and after treating with a water-immiscible liquid such as propylene dichloride in the absence of air, the starch is suspended in an oil and heated until converted to dextrin.

Generally speaking, the lower the acid-content of the 'mix' the higher the temperature employed for roasting to obtain the same degree of conversion, and in making white dextrins care must be taken to see that the colour does not turn yellow, due to some starch grains being very easily degraded at the higher temperature required to convert the bulk of the mass. A high acid-content, inasmuch as it assists conversion, is generally conducive to a high sugar-content. As mentioned elsewhere, the content of sugar increases to a maximum, sometimes to as much as 10 per cent., and then falls to the final value found at the end of the conversion. The longer the time and the higher the temperature the lower the sugar-content, a result that seems due in part to reversion of the sugar to dextrins, or substances akin to them, and in part to the evaporation of the catalyst.

If a sample to be matched contains more than 2 per cent. sugar, it may be inferred that it has been made by an acid process; the amount of sugar in excess of this figure appears to run roughly parallel to the amount of acid used in the conversion.

In the manufacture of dextrin, when the temperature of the roaster has reached about 110-115° C. (or about 125° C. in the case of British gum) a small amount of retained moisture will come off as steam above the heated mass; but suddenly there will be an evolution of a cloud of steam, the temperature of the mass will rise by 5-10° C., and dextrinisation proceeds very rapidly. It would appear that some sort of anhydride formation with the elimination of water occurs at this point. No reference appears to have been made to this phenomenon in the literature of the subject although it must have been observed, as it is very marked.

When tests, to be described more fully below, show that the conversion has reached the desired stage, the hot dextrin is discharged into the cooling apparatus.

M. D. Rozenbroek<sup>37</sup> has covered a process in which air-dry starch is treated with a gaseous acid so that some 0.5 per cent. is absorbed on the starch, and after allowing the starch to lie in the acidified state for some 12 hours it is 'roasted' at temperatures below 80° C., care being taken to maintain the moisture-content at that of the air-dry material.

**Cooling and Re-moistening the Dextrin**—To prevent the conversion of the dextrin proceeding further than is desired, it is cooled immediately it is discharged from the roasters. Uhland's apparatus for this purpose consists of shallow, circular iron pans one above the other, each having a stirrer attached to a common shaft, which passes through the centre of all the pans. The dextrin is swept by each stirrer to the pan below, and the whole is cooled by a current of air.

In another apparatus (see Fig. 59) the dextrin falls into a long cylinder, slightly inclined to the horizontal, which is cooled by water passing through an outer jacket, and is moved by a stirrer towards the discharge door, through which it passes to storage bins to await re-moistening. Still another apparatus is a tower in which the falling, hot dextrin meets a counter current of cold, damp air.

Dextrin, after roasting, is a very hygroscopic powder. As sold, it contains from 8 to 14 per cent. of moisture, and the further it has been converted the less water will it contain. In some factories the moisture is put into the dextrin by spreading the powder on to trays and exposing it on a rack to the air. This method requires a lot of floor space. Another method is to use some sort of apparatus in which the fine powder falls from a height against a counter-current of moist air,<sup>13</sup> or through a very fine mist produced by spraying water under about 5 lb. pressure through an atomiser-jet.

In one of these machines the dextrin can be re-moistened in 2-3 days, whereas by the tray method some 5-14 days may be required (see p. 421).

A further use for an apparatus of this type is that for spraying a chemical in aqueous solution which it may be desired to add to the dextrin. Potato dextrin, and other dextrans to a less extent, have an odour that is objectionable to some people. According to H. Kunz-Krause,<sup>10</sup> this odour is due to a non-volatile cyclic ester of myristic acid, dextrinozol, which can be separated from the dextrin by a steam-distillation and can be obtained by the extrac-

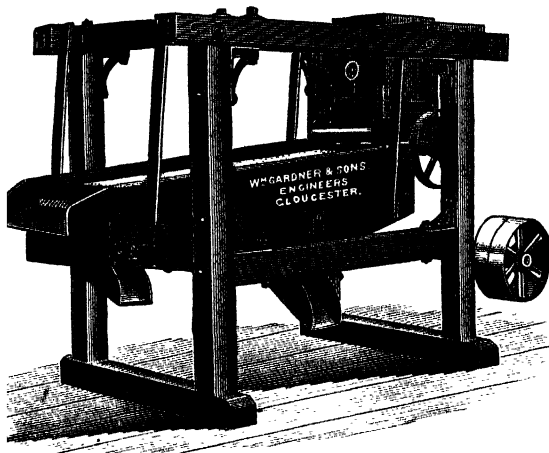


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Fig. 59.—Continuous rotary cooler for dextrin.



tion of the distillate with petroleum ether. In this connection it is interesting to note that A. Payen<sup>11</sup> as long ago as 1846 obtained a yellowish oil by distillation of saccharified starch with dilute sulphuric acid which had an intense odour and to which he attributed the odour of starch.

To render this smell less apparent or to destroy it, Pieper<sup>12</sup> proposes to pass ozonised air through the roaster during the conversion. If a small amount of sodium sulphite or bisulphite, or hydrogen peroxide, is added to the water in the re-moistening apparatus the odour and colour of the resulting dextrin are very much improved. The sulphur compounds also act as preservatives for solutions of the dextrin, but unfortunately tend to impart



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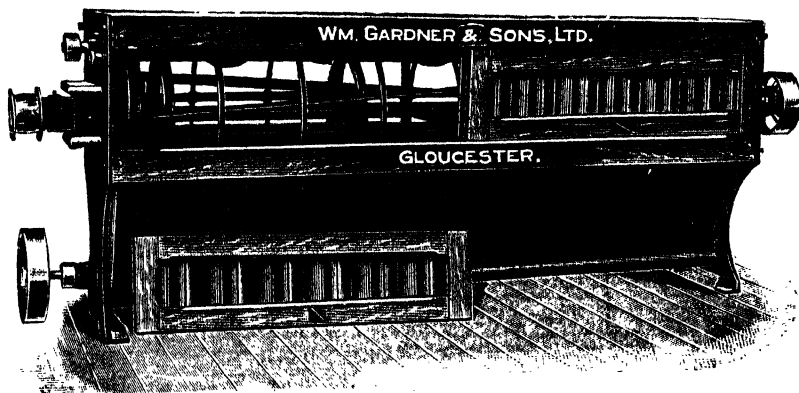
FIG. 60.—A shaking sieve.

a 'ropiness' to the gum so made. A further drawback to the use of reducing compounds for this purpose is that many dextrans are used in alkaline solutions, and the addition of alkali to the dextrin bleached in this manner restores its original colour. The use of oxidising agents for this purpose appears to be preferable, as the colour is not restored to anything like the same extent as when it is bleached with reducing agents.

**Grinding and Bagging-off Operations.**—If re-moistened in one of the machines mentioned above, the dextrin invariably contains lumps resulting from the presence of excess water. It is therefore ground in a mill and passed through a rotary sieve (Fig. 61) covered with silk of the required mesh, any lumps and coarse particles being automatically returned to the mill for further grinding. A shaking sieve (Fig. 60) is sometimes used for this



purpose. The powder falls into bins from which it may be transferred automatically to sacks. The dextrin thus obtained is often blended with other batches in order to obtain uniformity of output or to obtain products which have special, desirable properties that are not readily attainable by adjustment of the catalyst or of the roasting conditions employed. The average yield of dextrin from 100 tons of starch is about 90 tons.



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FIG. 61.—Sifting and bolting machine.

#### **Conversion of Starch to Dextrin by the Wet Process.—**

A very important process for the manufacture of dextrin is that involving the treatment of starch suspensions with acids or hydrolytic agents, the so-called wet process. The dextrins so obtained are always used, either for textile or for adhesive purposes. As previously mentioned, the sugar formed by the action of enzymes is maltose, whereas the action of acid to produce dextrins gives a certain amount of dextrose, which is more hygroscopic than maltose, and if present in excessive amounts causes cracking of the adhesive film with a consequent lowering of its strength.

**Acid Conversion in the Wet Process.**—Many tons of dextrin made by simply heating an aqueous starch suspension with an acid, such as hydrochloric or sulphuric acid, are made every month and marketed as adhesive pastes. Any of the hydrolytic agents used in the roasting process may be used, and when the hydrolysis has reached the required stage, as shown by viscometric measurements and the colour given with iodine, the action is stopped by cooling, and the acid present is neutralised. Some makers prefer to use acids which give white insoluble salts on neutralisation, such as sulphuric acid or phosphoric acid which

have white insoluble calcium or barium salts, as in this way a good white paste is obtained at the end of the reaction.

The action can be carried out at various concentrations; at the higher concentrations fairly viscous pastes are obtained which, after neutralisation, are strained and packed immediately. At lower concentrations, thin liquids are obtained which are evaporated to the required consistency after the action has been stopped. If the dextrans are to be used in coloured-paper work, it is preferable to use a lower concentration in order to obtain a completely neutral paste at the end of the reaction, the neutralisation being more readily controlled than with a thick paste. To obtain a product with superior odour, colour, and flavour, A. Schumann<sup>26</sup> treats starch with a 1 per cent. solution of an acid at room-temperature for about 24 hours, and after washing free from the acid, suspends it in water containing sulphurous acid; he then heats the suspension under 40-50 lb. pressure until reducing sugar is detectable. The pressure is then released, the solution of dextrin passed through animal charcoal, evaporated to dryness, and ground. Another process<sup>27-28</sup> employing heating under pressure is that in which equal weights of starch and water are mixed, acid to the extent of 0.5 per cent. on the weight of suspension added, and then heated under 20-30 lb. pressure. C. M. Higgins<sup>33</sup> obtains a solution of dextrin by suspending 5 parts of starch in 8 parts of water containing an acid catalyst, and maintaining the temperature at a point just below that at which the starch gelatinises; after a requisite period, he raises the temperature of the batch to above the gelatinising temperature, and finally neutralises the hydrolytic agent present.

This type of dextrin finds a ready outlet for many purposes in the stationery, paper-box making, and other trades dealing with paper goods in one form or another; it is useful for sticking the paper linings in cheap trunks and attaché cases and in certain textile dressings.

**The Conversion using Enzymes.**—The action of enzymes on starch has been discussed elsewhere (*v. pp.* 331, 466), and in the adhesives industry wide use is made of the process for producing dextrans intended for special purposes. Sometimes the dextrans so obtained are used as pastes without any further treatment after the action has been arrested, but in other cases dextrans produced by the roasting process are added in order to increase both the adhesiveness and the total content of solids of the final paste. Maize starch gives very smooth pastes by this method, and is used by certain manufacturers as the basis of a photographic mounting paste.

Conversion with enzymes is conducted by treating the starch paste (obtained by heating starch with water) with malt extract, or with an extract or preparation containing some other hydrolytic enzyme. Malt diastase works best at  $pH$  4.6, which may be allowed to rise to  $pH$  5.2 without detriment to the speed of the reaction. Takadiastase or similar enzyme may be used in place of malt extract and yields dextrans with good adhesive properties. Pancreatic diastase is most active when the  $pH$  value is around 6.9, and the activity of pancreatic amylase is increased by the addition of common salt.

In carrying out a conversion, the conversion-vat should be thoroughly cleaned before charging and copper or brass fittings should not be used, because traces of copper salts inactivate diastase. The temperature of conversion should be 65-70° C., in order to reduce the formation of sugar during the reaction. The production by this method of a product consisting chiefly of amyloextrin and maltose is described later (*v.i.*). The reaction is generally stopped when it has reached the required stage by heating for a short time to a high temperature to de-activate the enzyme, and by neutralising the batch so that the conditions are unfavourable for enzyme action. Occasionally some batches of starch are more difficult to convert than others (cf. p. 238), and this resistance appears to be due to an inherent property of the starch itself, and not to outside factors, as the resistance of a particular starch appears to depend largely upon the conditions and time of cultivation; but this difficulty is not often experienced in the ordinary course of factory processing.

Nitze<sup>29</sup> has attempted to shorten the process of obtaining dried dextrans by enzyme action by mixing potato starch with an equal weight of water and 1 per cent. its weight of malt diastase, and passing the mixture over hot rollers so that the starch is gelatinised, hydrolysed, and dried in quick succession. In a similar process, Buhtz<sup>30</sup> uses compressed yeast for conversion instead of diastase.

Stern<sup>31</sup> carries out the conversion, using diastase at a temperature of 70° C., which is just below the killing-point of the enzyme, and in this way the sugar-forming element present is retarded in its action, as previously mentioned. Stern treats a suspension of 30 kg. potato starch in 40 kg. water with about 5 litres of malt extract for 15 to 30 minutes at about 70° C., and dries the mass *in vacuo*. In this manner a mass containing 70 per cent. dextrin is obtained which can be further treated, according to another patent by Stern,<sup>32</sup> with yeast, which ferments the sugar present without affecting the dextrin, thus freeing the mass from sugar.

A good maize conversion<sup>1</sup> suitable for making an adhesive paste

for use in photographic mounting, may be made as follows: The tank is cleaned and 1000 lb. water run in, and the pH value adjusted by the addition of sulphuric or hydrofluoric acid to 4.6, using bromocresolgreen as the indicator, after which is added 1000 lb. of maize starch, the suspension being stirred from 15 to 30 minutes, or during the time the temperature is being raised to 55° C. At the end of this time, the pH value is again adjusted to 4.6, using acetic acid, 20 lb. of malt extract is mixed with 50 lb. of water and 28 lb. of this solution added to the tank, the temperature raised to 65° C. and maintained thereat for 15 minutes, the contents of the tank being efficiently agitated during the whole of the processing. At the end of these 15 minutes, another 7 lb. of the malt solution is added and the temperature raised to 69° C. for 15 minutes, then to 73° C., at which point it is maintained for 2 hours. Finally, the batch is heated to 95° C. and cooled to 73° C., when a test portion is withdrawn, and if the viscosity is not correct, another 7 lb. of malt extract solution is added and the conversion allowed to proceed until the required viscosity is attained. About 5 lb. of caustic soda solution (70° Tw.) is then added to neutralise the acid present.

The above conversion is mixed with yellow potato dextrans for the production of strong quick-drying adhesives for paper cartons. For cardboard tubes, or for a craddy tray gum, a potato starch may be used for making the dextrin. The amount of sugar present in such conversions is about 7-8 per cent. After the starch has been converted into dextrin by the action of enzymes, the mass may be dried by passing it round hot rollers.

## REFERENCES

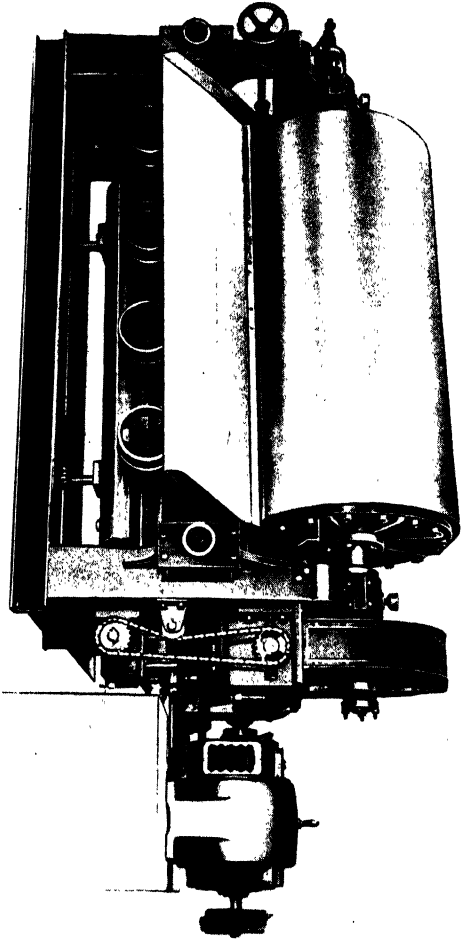
1. J. A. RADLEY, *Chem. Trade J.*, 1936, **98**, 21.
2. PERL and STEINITZER, G.P. 456,841.
3. A. E. WILLIAMS, *Ind. Chem.*, 1933, **9**, 52.
4. NATIONAL ADHESIVES Co., E.P. 383,778.
5. BÖHME, G.P. 252,827, 286,362.
6. J. A. RADLEY, *Manuf. Chem.*, 1936, **7**, 5.
7. H. WULKAN, E.P. 7032, 1910; U.S.P. 993,011, 1911; Austr. P. 44,009. (All lapsed.)
8. KRAUSE, G.P. 549,711.
9. HAAKE, G.P. 573,420.
10. H. KUNZ-KRAUSE, *Ber. Deut. Pharm. Ges.*, 1923, **33**, 149.
11. A. PAYEN, *Compt. rend.*, 1846, **23**, 487.
12. PIEPER, E.P. 9675, 1894. (Lapsed.)
13. UHLAND, E.P. 363,623, 1906. (Lapsed.)
14. V. G. BLOEDE, U.S.P. 61,991, 1867. (Lapsed.)
15. G. S. C. KIRCHHOFF, *Mémoires Acad. Imp. Sci. Petersburg*, 1811, **4**, 27.
16. BIOT and J. PERSOZ, *Ann. Chim. Phys.*, 1833, **52**, 72.
17. O. PHILIPP, *Zeit. Chem.*, 1867, **10**, 400.

18. BLUMENTHAL, G.P. 11,120, 1880. (Lapsed.)
19. FIELDING, E.P. 20,488, 1906. (Lapsed.)
20. CALICO PRINTERS' ASSOC., E.P. 19,499, 1903. (Lapsed.)
21. BROWNING and BARLOW, U.S.P. 773,469, 1904. (Lapsed.)
22. E.P. 16,362, 1914. (Lapsed.)
23. U.S.P. 1,159,591, 1,159,592, 1915. (Lapsed.)
24. F.P. 336,903, 1903. (Lapsed.)
25. V. G. BLOEDE, U.S.P. 1,324,332, 1919. (Lapsed.)
26. A. SCHUMANN, E.P. 5460, 1887. (Lapsed.)
27. W. McLAURIN, U.S.P. 1,283,839, 1915. (Lapsed.)
28. — U.S.P. 1,284,120, 1916. (Lapsed.)
29. H. NITZE, G.P. 407,789, 1920. (Lapsed.)
30. BUHTZ, G.P. 544,879.
31. STERN, G.P. 523,349.
32. — G.P. 409,499.
33. C. M. HIGGINS, E.P. 1885, 1900. (Lapsed.)
34. J. KATZ, *Rec. Trav. chim.*, 1934, **53**, 555.
35. S. P. AIGER, *Bull. Dept. Agric. Burma*, 1937, **33**, 5.
36. H. E. BODE, U.S.P. 2,156,488.
37. M. D. ROZENBROEK, E. Appl. 32,920, 27/12/1938.

#### ADDITIONAL REFERENCES

- F. LIPPMANN, *Zeit. Spiritusind.*, 1902, **25**, 237, 249, 269, 291, 304, 316. (Manufacture and testing of dextrins.)
- H. F. BAUER, *Eighth Int. Cong. Appl. Chem.*, 1912, **13**, 9. (Effect of acidity and time on the roasting of dextrins.)
- E. PAROW, *Zeit. Spiritusind.*, 1912, **35**, 507, 519. (Roasting processes for dextrin-making discussed.)
- M. FREIBERGER, *Farben-Ztg.*, 1913, **24**, 293. (Wet processes for dextrin manufacture discussed.)
- F. C. FRARY and A. C. DENNIS, *J. Ind. Eng. Chem.*, 1915, **7**, 214. (Dextrin-making using HCl.)
- S. E. WARDELL, *Amer. Gas Eng. J.*, 1917, **107**, 561. (Suggests gas is best means of heating roasters.)
- J. MORNINGSTAR, *Col. Trade J.*, 1918, **2**, 67. (General.)
- M. YANO, *J. Chem. Ind. Tokyo*, 1918, **21**, 865. (Dextrin from sweet-potato starch roasted with nitric acid.)
- W. A. DARRAH, *Chem. Met. Eng.*, 1924, **30**, 825. (Equipment and difficulties discussed.) U.S.P. 1,524,340, 1925. (Dextrin roasters heated by hot gases.)
- J. A. RADLEY, *Chemical Ind.*, 1936, **38**, 257. (Manufacture of dextrin by wet and by roasting processes described.)
- INT. PAT. DEVEL. Co., E.P. 453,132, 1936. (Vegetable proteins added to dextrin.)
- ANON, *Gel. Leim Klebst.*, 1934, 237. (Dextrin by acid process.)
- A. E. WILLIAMS, *Chem. Trade J.*, 1934, **95**, 273. (Oil process.)
- *ibid.*, 1936, **97**, 447. (General.)
- H. PRINGSHEIM, G.P. 279,256, 1913. (Use of *B. macerans* claimed.) (Lapsed.)
- K. MIYAJI, *J. Agric. Chem. Soc. Japan*, 1936, **12**, 851. (Dextrin produced as by-product of mannitol fermentation.)
- K. MYRBÄCK, *Svensk. Kem. Tid.*, 1937, 49,145. (Molecular weight.)
- STEIN HALL MFG. Co., U.S.P. 1,938,574, 1933. (Dextrin from grain.)
- HÖPPLER, *Gel. Leim Klebst.*, **7**, 75. (Concentrating dextrin solutions.)





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Fig. 62.—A drum dryer.

[Facing p. 253.]

## CHAPTER 7

## MODIFIED STARCHES

**Physical Treatment to Modify Starch.**—The effect of grinding a potato or a tapioca starch is to lower the viscosity of its solution, and it would appear that the longer the time of grinding, the greater is the fall in viscosity. The final point is reached when, on dissolving in water and allowing to stand, the solution forms two layers<sup>3</sup> (see p. 74).

G. Haake<sup>4</sup> has made use of this property to obtain soluble starch by subjecting starch to the shearing action of rollers revolving in opposite direction and nearly in contact. The most favoured physical means of producing starch swelling in cold water is by the action of heat on moist starch or on the pastes. E. Wulkan<sup>5</sup> heats starch to 110° C., and sprays water on it, so that small granules of paste are formed which dry almost at once. Schiedemantel<sup>6</sup> spray dries starch pastes in an inert or an active gas, as desired. Pfeiffer and Schwander<sup>7</sup> heat starch above the paste-forming temperature and drop on to it water, which may contain chemicals for decomposing the starch. Small globules are formed and the process is very similar to one covered by Mahler and Supf. The latter workers<sup>8</sup> obtain a cold-water swelling starch by taking the wet starch paste obtained in the manufacture of starch and passing it through heated rollers or drying it on drum-dryer (see Fig. 62).

R. W. G. Stutzke<sup>12</sup> modifies starch by forcing in under pressure in water through a heated tube and a spraying orifice into a drying atmosphere.

P. Petit and Richard<sup>9</sup> have prepared an ordinary starch paste for mechanical conversion into a solution of soluble starch by passing the paste six times through an atomiser-jet under a pressure of 2½ lb., whereby a limpid solution is obtained. Milk of starch is atomised in a stream of hot air and steam in one patent,<sup>10</sup> the starch becoming gelled. By using a modified spray-drier for this process a dry powder is obtained. A combination of some of the above effects has also been used,<sup>11</sup> the starch containing 25-50 per cent. of water being ground between hot rollers, which give a gelling, drying, and disintegrating action. Further instances of mechanical treatment to modify starch are given in the chapter on Adhesives. A rotary-beater machine is used by



W. Seck,<sup>1</sup> both with <sup>2</sup> and without swelling agents, for preparing cold-swelling starches.

Most of the above methods give products that swell and form pastes with cold water, but they are not very different from the pastes made by heating the untreated starch with water.

W. Seck and G. Fischer<sup>13</sup> have studied the effect of extremely high shear velocities, such as can be obtained with a Hurrell colloid mill, on potato, arrowroot, cassava, corn, wheat, rice and sago starch pastes. Great reduction in viscosity is noted, in every case amounting in some instances to 98 per cent. These high losses can be correlated with the swelling properties of the various starches, those giving the highest degree of swelling without disintegration suffering the greatest reduction in viscosity. The viscosity differences between the untreated pastes disappear to a large extent when the starch is subjected to mechanical treatment, and this viscometric equalisation is probably due to the natural viscosity of the starch, uninfluenced by swelling or structural effects.

#### REFERENCES

1. W. SECK, E.P. 467,098, 1937.
2. ——— E.P. 464,606, 1937.
3. C. L. ALSBERG and E. E. PERRY, *J. Biol. Chem.*, 1925, 63, 66.
4. G. HAAKE, E.P. 343,848. (Lapsed.)
5. E. WULKAN, U.S.P. 1,677,314.
6. H. J. BRAUN and H. SCHIEDEMANTEL, G.P. 401,361, 1922. (Lapsed.)
7. PFEIFFER and SCHWANDER, G.P. 445,557, 1924.
8. MAHLER and SUPF, G.P. 554,945. (See also G.P. 403,076, 1921.)
9. P. PETIT and RICHARD, *Compt. rend.*, 1926, 182, 657.
10. METALLGESELLSCHAFT A.G., E.P. 383,786. (Lapsed.)
11. INTERN. PAT. DEVEL. CO., E.P. 322,680. (Lapsed.)
12. R. W. G. STUTZKE, U.S.P. 1,516,512, 1924.
13. W. SECK and G. FISCHER, *Kolloid-Zeit.*, 1940, 90, 51.

#### ADDITIONAL REFERENCES

- J. A. RADLEY, *Manuf. Chem.*, 1936, 7, 246. (Soluble starches.)
- J. F. DEBUIGNE, F.P. 782,260, 1935. (Cold-swelling starch made in presence of gelatine, etc.)
- M. SAMEC and M. ZAKRAJSEK, *Kolloidchem. Beih.*, 1937, 46, 134. (Soluble starch from amylose.)
- G. LAQUEUILLE, *T.I.B.A.*, 1938, 16, 677. (General.)
- W. B. NEWKIRK, *Ind. Eng. Chem.*, 1939, 31, 153. (Industrial uses of soluble starches described.)
- SCHOLTEN'S CHEM. FABR., E.P. 494,927, 1938. (Moist starch and formaldehyde rapidly dried to give cold-swelling starch.)

- H. SCHORN and C. V. DAUMAS, U.S.P. 2,177,378. (Jets of superheated steam played on moist starch giving non-lumping cold swelling starch.)
- R. KISHORE and K. C. MUKHERJI, *J. Sci. Tech., India*, 1938, 4, 27. (Preparation of starch and soluble starch.)
- W. A. NIVLING, U.S.P. 2,204,615. (Soluble starch prepared by carrying out reaction in gaseous medium.)
- CORN PRODUCTS REF. CO., E.P. 531,267, 11/7/1938. (Starch suspension pasted with steam and ground in current of hot air to give more modified product than 'Amijel' or 'Mogul'.)

## PART III

## THE INDUSTRIAL APPLICATIONS OF STARCH AND STARCH PRODUCTS

## CHAPTER I

## ADHESIVES FROM STARCH AND DEXTRIN

THE field of adhesives is a very wide one, and to cover it completely from all points of view, including the theoretical and the manufacturer's, would be outside the scope of this volume; hence attention will be directed solely to the adhesives made from starch and dextrin.

**Purpose and Applicability.**<sup>1</sup>—One of the most important considerations affecting the manufacture of adhesives is the particular purpose for which they are intended. It might appear, for example, that if a starch paste affixes paper to glass firmly, it would serve in most trades which require to label bottles containing their products, but more mature consideration will show that it is not so.

Labels may be applied by hand or by machinery, and an adhesive which works well for the first purpose would not be suited to the latter, e.g. a tapioca starch paste (see p. 264) will work quite well for hand labelling, but on a machine, although it contains a lot of water, would not feed correctly, as it is too viscous and does not possess enough 'stick.' If it were to be thinned down to the required viscosity the large amount of water present would saturate the label and cause it to pucker on drying, and what 'stick' or tackiness the paste did possess previously would be seriously impaired.

A very tacky paste is not required in hand labelling, although in the machine it may be an important point, especially in a pick-up gum machine. Again, although the adhesive in this case must be tacky it must not at the same time 'fibre' or 'spin,' i.e. form long fine threads when two surfaces between which a portion of adhesive has been pressed are pulled apart. When an adhesive on a fast-working machine starts to spin, thousands of fine threads are very soon flying all over the place, which means that time is lost in stopping and cleaning the machine and replacing the poor batch of adhesive by a good one.

In this connection the following observation is of interest. A tapioca dextrin, fairly well converted, or a yellow potato dextrin,

will give solutions in water which can be made to fibre, but if potato and tapioca starches are mixed in the correct proportions (see p. 372), and together converted to dextrin, the product gives solutions which will not spin.

Sometimes the product has to resist water, as when used for affixing labels on champagne or wine bottles, and there does not appear to be any satisfactory starch adhesive to meet this demand, the addition of dimethylolurea to a starch glue might be of possible interest, as it has been patented for finishes on cloth to confer water-resistance. In other cases the addition of calcium or barium hydroxide confers some water-resisting power, but the effect is slight.

If a firm will accept returned empty bottles, the labels must be easily detachable when immersed in warm water or in the cleaning liquid of the washing machine; straight starch pastes serve very well here.

So far we have only mentioned adhesives for use between a non-absorbing and polished body and one that absorbs some of the adhesive, but when we come to deal with rough surfaces, such as a wood-to-wood joint, different requirements are met. In a joint between two rough surfaces all interspaces must be filled with the adhesive, which must also firmly unite those portions that are in contact, the area of which is very much less than the surface area of the interspaces.

Thus the efficiency of an adhesive to bind together two rough surfaces will depend very largely on its viscosity; it must be sufficiently fluid to penetrate the interspaces, and yet have enough body to give a thin but strong layer of adhesive when the joint is dry. With a comparatively rough surface the adhesive can 'key' itself into the material, and a rough parallelism between the strength of the joint and the strength of a thin film of the adhesive should exist as long as the adhesive is not stronger than the material it is joining. When other factors, such as rate of loading and relative humidity, elasticity, deformability, flexibility, stress and strain relations, are taken into account the determination of the tensile strength of a thin film of adhesive does give a good indication of its value in practice.<sup>2</sup>

F. Campo-Campino<sup>106</sup> has measured the pore size of a number of papers, and he points out that paper adhesives have to be formulated so that the penetrative properties have to counter-balance the pore size of the paper. Thus papers with small pore size require penetrative adhesives but those with large pores require a filming type of adhesive. Further, according to this worker, the pore size affects the rate at which the glue

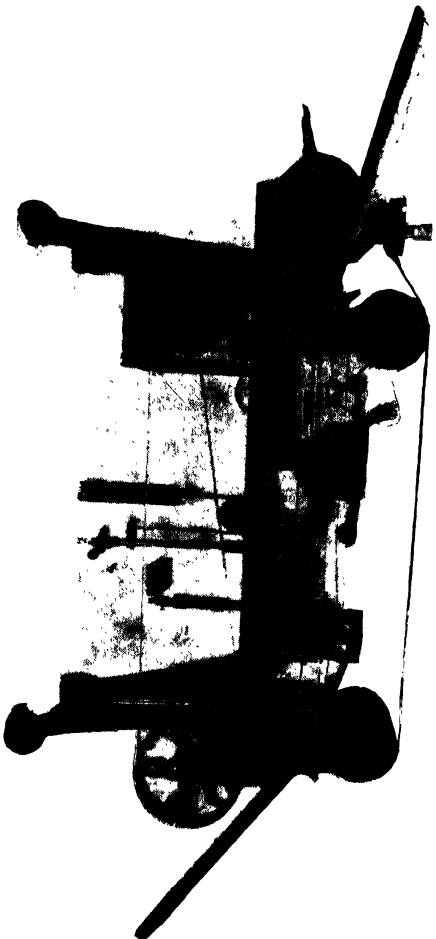
sets and also the behaviour of the wet glue films under pressure. The adhesive must be formulated, therefore, so that its properties compensate for these factors and for unequal porosities in the surfaces to be glued.

The term 'deformability' is used to define the property of an adhesive to adapt itself readily to stresses and strains, slow or sharp, which may be set up in a joint after it has been made. While the adhesive is drying and setting, strains are set up in the joint by swelling, shrinking, warping, alteration either in humidity or temperature, and it is the deformability of a good adhesive which allows it to accommodate itself to new conditions as they occur and yet maintain a firm bond between the surfaces joined. In some adhesives the internal strains set up on drying are so great that the film flies to pieces; an example of this kind is gum-arabic or gum senegal. If, however, glycerine, glucose or some similar agent is added to the gum it acts as a plasticiser and confers deformability on the film, which then dries without disintegrating.

So important is this property that when measuring the tensile strength of an adhesive film it is quite as necessary to note its deformability as its strength, if the figures are to be of value in the practical evaluation of the substance as an adhesive. Colloids in general show this property to a greater extent than pure substances and are generally superior to them as adhesives. The following figures illustrate this point: Isinglass, tensile strength, 13,000 lb. per sq. in., tough; starch, 7000 lb. per sq. in., ductile; sodium silicate, 600 lb. per sq. in., very brittle; gum-arabic, no value obtainable, very brittle. Gum-arabic splits up spontaneously on drying and, as stated elsewhere (see Dextrin, p. 288), dextrin pastes which crack on the surface should not be used to make adhesives for first-class work. If gum-arabic is plasticised, however, its strength immediately increases.

Rate of loading and humidity are important, and it has been found that long-continued stress on a joint, made between metals with recognised adhesives, reduces its ultimate strength. Another factor of interest is the thinness of the film of adhesive, as the joint-strength appears to increase with the thinness of the film over a wide range, providing the adhesive entirely fills the space between the two surfaces. A thick layer becomes less efficient as it ages, and this is especially important in tight wrappers, bottle-labelling, tin-labelling, and in sealing cartons. To obtain the best joint, therefore, the adhesive must entirely fill the space between the two surfaces and be present in a film as thin as possible. There appears to be some connection between the strength of certain joints and the mechanical properties of the





*Reproduced by courtesy of the Beth Label and Wrapper Machines, Ltd.*  
FIG. 63.—The ' Beth ' portable labelling machine.

[Facing p. 259.]

materials being joined, such as tensile strength, elasticity and compressibility, but this relationship is at present obscure and little understood.

**The Application of Adhesives.**—There are about half a dozen different ways in which adhesives in general can be applied. Many can be applied by hand or brush, and others may be spread on to a sheet of metal, such as zinc, by a mechanical method and the paper, etc., pressed into contact and removed. The last method is the chief one used in many gumming machines, and in order that the operation may be carried out at high speed much mechanical ingenuity and inventiveness have been shown in elaborating these machines. Most machines of this kind apply the gum by means of a roller (see Fig. 63), the thickness of the gum layer being determined by a doctor blade which scrapes excess glue off the revolving roller before it comes into contact with the material; in other machines brushes are used for the same purpose. When dealing with large surfaces spraying is very occasionally resorted to, but it introduces difficulties because viscosity and water-content must both be kept very low. The above methods are chiefly used with starch and dextrin adhesives.

For rubber, casein, or resinous adhesives, two other methods may be used; for example, two surfaces may be separately coated with the adhesive, which is allowed to dry and then pressed together with or without the application of heat. Alternately, a thin sheet of paper impregnated with the adhesive is placed between the two surfaces, which are then pressed together as above. This method is particularly suitable for resinous adhesives and a feature of the process is that no moisture is introduced.

The second method applicable to rubber and casein products is to make up two solutions and apply separately, or to mix just at the moment of application. Heat and pressure are often used to complete the reaction and form the joint.

The following hints in using adhesives are to be recommended; some of them may seem superfluous, but even so, in practice one finds that they are often ignored and lead to trouble :—

1. The surfaces to be joined should be as dry as possible, e.g. in bottle-labelling the glass portion should be dry, otherwise blistering and wrinkling of the paper label may occur and the joint become unsatisfactory.

2. The surfaces should be as clean as possible and be especially free from oily substances. Although this suggestion may seem obvious, many instances could be quoted to show that it is necessary. Cosmetic and pharmaceutical products of an oily or



greasy nature, which are being filled automatically into jars and bottles, are liable to be spilt. It needs only an extremely thin film of oil or grease on the bottle to bring about difficulties in the labelling machines.

3. Adhesives should be stored in a cool place, but not where they are liable to get frozen; this is especially important when the adhesive is a flour paste, as this type of product can be rendered absolutely useless by freezing (see p. 263).

4. If the air is unusually dry the containers of the glues can be covered with wet sacks, especially after they have been broached and some of the contents used.

5. Two different adhesives, bought in the ready-prepared state, should never be mixed, as each has been formulated with a fairly distinctive purpose in mind.

6. The adhesives used in machine work are preferably kept thick or 'heavy' in the glue-pots, but applied so that only a very thin film is transferred to form the joint. They should be tacky enough to produce a noticeable hissing on the machine when it is running, but should be so formulated that no 'webbing,' 'spinning' or 'fibring' takes place as the roller or glue-pad and the treated surface move apart. If the water-content of the adhesive requires some adjustment, the water should not be added to the glue-pot but the adjustment should be made prior to putting it in the machine, preferably by diluting it with a thinner mixture of the same adhesive.

7. Wherever possible, the two surfaces to be joined should be selected so that two hard surfaces do not come together, and at least one surface should be reasonably porous so that the adhesive can penetrate it and obtain a good 'keying' action. It is also advisable not to glue wrappers or labels too heavily to curved surfaces, owing to the strains set up within the film on drying.

**Theoretical Considerations.**<sup>2</sup>—When we come to consider adhesion from the theoretical aspect we are faced with several alternative theories. The one which appears to fit the practical data best postulates the existence of fields of molecular attraction between the two materials forming the joint, which are superimposed on the ordinary cohesive force between the molecules of the substance in the thin film of adhesive. In some manner, at present unexplained, these molecular attractions are transmitted through a film of adhesive at least 100 molecules thick. Thus, this theory suggests a chain effect, whereby a large range of molecular attraction is artificially set up.

Most of the common adhesives may be used for joining wood and metal surfaces, but if a strong joint is to be made between

two optically smooth surfaces, e.g. crystal faces, then true adhesion must be involved, i.e. the type of adhesion McBain and Hopkins<sup>3</sup> call 'Specific Adhesion,' in contra-distinction to that between rough surfaces where the substance is permeable to the adhesive. In the case of rough surfaces, such as a wooden joint, the glue can 'key' itself into the interspaces between the surface fibres. This type of joint is designated 'mechanical'. It is possible that it is only when pure molten chemical substances which actually wet the materials in the true sense of the word are used to form a joint that both specific and mechanical adhesion come into play. If one of the materials is porous mechanical adhesion usually predominates, thus explaining the above-mentioned rough parallelism between joint strength and the strength of the film of the adhesive itself; other factors, such as the tensile strength, compressibility and the elasticity of the materials being joined, contribute in this case to modify the weaker factor of specific adhesion. This theory may explain the value of adding a wetting agent to adhesives for joining smooth surfaces, in that the adsorbed air on the surface of the material is displaced by a solution of the adhesive and allows specific and mechanical adhesion to have fuller play than they would if the material were 'cushioned' with a layer of air.

Another theory to account for adhesive action assumes that molecular forces operate over a much wider range than that allowed for in the older theories of physics, but this explanation is less attractive than the one set out above, and has many less experimental data to support it.

**Flour Pastes.**—In the Textile Section it is mentioned that certain flours are used to make sizes in preference to starches, because they are considered to possess greater adhesiveness. This superiority is explained by assuming that the presence of gluten assists the adhesive action. Some of the recipes given in the Textile Section may therefore prove of interest to those interested in the use of flour pastes for adhesives.

Flour pastes can be used *inter alia* as the adhesive for lining cardboard boxes with paper, affixing wall paper, bill-posting and labelling, bookbinding, and in the manufacture of paper goods of various kinds where the requirements are not too stringent. In general, flour pastes contain relatively little solid matter and consequently they show a tendency to dry slowly and sometimes to cause 'puckering' of the paper used; in this event adhesives with a higher solid-content must be used; for example, those made from treated starches or dextrans.

In the lining of cardboard boxes, cheap attaché cases or travelling

trunks, an adhesive of the paste type can readily be used, and should be in the form of a very smooth paste quite free from lumps or any tendency to 'balling,' and its tackiness need not be pronounced. These pastes are also of value for the hand-labelling of bottles from which the labels are to be removed subsequent to use. For bottle-labelling by machine special types of dextrin adhesives are used. Pastes intended for use in affixing posters should be fairly weather-resistant and be tested for outdoor conditions. This test may be done by pasting a piece of the poster on to a brick or a board, drying slowly before a fire, and after wetting thoroughly by means of a fine spray of water, drying as before. A good poster paste should withstand several treatments without failing. Another property, which is preferable but not essential in a poster paste, is that of drying to give a fairly transparent film, so that coloured posters, or those with a black background, are not made unsightly with milky patches due to paste which has inadvertently become splashed on to the face of the poster.

Cereal flours generally form the basis of adhesive pastes; wheat flour and rye flour are those most favoured for a straight paste, but maize, barley and rice flours are also used. Very important is the stability of the pastes, which is assisted by the addition of an acid, e.g. acetic acid. The water-holding power of a starch paste appears to be indirectly connected with its stability, and this property is usually determined when a new batch of starch or flour is received in the factory. A test that is used in a number of factories is as follows: The samples to be compared are made into mucilages of the same concentration and under exactly the same conditions. These mucilages are allowed to cool, and when they have gelled, a cylinder of the same size is cut from each and stood on end on a piece of filter-paper lying on a sheet of glass, covered with a bell-jar and left for several hours. At the end of this time the diameters of the rings of moisture on the papers are compared, and the comparison gives a good indication of the best flour or starch, as the one with the largest ring has the lowest water-holding power and therefore would give the least stable paste.

Finely ground tubers can be used for making pastes intended for rough work; the author has successfully employed tapioca tubers, some batches of which were badly diseased, for this purpose. In one case some difficulty was at first experienced owing to the very poor stability of the paste, which showed syneresis and breakdown in a few hours, but this defect was entirely overcome by the addition of a little bleaching powder before making

the paste, the odour of the bleach being masked by the addition of a little oil of sassafras. It must be understood, however, that this type of product can only be used for very low-grade work, such as cheap board lining, and consequently commands a proportionately low selling price.

Generally, adhesive pastes are made with materials of good grade, and cleanliness throughout the whole of the processing should be one of the first considerations in the manufacture of first-grade products in order to prevent moulds developing on the surface of the finished product when it is stored in the factory. The temperature and time of storage of the pastes are also important. Pastes awaiting shipment should not be stored in such a position as to render them liable to freeze in cold weather, as on thawing, water rapidly separates from the paste leaving behind a crumbly, spongy mass quite useless as an adhesive (see p. 85). To prevent fermentation of pastes containing nitrogenous compounds, Recries Française<sup>98</sup> pre-treats starchy flour containing nitrogen with a current of air or inert gas containing 0.1 to 0.2 per cent. of the oxides of chlorine. Antiseptics for starch products are dealt with elsewhere (p. 364).

Aluminium sulphate<sup>99</sup> and glycerine are two compounds frequently added to flour pastes to improve their properties. The aluminium sulphate, besides acting as a preservative, also appears to increase the adhesiveness of the paste, and when this is dried exerts some influence on the moisture-resistance of the film. The glycerine is, as already mentioned, of value in preventing the film from becoming brittle and liable to fracture easily; besides rendering the film more pliable, it gives to the paste a smoothness in working properties.

**Adhesives from Starch.**—Starch forms the basis of a large number of adhesives varying from a simple paste made by heating starch with water to pastes made by complex processes entailing several treatments. Starch adhesives are used in many trades, and by making slight variations in the manner of carrying out a process, products widely different in character may be obtained. Using the same process, but varying the time or the temperature of the reaction, compounds are obtained which behave as thin-boiling or soluble starches at one end of the scale, and as thick, viscous and highly adhesive products at the other end.

Even the main starches of commerce, wheat, maize, tapioca, and potato, all behave in their own characteristic manner when submitted to the same process, the differences shown in some cases being truly striking, for example, those of the mucilages obtained by heating different starches with water. It is on these differences

that the use of various starches for the preparation of sizes and dressing-agents for textiles is based. Starches processed in the same manner may yield products so dissimilar that they may be used advantageously for different purposes.

The starches most commonly used for the manufacture of adhesive pastes are potato and tapioca, and of these the latter appears more suitable in several respects. Tapioca starch gives adhesives which are more viscous, smoother in working and more easily prepared, whilst the joints made from them are considered by some to show a somewhat higher tensile strength than those made from potato starch. The bitter odour and taste of potato-starch adhesives are also disadvantageous for some types of work.

The processes for preparing adhesives from starch, omitting those which give as end-products substances of a dextrinous nature or chemical derivatives of starch, may for convenience be classified under five main headings as follows:—

1. Treatment with caustic alkalis.
2. Treatment with other alkaline substances.
3. Treatment with acids.
4. Treatment with salts, oxidising agents or swelling agents.
5. Addition of various compounds to starch pastes formed by any of the above methods.

The effect of acids to produce dextrins and adhesives, and the modification of starch by means of oxidising agents, are dealt with elsewhere, and we shall consider them here only in so far as they are bound up with the other processes.

Of the straight starches used for producing adhesive pastes, tapioca, wheat and rye are perhaps the most widely employed and give the strongest and most adhesive pastes. Apart from certain lines of work indicated above, pastes made from starch and water without any special processing are not widely used, and we shall now pass on to consider the preparation of adhesive pastes by the methods outlined above.

**Treatment with Caustic Alkalies.**—The products obtained by the action of caustic alkalis on starch are known under a variety of names on the Continent; some of the names are mentioned in the Textile Section, and among other names are those of 'colle universelle,' 'Collodine,' 'colle du japon,' and 'colle froid'.

By the action of caustic alkalis on starch, adhesives can be obtained giving joints with a strength practically equal to or greater than that of the materials joined. Such joints cannot be termed insoluble, but they are only difficultly soluble and

resist moisture quite well. These pastes may be made by a cold process or by heating; those made in the cold are possibly not so stable as those made with heat because they tend to show some reversion.

In making cold adhesives the process may be carried out entirely at 15-20° C., and takes about 12 hours to complete. The mucilage may be neutralised either by the addition of an acid or a salt that reacts with the alkali, and sometimes with the starch as well. By using oxidising agents and heating the mass, soft, plastic and transparent adhesives are obtained, which will be dealt with later. The use of strong alkalis allows of the preparation of strong adhesives containing about 33-40 per cent. starch, which can replace dextrin adhesives for a number of purposes, and which are paler, cheaper, and capable of being diluted to a greater extent if necessary. In neutralising the alkali the process is generally allowed to finish slightly on the alkaline side, as this assists the stability of the paste, although it has a drawback in that it may give rise to staining when used with wood veneers; such preparations should not be heated when applying or drying.

Mérimée,<sup>4</sup> as far back as 1827, mentions the advantage obtained by using a weak caustic soda solution for making a starch mucilage for the sizing of paper. The first powdered preparation containing starch and caustic alkali, which when added to water gave an adhesive mucilage, appears to have been that patented by Marsden<sup>5</sup> in 1888. Marsden mixed powdered ammonium sulphate with his mixture of starch and caustic alkali so that on the addition of water the alkali gelatinised the starch and was then neutralised by the ammonium sulphate to give the neutral metallic sulphate, whilst the liberated ammonia gradually passed into the air.

J. Kantorowitz<sup>6</sup> appears to have been the next worker to take out a patent for the treatment of starch with alkalis. He treats the starch with caustic soda and after neutralising with hydrochloric acid precipitates the product either by adding magnesium sulphate or by keeping the mass at 20° C. for several hours. In a further patent he treats the starch with caustic soda in a concentrated solution of sodium sulphate. It should be mentioned that this treatment restrains the swelling of the starch and allows it to be readily separated from the liquor. Alcohol, acetone or mixtures of these with ether have also been used to obtain a similar effect.<sup>8-10</sup>

Simple as is the process of modifying starch with caustic alkalies, it appears to have been modified in approximately a dozen different ways. Leonhardt<sup>11</sup> pre-treats the starch with dilute acid prior to treatment with alkali, a process which is similar in

principal to that used by Perkins<sup>12</sup> in America many years earlier. The contents of Perkins' original patents have been the subjects of much litigation; the process as claimed by him gives excellent products, having great adhesive powers and excellent appearance.

Perkins uses cassava starch and an equal weight of water, and pre-treats with 2-3 per cent. sulphuric acid at 55° C. for 4-6 hours. Any other treatment leading to the formation of a thin-boiling starch can be used instead of the above method, and Perkins himself has covered the use of sodium peroxide.<sup>13</sup> The object of modifying the starch before making the adhesive is to produce a more fluid jelly, and a further method of achieving this end is to stir the jelly for five or six hours.<sup>14</sup> The stirring is continued until a sample withdrawn from the batch flows thinly and evenly off a spatula or rod.

The adhesive jelly, which is used extensively in wood-veneering, is obtained from the pre-treated starch by running into a slurry of practically equal parts of starch and water about 15 per cent. by weight of a 33 per cent. solution of caustic soda. More water can be used to suspend the starch if desired, but this will naturally lead to a final product having a lower solid-content and different adhesive strength. In this process the higher the temperature of the conversion, the less the amount of caustic soda required to bring about the required change.

The appearance of the mass changes quite suddenly at a certain point during the addition of the caustic solution; the white and extremely tough leathery mass first produced suddenly changes to a colourless jelly as the addition of the caustic is continued.

The old formula for the preparation of wood veneers, i.e. 1 pt. of tapioca starch to 2½ pts. water, was not entirely successful because, owing to the presence of the comparatively large amount of water present, the adhesive penetrated too deeply, causing weak joints (see p. 257). As previously stated, Perkins uses sodium peroxide to modify his starch before making the paste to obtain a more fluid product and thus allow a reduction to be made in the amount of water used. Another method<sup>100</sup> is to add barium peroxide and urea to the starch before making the paste, the urea stabilises the glue and tends to retard the evaporation of the moisture. The quality of the pastes made in the above manner improves with increasing concentration of the starch, with the efficiency of the stirring, and the rigid maintenance of the temperature throughout at 15-20° C.

The following formula will serve as a guide to the making of a paste of this type: 84.4 pts. tapioca starch, 0.5 pt. barium peroxide, 0.1 pt. soda ash, 5 pts. whiting, and 10 pts. urea are

well mixed, and 100 pts. of this glue base are heated with 120 pts. of water to 70° C. The addition of 2.5 pts. of caustic alkali is made in the usual manner. Using this method, sago, maize or even potato starch may be used to give a good veneering adhesive.

The addition of about 1 per cent. of potassium dichromate and of calcium peroxide to a starch base before forming the glue allows the use of a lower temperature and of less caustic alkali. The calcium salt is thought to improve the water-resistance of the joint. If desired, the potassium dichromate can be replaced by potassium pyroantimonate. Ferrous sulphate is also used as a catalyst.<sup>101</sup> In a recent patent by Perkins the viscosity of the final glue is brought within desired limits without any acid pre-treatment, to which reference has been made, by adding to the starch base a small amount of a copper salt to act as a catalyst to the action.

A very good adhesive may be made by suspending 150 pts. starch in 100 pts. water and adding 25 pts. caustic soda solution (36° Bé.) diluted with an equal volume of water, and stirring the mass for 60-90 minutes at a temperature between 15° and 20° C. If it is desired to neutralise one of the above pastes, the acid used should be but slightly diluted; thus, to neutralise and dilute a paste to a desired water-content the bulk of the water of dilution should be added first, followed by the acid diluted with a small amount of water, rather than the acid added to the whole of the water and then this added to the mass. A typical formula for a very slightly alkaline starch is the following: 140 lb. starch are suspended in 210 lb. water and 35 lb. caustic soda liquor (36° Bé.) diluted with 35 lb. water are added with constant stirring. When the mass 'comes across,' i.e. is thoroughly converted, 550 lb. of water, in which is dissolved 0.14 lb. borax, are slowly added, followed by 5 lb. hydrochloric acid (22° Bé.) diluted with 50 lb. water. If the alkali present is exactly neutralised, the viscosity of the resulting paste is lower, and it is preferable to omit the borax and slightly increase the starch/water ratio, as the thickening action of the borax is lost under these conditions.

According to Gröninger,<sup>15</sup> pre-treatment of the starch with triethanolamine allows a very smooth alkaline conversion to be subsequently carried out, minimising the formation of lumps resistant to the processing. Supf<sup>16</sup> obtains a dry powder capable of giving a viscous adhesive by treating starch with an equal amount of caustic soda solution (36° Bé.) at a few degrees above 0° C. Very little swelling of the starch takes place at this temperature, and what swelling does occur causes the absorption of all the water present. The practically dry powder is then freed



from alkali by washing with alcohol. Both the alkali and the alcohol in this process are recovered and used repeatedly. Among other attempts to obtain dry products are those of Pfeiffer and Schwander,<sup>17</sup> who spray caustic liquor of the same strength as that used by Supf on to potato starch which has been mixed with an organic liquid. Some 25-30 kg. of solution are used for 100 kg. starch. H. Bechhold<sup>18</sup> employs a somewhat similar process, but specifies that the organic liquid shall be insoluble or only slightly soluble in water. The use of chlorinated hydrocarbons or of hydroaromatic alcohols and ketones is excluded. He employs 3 per cent. of benzaldehyde on the weight of starch and dries the product at 50-70° C. O. Meyer<sup>19</sup> claims the use of cyclohexanone. He treats starch with 3 per cent. of cyclohexanone followed by aqueous caustic soda, the final paste being neutralised with oxalic acid. The paste obtained is white and very smooth and can be used for paper-lining work. Neutralisation of an alkaline starch paste tends to destroy the 'ropy' character of the paste, which becomes smoother and more 'bland'.

F. Riethof<sup>20</sup> mixes 3 per cent. amyl alcohol or 3-5 per cent. aromatic amine with the starch before the treatment with caustic and modifies the product so obtained by adding an acid, such as oxalic acid, or an acid salt, such as sodium bisulphite. The use of a chlorinated hydrocarbon is claimed by the Sächsische Klebstoffwerke<sup>21</sup> for the same purpose, the decomposition in this case being carried out by heating the mass to 30-35° C. for 25-30 minutes.

Henkel et Cie<sup>22</sup> consider that the use of these organic liquids leaves much to be desired and that organic liquids soluble in water should preferably be used. They employ an emulsion of trichloroethylene, some 4-5 litres being sprayed on to 100 kg. starch before treating it with 26.5 kg. caustic soda solution of sp. gr. 1.332.

The following formula gives excellent results with tapioca starch, and can also be used with potato starch, but in the latter case the resultant jelly gum is more adhesive although less stable, and more difficult to treat: 320 lb. tapioca starch are suspended in 500 lb. water in which is dissolved 0.65 lb. sodium bicarbonate; 80 lb. caustic soda liquor (36° Bé.) diluted with an equal weight of water are slowly run in and stirred for 12 hours at a temperature between 15° and 20° C. At the end of this time 38 lb. acetone are added followed by 6 lb. formaldehyde solution (30 per cent.) and 0.65 lb. ammonia-finished turkey-red oil; after stirring for a further 20 minutes at not too fast a rate the batch is run off.

In order to obtain a dry product, various workers have treated starch with dry caustic alkalis,<sup>23</sup> followed by the addition of

a powdered solid organic acid.<sup>24</sup> Mahler and Supf<sup>25</sup> grind 100 kg. starch with 5-6 kg. powdered caustic soda and neutralise with 6-11 kg. of powdered oxalic acid. On adding water, these products swell but have the drawback of usually forming lumps which are difficult to convert into the paste form; to overcome this, Henkel et Cie.<sup>26</sup> grind in the presence of a little water and then dry and re-grind. Another method<sup>27-28</sup> of conducting this process is to grind the starch with the dry alkali, then add moist starch, re-grind and dry. Better results are obtained if the starch added after the preliminary grinding is moistened with a mixture of alcohol and water. Pfeiffer and Schwander<sup>29</sup> treat starch with finely-divided alkali solution to which is added 10 per cent. of a water-soluble, volatile solvent to assist the drying process. To obtain a product free from any trace of colour, to be used more especially for the sizing of white goods rather than as an adhesive, Leonhardt<sup>30</sup> decomposes starch with alkali *in vacuo*, and when the modification is complete, passes ammonia or sulphur dioxide through the mass to destroy the colour. The product in this case is also a dry powder.

**Treatment with other Alkaline Substances.**—Milder alkalies may be used for the modification of starch, and patents have been taken out covering the use of a number of these compounds under varying conditions. According to one,<sup>31</sup> a paste similar to that obtained from wheat starch may be made by acting on potato starch with a solution of a mild alkali containing certain additions, such as alkaline persalts, peroxides, or neutral persalts. The starch is modified by this method to give a product approaching that obtained as soluble starch; for example, 100 kg. of potato starch are suspended in water to which is added from 0.5-2 kg. ammonium persulphate and 1 kg. ammonium hydroxide of sp. gr. 0.88. The product obtained from this conversion is a soft, lard-like, adhesive paste.

A painters' glue can be made from paste-like products that are obtained by modifying the starch with alkaline-earth compounds in the presence of such amounts of alkali compounds as are capable of being transformed by the alkali-earth oxides to alkali hydroxides in a concentration less than 2 per cent.<sup>32</sup> The following example illustrates this process: 100 kg. starch are treated with 14 kg. of sodium silicate (36° Bé.) and 4 kg. slaked lime. For the preparation of the painters' glue the results obtained by the above process are improved if a little rosin soap is added to the starch suspension and the modification carried out at room-temperature. Using only concentrated solutions of alkaline-earth hydroxides, Runge produces swelled starch by heating the

mixture, and after swelling has taken place, neutralising the alkali present with a solid acid, e.g. oxalic acid. In Runge's process,<sup>33</sup> 1 pt. of starch is heated to 70-80° C. with 3 pts. of a saturated solution of calcium hydroxide; after drying, the mass is ground with the requisite amount of oxalic acid to neutralise the alkali present.

Products which on drying split off alkaline hydroxides hydrolytically, but yet in solution have little swelling action on the starch, can be used to produce dry products of value as adhesives.<sup>34</sup> The adhesive qualities of the pastes may be regulated by controlling the amount of the alkali-producing substance added. Soda lime, sodium silicate and aluminate, etc., are among the substances used in this process. Paste-like products may be formed by the addition of water to the substance produced by the action of less than 30 per cent. sodium silicate on the weight of starch. To obtain this product, 200 kg. starch are suspended in 175 litres of water, and 60 kg. of sodium silicate (30° Bé.) are added. The mixture is dried on hot rollers and then ground to a fine powder. If glue-like products are required, the amount of silicate is increased until it exceeds 30 per cent. of the weight of starch. An additional patent substitutes the above-mentioned materials by barium hydroxide.<sup>35</sup>

In a further patent<sup>36</sup> the decomposition of starch to obtain adhesive pastes is carried out with alkali salts of weak inorganic acids, e.g. borates, aluminates or stannates, which have no influence on the starch at ordinary temperatures but cause it to swell on heating. Haake<sup>37</sup> neutralises a solution of borax with chlorine at 40° C. and suspends his starch in the solution until the particular modification required is obtained. The product is washed and dried, and gives thin-flowing solutions containing a high percentage of starch, which can be used either for sizing or for adhesives.

One outstanding advantage obtainable by the use of weak alkalies by any of the above methods, is that the reactants can be more easily mixed in with the starch than can strong alkalies, and little or no action takes place in the cold; but when the batch is ready the temperature can be raised and good homogeneous pastes obtained.

A number of products are obtained by first forming an alkali starch and then treating the solution with an alkaline-earth compound, which precipitates the alkaline-earth starch. In place of the alkaline-earth hydroxides, heavy metal salts, such as barium chloride, may be used and the resultant precipitate separated and dried.<sup>39</sup> By mixing this powder with a water-soluble alkali salt,

such as sodium sulphate, a cold-water swelling starch is obtained which gives adhesive pastes on the addition of water.<sup>41</sup> The precipitate obtained by treating alkali starch with an alkaline-earth compound may be treated with reagents to obtain derivatives containing barium, calcium, strontium, beryllium, magnesium, zinc, aluminium, iron, copper, or double compounds containing two of the above metals. The copper compounds obtained by this method are said to have disinfecting properties.<sup>40</sup>

The above compounds may be treated with chlorine and the chlorination followed by a treatment with an acid gas; the products obtained in this instance, however, are soluble starches. Magnesium chloride or calcium chloride may be used in the production of adhesives, that produced from magnesium chloride giving a very adhesive paste which is useful for paper work, or as an agent to carry the filler used in paper surfacing; when used for the latter purpose it shows very little tendency to absorb atmospheric moisture (see also p. 274). The calcium chloride process is fairly well known, and good adhesives may be obtained by treating 100 pts. of starch with 50 to 100 pts. of a highly concentrated calcium chloride solution at room-temperature or slightly above it, and adding to the mixture 50 pts. of animal glue to increase the 'pick-up' or adhesiveness. The mass is ground and dried and the powder so obtained dissolves readily in water to give an adhesive mass.<sup>42</sup>

**Treatment with Acids.**—Lintner made a soluble starch by acting on potato starch with 7.5 per cent. hydrochloric acid for several days at room-temperature. The modified starch gives a clear solution in water, but if a starch other than potato starch is used the solution is opalescent. The maximum action in the above case takes place during the first day and after 30 days the starch has undergone but slight further change.<sup>103</sup> The phosphorus-content and viscosity change but little during the last 29 days, and one-day and thirty-day products react in the same manner when treated with  $\beta$ -amylase. At the end of 30 days only about 4.5 per cent. of the starch dissolves in the hydrochloric acid solution.

When 15 per cent. hydrochloric acid solution is used the results are very different, in that with continued action greater amounts of starch go into solution in the acid, until after 30 days some 60 per cent. has dissolved. The viscosity decreases but little after the first day, but the phosphorus-content decreases progressively. In this reaction a fraction insoluble in hot water begins to form after the tenth day and increases in amount progressively with the length of time of treatment. It appears to be

similar to amylohemiacellulose, contains 1.3 per cent. ash, traces of phosphorus, but much silica. The dextrin in the acid solution can be precipitated with alcohol, gives a reddish-brown iodine reaction, and has a higher phosphorus-content than the original starch.<sup>103</sup> The use of a soluble starch made by the action of 15 per cent. hydrochloric acid on potato starch for 6 days has been suggested for the determination of the activity of malt extract in place of Lintner starch (see p. 516). The effect of cold dilute acids on starch has been extensively studied by M. Samec<sup>104</sup> (see also V. I. Nazarov<sup>111</sup>).

One of the earliest processes for treating starch with acids is that of J. Sellars<sup>43</sup> who, in 1865, neutralised the mass with soda after the acid treatment. By the action of vegetable and mineral acids starches may be modified so that their solutions range from mobile liquids to viscous and adhesive pastes. Some early treatments<sup>44-46</sup> involved heating the starch under various conditions of temperature and pressure with sulphur dioxide.

The 'in suspension' process is widely practised and embraces most processes in which a starch is suspended in a dilute acid solution and maintained at a temperature varying from room-temperature to just below that of the gelatinising point of the particular starch being processed. Such starches appear to be unchanged visually, but readily go into solution in hot water or dilute alkaline solutions. Klopfer<sup>47</sup> mixes rice starch with 0.5 per cent. lactic acid, and at the end of the treatment centrifuges it and dries the product. H. H. Lake<sup>48</sup> treats the starch with strong hydrochloric acid and dries at a low temperature, the product obtained being a thin-boiling starch. Duryea<sup>49</sup> suspends starch in a 1-2 per cent. aqueous sulphuric acid and maintains the temperature at 45° C. for 1 to 4½ hours; when the required modification has been effected the acid is neutralised, filtered off, and the product dried. Bergquist<sup>50</sup> adds hexamethylenetetramine or formaldehyde during this treatment and heats to 72° C. to obtain a product which gives a perfectly clear film on drying. Such a product may be used as an adhesive in, for example, poster work, where transparency of the dried paste is a desirable feature. Murphy<sup>51</sup> suspends starch in dilute sulphuric acid and passes in superheated steam until the mass liquefies, when the passage of the steam is discontinued, the acid neutralised with chalk, and the mass filtered hot. Browning and Barlow<sup>52</sup> spray hydrochloric acid on to dried starch maintained at 45° C.; B. Helferich and his co-workers<sup>53</sup> use anhydrous hydrogen fluoride at 20° C. and after 30 minutes remove the acid with a current of air. In later patents<sup>54-55</sup> both starch and cellulose are treated in this way,

the temperatures ranging from room-temperature to  $90^{\circ}$  C. H. Schenbach<sup>56</sup> treats starch with gaseous hydrochloric acid under pressure in the presence of an organic liquid, such as benzene, which is removed at the end of the process by filtration. Stutzke<sup>57</sup> sprays a starch suspension, which may contain acid, into a current of superheated steam or, according to another patent, a mixture of starch and water may be sprayed into hydrochloric acid vapour in a chamber at  $200^{\circ}$  C. In the latter process the starch is modified and dried simultaneously, as in this medium the partial pressure of the water vapour corresponds to the boiling-point of water at  $32.5^{\circ}$  C. Again, a wheat-starch suspension may be atomised in a chamber containing air at  $300^{\circ}$  C. at a pressure whereat water boils slightly above the gelatinising temperature of the starch. A number of other patents cover the treatment of starch by spraying with a small amount of acid, heating until the required stage is reached and then drying it on hot rollers. Methods embodying this principle effect great saving of time, labour and power.

The conversion of starch by means of acid either by the wet method or by dry heating is dealt with more fully in the chapter on Dextrin, and the reader is also referred to the section on Ethers and Esters of starch, especially to those portions dealing with the acetates and the xanthates, both of which are used commercially as adhesives.

**Treatment with Salts.**—In the preceding pages the use of certain salts like magnesium or calcium chloride for obtaining starch pastes has been mentioned, and we may now consider further the various uses to which metallic salts are put in the adhesives industry.

Several workers<sup>61-62</sup> have examined the effect of salts at different concentrations and at various temperatures on the swelling and gelatinisation of starch and, as will be seen, their results have found practical application. Courtonne,<sup>58</sup> for example, found that chlorides exerted most effect on the gelatinisation point, whereas sulphates exerted a retarding action. Thus we have the preparation of adhesive pastes like that of Möller-Holtkamp,<sup>59</sup> who treats a thick starch paste with calcium chloride and reboils the mixture, or the patent of Alexander,<sup>60</sup> who uses a high concentration of the salt to swell the starch. Mention has been made (see p. 265) of the addition of sodium sulphate to reaction mixtures to prevent swelling and gelatinisation taking place, thus allowing easy filtration and handling of the treated starch.

The effect of salts in general upon starch has not received the same amount of academic attention as that of acids and bases,

and the number of references in the literature is correspondingly small. It is now generally known, however, that certain salts have the power of dispersing or liquefying starch paste, or even gelatinising the raw starch when applied in concentrated solution. The value of this phenomenon in the study of the structure and physical chemistry of starch has been mentioned in the chapter on the Physical Properties of Starch. Salts have three main uses in adhesive work: they may be employed as swelling agents, as stabilising agents, and to impart transparency, adhesiveness, density or to increase the viscosity of the paste.

E. Meusel,<sup>72</sup> fifty years ago, noted that various thiocyanates, potassium acetate and calcium chloride in solution gelatinised starch at ordinary temperature and that the concentration of the salt plays an important part in the process. Small quantities of certain salts, even in concentrations as low as they occur in tap water, have been found by L. Eynon and J. H. Lane<sup>62</sup> to affect the viscosity of starch pastes adversely. With soluble starch an effect particularly noticeable is the regaining of gelatinising power if ordinary hard water is used to wash it during its preparation. This is explained by D. R. Nanji and R. G. L. Beazeley<sup>63</sup> by postulating the absorption of calcium to form the calcium salts of the amylophosphoric esters which constitute the amylopectin portion of the starch (see p. 37).

Commercially, however, a number of processes have been covered by patents and have as their object the production of starches which swell or dissolve in cold water, or the preparation of adhesives.

One of the first patents covering the use of salts for modifying starch was taken out by the Arabol Manufacturing Co.<sup>64</sup> In this patent, starch is heated with a strong solution of potassium or ammonium thiocyanate in alcohol, e.g. 100 lb. potato starch are added to 80 lb. of a 50 per cent. solution of ammonium thiocyanate containing 40 lb. of alcohol.

Neustadt<sup>65</sup> recommends treating 100 kg. of potato starch with 3 kg. of calcium nitrate, 1.5 kg. of sodium chloride, and 1.5 kg. magnesium sulphate, the mixture being ground after the solutions have been added to the starch. The swelling action of calcium chloride solution on starch is well known, and Wattecamp,<sup>66</sup> by mixing starch with its own weight of a concentrated solution of zinc chloride containing sodium chloride, obtains, after the mixture has been standing some time, a hard, gum-like mass which is soluble in cold water. According to his specification, 2100 gm. zinc chloride, 250 gm. sodium chloride, 1375 gm. calcium chloride, and 125 gm. ammonium chloride are dissolved in 3475 gm. water,

and to the solution 2000 gm. rice starch, 800 gm. potato-starch flour, and 500 gm. white dextrin are added.

A very transparent and syrupy paste may be prepared by suspending 100 lb. starch in 180 lb. water at room-temperature and adding 115 lb. calcium chloride to the suspension with constant stirring, which is continued for two hours. To increase the viscosity about 1 lb. borax may be added at the end of the process. This preparation offers no difficulties and special precautions are unnecessary. Adhesives of this type, containing more water, are widely used in the manufacture of wallpapers, as the binder for surfacing pigments, metallic powders, etc.; and magnesium chloride in equivalent amounts may also be used with excellent results for this type of work. R. Dulac<sup>98</sup> has found that the consumption of calcium chloride can be reduced by 13-15 per cent. if about 2 lb. of alum is included in the above formula. If borax, however, is used, more water and calcium chloride are required to obtain a paste of the same fluidity.

R. L. Datta and co-workers<sup>108</sup> have described the preparation of fluid office pastes and find that potato starch gives the best effect for this type of adhesive. The formulæ they give work well and are satisfactory for general office work. The starch, 100 pts., is sifted into 70 pts. of water containing 36 and 47 pts. of zinc chloride and calcium chloride, respectively, at a temperature of 65° C. The liquid is stirred during the addition of the starch and thickens to give a clear translucent gel which is then diluted with 800 pts. of hot water. Maize and wheat starches can be used but are not nearly so satisfactory as potato starch.

Kühl and Soltan<sup>67</sup> use neutral persalts to obtain strong adhesives with a glassy appearance; for example, they treat rice starch with 1 per cent. of a persalt, such as ammonium persulphate or sodium perborate, for 7-8 minutes at 3-5 atmospheres pressure. Ninety-six parts of starch and 4 parts of ammonium persulphate heated together for 2-3 hours at 45-50° C. give a good cold adhesive.

Stein<sup>68</sup> obtains modified starches and adhesives by treating starch at elevated temperatures with 1-2 per cent. of salts, such as potassium iodide, aniline hydrochloride or organic sulphonates, the products giving homogeneous pastes with cold water.

One feature of certain powders that swell in contact with moisture is that they form lumps when added to water. In some cases the powders have to be sifted on to the water, which is stirred during the addition, but even then small lumps are apt to form and give rise to a heterogeneous paste. J. Kantorowitz<sup>69</sup>



has overcome this difficulty in the case of certain soluble starches by mixing the starch with a salt-like alum or magnesium sulphate, which exerts some wetting action but retards the swelling. The effect of these salts in retarding the swelling action is also made use of in the manufacture of modified starches, the starch being processed in their presence and a product obtained which is easily handled.

Another patent, granted to Mahler and Supf,<sup>70</sup> although not coming strictly within this section, may be mentioned here owing to the similarity of some of the claims to those in the Kantorowitz patent. They add two kinds of materials to prevent lumping, (a) thickeners for starch pastes, such as alum, weakly alkaline reacting materials, tannic-, fatty- and resinous acids; (b) compounds which can precipitate starch from its solutions, principally magnesium compounds. These additions can be made to the starch or to the water. In a further patent<sup>71</sup> the same workers claim the use of soluble albumins, gums, dextrans, and pectin in the same manner.

Borax is widely used in the adhesive industry and particularly for adhesives containing dextrin. If borax is added to a starch paste the mass becomes like rubber and cannot be spread because it 'balls up' on the surface to which it is applied, in other words, it crumbles into little rounded masses while being spread. If the paste to which the borax has been added is treated with an acid, a workable paste is again obtained. One of the chief uses of borax is in the preparation of laundry stiffening mixtures; by stiffening the finished film it allows a higher glaze to be imparted by ironing or calendaring.

Henkel et Cie<sup>73</sup> use this property to obtain thicker solutions from the starches soluble in cold water that are obtained by grinding starch with caustic potash, water and alcohol,<sup>28</sup> and they also employ calcium borate<sup>87</sup> for the same purpose, except with the soluble starches made by the action of calcium halides. After obtaining calcium starch, this firm treats it with an alcohol-water mixture which eliminates the calcium and gives an adhesive product.<sup>74-75</sup> In another patent these calcium preparations are also treated with borax to solubilise them and produce adhesive pastes.<sup>76</sup>

In one patent, Grosvenor<sup>86</sup> employs trisodium phosphate, tribasic lead acetate or sodium aluminate, substances which have already been mentioned as being used in the preparation of adhesive pastes.

Borax can be used with lower-grade starches to make pastes with a higher viscosity than could otherwise be obtained, amounts

of 1-2 pts. of borax to 1000-10,000 pts. of starch effecting a notable increase in viscosity, the effect being greater the more nearly the reaction of the paste approaches neutrality. In a neutralised alkali paste, salt is added to increase the density. The addition of calcium chloride to such pastes renders them somewhat turbid and less viscous, and introduces the factor of hygroscopicity; and the same applies to the addition of magnesium chloride but to a more marked degree. The amount of sodium chloride added may vary widely for different purposes, between such limits as 10 to 50 per cent. on the weight of starch, leading to a great improvement in transparency, ropiness and density, but retarding the drying of the paste and giving low water-resistance. Such a paste can be diluted considerably and is quite stable, but is, of course, not meant to be employed for high-strength joints.

Dense and opaque adhesives which may be readily diluted with water, and are cheap, may be made by introducing sodium silicate into the formula. Such an adhesive may be made by suspending 130 lb. tapioca starch in 20 galls. water, adding 33 lb. caustic soda ( $36^{\circ}$  Bé.) diluted with 3.3 galls. water, and after forming the alkali starch, diluting with a further 20 galls. water. To this solution is added 43 lb. sodium silicate solution ( $35^{\circ}$  Bé.) and 0.1 lb. borax dissolved in 15 galls. water, and the mass stirred until homogeneous. The mass is next practically neutralised by the addition of 35 lb. hydrochloric acid ( $22^{\circ}$  Bé.) and diluted with 20 galls. water, which are added slowly and with constant stirring. The final addition is made of 1 gall. formaldehyde (15 per cent.). The paste so obtained is slightly alkaline and thus allows the borax to exert its maximum effect as stabiliser for the precipitated silica gel. It may be, however, that the alkaline nature of the finished product is undesirable, and then the water-content is kept lower, and the borax replaced by a little alum, which assists in neutralising the residual alkalinity and also increases the viscosity and water-resistance of the paste. Pastes made with sodium silicate are 'ropy,' and no work appears to have been done concerning its effect on the tensile strength of the joint made with such an adhesive. Most neutral salts increase both the viscosity and the density of the paste; although they generally stabilise the paste, there always remains the fact that they have no adhesive power of their own, and therefore their use leads to the production of weaker joints. Their tendency to increase the hygroscopic nature of the paste should also be considered in formulation. The compactness obtained by some salts can be obtained in several cases by the use of a small amount of soap (see p. 50).

**Treatment with Oxidising Agents.**—We have already dealt with the general effect of oxidising agents, used alone, upon starch, but if the action is modified by being carried out in the presence of an alkali the reaction can be stopped at a point at which the products obtained are not thin-boiling starches but adhesives that are cheap, rapid-drying and efficient. These adhesives may contain as little as 8 per cent. or as much as 50 per cent. of starch, and the joints obtained by their use are said to be excellent in their moisture-resisting properties.<sup>98</sup>

Tapioca starch is used, but potato starch may replace it if a somewhat inferior product is allowable. A feature of this process, as distinct from the manufacture of glues using strong alkalies acting in the cold, is the employment of higher temperatures, and the inclusion of formaldehyde, which appears to exert a specific effect on the finished joint made with such adhesives.

If adhesives with a solid-content in the upper range are required, more oxidising agent must be used. Thus for a paste with a water-tapioca starch ratio of 8-1, about 0.02 per cent. of 12-volume hydrogen peroxide is required, for a 6-1 ratio about 0.035 per cent., for a 3-1 paste about 0.35 per cent., whilst for a 2-1 ratio the amount used rises to 0.8 per cent. Hydrogen peroxide has the advantage over metallic hypochlorites of leaving no residue after the reaction. To make these adhesives, 1000 lb. of starch suspension of the required solid-content is made containing 6 lb. of lime or 0.45 lb. caustic soda liquor (36° Bé.), the hydrogen peroxide added and the temperature raised to 80-85° C. A strong reaction takes place; further reaction may be observed when a little formaldehyde is added to the paste while hot, a small amount of formic acid being formed which assists in neutralising the paste. When the water-starch ratio is 3-1, calcium chloride to the extent of 3.7 per cent. is added before the formaldehyde, and with a 2-1 ratio, lime is used in preference to caustic soda when the suspension is first made. The addition of 0.05 lb. of Marseilles soap to the suspension before heating gives a smoother paste. The solutions obtained as above should be quite watery when hot, and on standing should give a smooth lard-like paste.<sup>98</sup> A paste with a 3-1 ratio, or a 2.5-1 ratio, forms a valuable adhesive for cigarette papers or other thin paper, where the inclusion of too much water would induce puckering. Such pastes are also free from the defect of giving a brownish stain down the cigarette, and from the smell and taste of burnt sugar given by many dextrin adhesives. Here again potato starch may be used instead of tapioca starch, but such pastes have the usual strong odour associated with potato starch. In this case the alkali used is

sodium zincate, formed by the reaction between caustic soda and zinc sulphate, and the proportions in which these two substances are used determines the properties of the finished paste; the greater the amount of zinc sulphate used, the whiter the paste and the lower its final viscosity.

N. A. Spasskii<sup>109</sup> makes a bookbinding paste using bleaching powder and a potato or maize-starch suspension, but his formula has no new features.

**Treatment of Starch with Swelling Agents.**—We have considered several metallic salts which have the power of swelling starch and may now turn our attention to other compounds possessing a similar action.

Mauch,<sup>77</sup> and Schulze,<sup>103</sup> and Schaer<sup>78</sup> have examined the swelling action of chloral hydrate on starch. This compound exerts a very powerful effect and, as previously stated, it behaves similarly in presence of gelatine. Henkel et Cie claim the use of this compound in one of their patents.<sup>79</sup> They make the starch swell by treating it at a high temperature and pressure with urea, or thiourea, chloral hydrate, etc., in the presence of small amounts of liquids miscible with water, e.g. 100 kg. starch is treated with 2 kg. urea and 10 kg. alcohol and the mixture heated at 170° C. under 500 atmospheres pressure. Still another patent covers the use of water-immiscible amines and chloral hydrate.<sup>82</sup> E. F. Hoppler and J. W. Haake<sup>80</sup> heat various starches, grains, or tubers containing less than 25 per cent. moisture with urea, chloral hydrate, thiocyanates, calcium halides, or numerous other specified modifying agents, at the same time subjecting the mass to mechanical pressure.

Reference should also be made to page 49, where a number of agents which could be used in this manner are listed.

**Addition of Various Compounds to Starch Adhesives.**—Mention has already been made in previous sections of a number of compounds added to starch preparations to improve the working properties in one direction or another. It is extremely doubtful if one adhesive will ever be discovered that can be used to solve all the many problems in the adhesive field, for the property which has outstanding value in one set of circumstances may prove to be as big a drawback in another.

We thus find it necessary to add various compounds to a certain preparation so that from one base several adhesives for different classes of work may be obtained. Special types of adhesives show definite drawbacks, not so much in their use but in their preparation, and this applies also to the dextrin adhesives. Thus one drawback to a certain type of adhesive, which is sold in the powder

form and has to be dissolved in water before use, has already been mentioned as consisting in the formation of clumps of material which are very difficult to get into solution unless adequate facilities for heating, mechanical stirring, etc., are present, and often they are absent.

To overcome this tendency to form lumps on addition to water, the Jagenberg-Werke<sup>81</sup> treat the adhesive powder, in this case a dextrin, with a small quantity of a polyhydric alcohol, preferably an aliphatic glycol such as ethylene glycol, and heat the mass to about 80° C. The powder obtained goes into water quite smoothly and only about 1 per cent. of the agent is required. When dry, inorganic fillers are to be used; in an adhesive they should first be pasted with water, preferably containing a little soap, to prevent the formation of lumps.

The addition of urea has already been briefly indicated, and it is interesting to note that this compound is used in the manufacture of adhesives from casein, gelatine or glue as well as from starch. The viscosity of the solutions is reduced so that the content of solids may be increased and the same working consistency obtained as when no agent has been added and the original solid-content employed. With paper adhesives this tends to stop the crinkling of the paper, or the splitting of paper which has been stretched during the application of the adhesive, when the joint dries, as often happens when a paste of a high water-content is used. Reference may be made to the Physical Properties section of this book where this phenomenon is discussed more fully. Besides lowering the setting- or gel-point of the adhesive paste, urea is claimed to increase the tensile strength of the joint.<sup>83</sup> It is especially useful in certain classes of work in which paper is used or where non-rigid joints are required.

As urea stops the 'set-back' of adhesive pastes it forms a useful stabilising agent and, as before mentioned, its use allows a reduction in the degree of oxidising action or the amount of alkali and processing necessary for attaining a certain fluidity. It has a further peculiar action in retarding the initial evaporation of water from the glue. It does not interfere with the quick-setting action required in certain adhesives once this has started, and which is brought about by the use of less water, but it appears to delay the inception of the quick-setting action. This property has been found particularly advantageous in the preparation of glues for plywood, bentwood, and veneers. The insoluble products obtained by the action of formaldehyde on starch, described elsewhere, can be solubilised by the use of this agent, and thiourea would probably be quite as effective, if not more so, but its greater

price would prohibit its use on economic grounds. H. F. Bauer<sup>107</sup> has covered the use of about 5 per cent. of urea and 5 per cent. alkali metal acetates in re-moistening adhesives.

Thick pastes with smooth-working properties for paper work are obtained by the addition of a soap which acts as a thickener and gives the paste an unctuous consistency. A 10-12 per cent. starch paste containing 0.5 per cent. caustic liquor (36° Bé.) gives a good paste if 1.2-1.5 per cent. of soap is added before heating. If a low-grade starch which tends to be thin-boiling is used, the caustic soda solution may be replaced by 0.03 per cent. borax, which will body it up. By the use of hydrogen peroxide a higher ratio of starch to water may be obtained, the soap being added to the paste after the action of the peroxide is nearly complete.

Of recent years a remarkable class of compounds has been developed, chiefly for use as detergents in the textile industries and to 'wet out' textile fibres. The addition of a small amount of these wetting agents to aqueous solutions assists the solution to displace the top layer of air on a given surface, thus allowing the liquid to make contact with the substance. When one considers the difficulty of wetting certain foils used as wrappers or waxed papers, highly calendered surfaces, etc., the value of these agents for use in adhesives will be appreciated. Many of them are stable to acids, salt solutions, and hard water, thus further increasing their usefulness.

In one patent the products obtained from the action of sulphuric acid on the alcohols obtained from tallow, coconut oil, castor or sperm oils, are added to potato-starch pastes to improve the spreading properties of the pastes. For potato-starch pastes the sodium cetyl sulphonate, in amounts varying from 1-3 per cent., may be used. W. Schrauth<sup>84</sup> claims the use of 0.5-5.0 per cent. of the reaction products obtained from sulphuric acid and an aliphatic or cycloaromatic, saturated or unsaturated, alcohol containing eight or more carbon atoms. Henkel et Cie<sup>85</sup> use certain wetting agents stable to lime in the proportion of 0.1-1.0 per cent. on the dry base. The agents they claim may have in the molecule a long-chain alkyl group, or hydroaromatic group and a sulphonic, sulphuric ester, phosphoric ester, carboxylic, quarternary ammonium, ether or aminosulphonic group; for example, sodium dodecyl- $\alpha$ -sulphonate, disodium oleyl sulphate, or compounds of the formula  $R \cdot O \cdot CO \cdot CH_2 \cdot S \cdot SO_3 \cdot Na$ , where R stands for  $C_8H_{17}$  or its immediate higher homologues. To increase the smooth-working properties of cold-water soluble starches, they add 2 per cent. of the sulphuric

ester of a mixture of octyl, decyl, dodecyl, tetradecyl and cetyl alcohols, whilst for dextrans the addition of 2 per cent. of the sodium salt of dodecylmercaptoacetic acid is recommended.

Albumin derivatives of a salt-like character serve, according to the Sichel Komm-Gesellschaft and Stern,<sup>86</sup> to increase the spreading power and elasticity of starch adhesives when employed in amounts up to 10 per cent., calculated on the base of the starch used, e.g. the product obtained by the degradation of casein with hydrochloric acid may be used. In another patent of Stern's,<sup>88</sup> the paste obtained by the treatment of starch with sodium silicate is mixed with wetting agents, lecithin, or soaps, to improve the working properties; and the addition of 6 per cent. of naphthene soap has a favourable influence on the keeping properties of starch pastes according to the patent of Mahler and Supf,<sup>89</sup> who consider it superior in this respect to the fatty-acid or resin-acid soaps, for example, the ammonium resinate or rosin used by Grosvenor.<sup>97</sup>

Herth<sup>90</sup> obtains an increase in adhesive properties and an increased gloss in textile dressings by forming a colloidal dispersion of inorganic materials *in situ* from reagents which give a precipitate by double decomposition. As an example, 10 per cent. soda ash and alum is added to a corn-starch paste.

The addition to pastes of solvents for grease and waxes in order to obtain adhesives for waxed papers is practised by a number of firms; thus the Sichel Komm-Gesellschaft<sup>91</sup> adds toluene, xylene and similar solvents to the starch pastes, and after forming the emulsions find they are stable and of value for the pasting of waxed papers (*v.i.*).

Schluter<sup>92</sup> adds decomposed grain flour to his starch before forming the paste, and it will be seen later that in one of the processes for manufacturing textile sizes it is customary to allow a flour suspension to ferment for some time before making the size, the claim being that a better adhesive action is obtained. A further point of interest is that an adhesive of good standing is made by the controlled fermentation of gluten, as mentioned in the section on by-products. Another addition to starch in the preparation of an alkali starch adhesive is that made by Kreismann,<sup>93</sup> who incorporates a certain amount of potato pulp in the mixture.

The addition of borates to starch preparations has already been mentioned (see p. 276), but we may note here the addition of certain borates to starch preparations to increase their stability and smooth-working properties. If an alkali starch paste is made by, e.g., slurring 72 kg. potato starch in 140-150 litres of water

and running into this 15 kg. of 30 per cent. caustic lye with constant stirring and heating; then on treating this paste with 120 kg. barium chloride in about 18 litres of water the barium starch is precipitated and can be filtered off. The product is an insoluble powder and can be kept in the dry state in admixture with alkali salts, such as borax. When the mixture of barium starch and borax is added to water the inorganic constituents react to give an adhesive paste.<sup>94</sup> Sodium sulphate can be used in place of the borax to effect the decomposition, and instead of the barium starch the calcium, aluminium, zinc, and magnesium complexes may be used.

A further modification<sup>95</sup> of the above process is to employ, for the decomposition, soluble metal salts other than those of the alkalis, e.g. calcium, aluminium, iron or copper salts, and these may be added at the time of preparing the alkaline-earth starch. It will be seen that many alternatives are possible in this process; the adhesives obtained vary from glue-like adhesives to smooth pastes, depending upon the metals used and the order of use.

The addition of alum, glycerine, and other hydroxy-compounds of a hygroscopic nature has already been discussed (see pp. 263, 280). For compounds used as antiseptic agents and preservatives see page 364. We may now pass on to the consideration of dextrin adhesives.

**Dextrin Adhesives.**—A great many dextrin adhesives are in everyday use and can be formulated to give smooth pastes having little tackiness when first applied, but which after a slight exposure to air give good adhesion when pressed into contact with another surface, for example, photographic mounting pastes or poster pastes. Dextrin glues can also be made which have great tackiness, like the 'pick-up' gums used in labelling or sealing of cartons or packages.

To give body to a dextrin adhesive a white dextrin may be used as the base, and the solid-content and tackiness increased by the addition of dextrans which have been converted much further. For example, if a hand-sealing adhesive for cartons or paper is to be used, the tackiness of the paste need not be great, as the thin layer of adhesive is exposed to the air for an appreciable time before the joint is made. This condition is very different from that in which the adhesive is spread on by a machine and the joint made in a fraction of a second. In the second instance, tackiness is essential in order to hold the joint firmly together during the rapid progress through the machine.

A typical formula for a hand-sealing adhesive is as follows: A maize starch is converted by the action of malt extract until



the iodine test gives a slightly reddish-blue colour and the sugar-content is 5 per cent. This material can be dried round hot rollers and stored for further use. The dried material is soaked overnight in twice its weight of water and then 10 per cent. of a medium-cooking white dextrin is added, and the temperature raised to 80° C. Five per cent. of borax is dissolved in a little water and added to the mass, followed by 2.5 per cent. of caustic soda and a small amount of phenol to act as a preservative.

As an instance of the type of formula used to obtain an adhesive for sealing wrappers by means of a machine the following formula may be noted. Fifty parts of water are brought to boiling-point and 40 parts of a well-converted yellow potato dextrin dissolved in it, after which 4 pts. of borax are added, and when this has dissolved 2 pts. of caustic soda, 0.1 pt. phenol and 0.05 pt. turkey-red oil are added in that order. It will be noticed that in this formula the tackiness has been increased by using a well-converted dextrin, and that the water-content has been decreased from that used in the hand-sealing formula, being approximately 51 per cent. as opposed to the 57 per cent. in the previous instance.

Cardboard used to make tubes, etc., is more absorbent than the paper employed for wrappers, so that in an adhesive to be used, e.g. in making spiral tubes, the water-content has to be still further decreased in order to obtain a thicker gum which will not penetrate into the cardboard and thus be lost for the purpose of joining the two surfaces. For this type of work a very sticky gum is required, and the dextrin mentioned in the previous paragraph may be employed. The water-content in this case is reduced to about 48 per cent., thus limiting penetration. The deep colour of the adhesive is no drawback for this type of work, as it is practically the same as that of the cardboard itself.

The plant for the manufacture of dextrin adhesives is generally simple in design, and may be either a jacketed pan provided with a stirrer, or a vat heated by means of live steam passing into the glue itself. The latter type is very efficient, and care should be taken before starting manufacture to put at various heights graduation marks for indicating the weight of liquid contained in the vat, and then to pass in live steam until the temperature rises to 98-100° C. The increase in the amount of water from condensation of steam should be carefully noted and due allowance made for this factor in any mixings. The stirrers should be preferably of the gate type, revolving in opposite directions, geared to give 10, 20 and 40 revs. p.m. at will, depending on the size of the vat, as the peripheral speed will of course depend on this. The vats themselves should be preferably of wood, or of enamelled or lead-

lined iron in order that discoloration of the light-coloured adhesives made in it may be minimised. The mixers should empty by gravity—for viscous pastes through a gate valve, and thinner pastes through a large cock fitted with a straining cloth. The lid of the vat should fit well and be lined preferably with copper sheet, except for enzyme conversions.

Borax and caustic soda are often added to dextrin solutions, and it is essential that the borax should always be added first and allowed to dissolve before the addition of caustic soda. If these compounds are added in the reverse order the glue becomes 'burnt,' i.e. loses most of its adhesive characteristics and turns a very deep brown colour. The borax buffers the action of the caustic soda and is generally added to the extent of twice the weight of the alkali, whilst the total amount of inorganic matter often amounts to 15 per cent. on the weight of dry material present.

When a standard set of dextrans has been acquired from a reputable maker, further deliveries of any dextrin should be compared against the standard for viscosity in solution, colour, sugar-content, and 'set-back'. As explained on page 236, 'set back' is the term used to denote the stability of the solution, or the tendency of the solution to go cloudy or thicken on standing.

Sodium perborate<sup>105</sup> is often added to obtain a bleaching effect, and at the same time the amount of borax produced by its decomposition must be allowed for in the formula when borax and caustic soda are to be added. The treatment of glues with this agent is carried out by adding a cold solution to the glue at a temperature of less than 35° C. after all the other ingredients have been added.

It should be remembered that bleaching agents, such as sodium persulphate and especially hydrogen peroxide, cause a marked decrease in the viscosity of dextrin solutions. The same effect is obtained when formaldehyde is added to the hot mix (see, however, p. 272), but the use of sodium perborate does not show this effect so much, as the borax formed by the action tends to increase the viscosity and so rectify any drop in this value that may have occurred.

The addition of calcium chloride to a dextrin adhesive imparts an oily or 'lardy' appearance to pastes made from the mixture; it is one of the agents employed to give a slow-drying glue, glycerine being another. The solution containing calcium chloride and dextrin is often employed in adhesives for highly calendered papers, which are somewhat slow in wetting out, but the use of this agent is not entirely satisfactory for this purpose as sometimes there is a tendency for a discolouring stain to appear around the joint.

The sodium bisulphite which is added to dextrin adhesives sometimes acts as a bleaching and preservative agent, but its use tends to make the adhesive work badly on machines, owing to a strong tendency to ropiness. The same phenomenon is observed in the presence of salicylic acid, although, as already stated, this substance cannot be used as a preservative if the adhesive is liable to come into contact with iron, owing to the intense discoloration that follows contamination with iron salts.

Certain dextrans are supplied as powders containing all the ingredients necessary to make the adhesive, and all that is necessary is the addition of water. Some of these are called Arable Gums or Envelope Gums and are generally formulated on a fairly well-converted tapioca-dextrin base. They may contain up to 2 per cent. sodium bisulphite, or additions of borax and soda ash. On solution in water they yield yellow or brown, syrupy solutions which have excellent adhesive properties.

By careful processing, dextrin glues may be obtained containing gelatine, and such adhesives may be employed for joining cigarette papers that are used by those who prefer to make their own cigarettes. Pectin glue is another kind that can be used in conjunction with dextrin for this purpose.

For purposes in which the glue is to come into contact with the tongue, such as glues for envelopes or for stamps, a straight tapioca or maize dextrin may be employed; they have little taste or smell, whereas potato dextrin has a decided taste and an unpleasant smell.

For sticking waxed surfaces, several makers have evolved dextrin adhesives containing a solvent, like trichlorethylene, emulsified in them, but so far these mixtures have had no really outstanding success, whilst their odour constitutes a drawback for many purposes. Another problem confronting the adhesive maker is that of labelling tins, and the above type of adhesive has been used for this purpose. When the tins leave the factory where they are made the surface is covered with a very thin film of palm oil or a fat, which is applied at one stage during manufacture. The thin film of grease prevents the adhesive from wetting the metal, and although adhesion between the metal and label is apparently good when the joint is first made, on drying the label peels off or is very easily removed. Some makers claim to have overcome this defect by the addition of wetting agents to the adhesive, but for some time now many makers have used a base other than dextrin for this type of work; in some cases the difficulty has been side-tracked by using a label which extends right round the tin and is gummed to itself on the small overlap

left for the purpose. Most of the agents employed to pierce the film of grease generally cause rusting of the tin in time; caustic soda and nitric acid are old offenders in this respect and have therefore fallen into disrepute.

The type of dextrin known as Crystal Gum forms an excellent basis for adhesives to be used for delicate work. In some classes of work, such as lining boxes with coloured paper, the reaction to acid or alkali of the colour used on the paper has to be considered, some dyestuffs giving a change of colour in contact with acid, others with alkali. Crystal gum may be used for this kind of work, as it is generally neutral in reaction, free from starch, contains very little sugar, and very rarely contains chlorine or sulphur dioxide, both of which are very bad when present in adhesives used for colour work.

Crystal gum is generally made from potato starch, and although it is somewhat more expensive than ordinary dextrin, it is cheaper than other adhesives with similar properties and is very effective. It is sometimes used to make the wood filler in cabinet-making or in decorative work. It is used in conjunction with a filler, generally of the pigment or inorganic type. Barytes, china clay, kaolin or zinc oxide can be used where a white compound is required, but where a coloured filler is needed an ordinary dextrin is employed, as its colour is masked by the pigment. The following is an example of the type of formula used in this work: 2 lb. crystal gum are dissolved in 3-4 lb. hot water, and 5 lb. barytes and 2 lb. china clay are well mixed into the solution. Several ounces of driers are dissolved in 2 lb. boiled linseed oil and added to the hot crystal-gum suspension of the fillers. The resulting emulsion is then diluted to any required consistency with water. Synthetic resins are being used more and more, especially in the manufacture of moulded caps for bottles or jars used in the cosmetic and pharmaceutical industries. Many of these caps are lined with cork and are required to have labels affixed to them. A composition for affixing such cork linings may be made as follows: 35 lb. of crystal gum are mixed with 45 lb. of gypsum, and another mixture made containing 6 lb. powdered fish glue, 10 lb. casein and 3 lb. soda ash or borax. When required for use the crystal-gum mixture is added to water, followed by the second mixture.

One adhesive is made by mixing 2 lb. of very finely powdered casein with 1 lb. crystal gum containing 1 per cent. hydrated lime or borax. This mixture is used as a fixative for dentures; it is tasteless, odourless, and resistant to saliva and liquids in general. The adhesive properties remain for some time, as the alkali present

serves two purposes : it assists the casein to dissolve and retards the rate of hydrolysis of the crystal gum, which already has a slower rate of hydrolysis than the ordinary dextrans.

Mention should be made here of the importance of using dextrans having a low dextrose-content for the manufacture of adhesives. Dextrans produced by the roasting method contain a lower proportion of sugar than dextrans produced in other ways, as we have seen in Part II, Chapter 6, and the dextrans mentioned in the preceding formulæ come into this category. Dextrose is more hygroscopic than maltose, which is the sugar formed in the enzyme process. The content of sugar in a dextrin to be used in making an adhesive should be around 2-3 per cent. for yellow dextrans and 4-7 per cent. for white dextrans, including those made by the wet method using enzymes. The reaction in the last method is generally stopped at a point where the iodine test gives a purplish-blue coloration, and it is found that the sugar-content at this stage varies, according to the starch used, from 5-7 per cent.

A dextrin containing an excessive amount of dextrose gives adhesives showing the phenomenon of 'crystallisation,' i.e. of drying rapidly on the surface ; the internal strains set up in this surface film during drying are sufficient to cause it to fly apart and many minute cracks to appear. When a satisfactory source of supply of dextrin has been found it is therefore as well to get all supplies from this source in order to ensure uniformity of deliveries, and to check this by chemical and physical tests on every new delivery.

As explained earlier in this section, the elasticity of the film is an important point, but for high-speed work on automatic machines it is not advisable to add such agents as glycerine, ethylene glycol, etc., to attain this end, as these agents, being hygroscopic, tend to absorb moisture in humid atmospheres and give rise to trouble on the machines. Ammonium sulpho-ricinoleate may be added in place of these compounds for this type of work and does not suffer from the above defect ; further, owing to its wetting properties, the adhesion is improved. Glycerine, etc., and glucose may, however, be used for some types of paper work and give the film of adhesive an enhanced brightness and plasticity.

If two surfaces of low permeability are to be joined, one of which is delicate in construction, i.e. metallised labels on perfume bottles, the adhesive should contain as little moisture as possible and, as previously mentioned in regard to adhesives for coloured papers, be neutral. A straight dextrin adhesive containing approximately 60 per cent. solid matter, about 1.6 per cent.

formaldehyde, and a wetting agent should, after careful neutralisation with caustic soda, be found to give good results. A fairly soluble yellow dextrin will, of course, be employed in such mixtures in order to obtain a high solid-content with maximum workability.

The use of wetting agents has been mentioned several times and described for starch pastes; they may also be used for dextrin pastes. Varnished or highly calendered papers, and some metallic surfaces, are not easily wetted by straight dextrin-water solutions, but the presence of alkali in the 'mix' besides ensuring stability often improves the adhesion by assisting the adhesive to wet the surface. Alkali also tends slightly to erode some surfaces and thus gives the adhesive a chance to 'key' itself in. The propensity of alkalis to cause further corrosion after their first initial attack on the surface should be carefully watched, and also the likelihood of the gum being used for fugitive-coloured surfaces. Many wetting agents are neutral in reaction and very efficient in use; they may be employed not only for allowing a difficultly-wetted surface to be joined, but also to assist in the wetting of a dried gummed surface, such as labels or envelopes. The addition of glycerine assists the latter operation, but such labels, etc., are liable to stick together in a humid atmosphere, whereas the presence of a small but effective amount of wetting agent inhibits this action but readily allows the label, etc., to be moistened by the mouth or moistening pad.

A point to be remembered in the formulation of an adhesive for gummed labels and papers is that a low-priced adhesive may be just as effective as a higher-priced one that has been made from lighter coloured, and therefore probably more expensive, materials. The film given by the lighter-coloured adhesive appears thinner than a layer of the darker adhesive of the same thickness, and prospective purchasers of the paper may gain the impression that the darker adhesive has been more freely applied. Unless a light colour is actually demanded, therefore, it is preferable not to bleach the adhesive or to use expensive light-coloured materials in the formula.

The increase in viscosity imparted by certain additions to adhesives has been mentioned above, and it is sometimes necessary that the viscosity of dextrin solutions should be increased or decreased in order to suit certain working conditions. If very porous paper or other surfaces are to be joined, the viscosity of the adhesive should be such that too much of the adhesive is not absorbed and so lost for joint-formation, and this effect may be brought about by the inclusion of a certain amount of a fairly

good-grade gelatine in the mixture. To decrease the viscosity and allow a higher percentage of dextrin to be employed, the dextrin solution may be treated with formaldehyde. The amount of formaldehyde used may vary from 2-5 per cent., and even up to 30 per cent. in some cases, and the solution heated to 80° C. The colour of the solution is not darkened, nor are any other desirable characteristics of the film destroyed, in fact, a dextrin treated in such a way gives more lustrous and more easily-wetted films than a similar untreated dextrin. It should also be noted that many of the agents used to decolorise dextrin solutions tend to lower the viscosity, and this seems more apparent with the oxidising agents used for this work than with the reducing agents; hydrogen peroxide in particular markedly lowers the viscosity. It is interesting to recall that bleaching with oxidising agents is much more effective and lasting than bleaching with reducing agents, thus illustrating the more vigorous character of the former.

An *ageing test for envelope adhesives* is given by J. R. Adams<sup>110</sup> and may be of interest. This worker encloses 50 envelopes in a small air-tight tin box which is maintained at 60° C. for 72 hours. The test is claimed to give the same amount of discoloration in the adhesive as 10 months' natural ageing in a semi-tropical climate without changing the colour of the paper to a noticeable degree. Interruption of the test for only a few minutes, e.g. by opening the box to remove a few samples, delays the development of the maximum discoloration for 24 hours. A loose-fitting lid on the box delays the appearance of the maximum discoloration for a longer period than this. The number of envelopes in the box is immaterial. Those papers sized with glue are most susceptible to discoloration, while other classes of paper are practically unaffected.

Enough has been said to illustrate the principles of adhesive-making with dextrans and to give some idea of the many possible formulæ that are available, only a very few of which have been quoted above.

#### REFERENCES

1. J. A. RADLEY, *Manuf. Chemist*, 1934, May.
2. J. W. MCBAIN and W. B. LEE, *Third and Final Report, Adhesive Research Committee*, H.M. Stationery Office, London, 1932, 66.
3. J. W. MCBAIN and D. G. HOPKINS, *J. Phys. Chem.*, 1925, 20, 188.
4. MÉRIMÉE, *Bull. Soc. d'encouragement pour l'ind. nat.*, 1827, 118.
5. MARSDEN, U.S.P. 376,445, 1888.
6. J. KANTOROWITZ, E.P. 5844, 1896.
7. ——— E.P. 10,216, 1910.
8. ——— D.R.P. 157,896, 1903; U.S.P. 785,216, 1905.
9. ——— D.R.P. 158,861, 1903.

10. J. KANTOROWITZ, D.R.P. 160,259, 1905.
11. LEONHARDT, G.P. 408,523.
12. PERKINS, D.R.P. 282,699, 1911; U.S.P. 1,020,655, 1912.
13. — U.S.P. 1,020,656, 1912.
14. GROSVENOR, U.S.P. 1,200,488, 1916.
15. GRÖNINGER, G.P. 302,832.
16. SUPF, G.P. 351,370.
17. PFEIFFER and SCHWANDER, G.P. 432,961.
18. H. BECHHOLD, G.P. 564,302, 1936.
19. O. MEYER, G.P. 447,727; E.P. 286,377, 1926; U.S.P. 1,773,056.
20. F. RIETHOF, Austrian P. 128,641 and 112,647, 1928.
21. SÄCHSISCHE KLEBSTOFFWERKE, G.P. 474,602, 1924.
22. HENKEL ET CIE, G.P. 582,679, 1926.
23. L. WEISS, U.S.P. 1,474,129.
24. A. SINGER, E.P. 188,344, 1922.
25. MAHLER and SUPF, G.P. 508,160.
26. HENKEL ET CIE, G.P. 508,786.
27. — G.P. 478,538.
28. — G.P. 479,143.
29. PFEIFFER and SCHWANDER, G.P. 527,140.
30. LEONHARDT, G.P. 412,125.
31. MAHLER and SUPF, G.P. 364,314.
32. SICHEL and STERN, G.P. 349,280; G.P. 372,794.
33. RUNGE, G.P. 381,516.
34. SICHEL and STERN, G.P. 389,748, 1920.
35. — G.P. 415,092, 1920.
36. KLEBSTOFFWERKE COLLODIN, G.P. 414,979, 1920.
37. HAAKE, G.P. 547,421, 1926.
38. SOC. ANON. DES RIZERIES FRANÇAISES, G.P. 406,540, 1923.
39. E. STERN, *Zeit. angew. Chem.*, 1928, 41, 88; G.P. 519,300, 1924.
40. — E.P. 272,274, 1926.
41. — U.S.P. 1,661,201.
42. HEIM, G.P. 453,501, 1924.
43. J. SELLARS, E.P. 2810, 1865.
44. A. SCHUHMAN, E.P. 5460, 1887.
45. W. P. THOMPSON, E.P. 7272, 1891.
46. — E.P. 21,973, 1906.
47. F. A. V. KLOPFER, G.P. 528,109, 1930.
48. H. H. LAKE, E.P. 5617, 1893.
49. DURYEA, U.S.P. 675,822, 1901; U.S.P. 696,949, 1902.
50. BERGQUIST, U.S.P. 1,287,841, 1918.
51. MURPHY, U.S.P. 568,265, 1896.
52. BROWNING and BARLOW, U.S.P. 773,469, 1904.
53. B. HELFERICH, A. STÄRKER, and O. PETERS, *Ann.*, 1930, 482, 183.
54. K. FREDENHAGEN and B. HELFERICH, U.S.P. 1,883,676.
55. I.G. FARBENIND., G.P. 560,535.
56. H. SCHENBACH, G.P. 554,699; G.P. 566,515.
57. STUTZKE, U.S.P. 1,320,719, 1919.
58. H. COURTONNE, *Compt. rend.*, 1920, 171, 1168.
59. MÖLLER-HOLTkamp, U.S.P. 793,600, 1905.
60. J. ALEXANDER, U.S.P. 1,337,382, 1920.
61. REYCHLER, *Bull. Soc. chim. Belg.*, 1920, 29, 118.
62. L. EYNON and J. H. LANE, 'Starch,' W. Heffer & Sons, Ltd. Cambridge, 1928.



63. D. R. NANJI and R. G. L. BEAZELEY, *J. Soc. Chem. Ind.*, 1926, **45**, 215T.
64. ARABOL MAN. CO., F.P. 394,167, 1908.
65. NEUSTADT, G.P. 392,660, 1921.
66. WATTECAMPS, G.P. 444,576, 1925.
67. KÜHL and SOLTAN, G.P. 522,555, 1928.
68. STEIN, G.P. 390,478, 1919.
69. J. KANTOROWITZ, U.S.P. 1,677,348.
70. MAHLER and SUPF, G.P. 371,407, 1918.
71. — G.P. 389,023, 1921.
72. E. MEUSEL, *Jahresb. Chem.*, 1886, 2099.
73. HENKEL ET CIE, E.P. 359,756.
74. — E.P. 276,340.
75. — G.P. 308,616 and 406,820.
76. — E.P. 244,708, 1924.
77. R. MAUCH, *Archiv. Pharm.*, 1902, **240**, 166.
78. E. SCHAER, *Pharm. Centralhalle*, 1896, **37**, 540.
79. HENKEL ET CIE, G.P. 563,272.
80. E. F. HOPPLER and J. W. HAAKE, E.P. 346,224, 1930.
81. JAGENBERG-WERKE A.G., E.P. 441,658; F.P. 783,963, 20/7/1935.
82. CORN PROD. REF. CO., U.S.P. 2,165,834.
83. HOLZHYDROLYSE A.G., G.P. 605,016.
84. W. SCHRAUTH, U.S.P. 2,051,184; 18/8/1936.
85. HENKEL ET CIE, E.P. 432,486, 1934.
86. SICHEL KOMM-GES., G.P. 359,519.
87. HENKEL ET CIE, G.P. 554,988.
88. E. STERN, E.P. 447,810.
89. MAHLER and SUPF, G.P. 556,448.
90. HERTH, G.P. 395,647.
91. SICHEL KOMM-GES., G.P. 455,014, 9/10/1925.
92. SCHLUTER, G.P. 572,052.
93. KREISMANN, U.S.P. 1,490,330, 1924.
94. E. STERN, G.P. 519,300.
95. — G.P. 542,581.
96. GROSVENOR, U.S.P. 1,378,105, 1921.
97. — U.S.P. 1,311,965, 1919.
98. R. DULAC and J. L. ROSENBAUM, 'Industrial Cold Adhesives,' Griffin & Co., London, 1937.
99. G. E. CORSON, U.S.P. 1,977,514, 1934.
100. PERKINS, E.P. 427,880, 1935.
101. E. H. HARVEY, U.S.P. 1,790,346, 1931.
102. W. SCHULZE and C. BEYER, E.P. 466,287, 1937.
103. — *Biochem. Zeit.*, 1938, **292**, 141.
104. M. SAMEC, *Kolloidchem. Beih.*, 1912, **4**, 132; 1913, **5**, 141.
105. L. FACKLER, U.S.P. 1,618,150, 1927.
106. F. CAMPO-CAMPINO, *Paper Trade J.*, 1940, **110**, 120.
107. H. F. BAUER, U.S.P. 2,183,736, 1939.
108. R. L. DATTA, S. C. SEN and N. N. BOSE, *Manuf. Chem.*, 1940, **11**, 197.
109. N. A. SPASSKIĪ, *Poligraf. Proizvodstvo*, 1938, No. 1, 33; via *Chem. Zentr.*, 1938, **II**, 2547.
110. J. R. ADAMS, *Techn. Assoc. Papers*, 1939, **22**, 174; *Paper Trade J.*, 1939, **109**, No. 9, 31.
111. V. I. NAZAROV, *J. Applied Chem.*, U.R.S.S., 1939, **12**, 1745.

## ADDITIONAL REFERENCES

- F. HOŁT, *Paper Ind.*, 1935, **17**, 482. (General.)
- C. BECHER, *Gel. Leim. Klebst.*, 1934, **2**, 113. (General.)
- *ibid.*, 1935, **3**, 54 and 87. (General.)
- E. RINGINBACH, E.P. 465,301, 1937. (Starch pastes.)
- DUEROU, *Récherches et inventions*, 1935, **16**, 254. (General.)
- C. F. MASON, *Chem. Industries*, 1936, **39**, 171. (General.)
- HENKEL ET CIE, F.P. 816,967, 1937. Equivalent to E.P. 479,316. (Salts of sulphonic-acid derivatives of starch ethers.)
- F.P. 808,699. Equivalent to E.P. 478,299. (Salts of cellulose-ether carboxylic acids mixed with starch.)
- W. SCHRAUTH, U.S.P. 2,051,184. (Long-chain alcohols and their sulphates used in starch and dextrin pastes.)
- DEUTSCHE HYDRIERWERKE A.G., G.P. 653,186, 1937. (Aliphatic sulphuric esters used in starch pastes.)
- H. T. MAYER, *Gel. Leim. Klebst.*, 1938, **6**, 107. (Potato-starch adhesives of the neutral type.)
- P. KREISMAN, U.S.P. 1,667,073, 1921. (Potato starch, clay and aqueous alkali heated until semi-fluid glue obtained.)
- H. V. DUNHAM, U.S.P. 1,551,472, 1925. (Glue. Heats starch with water and alkali-saccharate.)
- P. D. COPPOCK, U.S.P. 2,181,782, 1939. (Dextrin rendered non-lumping in water by heating between hot plates under pressure.)
- J. F. WALSH and W. L. MORGAN, U.S.P. 2,170,271, 1939. (Thin-boiling starch by treatment with an amide-hydrochloride.)
- U.S.P. 2,170,272, 1939. (Acid salts of amides or amino acids claimed to thin starch.)
- F. OHL, *Kunstdünger u. Leim*, 1938, **35**, 309. (Practical suggestions on adhesives for use in cardboard industry.)
- N. SPASSKIĬ, *Polygraph Ind. (U.S.S.R.)*, 1937, No. 9, 29; *Chem. Zentr.*, 1938, **1**, 3294. (Alkali-starch adhesives described.)
- W. R. LONG, U.S.P. 1,633,840, 1924. (Hydrolysed maize starch mixed with caustic soda and borax to give a glue.)
- H. F. BAUER and D. M. HAWLEY, U.S.P. 2,188,329. (Dextrin, urea, chloral hydrate and glycerol used to obtain re-moistening gum.)
- E. R. EDSON and G. F. MACH, U.S.P. 2,192,585. (Envelope gum. Dextrin and urea.)
- M. SAMEC, G.P. 402,644, 18/2/1922. (J. B. KERB's reaction (see p. 90) used to obtain glues from starch.)
- P. B. DAVIDSON and J. R. ADAMS, U.S.P. 2,202,247. (Envelope glue from dextrin, water and dioxane.)
- R. DALE and J. F. WALSH, U.S.P. 2,094,558. (Dextrinising maize starch containing gluten.)
- R. M. HIXON and G. F. SPRAGUE, *Ind. Eng. Chem.*, 1942, **34**, 959. (Waxy starch of maize as a possible substitute for tapioca starch in adhesives discussed.)

## CHAPTER 2

## THE FOODSTUFF INDUSTRY

THE chemistry of starch is of great interest to foodstuff manufacture, as it constitutes an excellent raw material which can be used when an inert, neutral, edible filler is required, where liquids such as soups, etc., are to be thickened, or where gels are required, as with blancmanges, custard powders, etc.<sup>1</sup> As starch constitutes one of the major components of flour, its behaviour is of particular interest to the baking trade, whilst its presence contributes to the character of potatoes, when these are cooked.

The following chapter cannot assume to be more than a brief résumé of the immense amount of work done on starch in relation to foodstuffs, but the importance of the part played by starch in the properties of certain foodstuffs will readily be seen. Those interested in the chemistry of the potato should consult the paper by L. H. Lampitt and N. Goldenberg,<sup>63</sup> whilst the chemistry of cereals has been fully and authoritatively dealt with by D. W. Kent-Jones in his book 'Modern Cereal Chemistry'.<sup>36</sup> The manufacture of glucose, maltose and alcohol has already been discussed.

**Potato Products in the Food Industry.**—In Germany there are several thousand potato-alcohol distilleries and a similar number of starch and glucose factories. Besides glucose and starch a large amount of potato meal or flour is produced and is used in home-made pastries and bread. Its manufacture on the Continent is carried out either in large central factories, with an output of 100 tons or more a day, or in small portable plants.

Potato flour enables potatoes to be kept in a form which eliminates the loss usually met through frost, decomposition, etc., whilst the bulk is very greatly reduced.

The starch-content of edible varieties is generally about 14-17 per cent., of which 1 per cent. or so is usually unrecoverable. Industrial varieties contain from 18 to 23 per cent.—occasionally 25 per cent.—but are not suitable for food purposes in normal time owing to their poor flesh colour and high fibre content.

To make potato flour the tubers are well washed, pulped and then steamed under about two atmospheres pressure,

air being blown through the hot pulp at the beginning of the steaming process to remove any volatile impurities from the pulp.<sup>64</sup> This procedure improves the smell and flavour and at the same time removes those impurities which tend to hydrolyse the starch to glucose. At the end of half an hour the material is discharged into a vessel containing water where the peel and heavy impurities sink to the bottom and the rest of the liquor is dried in a band or other type of dryer, or in the most modern plants a spray dryer is used.

Potato flour will keep for many years and contains 8-9 per cent. proteins, 0.2-0.5 per cent. fat, 80-82 per cent. carbohydrates and 2.0-2.5 per cent. ash.<sup>65</sup> In some cases the moisture-content is allowed to rise to some 18 per cent.<sup>64</sup>

In the U.S.A. and Canada potato starch is blended with maize, tapioca or wheat to produce varieties of custard, blanc-mange and pie-filler powders. In cake mixtures, especially those of the waffle and sponge-cake type, a far superior product is said to be obtained by using up to 40 per cent. of potato flour to replace the wheat flour,<sup>65</sup> but a safer figure for shortbread, gingerbreads, cake and ordinary bread is probably 20 per cent. Some workers consider 50 per cent. can be added for bread-making, but the harsh flavour of the potato flour is accentuated. The consequent reduction in gluten is met by increasing the egg-content, although not in the same proportion. Potato starch is also used in soup and gravy powders.

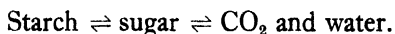
Moore and Partridge<sup>66</sup> record the use of boiled potatoes in bread-making to improve the whiteness of the bread. Contemporaneous usage in the U.S.A. calls for an addition of 10-20 per cent. of potato flour, with or without the addition of corn meal to reduce excess elasticity or 'dough tightness'. This practice permits the addition of greater amounts of water and the complete hydration of the gluten.<sup>65</sup> As an improver of crumb texture and colour the further claims have been made for potato flour that it has anti-staling properties and imparts a flavour that is valuable in the baking of brown, and especially rye breads. H. E. Cruz Monclova finds that good quality breads can be obtained when 20 per cent. of *cassava flour* is mixed with wheat flour, but more yeast than usual and potassium monohydrogen phosphate must also be included.

**Importance of the Storage History of Potatoes.**—According to J. Wright<sup>67</sup> and Barker<sup>68</sup> the sugar-content of potatoes may vary from 0.2-6.8 per cent. and is dependent on the storage temperature.

TABLE 6

Sugar.	Observer, Ref. No.						Additional Data.
	67.	78.	79.	91.	92.	93.	
	%	%	%	%	%	%	%
Reducing Sucrose .	0·91-1·99 0·27-0·3	— 0·04	0·72 0·67	0·05 0·14	0·17-0·35 0·51-0·6	0·3 0·2	— 0·1-0·26 <sup>84</sup>
Total	1·18-2·26	0·09-0·12	1·39	0·25	0·74-0·86	0·5	{ 1·25-2·28 <sup>85</sup> 0·26 <sup>88</sup>

When potatoes are stored the following reactions take place :—



Lowering of the temperature to below 4·5° C. leads to the formation of more sugar and at 0° C. 3 per cent. of sugar is the equilibrium concentration.<sup>63</sup> According to Barker<sup>69</sup> sugar formation can take place at temperatures as low as -20° C., and other workers have also studied the low-temperature storage of potatoes. Wright<sup>67</sup> found an increase in both sucrose and reducing sugars on low-temperature storage, and Barker<sup>70</sup> considers that sucrose, or possibly the fructose half of the sucrose molecule in the labile  $\gamma$ -fructose form, may be the respiratory substance. When the temperature is raised the sugar-content decreases<sup>71</sup> and may possibly be used up in respiration. The starch-sugar balance is not only dependent on the temperature but may be influenced, according to Barker, by its previous temperature treatment. He has suggested<sup>68</sup> that during cold storage an irreversible accumulation of a 'depressant' occurs which is developed at high temperatures. This would explain why storage at low temperatures depresses the respiratory action in a manner which subsequent storage at high temperature fails to overcome.

Prior exposure to higher temperatures increases the sensitivity of the starch-sugar balance, whilst a process of acclimatisation to intermediate lower temperatures has the opposite effect.

**Colour of Potato Chips.**—When potatoes are baked or fried the sugars present are caramelised and the coloration of the products obviously depends on the quantity of sugar present.

F. G. Denny and N. C. Thornton<sup>73</sup> confirmed earlier work that storage of potatoes at 5° C., at which sprouting was inhibited, induced the formation of so much reducing sugar that the chips made from the potatoes were too dark. Storage at

10° C. or above gives rise to sprouting if stored too long at this temperature. The storage of potatoes to be used for chip-making is therefore of great importance. A content of 5 mg. of reducing sugar per ml. of juice (4 mg. per gm. of fresh potato) is the optimal concentration. The total content of sugar is no guide in selecting potatoes able to give the attractive golden-brown colour when fried, but with American varieties the reducing sugar-content is an extremely good guide. Whether this holds for English and other varieties remains to be seen.

Thus we see that the previous temperature history of potatoes is of great importance when they are to be used for making chips. On baking or frying less losses are encountered than with other methods of cooking potatoes.<sup>74, 75</sup>

**Cooking other than Frying.**—Potatoes in their 'jackets' lose less than peeled potatoes when cooked,<sup>74-77, 100-106</sup> whilst steaming is more preferable in this respect than boiling.<sup>75-77</sup> If the potatoes are given a preliminary soaking in water or entered into cold water and brought to the boil, the losses are increased. In the case of boiled potatoes these losses are due to the leaching out of sugars, starch, nitrogenous compounds and mineral salts but the moisture-content is slightly increased.<sup>78, 79</sup>

Many workers have found the vitamin-C content of potatoes to decrease on cooking in an amount dependent on the method used.

On cooking, the cells of the tuber separate, causing mechanical disintegration, the starch is partially gelatinised, the pectins degraded, the cellulose becomes more digestible and the proteins coagulate. Sweetman<sup>80</sup> has discussed the physico-chemical changes taking place on cooking, and Personius and Sharp<sup>81</sup> have followed certain of the changes taking place by means of tensile-strength determinations and find that no loss of strength occurs at 50° C., but above this there is a progressive loss which reaches a definite value for each temperature. As the weakening occurs prior to the gelatinisation of the starch this takes place independent of cell separation. These workers confirmed this conclusion by gelatinising the starch in the potatoes by immersion in 40 per cent. chloral hydrate, 2N sodium salicylate or ammonium thiocyanate followed by a treatment with a 0.5 per cent. solution of ammonium oxalate to soften the tissues. Only when the starch-gelatinising agent and the tissue-softening agent were used in succession was it possible to obtain a 'cooked appearance,' either agent alone being ineffective in producing this effect. They concluded that, on cooking, gelatinisation of the starch and a marked decrease in the cell adhesion of the tissue takes place.

The texture of the cooked potato appears to be governed by some other factor as the chemical cooking produced the same effect as normal cooking when 'mealy' or 'soggy' potatoes were used. Sweetman<sup>75</sup> found no positive correlation between mealiness and the starch-content of potatoes and, so far, the cause of mealiness has not been explained. That it is a function of the damage to the pectins present was suggested by Sweetman,<sup>75</sup> but Barmore<sup>82</sup> obtained no correlation between pectin-content and mealiness in potatoes of equal starch-content.

Dumanski<sup>83</sup> found that the bound water increases from 0.567 gm. to 1.506 gm. per gm. dry solids on cooking, and it is possible that the resulting texture is dependent not only on the starch but also on the total, or bound, water of the partially gelatinised starch.

Rathsack<sup>77</sup> has introduced a Disintegration Index, Z, and a Texture Index, S, and found that Z is independent of, but S positively related to, the starch-content of the potatoes.

**The Gelatinisation of Starch.**—As stated above starch is insoluble in cold water, but when a suspension in cold water is poured into boiling water, or is itself boiled, the granules swell and, on cooling, forms a jelly when the concentration of starch present is high enough. Rice and maize starch both form 'short,' opaque white gels if heated above 75° C., whilst translucent rather adhesive gels are formed from potato or tapioca starches if these are heated to about 70° C. with five to ten times their weight of water.

Professor Sybil Woodruff has examined and photographed gels made at different temperatures from starches used in the Food Industry, and her photographs constitute excellent permanent records of the differences in physical appearance that otherwise could not be described adequately. The outline of a gel turned out from a mould gave good indications of its character, although differences were noted in opacity, tenderness and freedom from stickiness. The addition of sucrose, such as is made in some sweetened starch puddings, was found to lower the gel-strength.

Chapman and Buchanan<sup>62</sup> have studied the syneresis or slow separation of water from gels of maize, wheat, rice and potato starches and consider that neither the rate nor the time of heating the suspension affects the rigidity or the amount of syneresis in the resulting gels. The importance of this will not be overlooked by those interested in blancmange and custard powder manufacture.

When heating starch suspensions the opacity slowly decreases and at the temperature of gelatinisation it falls quickly to a

minimum value which is not affected by heating for a longer time or at a higher temperature. Heating to this point, however, is insufficient to produce good gels since, as explained above, all the granules are not fully swollen. If the heating is continued to about 100° C. well-formed gels are formed from 5 per cent. suspensions of maize, rice and wheat starches, but potato, arrowroot and tapioca starches give soft or quite fluid gels. The rice gel is the most translucent and tender, the maize the firmest and whitest, while wheat starches give a gel of intermediate characteristics. Potato starch gels are ropy, transparent and stick to the mould, arrowroot gives a still more transparent and softer gel, whilst tapioca starch does not give a gel at the concentration used.

The results show that to obtain the maximum strength of the gel it must be heated well beyond the temperature at which the opacity suddenly decreases. In ordinary cooking, of course, temperatures of 90-100° C. are readily reached. With potatoes properly cooked internal temperatures of 100-104° C. have been recorded, but if the internal temperature reaches only 97° C. the potato is underdone. In biscuits and muffins a temperature of 100° C. is reached, but in these cases, however, it must be remembered that the granules cannot be completely swollen as the amount of water is very limited. If starch containing about 45 per cent. moisture is heated to 90-100° C. the granules swell but do not gelatinise and remain as discrete particles.

When starch is examined microscopically between crossed Nicols prisms the granules appear strongly birefringent and the birefringency disappears when the granules gelatinise. Alsberg and C. P. Griffing<sup>2</sup> have examined breadcrumbs microscopically and found that although birefringent masses could be seen no individual birefringent granules could be detected. This is explained by Woodruff's observation<sup>3</sup> that the temperature at which birefringency disappears, i.e. at which the granules are swollen, depends on the amount of water available.

**The Effect of Various Factors on Gel-Strength of Starch Pastes.**—In making custard or blancmange powders another point of interest is that different strengths may be obtained by the use of different batches of the same starch, as Woodruff<sup>4</sup> has found that the ability of a starch to form a gel can vary according to the source of the starch. She found<sup>4</sup> that gels of maize starch made from corn which had suffered from heavy frosts before it is completely mature do not leave the mould as cleanly as usual but that the ability to form a gel did not appear to be noticeably affected.



Another important factor is the effect of the purification processes during manufacture of the starch. It is customary to steep maize in water saturated with sulphur dioxide (see p. 84) to reduce bacterial and fungoidal growth, to increase the speed of softening and to give the final product a good colour. Starch separated without the use of sulphur dioxide, however, gives stronger gels than that made using the normal sulphur dioxide treatment.

With wheat starch an improvement in colour together with an improvement in baking properties is obtained by bleaching, which can be carried out in about five different ways. The bleaching process is very akin to the sulphur dioxide treatment of maize, but Woodruff (*vide supra*) found that such treatment has little or no effect on the gel-forming properties of the wheat flour. This is probably due to the fact that the gelling power and the viscosity of maize-starch pastes are more readily affected by the addition of chemicals or the pre-treatment of the starch than is the case with wheat starch.

The effect of freezing on starch pastes is interesting (see also p. 106), and may hold some interest for food processors not only from the foodstuff point of view but also because they may be using starch pastes for adhesives in some part of the factory. By freezing and then thawing a starch paste the jelly-like structure is destroyed and a spongy mass results. With maize starch the water can be squeezed out readily by hand after which it will readily soak up water again without, however, giving the original gel. From wheat starch the sponge formed retains the water more firmly than that from maize starch and is not so tough (Woodruff<sup>5</sup>). When allowed to dry it gives a very tough, horny mass differing very markedly from that obtained by drying maize-starch 'sponge'.

It is interesting to note that the appearance and properties of the gels changed to a much greater degree when the pastes were frozen at  $-2^{\circ}$  to  $-3^{\circ}$  C. than when frozen at a much lower temperature. In this connection it must be mentioned that bread-staling occurs much faster at  $-2^{\circ}$  to  $-3^{\circ}$  C. than at any other temperature. At the temperatures given by solid carbon dioxide or liquid air the rapid formation of small ice crystals and the lack of facilities for orientation of the starch molecules probably explains the lesser change which takes place on freezing to these low temperatures. After freezing to such low temperatures the pastes, on thawing, have a consistency very similar to a freshly gelatinised paste, and microscopical examination shows that it has a very 'short' or 'brittle' appearance, whereas

paste frozen at  $-2^{\circ}$  to  $-3^{\circ}$  C. shows a ropy network. Woodruff has shown pastes frozen below this temperature gave the smooth form on thawing, but if this temperature is not reached then thawing produces the ropy form (see also p. 106).

With starch from different varieties of maize it has been found that the gel-strength of the pastes varies over a wide range, the starch from white corns giving stronger gels than those from the yellow types. The viscosity differences between these pastes are not very pronounced, but if a pectin-jelly-strength tester be employed to measure the gel-strength of these pastes wide variations are encountered. Thus a test based on a gel-strength would appear to be more valuable to the food manufacturer than one based on the more usual viscosity test which does not show the effect of poor growing conditions or of frost on the growing corn which is reflected in the gel-strength of the starch made from such corn. Another point of interest is that rapidly raising the temperature to  $95^{\circ}$  C. gives a firmer gel than slowly heating to that temperature.

**Uses of Starch in Various Foodstuff Preparations.**—Maize, tapioca, and rice starches or flours have all been used in the manufacture of *custard powders*, *ice-cream powders*, *cake powders*, *blancmange powders*, etc. Other substances, such as sugar, essences, and colours, are also added to meet special requirements. The addition of essences and colour is generally made by first preparing a small master batch, which is carefully incorporated with the rest of the mixing. If the colour is added as a solution, care must be taken to strain out any undissolved particles of dyestuff before it is added to the starch, and to dry the coloured starch at a suitably low temperature so as to avoid gelatinisation of the starch and the formation of small lumps.

Starch has also been used as a carrier for organic peroxides in bleaching flour,<sup>85</sup> and as a filler in *improvers and bleaching agents* for use in dough.<sup>86</sup> E. V. McCollum and O. S. Rask<sup>89</sup> have introduced a so-called solid form of lactic acid for use in baking powders and self-raising flours. It is made by mixing gelatinised starch paste with sufficient lactic acid to yield a product containing about 46 per cent. of the acid and roller-drying the paste at  $125-137^{\circ}$  C. *in vacuo*.

Maize starch is used to manufacture vinegar, the process being carried out on the same principle as that by which barley-malt vinegar is produced. The analytical figures for phosphorus and nitrogen given by maize vinegar are much below those generally accepted for a genuine vinegar from barley malt, whereas the figure for the extract and the original total solids are considerably higher.<sup>88</sup>

*Extract, or sugar, of malt* is widely used as a dietary preparation for the treatment of enfeebled digestions, and is administered as a strengthening accessory in conjunction with cod-liver oil, iron compounds, or quinine. It may be prepared by heating potato starch with water, adding 1-3 per cent. of malt extract, cooling, then adding a further 4-7 per cent. of 'green' malt, and carrying the reaction to the required stage.

The American Diamalt Co.<sup>87</sup> prepares a proprietary foodstuff by the following method: Maize starch is converted to dextrin by a preliminary treatment with hydrochloric acid in the usual manner. Hot water is then added to give a solution containing from 10-30 per cent. of solid matter, after which it is cooled to 75° C. and the pH value adjusted to between 4.7 and 5.2. An addition of diastase is made and the reaction allowed to proceed for 15 minutes, thereupon the mass is heated to 100° C. for 10-15 minutes, followed by cooling to 70-75° C., at which temperature it is maintained and more diastase added. This stage is allowed to continue until a portion of the solution withdrawn from the bulk gives a brown coloration with iodine solution. The solution is finally concentrated to a syrup of sp. gr. 1.39. This product consists principally of amylopectin, and 24-27 per cent. of maltose, calculated on the concentrated syrup.

Starch has been used as a comparatively inert filler in a number of preparations. One such product is that known as baking powder which is added to the materials used to make bread, cakes, etc., in order to leaven the product, i.e. to give off a certain amount of gas during the baking process and thus distend the mass to a honeycomb structure. One brand on the market is said to contain some 40 per cent. of rice starch. W. Gallay and A. C. Bell<sup>90</sup> have made a thorough study of the effect of potato, maize, wheat and rice starches in combination baking powders under various conditions of storage, and find that stability depends largely on particle size, so that rice, wheat, maize and potato starch mixtures were in that order of stability. Again, small wheat granules were found to be better than large granules, and finely-powdered wheat starch better than the coarsely-ground starch. The difference in stability imparted by the various starches becomes more marked as the particle size of the acid component decreases. Those interested in this problem are referred to the full details given in the original paper.

S. Medelsohn<sup>6</sup> has presented an excellent account of the function of starch in baking powder media. He points out that rice starch is more costly than other commercially available

starches and often contains residual adsorbed alkali which would affect the taste, but this would not be a drawback in baking powders. Wheat starch 'fines' have granules of approximately the same size as those of rice starch and are as efficient as the latter for stabilising purposes. Corn starch, however, keeps the constituents of the baking powder separated more efficiently than other starches, and this efficiency is so marked that it outweighs the effect of the greater surface which is presented by this starch and which makes for greater moisture adsorption than is the case with rice starch. It is interesting to note that maize-starch granules have an average size which is very close to the mean granule size of the other starches mentioned above.

Good, dry corn starch has for many years been employed in this type of work and looks likely to maintain this position in the face of competition from other agents suggested as fillers in patent literature, especially as the suggested substituents are, in most cases, more expensive in use.

In the above case it will be seen that a balance has to be struck between the surface area per unit mass, which affects the adsorption of moisture and hence the stability of the reactive mixture, and the granule size, which governs the effective separation of the components of this reactive mixture. Rice starch and wheat 'fines' present low surface areas, wheat starch a much higher area, whilst maize starch has an intermediate value, but the particle size of the latter affords a more efficient protective effect on the particles of the constituents of the mixture.

Pastes, sauces, and gravy powders are other culinary adjuncts which are sometimes thickened with starch.

In the manufacture of the so-called *clear-* or *hard-gums* in the *confectionery trade*, the actual vehicle for the glucose, colour and flavouring essences has long been gum senegal. Recently, however, a few firms have turned their attention to replacing this substance by starch, either the soluble or the ordinary variety. Satisfactory results are stated to have been obtained, but the choice of starch appears to be as important as the manufacturing process, no particular trouble being experienced, although long boiling times are generally required. Starch may also be used in Turkish Delight.

In making the clear-gums, the syrup is run into moulds and dried for several days. The moulds are formed by indenting the required shape in a packed mass of maize starch. After the gums have been dried to the correct moisture-content, the whole mass, gums and starch, are tipped out on to sieves, the gums being retained while the starch falls through; it is collected and

compressed again in trays ready to receive another impression to serve as a fresh mould. The condition of the moulding starch is very important. It should be cleaned and dressed regularly, with additions of new starch to replace losses, so that the general condition of the starch is always uniform. Where possible, starch that has contained 'short-stoved' work should be used next time for longer stoving, as this course assists to keep the starch dry. In this manner good work should be in a fit condition for crystallising with nothing more than a light brushing. N. I. Kozine<sup>111</sup> has found that the inclusion of potato starch in mayonnaises increases the stability, and allows a reduction in the oil-content without affecting the stability.

**Starch in the Baking Industry.**—The properties of the starch present play an important part in every process connected with bread-making. Ordinarily it is regarded as forming the source of carbohydrate which is first acted upon by the diastase present in the flour, after which the sugar so produced serves as the fermentation medium for the yeast which is added. Only a small amount of the starch takes part in this sequence.

**Moisture Absorption by Dough.**—Wheat starch in an atmosphere saturated with water<sup>7</sup> vapour at 21.5° C. absorbs at least 36 per cent. water and assuming that only the water is reduced in volume when starch swells, H. Rodewald<sup>8</sup> has calculated that it is under a pressure of 2821 atmospheres in fully imbibed starch granules. A. Maurizio<sup>9</sup> cites the work of Bouteuse, who has calculated that the distribution of water between the starch and gluten in flour is very nearly the same. Flours containing a higher proportion of gluten require more water in doughing and the distribution will be somewhat different.

In hard wheats the starch is present embedded in a relatively large amount of gluten and very few 'free' granules can be observed. In soft wheat flours, on the other hand, the gluten masses enclose less starch and a larger proportion of the starch granules are 'free'. These granules are readily accessible to and can readily be saturated with moisture. Thus the amount and the freedom of the starch in a flour plays a part in determining the moisture absorption although its influence is probably less than that of the gluten. If the absorptive powers of two starches in two samples of flour are different it follows that the water absorption will be affected.

Starch granules injured by the grinding processes swell more in cold water than do uninjured granules, and a flour that has been severely ground therefore shows an increase in moisture absorption over a flour made from the same grain but less severely.

milled.<sup>10, 11</sup> It should be noted that another factor entering into the greater activity of overground flours is the more thorough freeing of diastase present and the more intimate contact brought about between the diastase and starch. The gluten also contributes to this increase as its properties have been somewhat altered also, but when the grinding has been very severe the injury to the gluten is excessive and its moisture absorption decreases. Now injury to the starch granules has another effect inasmuch as the diastase which is present can act on badly injured granules in the same way as it does on gelatinised starch. Mangels<sup>12</sup> considers that variation in the diastatic activity of a flour is largely dependent on the susceptibility of the starch granule to attack by the diastase, so that a flour with damaged granules will ferment quicker than one in which all the granules are intact. Jones,<sup>96</sup> in recent work, has shown that the mechanically damaged starch which is produced during milling is the controlling factor in the diastatic activity of a flour, and the 'maltose figure' is proportional to the amount of mechanically damaged granules resulting from any milling process. In practice this has led to overgrinding of flours considered to be deficient in diastase as suggested by Alsberg.<sup>2</sup> It should be noted, however, that some workers consider that grinding wheat in too dry a state leads to the excessive formation of damaged granules, which become water-soluble, and that such flours show instability and poor keeping properties.

**The Influence of Other Physical Properties of Starch on Baking Quality.**—Le Clerc and co-workers<sup>13</sup> and Shollenberger and Coleman,<sup>11</sup> L. H. Pulkki<sup>14</sup> have studied the effect of different sized granules of normal flour on the baking properties. By bolting they obtained fractions of coarse, intermediate and fine particle size, the intermediate grade having the best baking qualities. E. Grewe and C. H. Bailey<sup>15</sup> found no correlation between the relative baking qualities and the size of the starch granules. E. Berliner and R. Rüter<sup>16</sup> found that the saccharification of starch and the flour from which it was derived was parallel but Swedish and Manitoba samples differed. The role of saccharification of the starch in baking will be discussed more fully below.

A number of workers have proposed to use viscosity measurements of flours under various conditions as an index of the baking quality of the flour. Lüers and Ostwald,<sup>17</sup> for example, propose to use the viscosity of a thin boiled flour suspension as an index of this property. The gluten would be altered by this treatment and further differences in the particle-size distribution of the

starch granules alone would give similar differences in the complete absence of gluten. There are great differences between strong and weak wheats by this method, but this method gives only a partial view of the problem.

A. Tasman<sup>18</sup> considers that useful information can be obtained by determination of viscosity in the presence of increasing amounts of acid and plotting the differences in viscosity to form a curve.

A. K. Kuhlman<sup>19</sup> examined the viscosity of starch washed from the various samples of wheat and tried to obtain some correlation between the figures obtained and the baking properties of the whole wheat. He prepared gels of different concentrations from the dried and powdered starches and found the viscosities of the gels from the different starches to vary. Those from soft winter wheats, which showed better baking qualities, gave starches which yielded gels having higher viscosities than starches from soft summer wheats of poorer baking qualities. Good samples gelatinised when treated with 0.4-0.5 per cent. caustic soda solution but poor samples required an alkali concentration of 0.7 per cent. This worker plotted the pasting curves of the various starches when treated with alkali and the samples from wheats of good baking properties were characterised by a sharp upward bend in the curve when the concentration of the alkali reached 0.2-0.3 per cent. This indicates that at this concentration of alkali an abrupt increase in the degree of peptisation of the starch takes place. On the other hand, starch from poor wheats did not show this effect, even at the higher concentrations of alkali.

A. Schulerad<sup>20</sup> had previously suggested following the viscosity of the starch paste by means of determining the suitability of rye flour for bread-making and pointed out that the properties of the starch vary with variety and age of the flour. Buchanan and Nadain<sup>21</sup> consider that the size of the starch granules in wheat flour is an important factor in determining the strength of the flour, inasmuch as the starch in the strongest wheats was found to have the smallest granule size. Alsberg and Rask<sup>22</sup> first thought that the difference in the viscosities of starch gels from various wheat samples might be varietal rather than due to the location of growing, but their work later convinced them that the answer was not so simple as significant differences in viscosity were found between starch gels prepared from different samples of the same variety of wheat. In general, winter-wheat starches gave higher viscosities than those prepared from summer-wheats, a fact confirmed by A. K. Kuhlman. Had these workers also determined the gel-strength of the pastes they prepared

it seems likely that, in view of Woodruff's work, better correlation between the figures might have been obtained. C. H. Bailey<sup>23</sup> has designed an apparatus similar to that of Caesar in order to measure the relative plasticity of pastes and doughs and to determine the effect of unit additions of water on the consistency of the dough. In another apparatus elaborated by this worker<sup>97</sup> the dough is extruded under pressure through a hole in a cylinder, the rate of flow being taken as an index of plasticity. The log rate of flow is a linear function of the water used in making the dough. Very precise temperature control is required with this method.

Vail and Bailey<sup>98</sup> have re-investigated the question of the proportion of water in dough which exists in the 'bound' state. They make use of an apparatus in which the dielectric properties of the dough are measured. They conclude that 35.5 per cent. of the water in dough exists in the 'bound' conditions as against the figure of 51.4 per cent. calculated by Skovholt and Bailey,<sup>99</sup> using a freezing-point depression method.

Some workers maintain that flours with similar water adsorptions may lose very different amounts of moisture during baking, and it is possible that the state and character of the starch present exerts an important effect on the moisture loss. A point in favour of this view is the effect of adding potato flour, or starch, which gives a moister appearing loaf. Potato starch, as is well known, behaves very differently from wheat starch on heating with water. The latter gives a mass of swollen granules but the former yields a large amount of colloiddally dispersed material and a larger number of fragmentary particles from the outer sacs of the starch granules.

From what has been said it is obvious that starch does play a definite role in the baking properties of flour, but it must be remembered that this role is subordinate to the part played by the gluten. As discussed on page 114 when considering the staling of bread the same statement would appear to hold true, whilst the action of the amylases present also has an extremely important bearing on the suitability of a flour for bread-making and baking processes. The staling of bread has been discussed on page 111 and also by Platt,<sup>84</sup> C. L. Alsberg<sup>107</sup> and L. P. Karacsonyi.<sup>108</sup>

**The Diastatic Activity of Flours.**—The baking qualities of flour are influenced in a most important manner by the diastase present. The type and distribution of the diastases are important factors having a very marked influence on the baking qualities of the flour. Other factors which contribute



to the diastatic activity of a flour is the extent to which the granules are attacked by the diastase and the proportion of injured granules present, as the latter are highly susceptible to attack.

In the Scotch process of bread-making a portion of the starch is gelatinised by scalding a portion of the flour and is thus rendered more susceptible to diastatic attack. As diastase is colloidal it is not likely to diffuse through other colloids, such as gluten, very quickly, and it is probable that a contributory cause as to why severe grinding apparently increases the diastatic power of a flour is that the diastase is thus brought into intimate contact with the starch by mechanical means and does not have to depend entirely on diffusion to reach the starch. A further factor is that the severe grinding also liberates the diastase that is mechanically held in the various portions of the grain, for its distribution in the wheat is by no means uniform.

In barley and wheat the scutellum of the embryo, the endosperm and the aleurone layer are the chief points of occurrence of diastase, the amount of diastase probably falling off as one proceeds from the outer to the inner endosperm<sup>24</sup> where the quantity is very small.<sup>25</sup> H. P. Wijsman<sup>26</sup> and Stoward<sup>25</sup> have shown that the diastase of the inner endosperm has but low sugar-producing activity. Thus, as this portion of the grain contains most starch, the diastase can only act on it if brought into contact mechanically or by diffusion. S. S. Elizavrova<sup>27</sup> has found that practically all spring varieties of *Triticum* have a high  $\beta$ -amylase activity whereas the  $\beta$ -amylase of winter varieties is low. This difference in spring and winter varieties was not found in *Hordeum*.

Early workers considered that the differences in the diastatic powers of flour were determined by differences in diastase content,<sup>28</sup> but F. A. Collatz<sup>29</sup> found that the starch of strong flours seemed to be more easily hydrolysed than that from weak flours. O. S. Rask and C. L. Alsberg<sup>22</sup> were among the first to consider that other factors might play a more important role than hitherto assigned to them, and, later, Mangels thought that the liability of the starch granule to diastase attack played a predominating part on the diastatic activity of a flour. L. A. Rumsey<sup>28</sup> considers that starch granules, themselves, show variations in susceptibility to attack, and A. H. Johnson and C. H. Bailey<sup>30</sup> have shown that by making starch readily available in the free condition, e.g. by addition of starch to flour, the rate of fermentation is increased. W. E. Stone<sup>31</sup> holds that the action of diastase cannot be very great as the water present during

baking is insufficient to gelatinise the starch in the commonly understood sense of the word,<sup>32, 33</sup> but the granules may be present in an extremely concentrated gel and in any event have undergone some change as to render them more susceptible to diastase attack.

The chief source of gas in dough is the maltose formed, and A. Ostrovskii<sup>34</sup> therefore considers it necessary in some cases to determine the amounts of  $\alpha$ - and  $\beta$ -amylase separately (see below). C. R. Jones<sup>37</sup> considers that in flours and intermediate stocks the maltose figure is an index of the number of damaged starch granules present. The maltose figure of the coarse particles, which may escape damage in milling, increases markedly after each rolling. In general, the maltose figure becomes higher the harder the wheat. This worker is another who considers that difference in diastatic activity between flours is not necessarily due to different diastase contents, or even to differences in the susceptibility of the starch to diastatic attack, but that the physical hardness of the endosperm plays some part, as this property influences the amount of damage done to the starch during milling (see also p. 305). L. H. Pulkki<sup>14</sup> considers wheat starch to have an outer, impermeable envelope which resists diastase action and which is not stained with Congo red. Its removal by grinding allows both diastatic attack and staining with Congo red to take place. Gortner and Hamalainen<sup>109</sup> consider the variable susceptibility of raw starch to diastatic attack to be due in part to an outer protein envelope and it will be remembered that Ling has also postulated an outer envelope around starch, his suggestion being it was hemicellulose (see also p. 46). The examination of mechanical damage of starch in flour is discussed on page 378.

With the clear recognition of the presence of the two amylases, at least in diastase, several workers have studied the effect of the individual components on the baking quality of flours and, as will be seen below, very important results have been achieved by Kent-Jones and Amos. E. G. Onishchenko<sup>35</sup> finds that an addition of  $\alpha$ -amylase to the dough in the course of mixing lowered the quality of the bread, and that the action of  $\alpha$ -amylase can be inhibited by increasing the acidity of the medium. Further studies, on the roles of  $\alpha$ - and  $\beta$ -amylases in bread-making, have been made by O. E. Stamberg and C. H. Bailey<sup>38</sup> on the relation of the overgrinding of flour to dough fermentation, by L. P. Karacsonyi and C. H. Bailey<sup>39</sup> on the baking properties of fractions, and on wheat flour by R. M. Sandstedt and co-workers.<sup>40</sup>

**The Chemistry of certain Baking Faults**—In certain goods aerated by yeast and other means a fault is sometimes encountered known as stickiness or streaks in the crumb. Of the bread produced in Eire some 60 per cent. is the so-called soda bread in which soured milk and sodium bicarbonate is added to make a slack dough which is then baked at a relatively low temperature. The trouble of the sticky crumb has been prevalent in Eire since Irish millers have been obliged by law to use a considerable proportion of native wheat, much of which has been somewhat out of condition and sprouted. In the case of the popular self-raising flour, when the harvest of English wheat has been a wet one, the presence of sprouted kernels has also resulted in a stickiness or streakiness in the cooked goods.

A certain amount of guidance on the process of a flour to give stickiness in the crumb has been obtained by the application of the maltose test devised by Rumsey<sup>28</sup> and extended by Kent-Jones<sup>41</sup> who then employed it as an index of the gas-producing power of a flour. The latter worker, expressing his results as percentages, found the maltose figures for English commercial flour to range from 1 per cent. to just over 3 per cent. For a flour to gas satisfactorily during fermentation Kent-Jones has suggested that the maltose figure (determined by Lane and Eynon's method<sup>43</sup>) should not be lower than 1.5 per cent. or over 2.3 per cent., as flours showing a figure over 2.3 per cent. are liable to give stickiness of the crumb in loaves or other baked goods.<sup>42</sup> In America the Blish and Sandstedt method<sup>44</sup> is generally used in which the flour is incubated at 30° C. in a buffer solution and the amount of maltose, as estimated by the alkaline ferricyanide method, is expressed as milligrams per 10 gm. of flour. In England and on the Continent the Kent-Jones method and nomenclature are used.

Many workers, J. G. Malloch,<sup>45</sup> M. J. Blish, R. M. Sandstedt, and Astleford,<sup>46</sup> C. F. Davis and D. F. Worley,<sup>47</sup> W. J. Eva, W. F. Geddes and B. Frissell,<sup>48</sup> C. F. Davis,<sup>49</sup> and R. A. Bottomley<sup>50</sup> consider that there is no exact relation between the maltose figure and gas production but that the former gives useful guidance as to the general gassing power of a flour and the likelihood of those defects due to incorrect diastatic activity developing. Too low a maltose figure generally indicates poor gassing capabilities under certain conditions, and E. A. Fisher, P. Halton and S. F. Hines<sup>51</sup> consider that a maltose figure over 2.3 per cent. indicated a strong trend toward sticky doughs but, as Kent-Jones has pointed out, there are many exceptions to this generalisation.

N. P. Kosmin<sup>52</sup> suggested that stickiness of the crumb might be connected with excessive enzymic hydrolysis of the starch so that the remaining starch was insufficient to bind all the water present. As dextrans are produced in this enzymic degradation Kosmin suggested a new criterion of flour quality, dextrinising capacity.

Various workers<sup>53-58</sup> find that starch is converted into maltose and dextrin by  $\beta$ -amylase which is, however, without action on raw undamaged starch and only attacks damaged granules.

As a result of  $\alpha$ -amylase action only dextrin is produced (see p. 476). According to M. J. Blish, R. M. Sandstedt and E. Kneen<sup>57</sup> there is some doubt whether the latter enzyme alone is capable of attacking raw undamaged starch. As  $\alpha$ -amylase is not so thermolabile as the  $\beta$ -form and is particularly active in the region of 64° C., a temperature which may occur for a comparatively long period in soda bread, considerable dextrinisation may occur during the baking. Thus a comparatively low temperature long-baking period is more likely to produce sticky crumb than a high-temperature, short-baking process in the presence of the same quantity of  $\alpha$ -amylase.

This explains why a high maltose figure does not necessarily involve the development of sticky crumb, as a distinctly high maltose figure may occur concurrently with a low  $\alpha$ -amylase activity and *vice versa*. It also explains the success of the Kent-Jones 'dextrin figure' test<sup>59</sup> in forecasting the tendency of flours towards the defect of sticky crumb.

The Kent-Jones test<sup>59</sup> is carried out as follows: mix 1.25 gm. of flour to a smooth paste in a 6 × 1 boiling tube with 3 ml. distilled water, using a thin glass rod, and incubate for exactly 30 min. at 62° C.  $\mp$  0.1° C. At the end of this time plunge the tube into a cold water-bath and allow to remain for 4 min. The flour paste must on no account be stirred or disturbed during the time from the inception of the incubation to the end of the 4 min. cooling period. To the cooled paste add 2 ml. distilled water and mix to a smooth paste, then add a further 20 ml. distilled water, mix and centrifuge. To 10 ml. of the supernatant liquor add 2 ml. of N/10 I sol. and make up to 100 ml. with alcoholic potassium acetate solution (equal volumes of industrial spirit and a solution of 4 gm. potassium acetate in 100 ml. distilled water). Allow to stand 5 min., then filter off the precipitated starch-iodide. Transfer 50 ml. of the filtrate to a glass evaporating dish and evaporate to a volume of 5-6 ml. on the water-bath, taking care not to carry the reduction in volume to below 5 ml. Transfer the liquid to a 250 ml. beaker, using

10-15 ml. of industrial spirit and then add more spirit to make up volume to 100 ml. Allow to stand 1 hour or preferably overnight and filter off the precipitated dextrin on a tared alundum crucible of medium porosity, washing the precipitate with alcohol, and finally ether. Dry for 1 hour at 100° C. and weigh. The result is reported to the nearest 0.5 per cent.

This technique can be applied to soda bread and self-raising flours without modification. D. W. Kent-Jones and A. J. Amos<sup>59</sup> find that many really good flours give dextrin figures around 5.6-5 or even lower, but a dextrin figure of 10.0 or lower shows the flour will be perfectly satisfactory. Flours showing dextrin figures in the region of 10-14 may be considered suspect and, depending upon whether they are baked for a long time in a slack oven or rapidly baked in a hot oven, they give unsatisfactory and satisfactory results, respectively. Flours with a dextrin figure over 14 are likely to give trouble, irrespective of the baking conditions.

The colloidal chemistry of baking has been discussed by W. Heupke,<sup>60</sup> whilst a review of the progress of research in the staling of bread has been given by W. H. Cathcart<sup>61</sup> and the controlled factors in baking which enhance the staling properties of bread are discussed on page 115. The evaluation of malt products for use in the bread-making industry has been surveyed by H. C. Freeman and W. P. Ford.<sup>110</sup>

## REFERENCES

1. J. A. RADLEY, *Food Manuf.*, 1941, **16**, 89, 105.
2. C. L. ALSBERG, 'The Role of Starch in Breadmaking,' 'Comprehensive Survey of Starch Chemistry,' R. Walton, Chem. Catal. Co. Inc. N.Y., 1928, p. 87.
3. S. WOODRUFF, *J. Agric. Res.*, 1933, **52**, 46.
4. ——— *Trans. Illinois State Acad. Sci.*, 1936.
5. ——— *J. Agric. Res.*, 1936, **52**, 233.
6. S. MEDELSON, *Food Manuf.*, 1938, Oct., 333.
7. H. RODEWALD, 'Untersuchungen über die Quellung der Stärke,' Lipsius and Tischer, Kiel and Leipzig, 1896, 70.
8. ——— *Landw. Vers. Sta.*, 1894, **45**, 201.
9. A. MAURIZIO, 'Die Nahrungsmittel aus Getreide,' P. Parey, 2nd Ed. 1924, **1**, 349.
10. C. L. ALSBERG and E. P. GRIFFING, *Cereal Chem.*, 1925, **2**, 325.
11. SHOLLENBERGER and COLEMAN, *U.S. Dept. Agric. Bull.* No. 1463, 1926.
12. J. G. MANGELS, *Cereal Chem.*, 1926, **3**, 316.
13. LE CLERC, WESSLING, C. H. BAILEY and GORDON, *Operative Miller*, 1919, **24**, 257.
14. L. H. PULKKI, *Cereal Chem.*, 1938, **15**, 749.
15. E. GREWE and C. H. BAILEY, *ibid.*, 1927, **4**, 230.

16. E. BERLINER and R. RÜTER, *Zeit. ges. Mühlenw.*, 1930, **5**, 134, 156 ; 7, 63.
17. H. LÜERS and OSTWALD, *Kolloid-Zeit.*, 1919, **25**, 82 and 116.
18. A. TASMAN, *Chem. Weekbl.*, 1930, **27**, 138.
19. A. K. KUHLMAN, *Zeit. ges. Getreid. Mühl. u. Bäcker.*, 1936, **23**, 128.
20. A. SCHULERAD, *Mühlenlab.*, 1926, **6**, 177.
21. J. H. BUCHANAN and G. G. NADAIN, *Ind. Eng. Chem.*, 1923, **15**, 1050.
22. C. L. ALSBERG and O. S. RASK, *Cereal Chem.*, 1924, **1**, 7.
23. C. H. BAILEY, *J. Rheology*, 1930, **1**, 429.
24. F. J. MARTIN, *J. Soc. Chem. Ind.*, 1920, **39**, 327T and 348T.
25. STOWARD, *Ann. Botany*, 1911, **25**, 799.
26. H. P. WIJSMAN, *Rec. Trav. Chim. Pays-Bas*, 1890, **9**, 1.
27. S. S. ELIZAROVA, *Compt. rend. U.R.S.S.*, 1940, **26**, 698.
28. L. A. RUMSEY, *Amer. Inst. Baking*, 1922, Bull. 8, 84.
29. F. A. COLLATZ, *ibid.*, 1922, Bull. 9, 72.
30. A. H. JOHNSON and C. H. BAILEY, *Cereal Chem.*, 1925, **2**, 95.
31. W. E. STONE, *U.S. Office of Exp. Sta.*, 1896, Bull. 34, 1.
32. WHYMPER, *3rd Rept. Brit. Assoc. Advanc. Sci.*, 1920, 61.
33. W. JAGO and W. C. JAGO, 'Technology of Breadmaking,' London, 1881, pp. 81 and 428.
34. A. OSTROVSKII, *Biokhimiya Khlebopecheniya*, 1938, **1**, 23 ; *Khim. Ref. Zhur.*, 1939, **2**, No. 3, 134.
35. E. G. ONISHCHENKO, *ibid.*, 1938, **1**, 39 ; *Khim. Ref. Zhur.*, 1939, **2**, No. 3, 61.
36. D. W. KENT-JONES, 'Modern Cereal Chemistry,' 3rd Ed., 1939, Northern Publ. Co., Liverpool.
37. C. R. JONES, *Cereal Chem.*, 1940, **17**, 133.
38. O. E. STAMBERG and C. H. BAILEY, *ibid.*, 1939, **16**, 319 and 330.
39. L. P. KARACSONYI and C. H. BAILEY, *ibid.*, 1930, **7**, 571.
40. R. M. STANDSTEDT, C. E. JOLITZ and M. J. BLISH, *ibid.*, 1939, **16**, 780.
41. D. W. KENT-JONES, 'Modern Cereal Chemistry,' 1st Ed., 1924, p. 255, 264.
42. — *ibid.*, 2nd Ed., 1927, p. 360.
43. J. H. LANE and L. EYNON, *J. Soc. Chem. Ind.*, 1923, **42**, 32T.
44. M. J. BLISH and R. M. SANDSTEDT, *Cereal Chem.*, 1933, **10**, 189.
45. J. G. MALLOCH, *ibid.*, 1929, **6**, 175.
46. M. J. BLISH, R. M. SANDSTEDT and G. R. ASTLEFORD, *ibid.*, 1932, **9**, 378.
47. C. F. DAVIS and D. F. WORLEY, *ibid.*, 1934, **11**, 536.
48. W. J. EVA, W. F. GEDDES and B. FRISSELL, *ibid.*, 1937, **14**, 458.
49. C. F. DAVIS, *ibid.*, 1937, **14**, 74.
50. R. A. BOTTOMLEY, *ibid.*, 1938, **15**, 509.
51. E. A. FISHER, P. HALTON and S. F. HINES, *ibid.*, 1938, **15**, 363.
52. N. P. KOSMIN, *ibid.*, 1933, **10**, 420.
53. J. S. ANDREWS and C. H. BAILEY, *ibid.*, 1934, **11**, 551.
54. J. W. READ and L. W. HAAS, *ibid.*, 1936, **13**, 14.
55. E. MUNZ and C. H. BAILEY, *ibid.*, 1937, **14**, 445.
56. M. J. BLISH, R. M. SANDSTEDT and D. K. MECHAM, *ibid.*, 1937, **14**, 605.
57. M. J. BLISH, R. M. SANDSTEDT and E. KNEEN, *ibid.*, 1938, **15**, 629.
58. P. S. OUGRIMOV, *Biochem. Zeit.*, 1935, **282**, 74.
59. A. J. AMOS and D. W. KENT-JONES, *Cereal Chem.*, 1940, **17**, 265.
60. W. HEUPKE, *Kolloid-Zeit.*, 1939, **89**, 29.

61. W. H. CATHCART, *Cereal Chem.*, 1940, **17**, 100.
62. O. W. CHAPMAN and J. H. BUCHANAN, *Ind. Eng. Chem.*, 1930, **18**, 190.
63. L. H. LAMPITT, N. GOLDENBERG, *J. Soc. Chem. Ind.*, 1940, **59**, 748.
64. ANON, *Chem. Age*, 1940, 267.
65. E. J. THOMAS, *Food Manuf.*, 1940, **15**, 33.
66. MOORE and PARTRIDGE, 'Analysis of Food and Drugs,' 4th Ed., 1918, p. 105.
67. J. WRIGHT, *J. Agric. Res.*, 1932, **45**, 543.
68. BARKER, *Proc. Roy. Soc.*, 1933, **112B**, 316.
69. — *D.S.I.R. Food Invest. Board Rept.*, 1931, p. 78. H.M.S.O.
70. — *Proc. Roy. Soc.*, 1936, **119B**, 453.
71. APPLEMAN and SMITH, *J. Agric. Res.*, 1936, **53**, 557.
72. BARKER, *D.S.I.R. Food Invest. Board Rept.*, 1936, p. 193. H.M.S.O.
73. F. E. DENNY and N. C. THORNTON, *Contrib. Boyce-Thompson Inst.*, 1940, **11**, 290.
74. LANGWORTHY, *U.S. Dept. Agric., Bull.* 468, 1917.
75. M. D. SWEETMAN, *Maine Agric. Exp. Sta. Bull.*, 383, 1936.
76. WINTON and WINTON, 'The Structure and Composition of Foods,' Vol. II, Chapman & Hall, London, 1935.
77. RATHSACK, 'Der Speisewert der Kartoffel,' Berlin, 1935.
78. McCANCE and WIDDOWSON, 'The Chemical Composition of Foods,' H.M.S.O., 1940.
79. CARPENTER, *J. Nutrit.*, 1940, **19**, 415.
80. M. D. SWEETMAN, *Amer. Potato J.*, 1933, **10**, 169.
81. PERSONIUS and SHARP, *Food Res.*, 1938, **3**, 513.
82. BARMORE, *ibid.*, 1937, **2**, 377.
83. A. DUMANSKI, *Kolloid-Zeit.*, 1933, **65**, 178.
84. PLATT, *Cereal Chem.*, 1930, **7**, 1.
85. U.S.P. 1,913,776.
86. U.S.P. 1,910,344.
87. AMER. DIAMALT CO., E.P. 382,517.
88. E. S. JAMIESON, *Analyst*, 1915, **40**, 106.
89. E. V. McCOLLUM and O. S. RASK, U.S.P. 1,771,342, 22/7/1930.
90. W. GALLAY and A. C. BELL, *Canad. J. Res.*, 1936, **14B**, 204.
91. SINGH and MATHUR, *Ann. Appl. Biol.*, 1937, **24**, 469.
92. WRIGHT, *U.S. Dept. Agric. Tech. Bull.* 507, 1936.
93. McCANCE, WIDDOWSON and SHACKLETON, *Med. Res. Council. Spe. Rep.*, Series No. 213, 1936.
94. J. M. NELSON and R. AUCHINCLOSS, *J. Amer. Chem. Soc.*, 1933, **55**, 3769.
95. NIKOLAEV, *Bull. Acad. Sci., U.R.S.S., Biol. Ser.*, 1939, p. 899.
96. C. R. JONES, *Cereal Chem.*, 1940, **17**, 133.
97. O. E. STAMBERG and C. H. BAILEY, *ibid.*, 1940, **17**, 37.
98. VAIL and C. H. BAILEY, *ibid.*, 1940, **17**, 39.
99. SKOVHOLT and C. H. BAILEY, *ibid.*, 1935, **12**, 321.
100. C. VON SCHÉELE, *Med. Kgl. Lantbruksstyrelsen*, 1930, No. 283, 5 ; via *Chem. Abs.*, 1931, **25**, 1702.
101. T. CHRZASZCZ, *Polish Agric. Forestal Ann.*, 1931, **25**, 45. (In German, 57 and 59.)
102. B. LAMPE, *Z. Spiritusind.*, 1931, **54**, 235.
103. C. VON SCHÉELE and G. SVENSSON, *Landw. Vers. Sta.*, 1931, **112**, 1, 104.
104. — and J. RAMUSSON, *ibid.*, 1936, **127**, 67.

105. W. TAEGENER, *Deut. Zuckerind.*, 1937, **62**, 69.
106. SPROCKHOFF, *Z. Spiritusind.*, 1930, **53**, 35.
107. C. L. ALSBERG, *Wheat Studies*, 1936, **12**, No. 6.
108. L. P. KARACSONYI, *Z. Unters. Lebensm.*, 1928, **56**, 479.
109. R. A. GORTNER and C. HAMALAINEN, *Cereal Chem.*, 1940, **17**, 378.
110. H. C. FREEMAN and W. C. FORD, *J. Soc. Chem. Ind.*, 1941, **60**, 6.
111. N. I. KOZINE, *Maslab, Jir, Delo*, 1937, **13**, 28(3); *Chim. et Ind.*, 1938, **40**, 975.

## ADDITIONAL REFERENCES

- J. G. MALLOCH, *Cereal Chem.*, 1926, **3**, 316. (Factors affecting diastatic activity of wheat flours discussed.)
- J. G. MANGELS and C. H. BAILEY, *Ind. Eng. Chem.*, 1933, **25**, 456. (Relative viscosities of wheat starches.)
- E. MUNZ and C. H. BAILEY, *Cereal Chem.*, 1936, **13**, 427. (Relation of amylase activity to gassing rate.)
- O. NELSON and G. A. HULETT, *J. Ind. Eng. Chem.*, 1920, **12**, 40. (The moisture-content of cereals.)
- R. M. SANDSTEDT *et al.*, *ibid.*, 1937, **14**, 17. (Factors governing diastasis in wheat flour.)
- R. C. SHERWOOD and C. H. BAILEY, *ibid.*, 1926, **3**, 163. (Control of diastatic activity of wheat flour.)
- C. L. ALSBERG, *ibid.*, 1927, **4**, 485. (Role of starch in flour.)
- J. S. B. HUTCHINSON, *Nat. Assoc. Review*, 13, Supplement, 1936. (Keeping qualities of bread.)
- C. H. BAILEY, 'The Chemistry of Wheat Flour,' Chemical Catalogue Co., New York, 1925.
- E. G. BAYFIELD, *Cereal Chem.*, 1934, **11**, 121. (Evaluation of flours by physical and baking tests.)
- H. J. BROWNLEE and F. L. GUNDERSON, *ibid.*, 1938, **15**, 257. (Oats and oat products.)
- J. BURTT-DAVY, 'Maize, Its History, Cultivation, Handling and Uses,' Longmans, Green & Co., London, 1914.
- F. A. COLLATZ and O. C. RACKE, *Cereal Chem.*, 1925, **2**, 213. (Effects of diastatic and malt extracts on doughs.)
- J. KÖNIG and F. BARTSCHAT, *Zeit. Unters. Nahr. Genussm.*, 1923, **46**, 321; Abstr. in *Analyst*, 1924, 187. (Estimation of rye in wheat flour.)
- O. E. STAMBERG, *Cereal Chem.*, 1939, **16**, 769. (Surface area of starch as a factor in water absorption of doughs.)



## CHAPTER 3

## THE PAPER INDUSTRY

FUNDAMENTALLY, the manufacture of paper consists in beating cellulosic fibres in a water suspension to a state of division suitable for deposition as a thin 'web' or layer, to be subsequently dried. The various means of producing the correct 'stock' from the different forms of cellulose used commercially will not be considered here, as we are only concerned with the role of starch in the paper-making process.

The sheets or web produced in the process mentioned are very water-absorbent, often possessing little strength, and the sole outlet for such paper is its use as the well-known blotting-paper. By the addition of resin and alum a sized paper is produced that finds extensive application in printing or writing papers. For these purposes a fairly high tensile strength is required, the most important factor being the surface, which must not readily 'wet out,' so that written or printed impressions appear sharp and legible and spread only in a direction normal to the surface of the paper. The surface of a writing-paper should allow the pen to travel smoothly; and to obtain a whiter and smoother surface, clay and other filling materials are used to close the interstices between the fibres. These spaces may also be partly closed by manipulation of the paper stock on the machine. An extension of this method involves the use of starch, glue, casein, or some other film-forming colloid, which is capable of rendering the fibres more water-resistant and assisting in the production of a so-called sized paper.

The use of these colloids as auxiliary sizing agents increases the tensile strength and stiffness of the paper by bonding the fibres together, holds down small fibres, or corrects the elasticity of the individual fibre, as understood in the paper trade, which would otherwise project from the surface.

When the sizing agents are added to the beater, the paper is known as 'Engine' or 'Pulp' sized, or the finished web may be given a coating of size, a process known as 'Tub' sizing.

**Engine Sizing.**—Resin is largely used in sizing papers, but its high prices in recent years have naturally directed attention to cheaper substitutes, and the use of water-glass (sodium silicate) in the pulp, followed by the addition of alum, was found to give a gelatinous precipitate which on drying was found useful in the

sizing. It could thus be used to replace a part of the resin normally employed. In passing, it may be noted that by the use of resin a porous paper may be produced which is, however, also suitable for writing on as the individual fibres are water-repellent, whereas a non-porous 'coated' paper is obtained by spreading on a medium of starch or glue together with mineral matter, e.g. china clay.

Cobb and his co-workers<sup>7-8</sup> found that when starch alone was used it slowed down sheet-formation and reduced the percentage solids present in the sheet after wet pressing, but in most cases increased the percentage of solids in the dry sheet. The comparatively high specific gravity and high water-holding power of starch account for these observations. The high water-holding power of starch also tends to increase the contraction of the starch on drying, but widely differing effects can be obtained by altering the water-holding properties of the starch. An ordinary starch, for example, swells and holds a very much greater amount of water than a 'soluble' or oxidised starch, thus causing a much greater contraction on drying; and when present in paper the unmodified starch paste gives a greater contraction of the sheet than a thin-boiling starch. It may in this case bring about a greater increase in sheet strength, but should it contract to a greater apparent volume than an equal quantity of thin-boiling starch, it would not be present as a continuous film but as a honeycomb structure containing air. In that case the sheet would not contract so much, and this drawback, together with the honeycomb structure, would weaken the strength as compared with a thin-boiling starch.

It has also been shown that the strength of a sheet containing an increased amount of starch passes through a maximum, an observation which confirms the well-known fact that starch is a poorer sheet-forming material than cellulose. Its action is similar to that of glues, which give a joint stronger than the glue itself (see Chapter on Adhesives).

A further development of the use of sodium silicate for sizing is due to H. Wredé<sup>1</sup> and termed 'mineral starch sizing'. Equal weights of sodium silicate and starch are heated together until the starch has gelatinised, care being taken not to boil the mixture, which would result in a lowering of the viscosity of the solution (see p. 52). This paste is added to the stock in the beater and when homogeneously distributed, alum is added to produce a precipitate.

According to Wredé, this method is very efficient for sizing papers intended for printing, and may be employed advantageously for certain writing papers for which the use of sodium

silicate and starch in the ratio of 1 to 4 is recommended. For printing papers, about 3.5-5.0 per cent. of starch, calculated on the dry weight of the pulp, is used, and if the final reaction after precipitating with aluminium sulphate is acid, a loss of 30-40 per cent. of the starch may occur. E. Fues, however, obtains practically quantitative precipitation of the starch by adding the aluminium sulphate in amount sufficient to give a final neutral reaction.

A. Lutz<sup>3</sup> has shown that if raw starch is added to the beater, 73.2 per cent. of it is retained by the paper, whereas with starch paste only 46.2 is retained. Blasweiler<sup>2</sup> has examined the mineral-starch-sizing of paper and compared the values obtained for strength, texture, etc., with those given by other methods of sizing. He concludes that this method gives a greater tensile strength as compared with raw starch, although the values shown by the latter are already high. The tensile strength of paper sized by this method is 27 per cent. greater than that obtained with an alkali-starch mixture containing equivalent amounts of starch and alkali. With loadings, the use of the mineral-starch size gave an increase of 15 per cent. on the amount of china clay retained, and an increase of 12 per cent. for talc, when compared with the use of starch alone. To obtain the best retention of loading agents, each particle of them should be coated with adhesive mixture, as a strong continuous film of adhesive gives the highest tensile strength to the paper, and if each particle has its own coating it stands a better chance of being retained when it comes into contact with a fibre. The starch 'mix' should therefore be added in two portions, one to the stock and one to the filler, before putting into the stock. If the filler is put in the beater with the raw starch, the loss of the latter often exceeds 50 per cent., depending on the amount of clay present and the 'freeness' of the sheet, because mechanical enmeshment of the particles is the only means of preventing loss, no adhesive forces coming into play when fibre, clay, and raw starch make contact.<sup>4</sup>

It will have been noticed that more starch is retained by the paper if raw starch is added to the beater than when it is first cooked to a paste with water. A starch swollen with sodium silicate, however, can be entirely precipitated in the fibres, because when the web is dried by passing it over drying cylinders, the raw starch mechanically retained by the fibres is heated sufficiently to gelatinise it. The largest granules are readily retained, but many of the smaller ones are lost in the back water, and this loss may amount to between 10-30 per cent. of the total starch. On bursting, the contents of the granules pass into solution in the

water retained in the web and spread over the fibres; on removing the water a strong continuous film over the surface is left. Some granules, however, escape gelatinisation and serve no useful purpose.

Treatment with sodium silicate swells the starch granules to many times their original volume, thus leading to greater mechanical retention, and, in addition, the glutinous nature of the swollen granules serves to increase their retention; thus the mineral-starch process gives little or no loss of starch in the back water. The addition of starch paste to the beater leads to a large loss, as only the empty sacs are retained in the web, the soluble amylose being carried away in the back water.

The properties of rice, wheat, maize and potato starches have been examined by Wredé,<sup>9</sup> who finds that wheat starch is absorbed by the fibres to the greatest extent but that maize is to be preferred on account of its greater gelatinising power and cheapness.

The so-called thin-boiling starches may also be used in the beater. The diffusion of the soluble portion of a raw starch when gelatinised on the drying cylinders is a function of the water present and of the amount of starch which dissolves in it at the gelatinising point. By the use of soluble starch the granules are retained as with raw starch, but on passing over the drying cylinders practically all of it passes into solution and spreads over the fibres and through the interstices of the paper. Furthermore, as the moisture rises to the surface of the paper, the very soluble starch comes with it and gives an excellent surface finish. The nearer the starch solution approaches a true colloidal solution, the better is the effect and the greater is the surface area covered.

W. A. Scholten's Chem. Fabr.<sup>10</sup> have covered the addition of a cold-swelling starch, in amounts up to 5 per cent., to the paper at any stage of its manufacture. If desired, it may be added in a dry state to the beater.

For this process the soluble starches produced by enzymes are preferable to the thin-boiling starches produced by acid treatment, as the latter are not so completely depolymerised as the former, and show retrogradation. For these reasons, thin-boiling starches made by acid-treatment do not give such a perfect film over the fibres as the enzyme-solubilised type, nor do they coat the fillers so effectively.

The use of soluble starch also eliminates a fault sometimes encountered, viz. that caused by a local concentration of starch which, on the drying cylinders, gives a spot that is transparent when examined in transmitted light, the so-called 'slime spots,'

'windows,' or 'shiners'. This fact is referred to again below in connection with the fancy effects that are obtained by deliberately causing 'shiners' to appear in the paper.

Papers sized by the mineral-starch process, although quite 'soft' to the tongue, give excellent printing surfaces. The use of resin size in conjunction with the mineral-starch size is considered by some to be essential for writing papers, the latter size acting as a partial substitute for the more expensive resin size.

The mineral-starch sizing, used as an auxiliary agent to resin sizing, acts as a protective colloid to the resin, which is precipitated in a more finely-dispersed state, and to a more homogeneous distribution of the resin over a wider surface. Here again the use of soluble starches gives the best protective effect, as a true colloidal solution of the starch is obtained with high protective powers. Wredé<sup>9</sup> considers that in sizing the starch should be thoroughly cooked with application of the warm paste to the fibres. The addition of a wetting agent increases the absorption of the starch and assists dyeing, but for papers requiring water resistance, e.g. posters, montan wax should be used.

The 'handle,' hardness, and capacity of the paper to take a high 'finish,' are all held to be improved by the addition of starch, and if for any reason the preparation of stock cannot be carried out to the desired extent, the quality of the sheet may be improved by adding starch. C. F. Cross and E. J. Bevan,<sup>6</sup> however, consider that starch is at best only a substitute for thorough beating, and in many cases is less economical.

**Tub Sizing.**—For the preparation of papers intended for use in the production of ledger books, bonds, and other high-class paper, the process of tub sizing is carried out to give the required finish. This process consists essentially in running the paper through a bath of the size, and drying.

Such papers are never used for printing, but only for high-grade writing papers. Certain pigments are incorporated in the paper, which is then tub sized, china clay being used in England and chalk in America, where supplies of china clay have to be imported. It follows, therefore, that in America a neutral or slightly alkaline size must be employed. The function of the pigment is twofold: it assists the rapid absorption of writing ink, and it gives a surface with a better appearance.

The use of starch for tub sizing has been followed by R. M. Cobb and co-workers.<sup>6</sup> The specific gravity of the various sizing agents must first be considered, and it will be found that more starch will have to be used than casein, the specific gravities of the two substances being approximately 1.62 and 1.26, respectively.

He and Lowe<sup>7-8</sup> give a very simple formula connecting the depth of penetration in cm. ( $d$ ), the paper-pore radius in cm. ( $r$ ), the surface tension of the starch solution in dynes ( $\sigma$ ), the contact angle taken up by the liquid in contact with the solid ( $\theta$ ), the time of penetration in seconds ( $t$ ), and the coefficient of viscosity in poises ( $\mu$ ) where  $\mu$  for water = 0.01006 poises at 20° C. This relationship takes the form  $d^2 = \frac{r\sigma \cos \theta t}{2\mu}$ . From inspection it

will be seen that, other things being equal, the degree of penetration of starch into the sheet at the press varies inversely as the square root of the viscosity of the starch dispersion. The use of thin-boiling starches is therefore indicated.

**Coated Papers.**—When the sheet is first formed and a size-filler mixture brushed on the product it is known as ‘coated paper,’ which probably offers by far the biggest outlet for starch in the paper-making industry; at least 50 per cent. of the coated paper made contains starch as the coating medium. The latest process is that covered by the Massey patents, which constitute a triumph of chemical engineering rather than of chemistry.

The fillers, e.g. china clay, lithopone, blanc-fixe or chalk, are used in conjunction with starches, generally modified by the hypochlorite process. Other modifying agents may be used and certain acids have been successfully employed by the author. The coating mixture is brushed on to a travelling sheet, and the product, after drying and calendering, has a highly finished appearance. This class of paper finds application in photogravure work, fancy cartons, and true art papers.

Shaw and co-workers<sup>11</sup> found that modified starches had not quite as strong adhesive quality as casein or glue, but all coatings containing 18 pts. of starch to 100 pts. of clay were well bound to the body papers. Starch had the best clay-suspending properties, and when coated papers were printed by the half-tone process all three coating mediums were equally good although the starch-bound coatings appeared to absorb somewhat more ink.

**Miscellaneous.**—When choosing a starch for paper-making freedom from specks is desirable, and, as stated above, if it is to be added to the beater as raw starch, a thin-boiling variety is preferable. If, however, the starch is gelatinised before adding to the beater, one which gives a stiff paste is best. On the Continent potato starch is widely used; in England maize starch is favoured on the price basis; and in America unmodified maize and tapioca are widely employed. For high-grade papers many manufacturers prefer to use rice starch, which they add to the beater in

the raw state. Owing to the small granule-size of this starch it can be uniformly distributed throughout the web, and on passing over the drying cylinders the granules burst and give a good tenacious paste. The price of this starch precludes its use for anything but the best grades of paper.

After the paper has been formed it may be required to bond together several plies of it to obtain thicker sheets. Bristol board and similar types of products, for example, visiting cards, show-cards, post-cards, or multiple wrappers for transport, are produced on this principle, and the adhesives chiefly used are based upon starch. The main points to be observed in using such adhesives are discussed in Chapter I, Part III, and the consistency, as will be seen, may vary from thick viscous pastes to free-flowing liquids, the former being applied by rollers and the latter by brush. The amount of water in such adhesives, and the viscosity, must be strictly controlled or warping on drying will occur, and too great a penetration will leave no adhesive between the interfaces to act as the bonding agent.

Some of these adhesives contain a little alkali or borax, and trouble may be experienced if a change is made from one grade of paper to a lower grade, the adhesive being found to lose its effectiveness after a part of the 'run' has taken place. The main reason for this behaviour, when it occurs, is that the lower-grade paper may have a more acid reaction, and the bleeding back of the acid substance into the slightly alkaline adhesive neutralises the acid and destroys the adhesiveness. The  $pH$  value of the adhesive has also to be carefully controlled when foils or coloured paper are used to cover cardboard, as corrosion and discoloration may occur in addition to the trouble mentioned above.

Starch is often added to paper to make it crackle, and novel effects can be obtained by varying the kind of starch, amount, and method of adding it. Fancy effects, e.g. paper appearing like parchment, and covered with blotches that appear transparent or less opaque than the main body of the paper, is obtained by putting an excess of unswollen starch into the beater, so that when bursting on the drying cans a large number of 'shiners' or transparent patches are produced by masses of the granules bursting in close proximity to one another.

Certain modified starches are met with in the paper trade, such as Paperine, Flourine, and Amijel, which impart special characteristics, such as increased 'burst' strength or apparent hydration, and permit of easy removal of water from the wet web of paper.

A. Frieden has pointed out that the use of starch in paper

making does not increase the bacterial content (see p. 364) even if no bactericide is used, and starch is probably superior in this respect to glue or casein.

To sum up, the paper industry consumes starch products as beater, calender, tub, and coating sizes, and as adhesives. Starch finds use in board mills to obtain a desired finish; in 'tag,' Bristol board and cover mills for obtaining increased strength, stiffness, finish, and formation of the multi-layer; in sulphite and rag-bond paper mills for improving the 'burst' strength; in Kraft paper mills for sizing, increased 'burst' strength, to impart rattle and a desirable finish; and finally, in book or printing papers for strength and 'fuzz' control. The control of 'fuzz' is important where a large quantity of paper has to be handled in a confined space, as the tiny fibres (or 'fuzz') fly off and affect the health of workers. It also has a large influence on the cleanliness of the printing as the speed of this is constantly increasing.

## REFERENCES

1. H. WREDÉ, *Wochbl. Pap.*, 1913, **44**, 835.
2. TH. E. BLASWEILER, 'Use of Sodium Silicate for Sizing Papers,' Constable & Co., London, 1926; *Chem. Zentr.*, 1925, **2**, 1235.
3. A. LUTZ, *Ber. Hauptvers. Verein. Zellstoff. Papierchem.*, 1907; *Papier Zeit.*, 1908, **33**, 314, 1022, 1062, 1098, 1142.
4. ANON, *Paper Trade J.*, 1927, Dec. 1st, p. 51.
5. C. F. CROSS and E. J. BEVAN, 'A Text-book of Papermaking,' 5th Ed. E. & F. N. Spon, Ltd., London, 1920.
6. R. M. COBB, D. V. LOWE, E. POHL, and W. WEISS, *Paper Trade J.*, 1937, **105**, 33; *T.A.P.P.I.*, 105.
7. R. M. COBB and D. V. LOWE, *Paper Trade J.*, 1934, **98**, No. 12, 43.
8. R. M. COBB, *ibid.*, 1935, **100**, No. 16, 42.
9. H. WREDÉ, *Papier-Fabr.*, 1928, **26**, 301.
10. SCHOLTEN'S Chem. Fabr., E.P. 380,674, 1931.
11. M. D. SHAW, G. W. BICKING, and M. J. O'LEARY, *U.S. Bur. Stds. J. Res.*, 1930, **5**, 1189.

## ADDITIONAL REFERENCES

- H. WREDÉ, *Papier-Fabr.*, 1922, **20**, 1429. (Discusses use of starch in paper-making.)
- H. WAGNER, *Chem.-Ztg.*, 1923, **47**, 249. (Discusses adhesives for the paper industry.)
- E. TROMP, *Papier-Fabr. Ver. Zellstoff-chem.*, 1925, **23**, 109. (Discusses use of starches in the paper industry.)
- J. TRAQUAIR, *Paper*, 1918, **21**, 68, 70. (The function of starch in beater-sizing discussed.)
- W. A. NIVLING, *Paper Trade J.*, 1922, **75**, 32. (Discusses the use of starch in paper sizes.)



- D. J. SAXL, *Paper Trade J.*, 1937, **105**, No. 13, 46. (Instruments for testing starch for use in paper-making are discussed.)
- C. G. WEBER, M. D. SHAW, and M. J. O'LEARY, *U.S. Bur. Stds. Misc. Publ.*, 1935, **M150**, 7. (Sweet potato starch is suitable for beater-sizing of paper.)
- J. STRASSER, *Paper Trade J.*, 1936, **102**, No. 17, 29. (General.)
- P. MURANYI, *Chem. Zentr.*, 1939, **110**, 1, 3477. (Starch treated with arylsulphonchloroamides used to size paper in conjunction with glue.)
- H. NOUVEAU, *Papier*, 1939, **42**, 383, 465. (Uses of potato starch and derivatives in paper-making.)
- J. ROBERTS, *Proc. Tech. Sect. Paper Makers' Assoc.*, 1939, **10**, part 2, 439. (Uses of various starches in paper-making discussed.)
- F. HOLT, *Paper Ind. Paper World*, 1939, **21**, 332. (General.)
- STEIN HALL MFG. Co., U.S.P. 2,197,754. (Laminated paper and paper-cloth made by applying ungelatinised starch in a starch gel to surface and then gelatinising *in situ*.)
- H. N. LEE, *Paper Ind.*, 1937, **10**, 785. (Photomicrographs in colour for investigating use of starch in paper-making.)
- A. H. KELLING (to Corn Prod. Ref. Co.), U.S.P. 2,171,796. (Preparation of modified starch for paper-sizing.)
- C. A. BRIGGS and J. L. MCCARTHY, *Pap. Trade J.*, 1942, Jan. 22nd. (Evaluation of starches for paper trade by viscosity measurement.)

## CHAPTER 4

## THE TEXTILE INDUSTRY

**Sizing of Yarns.**—The practice of sizing cotton, linen, or viscose rayon in the form of hank or warp has the primary object of causing the fibre to absorb an adhesive like starch or an allied product. This treatment imparts to the fibre a much greater tensile strength, and also resistance to the abrasive action of the parts of the various forms of machinery that are employed in making the yarn into a woven fabric.

The practice of efficient sizing can still be regarded as an art, and it is one which only in recent years has been approached in the true spirit of scientific inquiry. What was once the most empirical of operations, only carried out successfully by an operator of many years' practical experience, is gradually being based on some measure of scientific control. In spite of this technical progress, most sizing operations are controlled by practical experience rather than by scientific theory. Whilst the physical and chemical properties of starches and allied products are well known, the condition of application and the choice of the correct composition of a sizing 'mix,' are matters for practical experience.

This section deals solely with a brief résumé of the general principles underlying the practical usage of starch products in sizing, and standard works should be consulted if further practical details are required.

Reference to the section on 'finishing' will show that it is closely related to sizing, as similar materials are used for each operation. The main differences between the two processes are, firstly, sizing is only practised on warps and to some extent on yarn, and 'finishing,' for practical purposes, is the application to fabrics. An important difference between sizing and 'finishing' is that, generally speaking, the size must be removed before the woven fabric is in a fit condition to be dyed or properly 'finished,' whilst the 'finish' can with considerable advantage be regarded as permanent. In the case of fabrics that are to be dyed direct, the sizing material must be properly removed (see Desizing, p. 330), otherwise dyeing faults may occur owing to residual sizing materials acting as a partial 'resist'. For example, the evils attendant upon using paraffin wax as a component of a size for cotton warps, when

the woven fabric is subsequently to be dyed, are well known to many dyers.

**Sizing.**—There appear to be two schools of thought concerning the requirements of a sizing agent, one school maintaining that the size is best retained on the surface for obtaining the maximum effect, and the other that the size should penetrate the fibre and become a mechanical part of it. Those holding the latter view point out that starch retained on the surface is apt to be mechanically removed, or 'dust off,' in the weaving. Alternately, as high extensibility of the yarn minimises breakages, complete penetration gives the maximum extensibility with the same amount of size, bearing in mind that the size film is not so extensible as the fibre. A very dusty or 'flakey' size is, of course, very undesirable, and a size which combines a certain degree of penetration with adequate surface protection can be regarded as the most satisfactory from a practical point of view. In any case, the type of sizing employed depends upon the mechanical requirements of the yarn. Many agents other than starch products are in use for sizing various types of fibres, but starch paste, either alone or in a converted or solubilised form, is chiefly used on cotton and linen, and we shall confine our attention to these two.

Potato starch yields a more viscous paste, weight for weight, than maize starch, and this in turn gives a thicker paste than sago, which suffers from the defect that it breaks down on continued boiling to a much greater extent than the other two. The thicker the paste, the more of it is picked up by the fibres, as explained below, and allowance must be made for this fact when sizing with, for example, a sago paste, to get the same effect as with a potato-starch paste.

Generally, the strength of films obtained from starch size made by cooking the starch with water and then adding the softening agents is greater than the strength obtained by the use of modified thin-boiling starches that have received excessive treatment in manufacture, providing the same weight of starch is applied in each case (see below). It is safer, therefore, when a supplier of a satisfactory thin-boiling starch has been found, to obtain supplies from him rather than attempt to modify the starch in the works to match the quality used. A converted or modified starch is used to obtain penetration, because the viscosity of solutions of these preparations is much less than that given by untreated starch (see below). The degree of modification is sometimes expressed as the number of ml. of a starch solution of standard concentration that will flow from a pipette in a given time, e.g. 90 degrees thin-

boiling starch, etc. Such starches may be produced by oxidising agents (p. 180), acids (p. 271), or by enzyme action.

In sizing warps, the fibres lying side by side pass over beams to enter the hot size, and on leaving the 'sow-box' pass through rollers and over-heated 'cans' or hollow rollers to be dried. The threads which are stuck together are then separated. In another process the 'ends' or lengths of yarn are sized and dried simultaneously, but separately.

**Considerations influencing Sizing.**<sup>9</sup>—For sizing, the starch is used in all stages of solution or dispersion, from thin watery solutions to highly viscous 'solutions'. The viscosity of a starch solution is a good index of its practical value for many purposes, but care has to be taken, for example, in the case of potato starch where a higher initial viscosity is generally combined with a tendency to 'boil thin' and thus alter the amount of starch, as such, which is applied to the fibres because the amount of size picked up by a yarn depends on the viscosity and not on the concentration. A glance at Table 7 shows that the breaking load of films made from pastes of different starches at the same concentration depends on the film thickness which in turn appears to depend to a large extent on the viscosity of the paste.

As can be seen from the gelatinisation diagrams on page 58 the various starches do not gelatinise at the same rate, thus, for the same amount of boiling (providing the time of boiling is such to give less than the maximum gelatinisation possible with that particular concentration and starch), the extent of the gelatinisation and the viscosity, and hence the amount of size picked up by the cloth, may be different. Sago and tapioca gelatinise almost completely if the size is boiled for about two hours, and potato-starch size takes about the same length of time, although it is somewhat slower gelatinising in the early stages of the boiling, while maize is both slower and more difficult to gelatinise than the above starches, and the latter is also true of wheat flour. With maize and wheat, then, even thorough boiling does not give complete gelatinisation, so that the use of these two are probably confined to open cloths made from coarser yarns where a greater amount of size can be applied and, owing to the incomplete gelatinisation, is necessary in order to obtain good weaving.

Both potato and tapioca starches give more viscous pastes than sago starch even after prolonged boiling, and to obtain similar amounts of starch on the yarn in each case some 10 per cent. more sago starch should be used than in the case of the first

two starches. This increase in concentration increases the viscosity of the paste to a figure nearer to that of the other starches, so that nearly the same amount of size with a higher solid-content is picked up, giving a similar percentage of actual starch on the air-dried yarn. In stability, of the boiled size, tapioca is probably better and less variable than sago starch, whilst potato starch is more variable and liable to change than either.

In sizing, the warp enters the size box and picks up an amount of size depending on the viscosity of the paste, it then meets the squeezing roller where some of it is squeezed off; but the more viscous the size, the less is the loss in weight and the heavier the 'finish'. Other factors, such as softness of the pressure bowls, running speed of the machine, and the pressure between the bowls, influence the 'finish'. It should be noted that although up to a certain point increase in viscosity of maize and potato starches leads to an increase in the amount of size retained by the warp, after this maximum is reached there is an irregular falling off.

Another factor of importance in sizing is the rate at which cotton yarn is wetted by the size. The average sizing preparations only wet raw cotton imperfectly, and to produce spontaneous wetting of the yarn by the addition of wetting agents an excessive amount would be required. F. D. Farrow and S. M. Neale<sup>14</sup> have found that effective wetting of the yarn is promoted by using squeeze rollers and working 'at the boil.' The use of the higher temperature range is effective, not because of any marked alteration it induces in the surface tension of the size, but because it lowers the viscosity of the size and assists the escape of air bubbles.

Heavy pressure, slow running of the machine, or a double run through the sizing machine, lead to an increase in the area of warp threads covered by the size, all these factors favouring penetration. Drying on the hot cylinders or 'cans' also causes greater penetration than that obtained when the warp is collected wet, as the rapid cooling of the size in the latter case greatly increases the viscosity, which militates against penetration, the opposite effect being obtained in a short time on the drying 'cans'.

The effectiveness of a size depends upon its distribution and the adhesive character of the film, both factors which can be regulated to suit different conditions. By the use of hydrolysed starches the amount of solid matter in the solution can be increased without appreciably altering the viscosity or the penetrating power of the solution, leading to increased weight in the fibre and greater stiffness and bonding power of the solution.

M. S. Furry<sup>15</sup> has examined many of the mechanical sizing and finishing properties of a number of starches and her results are summarised below :—

TABLE 7  
MECHANICAL PROPERTIES OF STARCH FILMS AND SIZED CLOTHS  
ARRANGED IN ORDER OF DECREASING MAGNITUDE

<i>Film Thickness from same Concentration of Paste.</i>	<i>Extensibility of Film and Sized Cloth.</i>	<i>Folding Endurance.</i>	<i>Stiffness, Pliability and Stretch of Cloth.</i>	<i>Breaking Load at same Concentration of Paste.</i>
Potato Canna Sweet potato Rice Corn Dasheen Wheat	Potato Canna Sweet potato Rice Wheat Dasheen Corn <i>N.B.</i> —Glycerine and turkey-red oil increase extensibility, Borax decreases it.	Canna Potato Sweet potato Corn Rice Wheat Dasheen <i>N.B.</i> —Values decrease with increasing film thickness and with addition of auxiliaries.	Sweet potato Rice (a) Wheat Dasheen (b) Corn Canna (c) Potato <i>N.B.</i> —(a) Thicker film; stronger cloth obtained; (b) Better penetration and greater stiffness than with canna starch. (c) Stiffer cloth.	Potato Canna Sweet potato Rice Corn Dasheen Wheat <i>N.B.</i> —All give approx. same breaking load if conc. of pastes adjusted to give same film thickness. Breaking load increases with thickness.

Treatment of the starch with oxidising agents, acids or enzymes causes the films to be weaker, but as many of these products are of the 'thin-boiling' type a greater weight of starch per unit volume of size can be obtained. Thus, if the viscosity of a size made from a treated starch is similar to that of a size from an untreated starch the former will contain more starch, depending upon the extent to which it has been modified, and thus a thicker, and therefore stronger, film is obtained. As a greater amount of modified starch can be applied this more than offsets the loss in strength due to modification of the starch. If the starch is so modified that the viscosity of the size is low, and yet the concentration of starch in the paste is such that it offsets the loss in strength due to modification, then it penetrates better than untreated starch. The effect of humidity on the strength of starch films has already been discussed on page 79. Those starches showing lower folding endurance generally give stiffer cloths when used for finishing.

**The Effect of Auxiliary Agents on the Properties of Sizes and Finishes.**—A number of agents are added to sizes and finishes to obtain certain effects, more particularly from the point of view of 'handle,' or 'feel.' Small amounts of borax increase the strength of starch films but larger amounts weaken it. The increase in film strength is also obtained, but to a lesser degree,

by the addition of glycerine, but, in this case, increasing the amount of glycerine has a much lesser effect on the film strength than increasing amounts of borax whilst large amounts of glycerine have little more effect than small amounts.

Substances which are used to soften the films, such as tallow, castor oil, and glycerol, etc., cause a decrease in film strength if present in amounts over 4-5 per cent., a similar effect being shown at this concentration when waxes or soaps are incorporated. In the case of the last two auxiliaries the transparency of the film is also adversely affected. In small amounts soap and turkey-red oil appear to increase the strength.

**Desizing.**—For the sizing of warps on the slasher we have seen that various substances are used, e.g. starches, casein, gelatine, soaps and softeners, fillers such as clay, and certain inorganic salts. The presence of these substances may interfere with the subsequent treatments, such as bleaching, dyeing or printing, and in order that these processes may be more effectively carried out they are removed by the process known as 'desizing.' Efficient desizing should remove the whole of the size easily and without affecting the fabric in any way, and the place of desizing in the order of operations naturally depends upon the type of goods being produced by any one plant.

For removing starch sizes, the fabric is treated with agents to break down the starch molecule to a simpler type of molecule, e.g. that of dextrans or sugars, as these substances are more readily removed by washing than unchanged starch. For this purpose an inorganic acid, or an acid salt, may be employed. Boiling with water alone for a long time will bring about these changes, but the hydrolytic action is catalysed, or speeded up, to a marked degree by the presence of hydrogen ions. Although acids and acid salts can be used, saccharifying and liquefying enzymes are preferred by many workers, because they exert no deleterious action on the cloth. Other starch-solubilising compounds, e.g. certain swelling agents and oxidising agents, are also in use.

The use of diastatic enzymes in desizing fabrics is usually confined to textiles of the better grade, or to goods which are subsequently to be mercerised and dyed and, therefore, have to be quite free from extraneous matter if the fullest lustre is to be obtained. The best results in bleaching and dyeing depend upon efficient desizing of the cloth, as residual sizing agents make it impossible to obtain a well-penetrated and evenly-dyed material. It may sometimes happen that owing to incorrect dressing, the 'handle' or feel of a finished fabric is not what is desired, and in

such cases partial desizing or desizing followed by re-finishing may rectify the fault.

Hall has shown from Tschilikim's work that the complete elimination of starch from a sized fabric is difficult to accomplish by the older methods. Boiling a sized fabric in water followed by 14 hours' immersion at 30° C. did not remove the starch, nor did a 13 hours' treatment in a cold solution of a powerful wetting agent, nor 14 hours' treatment at 20° C. in a 0.4 per cent. solution of caustic soda. Treatment of the fabric, either with sulphuric acid, hot or cold, and strong enough to attack the fibre, still left some starch in the fabric. Complete removal of the starch, however, was claimed by the use of enzymes.

**Enzymes.**—Enzymes have been defined as catalysts produced by living organisms. Many chemical reactions can be brought about under ordinary conditions of temperature by their use where otherwise high temperatures and powerful reagents would be required. They obey the laws of catalytic phenomena and are heat sensitive. Amylolytic enzymes, which are fully discussed in Part V, destroy starch, giving soluble substances of lower molecular weight, and in desizing they perform the same functions as acids in eliminating starch from a sized fabric, but are more rapid in their action, and are readily controlled; they have the additional advantages of specificity and of having no deleterious action on the fabric if used in excess.

Concentration, temperature, and hydrogen-ion concentration all play important rôles in influencing the activity of any given enzyme, each one having its own set of optimum conditions (see Table 14, p. 456). On a commercial scale an important factor is the speed at which an enzymic preparation acts. It is insufficient to be told that a certain preparation will convert many thousand times its weight of starch; the time taken to bring about the conversion must also be stated. Enzymes increase the reaction velocity of an action already proceeding, and the amount of change brought about depends, up to certain limits, on the length of time the process is allowed to run. The rate at which such changes proceed depends on the concentration of the catalyst present.

As with ordinary chemical reactions, in the absence of a catalyst a rise in temperature speeds up the velocity of the reaction and up to a certain point this also applies to enzymes. Some of these substances have their optimum temperature in the region of 37° C., but some can withstand temperatures up to about 93° C. The majority are destroyed with extreme rapidity after the temperature has exceeded the optimum, but a few take some time



before they are thoroughly inactivated. Once they have been destroyed by heat they cannot be reactivated, and the deactivation by heat is much slower when dry powder preparations containing the enzyme are used. According to Gale, malt amylase loses 85 per cent. of its starch-degrading value in one hour at 60° C. and 72 per cent. in 30 minutes at the same temperature.

The *pH* value of the medium is another important factor influencing enzyme action and in some cases the optimum value varies according to the source from which the enzyme is obtained. Apparently the more these substances are purified the more rapid becomes their decomposition, and it may be that the impurities present have some stabilising or protective action on the enzyme. J. E. Evans,<sup>13</sup> for example, points out that the amylase preparation known as 'Rapidase' contains no albumins coagulable by heat, but these are present in other amylase preparations. This fact may account for the heat-resistance of Rapidase, and is indicative of the importance of the influence which may be exerted by extraneous substances on the reactions and activity of the enzymes.

Thus in one patent of Kalle & Co.,<sup>12</sup> enzyme preparations are activated by the addition of salts of phosphoric acid (except orthophosphoric acid), as little as 0.01 to 0.001 per cent. of pyrophosphoric acid being required. The action does not appear to be a buffering one, as it also occurs at the *pH* value which is optimum for the particular amylase. The use of imino-diacetic acids and their salts is also claimed<sup>17</sup> to give practically complete protection to desizing amylase preparations against the inhibiting effect of salts of heavy metals such as zinc and copper. A survey of the preparation and properties of amyloclastic enzymes is given in Part V (see p. 433).

**The Use of Enzymes in Desizing.**—The usual method of desizing is to run the fabric to be treated over the singeing rolls, then past a steam box to stop 'sparking' or into a box containing the desizing enzyme solution. Alternately, it may be passed into a bath of hot water which serves the double purpose of removing soluble inorganic salts and raising the temperature of the cloth to that of the desizing bath containing the enzyme, so that the temperature of the latter is not lowered by the passage of the cloth through it. After leaving the hot-water bath, the cloth immediately enters the desizing bath, and the process may be a continuous one or may be carried out on a jig. After treatment the cloth may be passed immediately to the washing machine or it may be piled and left overnight.

Generally 1 hour is allowed for desizing, but frequently the time may be extended to 10 or 12 hours. If the cloth is thoroughly 'wetted out,' so as to allow easy access of the diastatic solution to every part of the fibres, there is no reason why the desizing should not be completed in 15 minutes, provided the concentration of the diastase is somewhat increased. When a short time of treatment is desired it is preferable to work at a higher temperature. It should be noted that if the goods are allowed to lie in the piles overnight saturated with the diastatic solution, the actual temperature of the desizing is that of the room for the major portion of the time, as the temperature of the piles soon falls to that of its surroundings.

In the market preparations usually met with the amount of diastatic enzyme employed may vary between wide limits depending on the type of cloth, amount of size, and the activity of the preparation. About 5-8 lb. to 100 gall. of water may be used in some cases with a good malt, but a somewhat larger quantity may be required if the water is very hard, or the time is to be shortened by the use of a higher concentration at a higher temperature. When employing special preparations such as Rapidase, Polyzime-N or Diastafor, amounts in the region of 1-3 lb. per 100 gall. may be used. While standing overnight, the starch becomes thoroughly liquefied and readily washes out when the fabric is passed through the wash. After the desizing, the fabric may be prepared for dyeing or may be bleached. To ensure thorough penetration of the desizing liquor into a fabric of the tightly woven type, a wetting agent may be added to the bath; a heavy set on the pad-roller is desirable, the fabric being flexed as little as possible during desizing.

It sometimes happens that a fabric is given too harsh or too stiff a 'finish' to be suitable for sale, and then it may be stripped by a 1-2 hours' treatment with a warm diastase or protease solution, depending on the type of 'finish'. An alternative way of treating such a fabric is to pad it through a dilute solution of the enzyme, and either plait it into a box, or batch it up warm and allow to stand for a few hours. At the end of this time it is dried, either on a frame, or by passing it over drying drums or 'cans,' when the 'finish' is found to be much softer owing to the action of the enzyme on the stiffening agent. This method is considered valuable for blotch prints, which often 'finish up' too stiff, and it saves two operations.

**The Finishing of Textile Fabrics.**—Most fabrics before they are finished generally lack the properties that make them commercially desirable as assessed by appearance, 'feel' or 'handle,'

draping properties, and other physical characteristics. All textile fabrics, before they can be marketed, must receive some degree of 'finish,' which may range from a treatment with very weak solutions of starch or allied product through innumerable graduations to the 'back-filling' finishing method, for which a heavy, viscous 'starch mix' is used; this mix is pressed into the fabric, thus filling the spaces between the fibres which are themselves heavily coated at the same time. For the lighter type of 'finishing' the requirements may be quite the reverse, all the spaces between the woven threads being open, and individual threads being coated with a transparent flexible film through which any colour on the fabric can be seen to full advantage; for this purpose, the thin-boiling modified starches may be used.

The materials used in these processes, known as 'dressings,' are employed for stiffening, glazing, loading or weighting the fabric, and may be classified according to their origin or to the type of fabric to which they are applied, or, again, to the effect they produce.

The finishing of materials is an art in itself, and many manufacturers put the work of 'finishing' their textile materials into the hands of specialist firms. Much depends upon the mechanical aspect of 'finishing,' for the same dressing applied in different ways may give very different effects.

With dyed cloth the 'finish' plays an important role, but with a printed material the pattern is the most important part, although naturally the 'finish' must show this to the best advantage, and it is often said that a finisher is paid for his finger-tips, years of experience being required for a man to master the art.

It often happens that one dressing may be used for several fabrics, and then it becomes possible to classify these dressings according to the type of work for which they are used, for example, stiffening, glazing or imparting a soft 'handle'. For stiffening and glazing most of the common starches, dextrins, vegetable gums and gelatine may be used.

Shading ingredients (see p. 338) and antiseptics (see p. 364) are commonly incorporated in dressings, the former to improve the colour and the latter to prevent the growth of mould or mildew.

**Adhesive Dressings.**—Adhesive dressings are used when inorganic pigments are used as fillers, and their function is to retain the pigment and prevent 'dusting off'. Vegetable glue, sold to the trade under a variety of trade names, such as Japan Glue, Arabil, Tragacanthine, Gummi Germanicum, was at one time widely used, and appears as whitish, translucent solid

products, or in the liquid or paste form. It formed a substitute for gums, sizes and starch pastes in a variety of finishing processes. This class of compound is generally a treated starch, or a treated flour.

When making sizes enamel pans and rubber-coated stirrers are preferable, so that contamination with iron is avoided, for iron is detrimental to the colour of the product. The use of live steam is considered by some workers to give smoother pastes, but if this method is used the amount of water formed by condensation must be taken into account. If magnesium chloride is used as the swelling agent, then wooden vats heated by live steam may be used.

A vegetable gum, marketed under the name of Arabol, has been stated to contain approximately 55 per cent. dextrin, 25 per cent. maltose and 4 per cent. starch, the balance being moisture, and would appear to be an enzyme-treated starch.

'Apparatine' was first introduced by Gérard, who treated starch with caustic alkalies or carbonates.

The vegetable glue, made by the action of alkali on starch, is invariably neutralised, and instead of the mineral or organic acids generally used, a fat or vegetable oil which is readily saponified in the cold is sometimes used.

Dressings containing magnesium chloride have the useful property of absorbing moisture, so that even in the presence of a large amount of starch the fabric still has a fairly soft 'handle'; but care must be exercised, especially with goods for export trade, because storage in a place of high humidity will lead to a greater increase of weight than is desirable.

**Characteristics of Individual Starches.**—As starch is a very important ingredient of dressings for white goods, the properties of its different varieties are utilised with respect to the class of goods being dressed and the effect desired. The dressing may vary in consistency from a thin milky liquid to a thick, almost dough-like mass.

**Farina or Potato Starch.**—Of all the starches used extensively in the textile industry farina or potato is the most viscous and most variable in properties. On storage the viscosity often decreases and this does not appear to be connected in any way with the presence of soluble impurities, for when these are removed the same phenomenon is shown. Farina pastes are considered in the trade to 'boil thin,' i.e. the viscosity falls off rapidly after reaching a maximum. This, as explained on page 48, is due in a large measure to breakdown of the structure of the paste on prolonged boiling and stirring, but after the farina

pastes have been thoroughly boiled so as to obtain complete gelatinisation, the viscosities are higher than those of, for example, sago pastes of the same concentration, but variations in viscosity are greatly reduced.

Farina can therefore be used quite satisfactorily for sizing if tests are first made on the viscosity and the stability of the batch to be used, and the concentration and length of boiling of the paste controlled to give the best results. One method of overcoming the 'boiling thin' of starch paste is to make several successive batches of size which are immediately used after a short boiling. This, however, does not get to the root of the matter in that the product still has a variable viscosity and in use it is a competition between the sizer and the starch as to whether the former finishes the job before the viscosity of the latter changes. By adequate boiling and concentration adjustment the viscosity-variation is eliminated to a great extent. The passage of a potato-starch size or finish through a homogenising mill would probably overcome the 'thin-boiling' trouble and give a stable product.

To reduce the viscosity of farina pastes without lowering the solid-content, F. Ohl<sup>8</sup> recommends the addition of a boiling 2-5 per cent. solution of dextrin, instead of water, when making the paste.

Potato starch or flour acts as a filler that is also flexible, and imparts a soft 'handle' to the goods being used, where opaque effects are desired. In conjunction with borax it gives a 'finish' of increased brilliancy. In this respect Japanese starch is considered by some workers to be inferior to American starch, and from 1933 for a few years the Russians offered a very good product superior to many starches on the market, although it ceased to come into the country about 1938.

*Rice starch* penetrates, gives a glazed and transparent finish, and weights the material well. It is of chief interest to the laundry trade, where it is used for stiffening, as it does not become limp in a humid atmosphere as do other starches.

*Maize or corn starch* gives a hard board-like 'finish' and is used extensively, either alone or in conjunction with potato starch, for stiffening piece goods.

*Tapioca starch* is softer than maize starch and gives a more transparent jelly; 'finishes' from this starch are more flexible and tough than those made from maize starch, whilst dextrans made from it are the strongest of all dextrans (see p. 238).

No amount of boiling will reduce the harsh 'feel' characteristic of maize and rice starches, but maize starch, partly converted to

the soluble form by the action of hydrochloric acid, can be made to give a very similar effect to that obtained with wheat starch; the untreated starch, however, gives a thicker paste than wheat starch.

*Sago starch* has long been used in *cotton warp sizing*, and has been found satisfactory for several purposes, but in 'finishing' it is not widely used because, although it gives a thin, firm 'feel,' it tends to crack when folded. Bleached sago is reported to have been made in Lancashire 80 years ago.

When swelling or other agents are heated with starch suspensions degradation of the starch often ensues and, indeed, within limits, is often desirable in order to obtain the desired effect. If wheat starch is used, the 'mixes' will be found to require longer heating than those made with potato starch. In general, not less than  $1\frac{1}{2}$  hours, and not more than  $3\frac{1}{2}$  hours boiling are desirable. The addition of dextrans to starch pastes is inclined to give a harsh 'boardy' 'finish,' but used alone good dextrin gives one of the softest 'finishes,' providing it is free from unchanged starch. Starch may be modified to leave it with most of its properties unchanged, with the exception of the viscosity, which is greatly reduced, thus allowing effects to be obtained similar to those given by non-starchy materials.

Many of the dressings commonly used contain two or more different kinds of starch, which are often mixed in the dry state and gelatinised together. It is considered preferable by some workers to gelatinise the different starches separately, afterwards mixing the pastes and boiling the mixture for a short time to obtain a homogeneous mass. In this way, a more uniform gelatinisation of the starch granules is obtained. In a mixture of two starches, dry-mixed before gelatinisation, there is always a danger that the granules of the starch swelling at the lower temperature will form a gelatinous coating around the granules bursting at a higher temperature, thus insulating them and preventing their gelling. Although such a paste may appear normal when used as a dressing, it has the tendency to 'dust off' after some time.

*Wheat Flour*.—Kuo-Chun Chin<sup>16</sup> has examined samples of flour prepared with a silk bolter cloth and a metallic bolter and considers that the resulting flours have different properties which he ascribes to differences in particle size.

The method of preparing wheat-flour size is to steep the flour in water and allow fermentation to proceed. Various stages of fermentation may be distinguished, and the action stops when the ferments have used up all the available nutrient material, or are

destroyed by the products of their own metabolism. Alcoholic fermentation takes place, and is followed by the formation of acetic and butyric acids, etc., the gluten and nitrogenous constituents also being attacked and solubilised. The suspension is generally acid when finished, and if coloured goods that will not withstand acid are to be 'finished,' the size is neutralised with an alkali such as ammonia. The odour from a vat containing fermenting materials is not unpleasant, but should a foul odour develop, the vat should be discharged and thoroughly cleaned out, otherwise the next batch will also be contaminated. This trouble is generally due to the use of diseased or poor-quality flour.

Wheat flour fermentation can be shortened considerably if about 0.25 per cent. of washing soda on the weight of flour is added to the vat and the mixing well stirred while fermentation proceeds. At the end of the fermentation period an antiseptic is added and the stock diluted and mixed for boiling.

When using wheat flour prepared by this fermentation method about twice as much flour is required on the weaving warps as would be required if sago or farina were used. The handle and appearance of the finished materials are similar and the wheaten size is quite readily removed. As more nitrogenous material is present in this case it is wise to add some fungicide to prevent the growth of mildew which grows more heavily on this type of size than on those made from straight starches.

Firmness and the impression of solidity are obtained by the use of *wheat starch*, which penetrates well, and by beetling or calendering gives a good glazed 'finish'. A mixed starch, however, is generally used in beetle finishing.

**Tinting and Blueing Agents.**—To give the appearance of whiteness, blueing agents are often added to dressing for white goods, and soluble dyestuffs and pigments are sometimes added to the dressings for loaded coloured goods, especially if the ground colour is a heavy shade, in order to mask the whitish cast that would be given to the fabric by their use alone, so causing the dyeing to look 'bare'. Ultramarine is widely used for tinting white dressing, and the dressing should be neutral in reaction, because traces of acid will cause the ultramarine to discolour to a grey or brown shade, and thus defeat its own purpose. The neutralisation may advantageously be carried out with ammonia, and the presence of excess alkali is not harmful. Indigo Carmine and Paris Blue have also been used as blueing agents, but do not satisfy as many requirements of this work as does ultramarine. The amount of tinting agent employed depends on the particular

effect desired by the 'finisher,' who makes the addition to suit his requirements.

**The Suitability of Starches and Dextrins.**—Starches from some sources contain sulphurous acid either in the free or combined state; when the free acid is present it is inclined to 'tender' goods that are stored in a warm place, so that freedom from this compound is desirable. In white dextrins the presence of unchanged starch should be looked for, because it alters the working properties and affects the type of 'finish' obtained, even when present in small quantities. Freedom from acids and glucose is also often desirable if discoloration of the finished goods on storage is to be avoided. Where neutral dressings are required, it may be noted that some starches, such as maize and rice starch, are very frequently alkaline in reaction, and the substitution of a portion of potato starch in a 'mix' by one of these starches sometimes serves to neutralise the remainder of the acid in the mixture. Fillers, e.g. talc, barium sulphate, and china clay, are also frequently acid, which splits any soap present in the dressing to produce fatty acids which, on oxidation, give rise to undesirable odour in the goods.

**The Printing of Textiles.**—The reproductions of colour patterns on textiles, other than by weaving, may broadly be referred to as 'printing'. The usual procedure is to add dyestuffs, or their solutions, together with the required chemicals, to a paste prepared from starch, gum, albumin, or other substance which forms a mucilage with water or spirit.

These pastes are applied to the cloth in a variety of ways, by stencilling, by aerograph spraying, but chiefly by engraved rollers. The numerous requirements demanded of a thickener for printing are often contradictory, and each printer adopts the thickening which suits his own requirements.

For cotton, normal thickenings are used, but for other fibres, where wetting of the fibre is more difficult or other problems enter into the question, the natural gums are more generally used.

**Function of the Thickener.**—Thickenings may be divided into two classes, (a) those removed after printing, e.g. starches, British gums, vegetable gums; and (b) those used as fixatives and not removed after printing, e.g. casein and albumin. The thickener should serve for the following purposes:—

1. As carrier and diluent for the dyestuffs and chemicals required for developing or fixing the colour, at the same time preventing crystallisation or precipitation of the other ingredients both before and after printing the paste. The finer the dispersion of the



dyestuff or the greater is solubility in the colloidal thickener the better the results.

2. Provide a medium of such plasticity that will feed smoothly into the engraved portions of the roller, remain there until transferred to the cloth, and not spread along the fibres under the pressure of the printing process, or by capillary action, thus deforming the pattern.

3. Should delay chemical reaction between the constituents of the paste, e.g. a colour with its mordant, until the cloth is processed to bring about the reaction.

4. Should transfer completely from the rollers to the cloth.

5. Except where the thickening is used to fix the colour, e.g. printing with pigments, it should be easily removed on washing and so not alter the feel of the fabric.

6. Should be inert towards other constituents, non-hygroscopic, and cheap.

**Colour Value.**—The colour value obtained from printing a particular dyestuff depends, to some extent, upon the thickening agent employed, that having the lowest solid-content generally giving the best colour value. However, the colour value is generally outweighed in importance by that of getting the right effect to produce a marketable product, irrespective of any economy of dye which is effected by varying the thickening.

Mucilages, such as locust bean gum and tragacanth, which give fairly thick mucilages at 2-8 per cent., generally give better colour value than those which have a high solid-content, e.g. alkaline gum or gum senegal. Good colour value may be a false estimate in practical work, as it often entails increased running costs and lowered efficiency. It must be remembered that in printing fabrics the aim is to get the right effect, and the cost of dyestuffs and thickeners compared with the value of the finished printed fabric is very low, especially in fine work, e.g. silks. For the sake of a small saving, several 'pieces' of material costing perhaps £30 each may be spoilt.

Certain thickenings, e.g. those made from wheat starch, do not penetrate the fabric, and consequently the paste containing the colour lies on the surface, which receives all the colour present, and thus becomes intensely coloured. Compared with another thickening containing the same amount of dyestuff, and which penetrates, or 'strikes,' right through the material, and so transfers the colour to a much larger fibre area, wheat-starch thickeners appear to give good colour value, but only on one side of the fabric. If the second thickening is gum tragacanth, another factor comes into play. This thickening is generally made up

with 4-5 per cent. of gum, whereas wheat-starch thickening contains about 12-15 per cent. of solid matter. When the two are printed the amount of solid matter present between the fibre and the colour particles farthest from the fibre is about three times as much for wheat starch as for gum tragacanth. The dyestuff has therefore farther to migrate to the fibre in the first case, and thus less dyestuff will be fixed in the same time of processing. The poor penetration of wheat-starch thickening, however, outweighs this disadvantage.

The colour-mixer and the foreman in a print works know by experience how and when to alter a thickening to suit different styles of work, and often do so automatically without being able to explain exactly why. The actual comparison of thickness, with regard to general utility, has to be carried out over long runs on a large scale, as laboratory or pilot plant trials are of very little use.

**Starch Products used for Thickenings.**—The value of a thickening depends largely on its viscosity, plasticity, and the percentage of solid matter in the solution at working strength.

Starch thickenings, or those made from printer's flour, are usually prepared by pasting the materials to a sludge with water, adding more water and then 'boiling' the mass in a steam-jacketed pan with constant stirring. To prevent the mass sticking to the sides of the pan when heating, and to improve its working properties, it is customary in many print shops to add about a quarter of a pint of a vegetable or 'colour' oil to every gallon of the paste before boiling, the sides of the pan being rubbed with the oil and the rest added to the paste on charging. In some cases mineral lubricating oil is incorporated, but a saponifiable oil is preferable.

The keeping quality of neutral starch pastes is not high under normal conditions of storage, and is very greatly improved if a little acetic acid is added. All the usual precautions as to cleanliness of plant and elimination of contamination should be taken, and a good quality of starch should be used in preparing the thickenings. If these points are observed no trouble should be experienced, but should a batch become watery or 'go off,' the trouble cannot be rectified and the batch should be discarded.

Wheat starch has good, smooth-working properties, and at one time was probably the most widely used. Both it and maize starch are used at a concentration of about 12-15 per cent., i.e. 1-1½ lb. to the gallon. A mixture of maize and tapioca starches simulates the working properties of a wheat-starch thickening and has been used to replace this when the price of wheat starch was

high. Maize-starch paste by itself lacks 'body,' and does not work as well or keep as well as wheat-starch paste, and thus is rarely used alone.

Tapioca starch gives a strong stringy paste which cannot be used alone. As stated above, it gives a good thickening when used in conjunction with maize starch.

Printer's or wheat flour is generally used, together with maize or tapioca starch, and, owing to its gluten-content, has a better holding or adhesive power than the starches. It is widely used as a thickening for alizarine-dyed 'styles' in which alumina and iron mordants are used, and it is stated to be the best thickener for use with azoic styles.

**Disadvantages attendant on the Use of Starch Thickenings.**—Where an alkaline process is necessary to develop the colour, difficulty is experienced in using starch thickenings, the addition of caustic alkali causing them to form a rubbery mass, useless for printing. However, caustic alkalies can be incorporated into starch thickenings by pre-treating the dry starch with alkali of 90° Tw. and, after heating and constant stirring, diluting the mass with water to a workable consistency. Pastes made in this way keep very well, and are used in some print works for printing indigo. Alkaline carbonates are often used, and here the same effect is to be noticed, but it is not so pronounced.

After printing and fixing the colour the thickening is often removed by washing the goods. Starch thickenings are not readily removed by washing, and impart a harsh feel to delicate fabrics. At one time a harsh or 'boardy' finish was in demand, but this demand has now decreased and smooth soft finishes are required, so that use cannot always be made of the excellent working qualities of starch thickenings.

**Thickenings of British Gums.**—As pointed out elsewhere (p. 235), a large range of British gums is on the market, and they are widely used for thickenings. They possess good working properties, and their stability to alkalies varies inversely with the content of unchanged starch. The availability of a wide range of these products renders easy the selection of one suitable for any particular type of work. British gums have the further advantage over starch in that they are readily and completely removable from the finished printed design, leaving the cloth with the required soft 'handle'.

Several grades of British gum are used in the printing trade, and vary from 'lightly calcined' gums to those which have been 'highly calcined'. A lightly calcined British gum may contain from 40-50 per cent. of unchanged starch, and would be used in

thickenings at a concentration of about 20-30 per cent., the product being paste-like in consistency. Moderately calcined gums may be used with alkali carbonates, which do not cause much variation in their working properties, but they cannot be employed with caustic alkalies as the pastes obtained are unworkable on the machines.

Well-calcined gums can be used with both caustic carbonates and alkalies, as little or no unconverted starch is present; pastes made with them containing about 50 per cent. solid matter have a semi-fluid consistency.

A very highly-calcined gum, known as Senegal Gum Substitute, is used in silk printing, and is probably the last member of this group of products to be of practical value. It contains no starch and consists of a mixture of the lowest members of the family analogous to the dextrans. A workable paste, which penetrates well into the fabric, is obtained when a mucilage is prepared containing about 70 per cent. of solid matter.

The following recipes may serve as an indication of the type of starch and British-gums thickenings used in printing textiles: 350 pts. of wheat starch are suspended in a 1000 pts. of water and 1000 pts. of an 8 per cent. gum-tragacanth mucilage are added. The mixture is boiled for half an hour with constant stirring, cooled and strained through a strong cotton cloth. A paste made in this way is much superior to that obtained by boiling the starch separately and then mixing with the gum-tragacanth mucilage, the paste being even and possessing smooth-working properties.

Thickenings for use with basic colours generally contain acetic acid, as the following typical recipe shows: 630 pts. of maize starch, 320 pts. tapioca starch, 4000 pts. water, 2000 pts. of an 8 per cent. gum-tragacanth mucilage, and 480 pts. of acetic acid (80 per cent.) are boiled together with constant stirring for 40 minutes. Before the mass is put in the pan, the sides are oiled, as previously mentioned, with 80 pts. of a vegetable oil (which is sometimes diluted with a little mineral oil for cheapness) to prevent the paste sticking to the sides and to improve the smoothness of working on the print machines.

Certain colours, such as vat dyestuffs, are reduced on the cloth to the leuco state during the processing which follows the actual printing, or in certain cases they may be reduced before printing. As the printing paste must be alkaline, thickeners stable to alkalies, such as British gums, are employed. The following illustrates the type of mixture used in such a thickening: 2000 pts. of a moderately calcined British gum are boiled for 20-30 minutes in

3600 pts. of water, and 1670 pts. of potassium carbonate added; the mixture is boiled for a further 10 to 15 minutes and then allowed to cool. When cold 500 pts. of glycerine and 980 pts. of sodium formaldehyde-sulphoxylate are well mixed into the thickening.

J. Pokorny<sup>1</sup> gives a recipe for a thickening to be used for printing on cotton. It is made by boiling 145 pts. of a dextrin, 52 pts. of maize starch, 104 pts. tapioca starch and 2 pts. of stearic acid in 900 pts. of water, then adding 700 pts. of resorcinol. A considerable drop in temperature is observed when this addition is made, and the pasty appearance of the mass disappears on stirring. After 48 hours it becomes resinous or rubber-like and must therefore be used soon after being made up. The film produced by drying at 95-120° C. is claimed to be insoluble in alcohol, water, benzine, and ether.

Other starch derivatives on the market have a limited use for certain types of work. There are a number of patents covering the use of cellulose and starch derivatives for various purposes, but in the actual manufacturing processes cellulose is generally used in preference to starch to make the product which appears on the market. Of these the so-called Colloresins are probably the best known<sup>10</sup>; they are cellulose compounds containing about 23 per cent. of the methoxyl group. Bayer<sup>2</sup> has covered the use of acetyl cellulose in admixture with boric or glycollic acid<sup>3</sup> as a printing-paste thickener, the preparation and use of the products obtained by the action of ethylene oxide on starch, dextrin, gum tragacanth, etc., and of the water-soluble cellulose ethers which are suitable as thickeners.<sup>4, 5, 6, 7</sup>

The printing of textile fabrics is an art, and there are a number of points which cannot as yet be rationally explained. Although work has been done recently to evaluate different properties of the printing paste,<sup>11</sup> and to co-ordinate them with the working properties of the paste when used in large-scale work, the results of these investigations are at present too immature to warrant detailed discussion. Rule-of-thumb methods still persist and adjustments to the printing pastes are made in an almost instinctive manner by the colour-mixer. However, despite our lack of scientific knowledge of the subject, many varied and very beautiful coloured effects are obtained.

#### REFERENCES

1. J. POKORNY, *Kunststoffe*, 1927, 17, 31.
2. BAYER, G.P. 291,802.
3. — G.P. 292,589.
4. — G.P. 368,413.

5. I. G. FARBENIND., E.P. 279,864.
6. BAYER, G.P. 363,192.
7. I. G. FARBENIND., E.P. 359,618.
8. F. OHL, *Gel. Leim u. Klebs.*, 1928, **1**, 4138.
9. F. D. FARROW and E. JONES, *J. Text. Inst.*, 1927, **18**, T1.
10. M. KERTH, *Textilber.*, 1937, **18**, 378.
11. S. N. GLARUM, *Amer. Dyest. Rep.*, 1934, **23**, 175; 1936, **25**, 150; 1937, **26**, 124, 437; 1938, **27**, 14, 308.
12. KALLE & Co., E.P. 399,998, 1933.
13. J. E. EVANS, *J. Soc. Dyers Col.*, 1933, **49**, 250.
14. F. D. FARROW and S. M. NEALE, *J. Text. Inst.*, 1925, **16**, T209.
15. M. S. FURRY, *U.S. Dept. Agric. Tech. Bull.*, No. 674, 1939.
16. KUO-CHUN CHIN, *Compt. rend.*, 1940, **210**, 581.
17. KALLE & Co., E.P. 521,468, 21/11/1938; G.P. 1/12/1937.

## ADDITIONAL REFERENCES

- K. GEHARD, *Zeit. angew. Chem.*, 1909, **22**, 2484. (Lightfastness of dyestuffs decreased by starch but increased by dextrin in finishes.)
- S. R. TROTMAN, *J. Soc. Chem. Ind.*, 1911, **30**, 1294. (Loss of colour and tendering of fabrics may be due to faulty starch in the finish.)
- P. BEAN, *J. Text. Inst.*, 1915, **6**, 223. (Full discussion of various starches used in sizing.)
- W. R. CATHCART, *Text. World*, 1921, **59**, 2895. (Penetration of size into fabrics shown by photomicrographs.)
- W. B. NANSON, *Cotton*, 1921-22, **86**, 161, 243. (Notes a large fall in viscosity of potato-starch pastes on boiling.)
- G. SMITH, *Col. Trade J.*, 1921, **8**, 147. (General.)
- W. B. NANSON, *Cotton*, 1923, **87**, 661, 700, 721. (General.)
- W. R. CATHCART, *ibid.*, 1924, **88**, 818. (General. Sizing.)
- C. H. HARPER, *ibid.*, 1924, **88**, 265. (Glazing yarn. General.)
- W. B. NANSON, *ibid.*, 1924, **88**, 811, 1116, 1126; 1925, **89**, 354. (Textile printing. General.)
- W. A. NIVLING, *ibid.*, 1924, **88**, 800, 863. (Preservatives for sizes recommended. Size should be fluid when it reaches the last squeeze-rolls.)
- J. WOODMAN, *Text. Col.*, 1924, **46**, 717, 783; 1925, **47**, 232, 640, 736, 805. (Sizing value of starches discussed.)
- H. SEYDEL and A. H. REINERS, *Text. World*, 1925, **67**, 2986. (General. Sizing.)
- R. HART, *Amer. Dyest. Rep.*, 1936, **25**, 231. (Warp sizing.)
- P. L. MANN, *ibid.*, 1937, **26**, 177. (Recipes for sizing.)
- C. P. WALKER, *ibid.*, 1935, **24**, 374. (Use of Aktivin for solubilising starch.)
- L. F. GLEYSSTEEN, *ibid.*, 1938, **27**, 14; *ibid.*, 1939, **28**, 280. (Printing thickeners.)
- J. E. EVANS, *J. Soc. Dyers Col.*, 1935, **51**, 319. (Desizing with enzymes.)
- G. F. DALENOORD, *ibid.*, 1932, **48**, 275. (Uses of starch in textile trades.)
- J. A. KIERNAN, *ibid.*, 1937, **53**, 379. (Printing thickeners.)
- A. MOLNAR, *Mell. Textilber.*, 1936, **17**, 234. (Enzymes for desizing.)
- KEHREN, *ibid.*, 1935, **16**, 875. (Enzymes for desizing.)
- V. P. SEYDEL, *Cotton*, 1936, **100**, 74, April. (Chemicals used in warp sizing.)

- J. WAKELIN, *Text. Col.*, 1938, **60**, 302. (Permanent finishes.)  
ANON, *Text. Manuf.*, 1937, **68**, 376. (Starches for finishing.)  
P. COLOMB, *T.I.B.A.*, 1937, **15**, 223. (General.)  
L. H. and J. FISHER, *Deutsch. Textilwirtschaft*, 1937, **4**, 11. (Detailed review of starch for textile work.)  
F. SICHEL, *Brit. Appl.*, 5584/38. (Cellulose-ether carboxylic acids as sizing agents.)  
SOC. POUR L'IND. CHIM., BALE, F.P. 809,932, 1937. (Urea-formaldehyde resins used with starch for sizing.)  
ULTRAZELL G.M.B.H., E.P. 472,473, 1936. (Fluorescent substances added to finishes to increase whiteness.)  
INTERN. PATS. CO., F.P. 819,374, 1937. (Halogenated starches as thickener in textile work.)  
I.C.I., E.P. 472,389, 1936. Equivalent to F.P. 816,387. (Fastness of starch improved by treatment at 90-140° C. with quaternary ammonium compounds.)  
PATENT CO. LTD., F.P. 810,688, 1937. (Diastase on hydrolytic products of starch used for sizing.)  
I.G. FARBENIND., Anm. I. 47,634, IVc/8k. (Treatment with chloral hydrate and urea to increase fastness of starch finishes to washing.)  
STEIN, HALL & Co., U.S.P. 2,083,982, 1936. (Urea, starch, and water for weighting textiles.)  
FIRMA ARNOLD H. WIVE, G.P. 647,997, 1937. (Laundry starch.)  
A. E. WILLIAMS, *Chem. Trade J.*, 1934, **94**, 190. (Starch for textile purpose.)  
J. A. CLARK, *Dyer*, 1934, **71**, 24. (General. Finishing.)  
F. OHL, *Spinn. u. Web.*, 1935, **53**, (26), 8. (Desizing.)  
ANON, *Textilber. (Eng. Ed.)*, 1930, 154. ('Quellin' described.)  
A. KOSEK, *ibid.*, 1935, **16**, 23. (Testing printing thickeners.)  
S. M. NEALE, *J. Text. Inst.*, 1927, **18**, T25. (Mill practice of heavy sizing.)  
F. KRONBERGS, *Latvij. Univ. Raksti.*, 1934, **2**, 385. (Surface tension and viscosity of printing thickeners.)  
LAUCKS, U.S.P. 2,098,083. (Urea-formaldehyde-resin starch as sizing agent.)  
E. PEZOLD, *Textilber.*, 1936, **17**, 222 ; 1938, **19**, 516. (Recipes for printing thickeners.)  
J. RIERE, *T.I.B.A.*, 1938, **16**, 321. (Identification of starches in finishes and thickeners.)  
JACOBY, *Amer. Dyest. Rep.*, 1938, **27**, 349. (Factors affecting colour value of print pastes.)  
DU PONT, U.S.P. 2,148,951. (Starch ethers and esters as printing thickeners.)  
E. M. MULLER, G.P. 671,259. (Printing thickeners.)  
E. V. PEZOLD, *Melliand. Textilber.*, 1938, **19**, 516, 593 and 743. (Comparison of newer textile printing thickeners.)  
I.G. FARBENIND., F.P. 766,119. (Formaldehyde on starch in presence of acid to give resistant finishes on textiles.)  
NORDDDEUTSCHE KARTOFFEMEHL-FABRIK. M.B.H., G.P. 666,252, 1938. (Wetting agents dried into starch. Products used in textile industry.)  
G. S. RENSHAW, *Chem. Age*, 1939, **41**, 123. (Desizing with enzymes. General.)

- KLEBSTOFFWERKE, 'Collodin,' G.P. 414,979, 1922. (Sizes, by heating starch with alkali salts of weak organic acids which only dissociate and act at higher temperatures.)
- J. STRASCHNOW, E.P. 518,510. (Acid-roasted, powdered manioc root for printing thickness.)
- A. E. WILLIAMS, *Text. Colourist*, 1939, **61**, 766. (General.)
- L. MAYER, *Textilber*, 1940, **21**, 176. (Various types of amylases and their use in desizing discussed.)
- J. SÉNÉCHAL, *Rusta*, 1939, **14**, 333. (Various methods of testing starch to be used for sizing described.)
- H. REHMANN, *Monats. Textil-ind.*, 1940, **55**, 38. (General. Sizing.)
- J. and J. TAKAMINE, U.S.P. 1,660,458, 28/2/1928; Appl. 17/12/1921. (Desizing with mould diastases.)
- L. MAYER, *Textilber.*, 1940, **21**, 176. (Desizing with amylases discussed.)
- J. PORZKY, *Z. ges. Text. Ind.*, 1936, **39**, 198. (Enzymes in desizing.)



## CHAPTER 5

## MISCELLANEOUS USES OF STARCHES AND DEXTRINS

**The Soap Industry.**—The use of starch as a filler in soaps has been suggested often, and appears to be practised to a fair extent in South America. In Germany it has also received attention, as a consequence of the national need for reducing the fats and oils in soap, minimising the blocade and finding an outlet for home-produced starch. It is known as a 'Verschnitt' agent,<sup>21</sup> a term introduced by E. Jaeschke to indicate that it is neither an adulterant nor, strictly speaking, an improver. Some 50,000 tons of potato starch were consumed annually in Germany before the 1914-1918 War for this purpose; to-day soluble starch is being used.

Rice starch is added to toilet soaps in some countries, but in Germany potato starch, and in Guatemala, Yuquilla starch are used. Those who advocate the use of starch in soap give the maximum amount that can be added to most kinds of soap as 15 per cent., and they consider these concentrations harmless, except for white soaps, in which the inclusion of excess starch may affect the colour.<sup>22</sup> The opponents of this suggestion point out that starch has little or no detergent properties, and therefore its inclusion is pure adulteration. On the other hand, the inclusion of starch does not appear to affect lathering, or indeed any other property, to a marked extent; in fact, some claim that the incorporation of starch in soap improves both the stability and volume of the lather.

Starch alone swells in water and forms an adhesive paste on heating, the colloidal solution having the protective action and strong adsorbent properties usually associated with long-chain organic colloids. There is, therefore, some ground for expecting starch to be innocuous to soap, even if it does not increase its detergent power.

In Germany the best grades of ordinary starch and flour are known as Hochfein, Superior, and Prima, in descending order of quality. These are of high gloss and maximum whiteness, free from chlorine and acid, and contain 0.25-0.5 per cent. of mineral matter. The moisture-content should not exceed 20 per cent. and not more than a trace of combined chlorine or iron should be present. Kroner and Steinhoff<sup>20</sup> have elaborated another grade of starch, called by them 'Industrial Starch,' which, although indistinguishable from ordinary starch, has a lower ash-content

of a different constitution ; its physical properties are claimed to be somewhat out of the ordinary, enabling 25-30 per cent. of it to be incorporated in soap. The inclusion of 20 per cent. of this new starch was found to reduce the water-loss of a coconut-oil soap from 11.9 to 4.9 per cent. over four weeks. The authors also confirmed the claims for increase in lather number and stability of the lather.

In addition to those who criticise the inclusion of starch in soap because it is merely an adulterant without detergent power, other workers maintain that its inclusion is detrimental,<sup>23-24</sup> especially if the soap is to be used to wash fabrics printed or dyed with vat colours, on the ground that these colours are reduced by the combined action of the starch and its by-products in the presence of the alkali. Such an action would render the dyestuff soluble and therefore loose to washing.

Some workers prefer the use of starch to that of sodium silicate. Kroner and Steinhoff<sup>20</sup> have shown that by heating starch for periods varying from  $\frac{1}{2}$  hour to 12 hours in the presence of a 2 per cent. soap solution, either alone or with sodium silicate or soda lye, no measurable amounts of reducing substances were formed which could be detected by Ost's test or by Fehling's solution. Despite the elaborateness of the tests performed by these authors, further work on a large scale is needed before the matter can be settled, but the balance of evidence suggests that the inclusion of starch is practically harmless, and that it offers certain advantages which will not be overlooked by the trade, especially in Germany, where soap-makers are being encouraged to economise on fats wherever possible (see also W. Schültze<sup>11</sup>).

For toilet or milled soaps, the starch is added in powder form to the chips before milling. If desired, it can be used in the manufacture of soap flakes, but its use in soap powders is un-economic, although it may not be so for shaving powders. The following example illustrates the composition of a shaving powder containing starch :—

Castile soap powder . . . . .	800 pts.
Maize starch . . . . .	200 „
Cassia oil . . . . .	6 „
Caraway oil . . . . .	1 pt.
Geranium oil . . . . .	3 pts.

The powders are first mixed, the oils are added, and the whole thoroughly re-mixed.

A product which is claimed to have detergent properties is prepared, according to A. L. Sodergreen,<sup>1</sup> by heating corn-flour to 120° C., or lower, in the presence of moisture to break down

the protein matter and to hydrolyse the starch. The mixture is then made alkaline with caustic soda and reheated under pressure to saponify the oil present, any excess caustic soda being neutralised finally with carbon dioxide under pressure.

Although laundry work does not come under the heading of 'Soap Industry' it is convenient to mention here the use of starch in the laundry. For imparting a glaze, or as stiffening, rice starch is used in conjunction with waxes, e.g. paraffin wax, which are often added in the form of an emulsion. Soluble starch, dextrin or glucose all find use as the carrier and adhesive in the making of 'laundry blues,' a pigment dyestuff like ultramarine being the actual tinting agent and imparting an apparent whiteness to the cloth.

**Laundry Starches.**—There has always been considerable reticence on the part of laundrymen, in common with textile finishers, on their use of starch. The final effect in both industries is largely influenced by the skill of the operative. Even using the same materials in ostensibly the same manner sensibly differing results are often observed. Since the housewife is largely influenced in her buying and laundering of textile fabrics by such factors as appearance and handle, the reason for a desire for secrecy becomes apparent. Nevertheless, it has been possible for suppliers to institute some measure of co-operative research, and the following brief résumé of the application of starch to laundry work has been written by Mr. J. M. Faulds.

For a considerable number of years rice starch has been the most widely used material both for commercial and domestic work, despite its higher price. There are good reasons for its popularity although it must be stated that the cost of starch in a laundry is relatively unimportant. It has been found that the small granules of rice starch have a good penetrative power, besides imparting a good flexible glossy finish to the article. Furthermore, with personal linen it is more resistant to the effects of heat and perspiration from the body.

With the trend to less heavily filled fabrics, and a wider range of finishes, ideas on starching gradually modified. Again, the need for disposal of surplus quantities of other varieties of starch, including maize and wheat principally, led the makers of these to investigate their possible applications to the laundry industry. Various ingredients were added in endeavours to improve the natural qualities of the various starches, and the writer spent some considerable time in an experimental laundry plant investigating the use of these for laundry work.

It was found that practically all starches commonly occurring

in practice could be used. With maize starch a characteristic 'boardy' feel was usually obtained; whilst wheat starch gave rather a soft effect. Blendings of these two were then tried with very good results.

In view of the trend towards adding the starch to the washing machine after the final rinse, and the importance of the degree of gelatinisation achieved on calendaring, particular attention was paid to this procedure. It was found that, contrary to general supposition, only a fraction of the granules were burst when heat was applied, and this was found to apply even when the starch paste was prepared in a boiler. The behaviour of soluble starches and dextrans was investigated and it was found that some of these gave very pleasing results, which is understandable in view of the large use of these types of starch derivatives in textile finishing. In general the modified forms of starch have lower gelatinisation temperature ranges, and possess greater penetrating power, the latter property imparting a fullness to the article.

The practice of adding dry starch to the washing machine is rather wasteful as an appreciable quantity is lost when the water is run off. Further, it is not at all certain that each article picks up starch in its rumpled state uniformly. The use of wetting agents is often beneficial.

The addition of borax to laundry starches has always been largely favoured and undoubtedly is responsible for a considerable improvement in the results obtained. The quantity added may vary within the limits of 1-17 per cent. on the weight of starch, depending on the other constituents present. The mechanism of the action of borax on starch is as yet unknown but work is proceeding to elucidate the matter. Starch-borax compositions are often designated as 'gloss starch,' although trade descriptions seldom give accurate indications of composition.

Other mineral additions to laundry starches include china clay, chalk and some of the other filling materials commonly used in textile finishing. These may be added with a view to making good any large loss in weight during washing. This loss is not experienced to anything like the same extent with linen as with cotton, and even in the case of the latter the modern tendency is towards pure finishes. If such a mixture be used, the starch should bind in the filler which should not dust off on rubbing the laundered fabric. These fillers are always very much cheaper than starch and a buyer can be guided by an analysis of the ash-content of a brand under consideration.

The last type of addition to be considered is that of fatty matter. The choice often consists of tallow or stearin. A very pleasing effect results from the use of either of these two substances. One point deserving special attention is that of temperature. The melting-point of tallow and of stearin are both below the gelatinisation temperature range of commercial starches. Consequently, if the iron or calender is sufficiently hot to secure even partial gelatinisation some harm may be caused through melting and darkening the fatty matter present. The use of modified starches enable lower temperatures to be used on the calender and the difficulty is largely removed. If dry starching is used there is a tendency for the fatty matter to separate from the starch if the two have simply been dry mixed in manufacture. Even although an emulsion of the fat is added to an agitated suspension of the starch the two are found to separate during casting. It has been found possible, however, to incorporate the fatty matter with the starch in such a manner that when the mixture is thrown over the cage in the washing machine there is uniform dispersion and pick-up by the articles.

Finally, a word should be said about what is termed 'sticking.' This trouble is due to excessive starch being left by the articles on entering the calender on the lip of the machine where pasting and drying of the starch take place to be followed by charring. The brownish flakes peel off and become attached to subsequent articles. It must be borne in mind that all starches exhibit this tendency which can, however, be reduced. The most effective method of prevention is to fit squeeze rollers to remove surplus starch, before the heated bed is encountered. There has been considerable controversy over this subject and much work remains to be done. Undoubtedly routine practices in a laundry are largely responsible for the degree of trouble experienced from this source.

**Cosmetic and Pharmaceutical Uses.**—The use of wheat, maize and rice starch is specified in the British Pharmacopœia and that of maize starch in the American Pharmacopœia. Starch is used as a diluent and carrier in many of the toilet powders of to-day, e.g. the so-called Violet Powders, which consist essentially of perfumed starch powders, deodorant powders (*v.i.*) containing salicylic acid, boric acid or alum, and dusting powders containing zinc oxide, salicylic acid and starch.

In making these toilet powders care should be taken to ensure that the starch is perfectly dry before mixing. A basis of rice starch is widely used and is considered better than wheat starch, which has a bluish-white tone, or potato starch, in which the

granules are so large that the powder has a coarse appearance. A further drawback to the use of potato starch as compared with rice starch is the high gloss and inferior colour it imparts, but it is sometimes used for cheap goods.

In the manufacture of face powders, starch finds itself in competition with a number of other substances. The best face powders contain 50 per cent. or more of rice starch to which maize starch is often added in the manufacture of cheaper products. The question whether it is preferable to use starch instead of other carriers in face powders is debatable. Some medical men claim that starch may be rendered acid by contact with perspiration; also that when it works into the pores, the acid and moisture in the perspiration cause the small granules to swell. This swelling enlarges the pores, thus coarsening the skin and rendering the penetration of larger granules easier on subsequent applications. To overcome this alleged defect one manufacturer first heats the starch with water to swell the granules, and then, after drying, grinds the mass to reduce it to a fine powder, at the same time incorporating a wax to increase the water-resistance of the powder. By this method he claims to produce a powder with satisfactory properties. It may be pointed out, however, to those who oppose the use of starch on the grounds stated above, that the powder is invariably applied in a thin layer, and is washed off and renewed after a relatively short contact with the skin, so that the part played by starch in beauty skin-treatments is not a harmful one. The mineral vehicles employed, such as talc, zinc oxide, magnesium or calcium carbonate, kaolin or kieselguhr, and the stearates of zinc and magnesium, are also open to the same objection, viz. that they may stop up the pores of the skin, although, of course, they do not swell in contact with water. A formula for a compressed tablet or 'compact' is as follows: rice starch 250 pts., talc 450 pts., china clay 250 pts., zinc white 50 pts., are well mixed together with a very small amount of binder, such as gum senegal or gum-arabic, and then compressed. The starch itself can sometimes be used as the binder, e.g. for certain medicinal tablets to be mentioned below.

Starches are rarely, if ever, put into infant powders or perspiration powders, although E. Donath<sup>2</sup> uses starch among other carriers for such powders. The powders claimed by Donath contain amylolytic or proteolytic enzymes, such as pepsin, pancrease, and urease, together with water-soluble compounds which are claimed to activate the enzymes in the presence of water, e.g. boric acid, sodium benzoate or salicylate. In making nursery powders, Donath uses enzymes which are activated by

compounds having an alkaline reaction in water, e.g. sodium salicylate.

Creams and mucilages are often made up on a base of glycerine and starch, or glycerate of starch. According to the preparation in the British Pharmacopœia, 20 gms. of starch, 130 c.c. of glycerol, and 130 c.c. of water are heated with constant stirring until a translucent jelly is obtained. This mucilage gives relief when applied to chapped hands or chilblains, and is used in admixture with zinc oxide as a cosmetic. Glycamyl or Plasma are other names by which glycerate of starch is known.

Starch serves two distinct purposes in the making of pills: it is used as a coating and dusting agent, and as a binder for the materials of which the pill is composed. The pills may be coated with a mixture of syrup and starch paste and then rolled in a mixture of powdered starch and sugar. As a binding agent it sometimes appears in grey pills or in phenolphthalein pills. In the manufacture of aspirin tablets starch is used for a specific purpose: it is dried until as little moisture as possible remains, mixed with the acetylsalicylic acid and other components, and the mixture compressed into tablets. When the tablet is swallowed, the starch rapidly absorbs moisture and swells, thus setting up an internal stress which causes the tablet to disintegrate completely. San-Gri-Na, which is a proprietary remedy for obesity, is stated to contain 11 per cent. arrowroot starch, a vegetable extract, and phenolphthalein.

Instead of using starch iodide for treating wounds (see p. 133), certain preparations of formaldehyde and starch, known as Amyloform and Euformol, can be used. The latter is a dextrin-formaldehyde product. Both substances are also sold for the treatment of colds.

Certain insect powders, such as beetle powders, sometimes contain starch as the carrier for the borax, sodium chloride, or other effective agent.

Starch has sometimes been suggested for, and used in, the making of depilatories, maize or potato starch being used instead of talc or other inorganic carriers for the metallic sulphide, which is the effective agent; it has, however, the disadvantage that it forms stodgy masses on the addition of water and sulphide, so that it is preferable to use a lighter grade of magnesium carbonate as the carrier.

**Horticultural Uses.**—Insect powders have already been mentioned, and in addition, starch or dextrin are sometimes used in the preparation of horticultural sprays. These sprays contain a killing agent for pests or fungi, together with a substance to

cause the solution to penetrate cracks or spread over the leaves which, normally, are not easily wetted. Soluble starch, or more usually a yellow dextrin, is added to make the dissolved material adhere to the treated portions when the liquid medium evaporates. Although some people have used the feeble wetting-out properties of the dextrin to cause the liquid to spread effectively, and have tried to dispense with the use of a synthetic wetting agent, it is decidedly preferable to include the latter. In emulsions containing soap, the emulsifying agent also acts as wetting agent, and the sole function of the dextrin is that of an adhesive.

**Fire-Proofing Preparations.**—To obtain non-inflammable fabrics, J. Benoid<sup>3</sup> used a maize or rice-starch sizing containing sodium tungstate, magnesium carbonate and sulphate, together with several other ingredients usually used in fire-proofing. H. Becker<sup>4</sup> uses ammonium salts and aluminium sulphate in a starch preparation for the same purpose, whilst still another preparation<sup>5</sup> containing talc, sodium hyposulphite, sodium chloride, and borax has also been suggested. According to H. J. Henk,<sup>32</sup> starch for certain purposes can be fire-proofed with various mixtures of aluminium and ammonium salts containing borax.

**Explosives and Fuels.**—Rice starch has been used as a crystallising and binding agent in moulded Black Powder explosives.<sup>6</sup> Abelite is said to contain starch, ammonium nitrate, T.N.T. and sodium chloride; Ammonia Dynamite Pulverent, 20 per cent. nitroglycerine, ammonium nitrate, sodium nitrate and 19 per cent. rice starch or flour; whilst Ammonia Dynamite (French) is said to contain wheat starch. Bobbinite, a type of gunpowder, contains starch and paraffin wax. The different varieties of Carbonites, which are used in coal mines for blasting, contain wheat or rice starch; Foerdite and Fractonite contain 4 per cent. dextrin, and Gesilit Nos. 1 and 2 are said to contain 39 and 21 per cent. of dextrin, respectively. Fulgurite contains 40 per cent. of wheat flour, and Gelatinwetterastralite 8 per cent. of potato starch. The explosive properties of nitro-starch are well known (see p. 142).

According to A. Schrimppff<sup>33</sup> two kinds of nitro-starch explosives are used in America. For one kind, nitro-starch is mixed with sodium nitrate and an oil, a typical composition consisting of 50 per cent. nitro-starch, 47.5 per cent. sodium nitrate, impregnating oil 1.5 per cent. and sodium bicarbonate 1.0 per cent. This mixture has a high brisance and is readily detonated, making it suitable for chamber blasts and quarrying limestone or granite. The other type contains a large amount of ammonium nitrate



mixed with the nitro-starch together with oxidisable materials such as T.N.T., coal dust and aluminium powder, together with a little oil. Explosives of this type have a lower brisance and are more difficult to detonate; they also give rise to larger amounts of gaseous products. Some of these mixtures are used in fiery mines. Investigations made at the Pittsburg experimental station on the sensitising of ammonium nitrate by nitro-starch are described by this worker (see also J. B. Bronstein<sup>12</sup>).

Rice starch finds some outlet as a binding agent in the manufacture of match-heads,<sup>9</sup> and has also been used in the manufacture of fireworks. In match manufacture the most important adhesive used to bind the chemicals forming the tip is a good grade hide glue, but starches and dextrans are also of importance. Not only do they function as adhesives, thereby reducing the amount of the more expensive hide glue, but they also act as thickeners and fillers which are readily oxidised and take part in the combustion when the match is struck. In most cases a satisfactory amount of starch or dextrin pastes to add to the mix is about 13-14 per cent. on the batch volume. Dextrans are considered to be better than starch for this purpose by a number of match-makers but, they are, of course, more expensive.

A further use for rice and other starches<sup>7</sup> is to stabilise the colloidal catalysts used in the hydration of olefines, and to stabilise coal-dust suspensions in oils that contain combustion catalysts and are used for fuel oils.<sup>8</sup>

Spent water from coal-washing contains very finely divided coal dust that is difficult to separate from suspended clay and other impurities, but the filtration and removal is facilitated by the addition of starch, which flocculates the particles and carries them down with it as it settles. By suitable selection of equipment, this method has been found practicable and effective for full-scale work. The starch for this work is often modified with zinc chloride and its efficiency is greatly increased by carrying out the precipitation under alkaline conditions. The well-known 'Unifloc Process,' for example, embodies the use of lime as the alkali (*v.i.*).

L. Marton<sup>10</sup> proposes to utilise waste coal dust in the manufacture of fuel briquettes in which the particles are cemented together, using starch. Such briquettes, however, suffer from the drawback of disintegrating when they become damp, and to overcome this, it has been suggested to use skimmed milk and to treat the briquettes subsequently with formaldehyde.

**Some Unclassified Uses.**—It remains to consider some isolated uses of starch in a few widely different industries. Starch

is used as a dusting powder for moulds in foundry work and has been used in oilcloth manufacture. A dimethyl derivative of starch has found use as a creaming agent for rubber-latex creaming,<sup>14</sup> whilst according to another patent,<sup>15</sup> the backs of carpets may be finished with an aqueous, compounded, rubber-dispersion containing more unpeptised starch than rubber. It is also used as a dusting agent to eliminate tackiness in certain rubber articles, such as mackintoshes.

In conjunction with soap bark, the use of rice starch has been claimed<sup>16</sup> for the manufacture of porous building blocks from hydraulic cement (see also K. Schenkel<sup>31</sup>); and a further use, of interest to the building engineer, is that for making bitumen and tar emulsions, in which it acts as the emulsifying agent.<sup>17</sup>

A. V. Petrov<sup>18</sup> has examined the use of starch for speeding up the separation of the sludge formed in the manufacture of caustic soda by the lime process. He claims that the addition of 0.01 per cent. of starch increases the speed at which the carbonate formed in the process settles in the tanks. J. O. Samuel<sup>34</sup> produces starch gels for flocculating suspended matter by agitating modified starch at 70° C. with a concentrated aqueous solution of a neutral hydrated metallic salt, e.g. zinc, calcium, magnesium or lithium chloride or a thiocyanate until a thick gummy mass is obtained (see also pp. 50, 51).

The U.S. Navy Standard Boiler Compound—used for softening boiler feed-water—consists of 76 per cent. soda ash, 10 per cent. trisodium phosphate, 1 per cent. starch or dextrin and 11 per cent. water together with sufficient cutch to yield 2 per cent. tannic acid.

Starch finds occasional use in the leather industry for finishing such leathers as sole leathers and the grain side of wax kips.

In the common electric dry-battery cell starch is used for several purposes. The label may be surfaced with starch and affixed to the battery with an amylaceous adhesive; the paper used for lining the walls of the cell may be coated with rice starch that has been treated with ammonium chloride and zinc chloride<sup>19</sup>; and the electrolyte in these cells may be carried in a mucilage, of which a rice-starch paste may form the basis. The Industrial Research Bureau of the Indian Government have recently found that the starch content of the electrolyte in dry cells can be reduced considerably without any adverse effects. W. D. Staley and A. J. Helfrecht<sup>29</sup> have studied the gelatinisation of maize starch in dry cell electrolytes. A mixture of 2 pts. of starch and 1 pt. of corn meal was added to solutions of zinc chloride or of zinc and ammonium chlorides at 10° C. and after thorough

mixing the time taken to gelatinise at 18° C. was determined. With 35 gm. of the cereal in 80 c.c. of zinc chloride solution no gelatinisation takes place unless the zinc chloride concentration reaches 35 per cent. The setting time decreases to a minimum as the concentration of zinc chloride is increased to 42 per cent. and then increases rapidly above this concentration. Ammonium chloride is less effective in promoting swelling, and similar results to those above are obtained with solutions in which some zinc chloride is replaced by ammonium chloride. They recommend making two solutions, one containing most of the zinc but little of the ammonium chloride and the other containing most of the ammonium chloride, a little zinc chloride and all the cereal and then mixing in suitable proportions to give a solution which will set in a convenient time for the preparation of dry cells.

Certain foodstuffs, such as eggs, oranges, and meat, are often stamped or marked to designate their grade or origin; and sacks used for packing many substances are stamped or stencilled for a like purpose. Many of the marking inks on sale for this purpose consist primarily of an aqueous solution of a dyestuff to which is often added a yellow dextrin or thin-boiling starch to act both as thickener and adhesive.

An interesting use of rice starch is in the manufacture of luminous paints. Very pure starting-materials are required to produce the finer grade of product, and the presence of a minute amount of impurities often alters the colour or the intensity of the luminescence. Sulphides of heavy metals form the basis of many of these luminescent products, in the preparation of which certain metallic compounds are heated with starch. The starch acts as a reducing agent, and rice starch is generally preferred to potato starch because it is less likely to contain traces of iron compounds, which would adversely affect the quality of the luminescence. Anhydrous starch has been suggested for use for preparing fillers and thickeners in paints, lacquers and polishing compositions.<sup>35</sup> The dehydrated starch is used to adsorb a water-immiscible liquid such as propylene dichloride and then mixed with a strong swelling agent. S. E. Stockman<sup>36</sup> suggests the use of corn starch in glazing liquids to prevent running and for use in various woodfillers. Air-dried starch heated with formaldehyde in the absence of air until the mass no longer gives a blue colour with iodine is claimed<sup>37</sup> to give a product which can be moulded at high temperatures to a transparent body.

M. M. Nurkass<sup>30</sup> claims that printing rollers of outstanding durability can be made from 25 pts. starch and 25-30 pts. of magnesium-glycerine syrup. This syrup consists of 11 pts.

of glycerine and 1.6 pts. of magnesium chloride. After filtering the starch-syrup mix and allowing to stand for 24 hours it is cast into moulds. The hardness of these rollers are said to decrease as the syrup-content is increased.

## REFERENCES

1. A. L. SODERGREEN, U.S.P. 2,049,476, 4/8/1936.
2. E. DONATH, E.P. 378,888.
3. J. BENOID, E.P. 14,205, 1906.
4. H. BECKER, E.P. 20,460, 1906.
5. A. J. JARMAN, *Scientific American*, 1910, **103**, 364.
6. U.S.P. 1,913,344.
7. E.P. 413,043.
8. E.P. 408,951.
9. U.S.P. 1,831,760.
10. L. MARTON, E.P. 21,755, 1904.
11. W. SCHÜLTZE, *Fette u. Seifen*, 1938, **45**, 522.
12. J. B. BRONSTEIN (to Trojan Powder Co.), U.S.P. 2,170,629.
14. RUB. PRODS. RES. ASS., E.P. 437,758.
15. ANM. I. 50,012, IVc/8k (3).
16. U.S.P. 1,937,472.
17. E.P. 387,657. (Lapsed.)
18. A. V. PETROV, *J. Appl. Chem. U.S.S.R.*, 1936, **9**, 34; via *Chem. Abst.*, 1936, **30**, 6140.
19. U.S.P. 1,911,400.
20. KRONER and STEINHOFF, *Seifens. Zeit.*, 1936, **63**, 272.
21. G. KNIGGE, 'Soap, Perf., Cosmetics,' 1936, 316.
22. H. NITSCHKE, *Seifens. Zeit.*, 1935, **62**, 840.
23. K. KIEFER, *ibid.*, 1936, **63**, 413.
24. K. L. WEBER, *ibid.*, 1936, **63**, 189.
25. P. BRETTSCHEIDER, *ibid.*, 1936, **63**, 311.
27. N. I. KOZINE, *Maslob, Fir, Delo*, 1937, **13**, 28 (3); *Chim. et Ind.*, 1938, **40**, 975.
28. L. ZAKARIAS, *ibid.*, 1936, **36**, 1095; *Chem. Trade J.*, 1937, p. 27. (Starch solutions for metal degreasing.)
29. W. D. STALEY and A. J. HELFRECHT, *Amer. Electrochem. Soc.*, 1928, **53**, April.
30. M. M. NURKASS, *Polygraphic Ind. (U.R.S.S.)*, 1939, No. 6, 44.
31. K. SCHENKEL, E.P. 285,470, 25/11/1927.
32. H. J. HENK, *Seifensieder-Ztg.*, 1939, **66**, 141.
33. A. SCHRIMPF, *Zeit. ges. Schiess- u. Sprengstoffw.*, 1930, **25**, 273.
34. J. O. SAMUEL and UNIFLOC REAGENTS LTD., E.P. 516,294, 29/6/1938.
35. U.S.P. 2,165,834, 1939.
36. S. E. STOCKMAN, *Amer. Painter and Dec.*, 1939, **16**, No. 6, 25.
37. SCHOLTEN'S AARDAPPEL MEELFABR., Dutch P. 46,185; *Chem. Zentr.*, 1940, **111**, I, 142.

## ADDITIONAL REFERENCE

- J. SERBERLICH, *Modern Plastics*, 1941, **18**, No. 7, 64 and 98. (Use of starch in film-forming compositions and plastics reviewed.)

## CHAPTER 6

UTILISATION OF THE BY-PRODUCTS OF STARCH  
MANUFACTURE

BY-PRODUCTS are obtained in the manufacture of the different varieties of starch, and some of them present the problem of their disposal ; others, on the other hand, may be commercially valuable and be worth recovering.

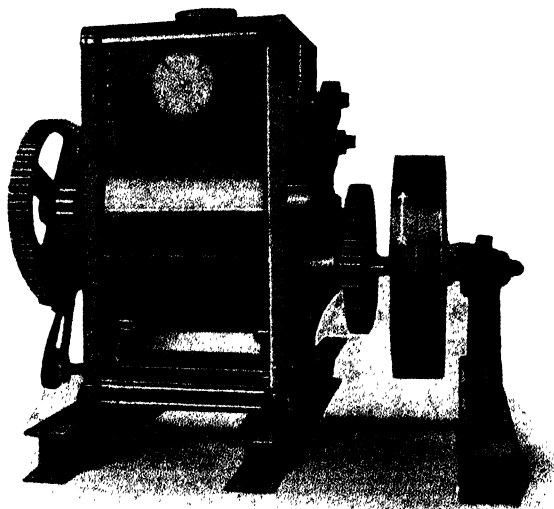
The by-products obtained in the manufacture of maize starch are cattle-food, consisting of dried gluten, corn-bran and germ-oil meal, and corn-oil. The last-named is obtained in a yield of about 2.5-3 per cent. on the weight of the maize processed, and is used in several industries where a semi-drying oil is required. Steeping-water from the preliminary washing contains mineral salts and water-soluble proteins, and after concentrating from approximately 5-18° Bé., it is sprayed on the cattle-food whilst this is drying. In some cases it is still further evaporated and used as a nutrient medium for yeast and bacterial cultures, or for purposes where a non-coagulable water-soluble protein is required. The soluble products from the steep-water are removed by W. Sage<sup>1</sup> by precipitation with lime, transferring the liquor to a settlement tank, and after expressing surplus liquid from the sediment, drying the pressed product.

The maize gluten has a number of interesting possible uses in industry, and several grades are available commercially. One grade is known as carbohydrate-free, another grade is free from both carbohydrates and from the portion which is soluble in aqueous alcohol, whilst the third grade consists of the portion soluble in aqueous alcohol, the so-called 'Zein'.

The carbohydrate-free gluten is also produced in several grades, e.g. an oil-free grade, a bleached grade, and an oil-free and bleached grade. The various grades differ slightly from one another, but as a group their chief interest lies in their value as fillers or bases for plastics. They can be incorporated with both natural and synthetic resins or with cellulose derivatives, and are, in general, light in colour and thermoplastic. They react with formaldehyde or phenol, and if free from the portion soluble in aqueous alcohol are almost completely dispersed in dilute alkaline solutions. The percentage composition of the carbohydrate and oil-free protein is approximately as follows : moisture 11, protein

76, ash 1.2, fibre 4, oil 1.1, starch nil; the alcohol-soluble portion amounts to about 36 per cent.

The fraction which claimed early attention because of its solubility in aqueous alcohol—the Zein—was isolated by Gorham in 1821. It is available only as a by-product of corn processing, and its potential yield is 1 lb. per bushel of corn. The commercial product contains <sup>6</sup> about 90 per cent. of matter soluble in 80 per cent. alcohol, 8 per cent. moisture, 0.4 per cent. ash, and 0.4-0.6 per cent. oil; it is marketed as a white, tasteless, odourless, amorphous solid. It is said to be resistant to light, is



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FIG. 64.—A pulp-pressing machine.

thermoplastic, has a high electrical insulating value, and is non-inflammable. It is unaffected by anhydrous alcohols or by organic solvents in general, whilst dilute acids and weak alkalis do not dissolve it. Solutions, can, however, be obtained with aqueous alcohols, dilute solutions of strong alkalis, molten phenol, or strong solutions of urea. It is also claimed <sup>6</sup> to be compatible with many cellulose derivatives, plasticisers, natural and synthetic resins, and can be used in the preparation of stable glass-like plastics. Modifications are also used as adhesives and in the preparation of coating compositions for paper, etc.

E. Bartow and W. W. Walker <sup>9</sup> have discussed the recovery of compounds from corn starch steep-water and consider it a very likely source of inositol.

The by-products from wheat-starch manufacture are gluten and, as in the case of every other starch, waste water containing soluble and colloidal impurities. The disposal or utilisation of waste water is important in every starch factory, and various processes have been put forward for its use. One early suggestion<sup>2-3</sup> was that it should be pumped on to meadow-land, like the effluents from sewage farms. An artificial manure was obtained by G. de Claubry,<sup>4</sup> who treated the effluent with milk of lime and a tannin solution. Precipitation with lime was also used by Markl.<sup>5</sup>

By far the most important by-product from wheat-starch manufacture is gluten, which is used in preparing diabetic foods, for feeding cattle, sometimes as a thickening agent in textile printing, and as a size or adhesive. The gluten for technical purposes is largely supplied from the factories operating the Martin process, whereas that for food uses is generally obtained by the Fesca process, which yields a product containing all the non-starchy matter in the wheat. As moist gluten tends to decompose after a day or two, it must be dried before it is stored. Fermentation under controlled conditions gives a size which is without any offensive odour.

Sodium glutamate, which has a taste very like meat extract, is used in the preparation of certain soups and gravy flavourings, and can be used in water as a substitute for beef tea. It can be made by dissolving gluten in an acid, preferably hydrochloric acid, at a temperature below the coagulation point of the protein, adding a small quantity of finely granulated tin, and then heating the mixture under pressure with steam. The tin accelerates the hydrolysis and removes any arsenic in the acid. The solution is filtered, concentrated, and the glutamic acid hydrochloride is allowed to crystallise. The crude hydrochloride is neutralised with slight excess of caustic soda, which precipitates any tin present, the sodium and ammonium chlorides are separated by evaporation and crystallisation, and the mother-liquor is added to alcohol to precipitate the sodium glutamate.

Corn Products Refining Co.<sup>8</sup> utilise maize gluten for the preparation of pure amino-acids. The protein is hydrolysed by boiling with 20 per cent. hydrochloric acid and humin substances are removed by evaporation and filtration. The *pH* value is then adjusted by the addition of caustic soda, and by successively adjusting the *pH* value to the iso-electric point of each amino-acid present these can be separately precipitated relatively pure. Care must be taken to control certain factors such as the density of the solutions and the crystallising temperatures. Tyrosine is precipitated at *pH* of 2.4-3.4 followed by leucine at *pH* of 6-7.

Two lb. of the former and 15 lb. of the latter are obtained from 100 lb. of maize gluten.

From potato-starch manufacture is obtained the exhausted pulp which, in some factories, is treated with lime to allow easier separation of the water, which is present to the extent of more than 90 per cent. After the moisture has been expelled from the pulp by means of pulp presses (see Fig. 64), or other mechanical means, the solid cake is used as cattle fodder. Another method, which is stated to give a more palatable fodder for cattle, is by controlled fermentation of the exhausted pulp in earthen trenches. W. Ostwald, H. Erbring and A. Siehr<sup>10</sup> have covered the use of potato protein in soaps and detergents. The protein is removed from the factory waste waters by blowing air into the liquid when the foam so formed contains all the protein present and can be removed and the protein recovered. The use of starch, itself, in soaps is discussed on page 348.

The utilisation of rice oil for olein and stearin manufacture has been dealt with by S. Ueno.<sup>7</sup>

#### REFERENCES

1. W. SAGE, U.S.P. 1,187,392, 1916. (Lapsed.)
2. BURGGRAF, *Dingl. Poly. J.*, 1835, **56**, 46.
3. M. MAERCHLER, *Zeit. Landsw. Central Prov. Sachsen*, 1876, 7.
4. G. DE CLAUBRY, *Dingl. Poly. J.*, 1837, **63**, 465 ; 1841, **80**, 399.
5. MARKL, *ibid.*, 1874, **214**, 225.
6. ANON, *Ind. Eng. Chem.*, 1937, **29**, 673.
7. SEI-ICHI UENO, *J. Soc. Chem. Ind. Japan*, 1937, **40**, 200.
8. CORN PRODUCTS REFINING CO., E.P. 528,162.
9. E. BARTOW and W. W. WALKER, *Ind. Eng. Chem.*, 1938, **30**, 300.
10. W. OSTWALD, H. ERBRING and A. SIEHR, G.P. 660,992 ; *Deut. Parfüm. Zeit.*, 1940, **26**, 185.



## CHAPTER 7

## ANTISEPTIC AGENTS AND PRESERVATIVES

THERE are two distinct conditions that may arise to worry those concerned with the manufacture and applications of starch and flour preparations, and cause spoilage of materials at some stage of the process. The first of these is bacterial action and the second is fungoid growths or mildew, and of these the second is probably the more troublesome.

Bacteria are found in starch, sometimes in large numbers, although starch is far from being an ideal or complete food for them. The bacterial count is greatly influenced by the chemical processing it has received and on the air, water and equipment with which it comes into contact. A. Frieden<sup>16</sup> has published data showing the wide fluctuations which occur in the number of bacteria in corn starches. In general, oxidised starches have a lower bacterial count than thick-boiling starches, whilst unmodified products dried by slow methods in the presence of unlimited and untreated air contain more bacteria or spores than the same starches dried by modern methods.

Tapioca flours have a surprisingly low and uniform bacterial content. Frieden<sup>16</sup> classifies the micro-organisms in starch into three groups, namely, (1) general, including moulds, (2) *B. coli*, (3) thermophilic bacteria. The number of bacteria tends to decrease when the starch is stored in a cool dry place, but there is an increase in bacterial activity when the starch is mixed with water prior to using it for processing. The above worker recommends the use of a combination of benzoic acid and paraformaldehyde to restrain bacteria, while he considers that copper salts are the most effective agents against moulds and yeasts (see, however, below).

Mildew is propagated or spread by the minute spores of the growth being carried by the air from place to place, and thrives on a wide variety of diets. Moisture and warmth increase its rate of growth and allow it to make full use of any favourable conditions it may find.

It cannot be emphasised too strongly that scrupulous cleanliness should be observed from the beginning of the processing to the final packing of the goods. When adhesives or sizes, etc., are to be transported the barrels should be washed out with water containing weak alkali and 1 per cent. of formaldehyde, and then

thoroughly steamed with live steam. A pipe-line with side pipes can readily be fitted up in most factories so that a batch of barrels may be steamed at once, the steam being passed into the bung hole of the inverted and slightly tilted barrels. It is preferable to carry out the steaming immediately before use, so that the barrels have enough time to dry but not enough to become contaminated. If this cannot be arranged they should be stored, preferably in a dry place in an inverted position until required. Beechwood casks should be waxed after cleaning, for this assists greatly in reducing the possibility of mould spores gaining foothold.

The remarks on absolute cleanliness apply also to the condition of the vats or boilers, and apart from the danger of infection of batches, the factory in general should be kept clean in the interests of safety and efficiency of the workers.

In the preparation of sizes the temperature is generally between  $95^{\circ}$  and  $100^{\circ}$  C., and many of the spores of moulds, etc., introduced with the sizing material, or already present in the vat in the residue from previous batches, survive and remain viable, probably because the colloidal nature of the medium tends to protect them. During the sizing operations the size is passed from the vat in which it is prepared to the 'sow' or size box on the sizing machine, and losses are replaced every six or seven hours. The vat usually contains some residual size, which constitutes a good medium for mould growth, as its temperature falls below  $40^{\circ}$  C. in the interval between making two batches.

The flora remaining viable in the residue would be chiefly butyric-acid bacilli, which are capable of hydrolysing and souring the starch. Further contamination may be caused by air-borne spores. The latter also possess hydrolytic properties, and are thus capable of rendering the medium suitable for further attack by other microflora introduced with the flour or starch itself.

In the size box on the machine the temperature at which the size is maintained during the operations would check the development of the organisms present, but these would be transferred to the cloth with the size, and when the moisture-content of the cloth had risen on storage to 8-10 per cent., the *Aspergillus* and other fungi could readily develop.<sup>3</sup> Sometimes uncooked starch or flour is added to the size box, and this introduces another source of infection.

The fermentation of wheat flour to obtain a sizing material is mentioned elsewhere, but it may be mentioned here that when this process is carried out in the presence of an antiseptic, e.g. 8-10 per cent. zinc chloride, the process is termed 'steeping'.

Bean and Scarisbrick <sup>4</sup> consider this process preferable for avoiding loss of starch, but on this point there is still some controversy.

The fermenting paste is stirred from time to time in order to introduce air, which retards the growth of putrefying organisms. In the first stages of the fermentation, yeasts and bacteria increase enormously, and the sugar present is consumed with the formation of gas, but after about a week lactic-acid bacteria predominate, and most of the other organisms present are suppressed by the organic acids produced.

A sized cloth may harbour organisms derived from three sources: those applied with the size, those deposited from the air, and those already present in the cloth before processing, especially when grey goods are sized. The moulds present are chiefly of the *Aspergillus* and *Penicillium* type, and certain *Fungi Imperfecti*.

Morris <sup>5</sup> has evaluated the suitability of various sizing material as nutrient media for *Aspergillus* species, taking a strong wheat flour, i.e. one with a high protein-content, arbitrarily as 100, and his results are reproduced below:—

Rice flour . . . . .	108	Soluble starch . . . . .	85
Cassava flour . . . . .	106	Maize starch . . . . .	82
Maize dextrin (acid process) . . . . .	100	Sago . . . . .	78
Maize dextrin (heat) . . . . .	98	Farina . . . . .	76
Potato dextrin (diastase) . . . . .	97	Cassava starch . . . . .	74
Potato dextrin (acid) . . . . .	90	Soft-wheat-flour fermented 10 weeks . . . . .	72
Wheat starch . . . . .	89		

Fungi other than those used by Morris might not give the above sequence, but generally speaking starches are more resistant to the propagation of moulds than are flours. This is also generally true of adhesives made from these two types of material. Adhesive pastes exposed to the atmosphere in a damp place for any appreciable time become covered with a layer of mould-growth, of which the *Aspergillus* and *Penicillium* genera and species of *Bac. mesentericus* preponderate. Below the layer of mould-growth is generally a region which soon shows signs of fermentation and acid-production. Pastes showing even a little mould-growth are generally rejected so as to eliminate possible contamination of other products which may be made in the factory, and also because of the unsightly appearance of a mouldy paste.

The common preserving agents used in the textile industry are zinc chloride or sulphate, barium chloride, sodium chloride, phenol, formaldehyde, cresylic acid, salicylic acid, and magnesium chloride.

Phenol and cresylic acid suffer from the drawback of possessing

a very well-defined and penetrating odour, which renders them objectionable for certain uses in the textile trade, or for adhesives to be used in the packing of foodstuffs. Salicylic acid has but a slight protective action, and is expensive. It also has the defect of forming a highly coloured compound in the presence of traces of iron salts, which may contaminate the batch by solution from the apparatus or machinery used. The unsightly appearance conferred on a paste by this coloration renders it quite unfit for sale.

Aluminium chloride has been used as a mould-preventive, but it is difficult to see any advantage it possesses; when used in quantities sufficient to prevent mould-growth, it can have a very pronounced tendering action on a textile material. Magnesium chloride has been used in textile dressings for at least three purposes: to assist in solubilising the starch, to weight the material, and by virtue of its hygroscopic properties to retain moisture, so that it imparts a soft 'handle' to the 'finish'. It also possesses some anti-mildew action. Its use has to be watched, however, when it is to be applied to textiles which are to be subsequently singed as, in this case, tendering of the fibre is very liable to occur.

Formaldehyde and hexamethylene-tetramine are good preserving agents, but the latter is somewhat too expensive for general use. In a large number of adhesives and pastes, where odour is not a drawback, the addition of  $\frac{1}{2}$  per cent. phenol and  $\frac{1}{4}$  per cent. formaldehyde to the 'mix' is to be recommended. Formaldehyde is valuable in that it can be used in acid or alkaline pastes, and appears to form some kind of loose compound with the starch (see p. 116) and with any protein matter present. It is therefore valuable for use in flour pastes containing a marked percentage of protein matter, i.e. those from strong flours. In flour pastes used as adhesives its employment tends to increase the strength of a joint, because salts are, if anything, harmful to joint strength, especially if they are unduly hygroscopic, and formaldehyde is free from this drawback.

Beta-naphthol is widely used and can safely be recommended as a component of 'difficult' mixtures; it also has the advantage of having a smell less marked than that of phenol. The addition of 0.25 per cent. usually suffices.

Boric acid and borax do not appear to be such good agents as one would expect, and adhesives containing them sometimes show quite a bad mould-growth, especially products made from flour or those containing some nitrogenous material, such as maize or wheat products. Generally, they have been added to the mix for quite a distinct purpose, apart from any possible antiseptic value (see pp. 275, 276, 285). Dimethylglycol, eucupinotoxin, furoic acid,

sodium furoate, furfural, hydrofuramide, trimethylene and propylene glycols, and thymol are also strongly protective against mould-growth, but with most of these the cost is prohibitive.

Oil of sassafras and terpineol are used by some firms, and here again several functions are combined. They are generally used where it is necessary to mask the odour of any particular 'mix,' such as the odour of formaldehyde and phenol which have been added purely as anti-mildew agents.

In the paper industry the principal operations in the mills, especially the drying operations, tend in the main to reduce the bacterial count and in the finished paper the starch present tends to reduce, rather than maintain, the number of micro-organisms present.<sup>16</sup>

Sodium ortho-phenyl phenate has been claimed to be a good protective agent, and E. C. Britton and L. E. Lindley<sup>1</sup> suggest the use of a polychlorophenol, e.g. 2-4-5 trichlorophenol, in the presence of alkali. An I.G. Farbenindustrie patent<sup>2</sup> covers the use of 1 pt. ortho-benzyl phenol in 2 pts. of glycerol diacetate, the mixture being added to the 'mix' to the extent of 0.1 per cent. to prevent either mould or bacterial growth. The last-mentioned antiseptic is claimed to be odourless, a claim that cannot be made for the other compounds listed. Morris<sup>7</sup> has also examined a number of compounds which, although efficient fungicides, possess undesirable properties militating against their use commercially. In his second paper<sup>7</sup> this worker concludes that the common antiseptics do not appear to be effective in the amounts usually employed. He tested 32 antiseptics with a range of common mould fungi and found that the most efficient of the new materials tested were thallium carbonate and *p*-nitrophenol, the use of the latter being restricted to acid media owing to the colour it gives in the presence of alkali.

Finally, mention must be made of a compound of proved worth in the textile industry marketed by Imperial Chemical Industries<sup>6</sup> under the name of Shirlan. It is a colourless, odourless, non-toxic, stable and very effective agent, and its use may confidently be recommended.

The following list shows the preservative agents in common use and the approximate amounts which are met with in commercial adhesives or dressings:—

Alum . . . . .	1-100 to 200	Phenol . . . . .	1-200 to 1000
Acetic acid . . . . .	1-1000 to 10,000	Salicylic acid . . . . .	1-1000
$\beta$ -naphthol . . . . .	1-250 to 1000	Shirlan . . . . .	1-1500
Boric acid . . . . .	1-200	Sodium phenate . . . . .	1-200 to 1000
Cresol . . . . .	1-1000	Sodium salicylate . . . . .	1-1000
Formaldehyde . . . . .	1-400 to 20,000	Zinc sulphate . . . . .	1-250
Paraformaldehyde . . . . .	1-400 to 20,000		

As previously mentioned, oil of sassafras and terpineol are used as masking agents for the odour of the adhesive or size or of one of the constituents. The addition of lemon-grass oil is sometimes made with the same object, and that of oil of cloves and oil of cinnamon imparts a freshness or a 'clean' smell, which may have some psychological effect, as these oils are often included in the formula for wallpaper adhesives.

To prevent butyric, acetic and lactic fermentations in starch solutions the use of soluble fluorides has been advocated,<sup>17-22</sup> an acid medium giving the maximum effect.<sup>21</sup> Effront considers that hydrofluoric acid and soluble fluorides do not affect amylase and that the apparently favourable effect exerted by fluorides on the action of this substance is due to the restraining action they exert on butyric and lactic ferments. The author and other workers, however, find that sodium fluoride does affect the activity of certain amylases (see p. 447). H. Cluss and H. Feber<sup>22</sup> consider aluminium chloride the most effective in this respect.

It may be of interest to mention here that Barton-Wright and Tomkins<sup>24</sup> conclude that the critical moisture-content of flour for fungoidal growth is 16 per cent., and they recommend a figure of 15 per cent. as being safe and that it should not be stored in an atmosphere having a relative humidity of 80 per cent. R.H. It may be noted that 14 per cent. moisture is the maximum content allowed by the Ministry of Food for Security Stock.

To sum up, the requirements to obtain a product which will keep well are : scrupulous cleanliness in every stage of manufacture and packing of the preparations ; the use of zinc salts, formaldehyde, phenol, or preferably  $\beta$ -naphthol ; or where the profits can stand the expense, the use of some of the more modern synthetic agents, such as Shirlan, which are now well-established on the market.

For the preservation of starch solution, to be used as an indicator in various titrations, mercuric iodide,<sup>8, 12, 23</sup> oil of cassia,<sup>9</sup> 1 per cent. benzene,<sup>10</sup> 2 per cent. benzoic acid,<sup>11</sup> traces of sodium hydroxide,<sup>13</sup> metallic mercury in the reagent bottle<sup>14</sup> and carbon disulphide<sup>15</sup> have all been suggested. For iodometric titrations 0.5 c.c. of 2 N HCl per 50 c.c. of starch solution is recommended by N. Kano,<sup>15</sup> but the author considers the addition of a little thymol to be the most effective and it has no effect on the titration.

#### REFERENCES

1. E. C. BRITTON and L. E. LINDLEY, U.S.P. 1,946,057.
2. I.G. FARBENIND., E.P. 396,737, 1932.

3. D. ARMSTEAD and S. C. HARLAND, *J. Text. Inst.*, 1923, **14**, T275.
4. P. BEAN and F. SCARISBRICK, 'The Chemistry and Practice of Sizing,' Hutton, Hartley & Co., Manchester, 1921.
5. L. E. MORRIS, *J. Text. Inst.*, 1926, **17**, T1 and T23; 1927, **18**, T99.
6. BRIT. COTTON IND. RES. ASS., R. G. FARGHER, L. D. GALLOWAY, and M. E. PROBERT, E.P. 323,579.
7. L. E. MORRIS, *J. Text. Inst.*, 1927, **18**, T99.
8. M. MUTNIANSKI, *Zeit. anal. Chem.*, 1897, **36**, 220.
9. F. MOERK, *Amer. J. Pharm.*, 1904, **76**, 476.
10. F. N. ALCOCK, *Pharm. J.*, 1907, **79**, 121.
11. D. HELD, *Archiv. f. Hyg.*, 1915, **84**, 289.
12. ANON, *Chemical Abstracts*, 1917, **11**, 2648.
13. POLLITZ, *Zeit. angew. Chem.*, 1917, **30**, I, 132.
14. A. JUNK, *Chem.-Ztg.*, 1919, **43**, 258.
15. N. KANO, *J. Chem. Soc. Japan*, 1921, **42**, No. 11.
16. A. FRIEDEN, *Paper Trade J.*, 1940, **110**, TAPPI, 233.
17. SOC. GEN. MALTOSE BRUXELLES, *Zeit. Spiritusind.*, 1889, **12**, 291.
18. J. EFFRONT, *Bull. soc. chim. Paris*, 1890, series 3, **4**, 337, 627.
19. — *ibid.*, 1891, série 3, **5**, semestre 1, 149, 734.
20. — *Bull. Soc. d'encouragement pour l'industrie nat.*, 1891, **90**, 642.
21. — *Moniteur scientifique du doctor Quesneville*, 1892, **39**, 81; via *J. Soc. chem. Ind.*, 1892, v. **11**, 931.
22. H. CLUSS and H. FEBER, *Zeit. Spiritusind.*, 1898, **21**, 2.
23. R. C. TERRY, *Chem. and Ind.*, 1941, 155.
24. BARTON-WRIGHT and TOMKINS, *Cereal Chem.*, 1940, **17**, 332.

## ADDITIONAL REFERENCES

- M. VAN HAUWAERT, *Natureset. Tijdschr.*, 1936, **18**, 187. (Iodoform greatly retards diastatic hydrolysis, benzenoid hydrocarbons do not.)
- J. DARTOIS, *T.I.B.A.*, 1939, **17**, 25. (Fungicides for textile industry.)
- J. BRUYNE, *Bull. Ass. anc. El. Inst. sup. Ferm.*, 1938, **39**, 203. (Disinfectants for use in fermentation industries compared.)
- H. STADLINGER, *Kunstdüng. Leim.*, 1932, **29**, 138; *Chem. Zentr.*, 1932, ii, 160. (Preservatives for adhesives discussed.)
- CORN PROD. REF. Co., E.P. 533,023, 22/8/1938. (Free chlorine used to destroy thermophylic bacteria.)

## PART IV

### THE EXAMINATION AND ANALYSIS OF STARCH AND ITS PRODUCTS

#### CHAPTER I

#### GENERAL EXAMINATION OF STARCHES

ACCURATE analysis of commercial grades of starches may be needed in the food, textile, or adhesive industries ; detection and determination of starch in various finished products may also be required. In practice, the examination of a commercial starch consists in determining the contents of moisture, protein, mineral matter, fibre, and fat, and calculating the amount of starch present by difference. This method is not adaptable to agronomical, horticultural, or botanical problems, which require the direct determination of the starch itself. Many methods are available, but their very number is evidence that each is of limited application, either because of insufficient accuracy or because of the difficulties encountered with various materials. The determination of starch often constitutes a separate problem with each type of material encountered, e.g. the extraneous matter in different plants may vary widely in nature and amount. The starch in such cases may be accompanied by cellulosic compounds, gums, pentosans, glucosides, or hydrolytic products of starch, all of which may give rise to products which will affect the result of analysis.

The starch manufacturer and the buyer whose raw material is starch includes everything that occurs in the starch granule as 'starch,' and is interested in the amount and physical condition of the 'starch' in the product. On the other hand, botanical and other workers consider starch to be that portion which has certain physical characteristics and yields glucose on hydrolysis. From this point of view, the theoretical yield of glucose may be calculated from the various formulæ for starch. The larger the number of glucose units in the molecular chain of starch, the nearer does the factor for converting glucose to starch approach 0.9. The work of Haworth,<sup>2</sup> Richardson, and Freudenberg<sup>1</sup> indicates that the chain is long, so that this factor is probably close to the truth.

Commercial starch or dextrin should be examined for appearance (including microscopical examination for the detection of



adulteration), moisture, mineral matter, protein, fat, and starch. In dextrins the degree of conversion, which is judged by solubility, stability, and iodine reaction, is determined along with the sugar-content.

There appears to be but little evidence as yet as to whether starches from different varieties have identical properties. Kavcic,<sup>63</sup> working with potato starch, has shown that some of the physico-chemical properties from four varieties were substantially the same. He determined phosphorus-content, ash-content, viscosities, iodine adsorption, gelatinisation temperatures, etc. Winton and Winton,<sup>64</sup> find that the starch-content of potatoes depended considerably on local environment, and S. Woodruff (see Section 1) has shown the influence of environment during growth on the properties of maize starch. The biochemical and anatomical properties of starch from different varieties of potatoes have been described by I. A. Veselovskii.<sup>65</sup>

**Appearance.**—Starches should have a good white colour, be lustrous, and, if required for some of the finer technical uses, free from specks or dirt, but inferior grades from this point of view are acceptable for some kinds of work. This also applies to dextrins, but the colour in this case will depend on the extent of conversion, the acid used, and the starch employed.

Dextrins made from maize or wheat starch are matt, whereas potato dextrins often appear lustrous except in the well-converted type of product. For starch and dextrins needed in first-class work, the absence of specks may be a criterion of cleanliness. A sheet of glass with a 10 cm. square marked on it is laid flat on the top of a layer of starch or dextrin spread out on paper, and the number of specks enclosed in the square determined. For potato starch the best prima grade should not contain more than 30 visible specks, medium prima 27-170, inferior prima 145-450, and starches not classed as prima 700-800.

Prima starch should be pure white, but for some purposes a faint tinge of blue or yellow is not a defect. It should not have a musty, sour, or putrid smell. The lustre of the highest grades is due to the large size of the particles, or sometimes to processing with hypochlorous acid.

**Microscopical Examination.**—Starches may be mounted in water, but dextrins, being soluble to varying degrees in this medium, should be mounted in a polyhydric alcohol such as glycerine. Microscopical examination of dextrins often gives a clue to the nature of the starch used. In one case the author found it of great value in clearing up a problem which would not

have yielded readily to other methods: A potato dextrin which had hitherto shown good 'working properties' exhibited a strong tendency to 'spin' or 'fibre' (see p. 256), and microscopical examination disclosed the presence of about 40 per cent. of tapioca dextrin. The price of tapioca dextrin at this time was considerably higher than that of potato dextrin, so that the addition was not made with the object of adulteration, but was a mistake on the part of the manufacturer, who should have admixed only a small percentage of tapioca starch before roasting to give it the characteristic properties of this grade.

According to E. Berliner<sup>52</sup> clove oil is a valuable mounting medium as it makes the fissures in diastatically corroded starch granules very prominent.

In examining mixtures of starches, two methods are generally employed: (1) differential staining of the components; or (2) differential swelling in various reagents.

*Staining Methods.*—Whereas Metachrom Red G. Agfa does not colour cereal starches,<sup>3</sup> it stains potato starch a bright golden yellow. The sample of starch to be examined in this way must be exactly neutral to litmus in reaction because, in the presence of acid, wheat starch is also stained. E. Unna<sup>4</sup> proposes a test, based on differential staining, to distinguish between potato, rye, and wheat starches. The suspected mixture is suspended in a 3 per cent. phenol solution for 24 hours and then a drop is transferred to a slide and allowed to dry. The slide is immersed in a solution of a mixture of Soluble Blue, orcein (a carmine-red crystalline powder obtained from lichens, species *Roccella* or *Lecanora*), and Eosin in aqueous alcohol for 10 minutes, and after washing is immersed for 15 minutes in a solution of Safranine. It is then removed, washed, and immersed in a 0.5 per cent. solution of potassium dichromate for 30 minutes and again washed, first with water then with alcohol, and finally with xylene; finally it is mounted in Canada balsam. In this manner the potato starch is stained dark red; wheat starch, pink; rye starch, yellow to brown; and the gluten, blue. Potato starch is also detected by its characteristic behaviour when treated with Löffler's solution of Methylene Blue and examined under polarised light.<sup>5</sup>

A. P. Schulz and G. S. Steinhoff<sup>6</sup> suggest a mixture of Methyl Orange, Fuchsin and Methyl Green for the identification of potato starch which is stained blue. Neutral Red colours this starch pink. They consider that Metachromate G. or Congo Red is the most satisfactory if the starch is in a paste form or if the grains are corroded. No sharply distinguishing colour test for starches

other than potato starch is available, but they can generally be identified by the above dyestuffs augmented by Safranine and Thionine. To detect potato starch in bread G. Schutz and L. Wein<sup>24</sup> use Thionine, which stains potato starch lilac, the rest of the potato substance reddish-violet or blue, but is without effect on wheat or rye. W. Neuwohner<sup>57</sup> and Klauss<sup>58</sup> both use iodine/potassium iodide solution for the detection of potato meal in wheat meal as the granules of potato starch stain with a greater intensity of colour than wheat starch granules. The examination of wheat starch by staining methods is more fully dealt with on page 378.

*Swelling Methods.*—W. H. Symons<sup>7</sup> suggested stirring the starch with alkalis at various concentrations and noting the number of grains swollen at each concentration as a means of distinguishing between the various starches. He measured the concentration of sodium hydroxide solution required to gelatinise the majority of granules and found concentrations between 0.5 and 1.5 per cent. to be most suitable: 0.1 gm. starch was stirred with 1 ml. of the caustic soda solution and examined under the microscope. The following percentage concentrations are those which just swell the majority of the granules of the starches named: potato, 0.7; oat, 0.8; tous les mois, wheat, and sago, 0.9; maize, rice, and cassava, 1.0. Later, K. Baumann<sup>8</sup> used the fact that maize starch is less readily swollen by 1.8 per cent. potassium hydroxide solution than wheat or rye starches, and that the latter are ruptured more readily than the wheat starch. After the gelatinisation has taken place the maize starch can be made more apparent by staining with iodine.<sup>9</sup> Rye starch<sup>10</sup> is also differentiated from other starches in that the granules gelatinise faster when suspended in a 9 per cent. solution of sodium salicylate. At the end of an hour the larger granules have swollen and show no cross with polarised light. J. A. Radley has found that 38 per cent. formaldehyde solution swells potato, tapioca, wheat and maize starches in that order, the swelling of the first two being very much more rapid than is the case with the last two.

Identification of the starch by microscopical examination is readily carried out and, owing to the characteristic shape of the granules, adulteration is readily detected. Adulteration of one starch with another is sometime made more difficult owing to similarities between the two starches; wheat starch adulterated with small quantities of rice starch is a case in point.

Destruction of the structure of the starch, e.g. by any of the methods already described, practically eliminates the use of

the microscope as a means of investigation. Fortunately, Giri and Bhargava<sup>11</sup> have found a method of narrowing down the possibilities of a sample being a member of a particular group. The method depends on the formation of characteristically coloured zones when starches are impregnated into an agar medium, hydrolysed with amylase, and then flooded with iodine solution: 0.8 gm. of the sample to be examined is added to 100 ml. of boiling water and, after boiling 1 minute, the liquid is filtered through muslin and then added to an equal volume of a 1 per cent. agar medium, which has been adjusted to pH 4.6 by the addition of 0.2 N acetate buffer. If salivary amylase, malt amylase, or takadiastase is to be used, the pH value is adjusted, before mixing, to 6.8 by means of N/15 phosphate buffer. The solution is then plated out into petri dishes, one drop of the amylase solution dropped into the centre of the plate and allowed to diffuse for 24 hours at room-temperature. The concentration of the amylase should be 1 in 5 for saliva and 0.1 per cent. in the case of takadiastase.

After 24 hours the surface of the solution is flooded with N/200 iodine solution, and a few minutes later the coloured zones may be examined. Various cereal starches and flour have characteristic zones, and the use of different amylase preparations gives a still further differentiation, as the diffusion zones developed by each type vary in width and intensity of colour with the source of the amylase. Wheat starch with salivary amylase gives a central colourless zone surrounded by a deep blue zone. Malt amylase, however, gives a central zone of deep green surrounded by a diffused violet zone, whereas takadiastase produces a central blue zone surrounded by an edging of violet. Maize, jowar, and bajri behave in a similar manner. Rice, ragi, and barley, however, give with malt amylase a deep blue instead of a green central zone.

A similar method for the examination of dextrans that have been treated with water would be of great value.

For examining untreated starches, a microscope having a  $\frac{1}{8}$ -in. objective, and fitted with a micrometer eyepiece, is desirable. Polarising and analysing Nicol prisms and a selenite or 'polarised' plate for examinations in polarised light are also highly desirable. For the latter purpose, the sample is mounted in dilute glycerol or Canada balsam. The field should be well filled but not overcrowded. The size and shape of the granules should be observed, and the position of the hilum and the concentric rings round it should be noted, if they are present. The appearance of granules in polarised light also gives valuable information, the granules of

some starches showing a dark cross or V, which disappears if the granule is fractured by grinding or heating in water, although on moistening a dry granule the markings become much more distinct, due to an increase in internal pressure brought about by the swelling of the interior, but not the exterior, of the granule.<sup>12</sup>

Lack of contrast is a drawback to microscopical work and the loss of refractiveness of starch on hydration reduces the contrast between the object and the field. This constitutes, perhaps, the greatest difficulty met with when trying to photograph a field under examination. Resort to staining is sometimes made, but it is not as a rule advisable, because it often leads to the formation of artifacts.

Generally, the size of the granule is expressed as the length of its longest axis in microns ( $\mu = 1/1000$  mm.), and the size of the largest and smallest granules should be noted as well as the average size of the mass of the particles.

The position of the hilum varies in size, shape, and position for various starches, and so aids identification when examining mixtures. When dried to a low moisture-content, the hilum often appears as a star-shaped crack, the type of crack varying with the type of starch. The striations, if present in a granule, are made more pronounced if the starch is treated with dilute chromic acid solution and examined with oblique illumination.

**Potato Starch.**—The granules of this starch vary greatly in size; the largest are often egg-shaped, and the majority are flattened ellipsoids, but the smallest may be perfectly spherical. The granules generally occur singly, although compound granules containing two or three units are seen on rare occasions. The size varies from  $15\mu$  to  $100\mu$ , but O. Hoyer,<sup>56</sup> who has examined samples from many sources, considers the upper limit to be  $121\mu$ , and in the trade the starch with the highest average granule size is considered the best grade for some purposes.

O. Saare has measured the average length and breadth of granules of various grades of potato starch and has found the following values: best quality  $35.5\mu$ , superior prima  $32.8\mu$ , second product prima  $21\mu$ , secunda  $16.9\mu$ , tertia  $12.5\mu$ .

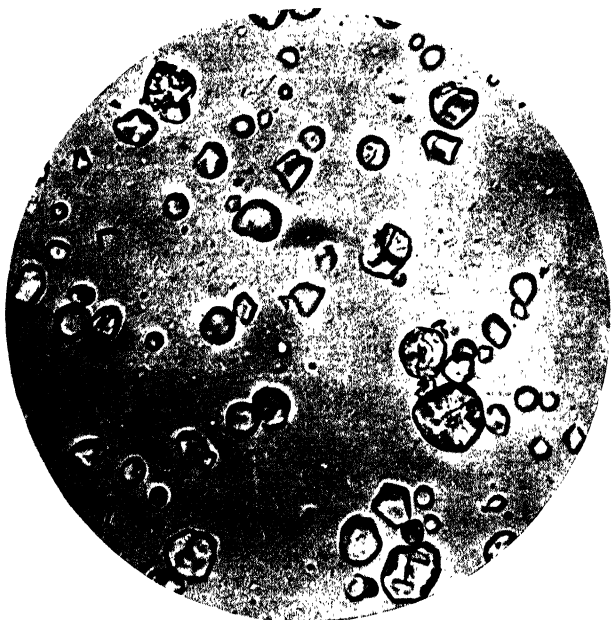
The cross seen with polarised light is well-defined but irregular in shape, and is centred in the hilum, which may be seen as a black dot or sometimes as a small split, and eccentrically situated near the smaller end of the granule. On rotating the analyser through an angle of  $90^\circ$ , so that the 'field' is light, the grains appear dark and the cross appears light. The cross appears in all grains, the very smallest showing it quite distinctly.

When gelatinised the granule opens up partially along the



Photomicrograph No. 13.

No. 13. Yellow potato dextrin swelling in aqueous glycerine. Note the method of dissolution (see pp. 30 and 377).  
No. 14. Thin-boiling maize starch made by oxidation. Granules easily crushed. Compare with acid-treated wheat starch, No. 4.



Photomicrograph No. 14.

[Facing p. 376.]



median line, the parts of the granule on both sides of the opening elongating as gelatinisation proceeds to give as a final result a ring of more or less irregular shape which shows no cross in polarised light. O. A. Sjoström<sup>13</sup> has noted that the hydration develops peculiar units of rounded form, arranged in rather an irregular manner, and often showing individual striations which are usually obliterated with increased swelling at higher temperatures.

The granules of thin-boiling starches made from potato starch tend to swell more in relation to the original size than do those made from tapioca or maize starches. The phenomenon shown by the unmodified granules can still be observed in the thin-boiling type. With potato dextrin mounted in water and glycerol the peeling off of layers can be observed (see Photomicrograph No. 13), and the difference between the mode of disintegration of potato dextrin and thin-boiling potato starch is greater than with cereal starches.

According to V. Vilikovski<sup>55</sup> very dry seasons diminish the size of the granules formed in the potatoes, and to obtain large granules potash fertilisers should be used.

**Wheat Starch.**—Wheat granules are thin and lenticular in form; perfectly circular grains are much more rare than in rye starch, and do not show any distinct striations. The hilum is only observed in a small number of grains, and appears as a dot situated eccentrically. Different varieties of wheat yield starch in which the limits of size of granules vary to a marked extent. The large granules may vary from  $25\mu$  to  $35\mu$  with a centric hilum, whereas the smallest granules are from  $2\mu$  to  $8\mu$ , and show no hilum. The upper limit for size is, according to O. Hoyer,<sup>56</sup>  $55\mu$ .

When heated in water the granules swell, and near the boiling-point they assume a peculiar and characteristic curved shape, which has been used by Sjoström<sup>13</sup> to identify wheat starch in several technical products where identification by other means would have been impossible. Rye starch shows a similar behaviour.

Brucknor and Thomas<sup>26</sup> detect maize starch in wheat flour by observing under the microscope the action of 0.16 N potassium hydroxide on the flour, the maize starch being unaffected. They have also elaborated a biuret test to distinguish between the two starches. E. Vogt<sup>43</sup> uses a stain of Congo red in indian ink to detect adulterants of wheat flour, whilst T. E. Wallis<sup>44</sup> detects wheat in barley starch by noting if any granules have a diameter greater than  $40\mu$ , as some wheat starch granules exceed this size. N. I. Ozolin<sup>49</sup> gives a colorimetric method for detecting corn flour in admixture with wheat flour, whilst the detection of rye flour in the latter is dealt with by C. Schweizer.<sup>50</sup> The turbidity



produced when an aqueous extract of flour is treated with ferric chloride and kept for 2 min. in a water-bath is used by H. Kühl<sup>51</sup> to detect rye in wheat flour. J. König and F. Bartschat<sup>68</sup> have dealt with the estimation of rye in wheat flour, and R. Strohecker<sup>69</sup> has described the identification of these two flours by means of their aqueous extracts.

**Wheat Flour.**—It is very important with certain wheats (see p. 305) that mechanical damage to the starch be detected. The type of damage which is found associated with an increase in maltose figure (see p. 309), due to one form of rolling as opposed to another, is not obvious, but such damage is nevertheless highly characteristic.

Damaged granules do not appear cracked but have a curious flat appearance and a thin faint outline. Owing to their attenuated appearance C. R. Jones<sup>66</sup> has termed them 'ghosts,' and they are more easily detected in flour by suitable differential staining. Aqueous Congo red solution (0.1-0.35 per cent.) does not stain sound starch, whilst the 'ghosts' are all stained uniformly an orange-pink, the gluten is coloured a bright brown. Care is sometimes required (especially with low-grade flours) to avoid confusion arising from the presence of endosperm cell-wall tissues which stain a vivid pink. With large particles the outer margin of the gluten is heavily stained but the interior escapes.

Staining by irrigation is not to be preferred, as it can lead to uneven distribution of the stain over the field and tends to derangement of the field under observation. It is thus preferable to make up the preparation from the start with the stain solution. A few important features of this staining are that it is uniform, the starch granules either staining alike or not at all. Every stained granule is uniformly stained and has the same tint. Very occasionally a granule is seen which is sound on one side but swollen to a 'ghost' on the other, rather like an oyster shell. One side has the 'solid,' strong outline of sound starch, whilst the other has the usual faint enlarged 'ghost-like' outline. In such a case the 'ghost' portion stains uniformly and the other remains unstained. Except in these rare cases the 'ghosts' are uniformly stained around the circumference. The zone of stained material in any one 'ghost' increases in width uniformly from the circumference inwards as the colour is taken up evenly until, at last, the unstained point at the centre disappears, leaving the 'ghost' uniformly tinted. Congo red stains thus very rapidly and is not easily followed. It is slower and more easily followed if dilute iodine solution is used. An iodine/potassium iodide

solution of more than a certain concentration stains starch, sound and damaged alike, a deep purplish-blue. If 0.02-0.03 per cent. iodine in 0.07 per cent. potassium iodide is used the 'ghosts' stain intensely blue but the sound granules never stain more than a faint purple. The staining process is reversible and the colour, due either to iodine or Congo red, can be removed by irrigation with pure water, the colour disappearing from the circumference first and the centre last. This process of staining and removal can be repeated many times on the same granules, but after several repetitions the intensity of staining of the 'ghosts' falls.

The microscopic appearance of the 'ghosts' suggests that they are formed by a peripheral cleavage between upper and lower dish-shaped skins of the initially sound lenticular granules. Once the peripheral cleavage is brought about the stain enters uniformly from all peripheral points and colours the inner starch but not the skin. The repeated staining and irrigation leading to a weakening of the intensity of coloration suggests that the inner starch slowly dissolves away.

Another form of damage which may be observed in starch granules is shown by the presence of fissures in the granules. It should be pointed out that the 'ghost' form is specifically the result of flour-milling processes generally. The cracked granules behave as sound granules when treated with the above stains, but C. R. Jones<sup>66</sup> considers that on long standing in contact with the stain they do eventually take up the stain and become uniformly coloured.

It will be seen later that on germination of the grain, fissures, or 'pits,' appear in the granules (see p. 440), but even when this 'pitting' has reached an advanced stage the granules do not stain with Congo red. This suggests that in ordinary damage of wheat starch it cannot be a question of removal of an outer resistant envelope as suggested by Pulkki (see pp. 46, 309). The weak iodine solution, mentioned above, will stain them to a deep purplish colour, not the pure blue shown by the 'ghosts'. Granules heated in water to just above the gelatinisation point appear, stain and behave similarly to 'ghosts'. Making up a suspension of flour containing 'ghosts' with a highly diastatic malt extract solution containing Congo red the 'ghosts' practically disappear in ten minutes at room temperature.<sup>66</sup>

**Rye Starch.**—Excepting that they are larger and thicker, the granules of this starch appear similar to those of wheat, the majority being spheroidal in shape, and among the smaller granules bell-shaped ones are sometimes seen. The granules

show very fine striations, radial from the hilum as well as concentric, and the size may go up to  $50\mu$ , although the average diameter is of the order of  $40\mu$ , 6.5-9.0 per cent. having a diameter greater than this. J. G. A. Griffiths finds that 5-11 per cent. of the granules of diameter greater than  $10\mu$  had stellate hila.<sup>26</sup> In a few granules the hilum may be composed of three fissures instead of four.

With gelatinised, thin-boiling rye starch, indications of a micellar structure can be seen if an immersion lens is used.<sup>13</sup>

Rye flour is detected in wheat flour by the precipitation of characteristic threads from the flour extract when acetone is added, and bean flour coagulates ferric oxide sols in a similar manner to aleurone cells.<sup>52</sup>

**Barley Starch.**—The granules of barley starch may be bulb-shaped, elliptical, or circular in outline. The large granules vary in diameter from  $20\mu$  to  $35\mu$ , and the smallest granules from about  $2\mu$  to  $6\mu$ . The hilum is absent, but in the largest grains some show striæ, but the hilum is observable even less often than in wheat starch. Barley has a large number of very small granules, whereas wheat contains comparatively few. The black cross in polarised light is generally very indistinct.

**Maize Starch.**—Maize-starch granules vary in size from  $10\mu$  to  $25\mu$ , and are usually polygonal, although many rounded granules from the interior of the endosperm are found. If the corn is of a soft and mealy variety the starch obtained from it contains a preponderance of rounded granules having an average diameter of  $13\mu$ - $15\mu$ . Pickens and Englis<sup>71</sup> find that the granules of hard corn starches are smaller and less rounded than those from the soft corn. The hilum is always strongly marked, and is starred with fissures, but no striæ can be observed. The polarisation crosses are distinct.

In thin-boiling maize starch, the structure is not greatly altered. With 50-fluidity granules the characteristics of an untreated pasted granule are largely retained, but the granule is smaller, and cracks in the outer layer are more pronounced, becoming very pronounced in a 75-fluidity starch. In a 90-fluidity starch the radial cracks are deeper and disintegration begins to take place along concentric lines of cleavage.<sup>13</sup> The increase in volume of these granules is much less marked than in the other grades. Using an oil-immersion lens of high magnification, Sjostrom<sup>18</sup> was able to distinguish concentric rings of micellæ of about  $0.3\mu$  in diameter when a chlorinated starch was examined. The photograph by Radley and Young of untreated maize starch, however, shows the concentric rings quite clearly in several granules. Maize may

be detected in wheat flour by its swelling in paraldehyde<sup>52</sup> or by its resistance to staining by mucicarmine.

**Cassava Starch.**—The granules of cassava or tapioca starch are similar to maize starch in average size, but variations are encountered, depending on the origin of the starch. They are round, with a flat surface on one side that contains a conical pit extending to the excentric hilum, which is well defined; some granules are practically circular. In polarised light a well-defined cross is observed. The smallest granules range from  $5\mu$  to  $15\mu$ , whilst the largest measure from  $25\mu$  to  $35\mu$  with intermediate granules of  $15\mu$  to  $25\mu$ . Apart from the truncated shape, this starch has very few characteristic features. J. A. Radley has noted the peeling off of layers from tapioca dextrin similar to that shown by potato dextrin. These layers are less in number than in the case of potato dextrin, and not so easily observed. They served, however, to distinguish between a dextrin and a soluble starch. A 60/40 ethylene glycol/water mixture forms a good medium for this type of work.

F. Robertson Dodd<sup>61</sup> found some pods of linseed grown in a wet summer contained starch granules which were indistinguishable from those of tapioca. Generally the starch-content of mature linseed is negligible, and when starch is found it is usually taken as evidence of adulteration. This worker<sup>62</sup> has also called attention to the similarity of the little-known starch—that from unripe tomatoes—to immature linseed and to sweet-potato starch.

**Rice Starch.**—The granules of rice starch have the smallest size of the ordinary starches, the diameter varying from  $3\mu$  to  $5\mu$ . They resemble oat starch somewhat, but are uniformly smaller, and they are definitely polygonal. The centric hilum is difficult to see, and striæ are visible only after treatment with dilute chromic acid. Compound grains composed of several granules are sometimes observed, the whole having an angular outline. They do not appear to show any cross in polarised light.

M. Wagenaar<sup>70</sup> detects particles of rice in wheat, rye, barley, oat and buckwheat flours by staining with Fuchsin S. This acid dyestuff is adsorbed by the protein granules of the rice, and the peculiar distribution of these stained granules in the rice grain gives a characteristic appearance to the particles of rice flour.

**Pea Starch.**—This starch has special features which are characteristic of the leguminous starches. They are translucent, rounded in outline and irregularly reniform in shape, whilst some are more or less elliptical. Both ends of the granule are similar, unlike the egg-shaped granules of some potato granules.

The granules are strictly uniform in size, and the hilum is a dark slit lying in a shallow depression running along the long axis. Unless fractured, the granules never show a stellate hilum, and where the hilum is absent the depression can still be seen. Concentric striæ are visible, and with polarised light a dark V is seen at each end of the granule, the apex of each touching the end of the hilum. According to Berliner<sup>52</sup> the cleft hilum is not preformed but occurs only in water or other starch-swelling media as a consequence of the sudden expansion of the granules.

It is most valuable to compare samples under examination with those of known origin, much more being learnt in this way and by practice than from any amount of description. A most complete set of photomicrographs of starches has been published by Reichart,<sup>36</sup> some 306 starches in ordinary and polarised light being shown, and the reader is referred to this work if unable to obtain genuine samples of starch for examination. In addition, the reader is referred to the photomicrographs of modified and pasted starch published by Sjostrom<sup>13</sup> and S. Woodruff.<sup>37</sup>

The average buyer or user of starch is generally assured of the authenticity of his materials. He is then interested in the value, which is obtained by determining the moisture and other extraneous matter present, the latter often giving information on the manufacturing history of the sample.

**Moisture.**—The moisture-content of a starch is of importance, as the buyer does not want to pay for more water than is normally present in air-dry starch. In air-dry wheat starch the moisture-content is usually around 13 per cent., whilst that of potato is between 18 and 22 per cent. Should a greater percentage be present in the latter, the price should be adjusted so that moisture in excess of 22 per cent. is not paid for. When determining moisture by finding the loss on heating, it must be borne in mind that most starches gel between 57° and 67° C., and thus it is advisable to keep the temperature at 40° C. for some hours until the greater proportion of the moisture has been driven off. The temperature may then be raised to 120° C. for 4-6 hours. The author has found it advantageous to add 5 ml. of absolute alcohol to every 10 gm. of starch taken, the time of drying thus being reduced.

Sprockhoff<sup>27</sup> gives the official German method in which 5 gm. of starch are heated for 1 hour at 50° C., and then for 3 hours at 120° C. He also describes a method requiring only 1 hour, claimed to be accurate to 0.2-0.4 per cent., in which 10 gm. are plated in a shallow dish that is suspended on a wire through the top of an oven and attached to one arm of a balance. After

heating for 15 minutes at 90-100° C., the temperature is raised to 140-150° C. for 25-30 minutes, and the loss of weight determined.

Saare<sup>14</sup> gives a rapid method for estimating moisture in potato starch which is claimed to be accurate to 0.5 per cent. : 100 gm. of starch in a graduated flask are made into a suspension with distilled water, the volume made up to 250 ml. at 17.5° C., and then weighed. If S represents the weight of the starch plus the water in the flask then the water-content of the starch is given by the formula : per cent. water =  $\frac{289.4 - S}{0.394}$ . The weighing should be done as accurately as possible, as a difference of 0.1 in S represents a final difference of 0.25 per cent. water.

J. A. Radley has made use of the hygroscopic nature of absolute ethyl alcohol to determine the moisture-content of potato starch by finding the specific gravity of the supernatant liquid obtained by shaking up known weights of alcohol and starch ; the results were in close agreement with those obtained by other methods. It is possible that the refractometer could be used with advantage in this method.<sup>15</sup>

T. H. Fairbrother and R. J. Wood<sup>28</sup> find that distillation with tetrachlorethane or carbon tetrachloride allows the moisture in flour to be estimated in 20 minutes with an accuracy of ± 0.5 per cent. They also confirm the claims to accuracy and speed of the Burton and Pitt method,<sup>29</sup> in which the moisture present is determined from its effect on the dielectric constant of the material under examination. Although their results were obtained with wheat flour these methods should be applicable to the determination of moisture in starches and dextrins.

It seems possible that more use might be made of the azeotropic mixtures which various solvents form with water for the determination of the latter. The few selected solvents below show properties which are suggestive for this work.

TABLE 8

<i>Added Liquid A.</i>	<i>B. Pt. A.</i>	<i>B. Pt. of Azeotrope with Water.</i>	<i>Wt. per cent. of A.</i>
Benzene . . .	80.2° C.	69.25° C.	8.83
Cyclohexane . . .	80.75° C.	68.25° C.	8.33
Toluene . . .	110.7° C.	84.1° C.	19.6

The greatest amount of water in any azeotropic mixture is probably that formed by 1-octanol which contains 99.4 per cent. water by volume or 90 per cent. by weight.

Using a pyridine solution of methyl magnesium iodide, T. Zerewitinoff<sup>16</sup> has estimated the amount of methane liberated by the addition of starch, and finds close agreement between the values obtained on vacuum drying by this method. J. F. Hoffmann and J. H. Schulze<sup>17</sup> suspend the starch in a mixture of turpentine and toluene, distil off water and measure it. S. Maquenne<sup>18</sup> noted that drying at 120° C. for 1 hour, followed by 2 hours at 100° C. in a current of dried air, gave results which were lower than those obtained by the usual methods, the difference being as large as 1 per cent. of the weight of sample taken.

**Mineral Matter.**—The determination of mineral matter will often show whether a starch has been ‘chemicalled,’ as well as the inclusion of sand or dirt: 5 gm. are ignited in a dish in a muffle furnace until the ash is white or very light grey in colour. A high-grade potato starch will have an ash-content of about 0.2-0.4 per cent. on the dry starch, whilst 0.15 per cent. is generally found for wheat or maize starches, but the figures may rise as high as 0.5 per cent. if it has been ‘chemicalled’. Low quality tapiocas generally have a high ash-content of a brownish colour, whereas the ash of high-grade tapioca is less and is white in colour. The amount of ash, however, cannot be used as a guide to quality. H. Tryller<sup>19</sup> considers that the presence of calcium in the mineral matter may be ascribed to the washing water, but it must certainly be borne in mind that some factories use calcium bisulphite in the manufacture of the starch and there is a possibility of this being a contributory cause of the presence of calcium. With certain ‘prepared’ starches the chemicals mentioned in the various sections should be looked for, due regard being paid to the behaviour of the starch in use which will give an indication of substances likely to be present.

When determining phosphorus in starch the results are sometimes low when incineration is employed. G. Steinhoff<sup>67</sup> overcomes this by destroying the organic matter with concentrated nitric acid containing 5 per cent. potassium permanganate. This is later removed with hydrogen peroxide and the phosphorus precipitated as phosphomolybdate. This is dissolved in 1 per cent. ammonia, the excess of this being fixed with neutral formaldehyde, and then titrated with 0.1 N caustic against phenol phtalein.

**Fat.**—According to T. C. Taylor and J. M. Nelson,<sup>20</sup> a small amount of the fat contained in maize starch seems to exist in combination with the starch and is not removable by solvents (see p. 98); however, on hydrolysis it is set free and appears as palmitic acid, etc. Further work by Taylor and L. Lehrmann<sup>21</sup>

has shown that the combined fatty matter has approximately the following percentage composition: palmitic acid 24, oleic acid 40, linoleic acid 36. The above workers find that the percentage of combined fatty matter in maize starch is approximately 0.5, in rice starch 0.83, in sago starch 0.11, and in cassava starch 0.12.

For the determination of uncombined fatty matter the starch is dried, and a known weight, which should be as large as possible, is extracted with ether or petroleum ether in a Soxhlet apparatus.

**Determination of Nitrogen and Protein.**—The proteins in a given sample of starch may be calculated by carrying out a Kjeldahl nitrogen determination and multiplying the figure for nitrogen by 6.25.

The following represent typical results of the analyses of various starches :—

TABLE 9

<i>Component.</i>	<i>Wheat.</i>	<i>Maize.</i>	<i>Tapioca.</i>	<i>Potato.</i>
Moisture	11-15 per cent.	12-15 per cent.	9-18 per cent.	18-22 per cent.
Protein .	Less than 0.5 per cent. in good grades.	0.1-0.2 per cent. Inferior grade, 1.0 per cent.	0.2-1.0 per cent.	Nil.
Ash .	0.25 per cent.	0.1-0.2 per cent. in 'unchemic-alled'; 'chemic-alled' 0.3-0.5 per cent.	0.1-0.8 per cent.	0.2-0.3, not over 0.5 per cent.
Fat .	0.15 per cent.	0.5-0.75 per cent.	0.1-0.4 per cent.	Nil.

**Acidity.**—The acidity of starches is sometimes determined and a qualitative test is carried out by spreading a small heap of starch on a plate and moistening it with two or three drops of purified litmus solution.

For a quantitative estimation, Saare shakes 25 gm. of starch with 25-30 ml. of water and titrates with N/10 caustic soda solution against phenol phthalein. Under these conditions he classes as 'technically free' those requiring less than 3.75 ml. of N/10 alkali, whilst those requiring 5.0, 5.0-8.0, and over 8.0 ml. are classed as faintly acid, acid, and strongly acid, respectively. J. Mayrhofer has pointed out the advantages of electrometric



titration.<sup>30</sup> L. Pickens and D. T. Englis point out that  $pH$  value and titrable acidity are of little value in classifying starches.<sup>71</sup>

According to H. Tryller,<sup>22</sup> the acid reaction is due to several components, such as sulphur dioxide, and propionic acid in inferior starch, but is chiefly related to the amount of amylo-phosphoric acid present as hydrolysable salts (see p. 87). The results obtained by titration are often not a measure of the acidity of a sample, since a starch containing a small amount of a strong acid would be more acidic yet give a lower titration value than one containing a higher proportion of weaker acids. If it is necessary to neutralise the starch at some subsequent stage of processing the latter figure is important, but for determining the quality of the starch C. Schéele<sup>31</sup> considers the  $pH$  value of the starch is of most value, and gives a method of determining this. A complete picture would, of course, be obtained by determining the electrometric titration curve which would give all these figures in one diagram.

For starches to be used in foodstuffs, the amount of  $SO_2$  present should be determined, and should not exceed 100 parts per million as laid down in the Food and Drugs Acts.

**Gelling and Water-holding Power.**—The gelatinising power and the degree of tenacity with which a starch paste retains its moisture is a measure of its superiority, the lower grades going thin after a short time whilst good grades give a paste which retains its firmness for a very much longer period. This property may be measured in two ways; the first described below is due to Schreib, and the second is a method used in a number of factories.

*Schreib's Method.*—Four grams of starch are suspended in 50 ml. of cold water and heated to boiling with constant stirring. When the liquid begins to boil the flame is removed and the stirring continued. The starch should not be boiled for longer than one minute, and when cold the jelly should not flow when the container is inclined.

In the second method, which utilises the principle of capillary attraction, 16.6 per cent. starch pastes (dry weight basis) are made with the samples in dishes of the same diameter. When cold and set the paste is transferred to a filter paper, still keeping it in the moulded form, and put aside in a place free from draughts. All the samples should be made up under identical conditions of temperature, stirring, etc. The size of the water-rings formed on the papers is examined after 1, 12, and 24 hours; the sample showing the largest ring is graded as the worst starch and that with the smallest as the best.

Direct determinations of viscosity on starch pastes do not give very concordant results, as the method of preparation can play a big part.<sup>41</sup> Instead of gelatinising the starch in water, W. F. A. Ermen<sup>23</sup> swells 1-2.5 gm. of starch in 245 ml. water, containing 1.5 gm. caustic soda, makes up to 250 ml. when swelling begins, and after several hours determines the viscosity of the solution with a Redwood viscometer. The results clearly indicate differences in the manufacturing history of samples, and different starches are readily distinguished from one another. Some of the methods discussed in the chapter on Physical Properties, especially in the section dealing with the rigidity of starch pastes, could no doubt be adapted to factory control or routine examination of samples being purchased.

W. Ekhard<sup>32</sup> has described in full an apparatus for determining the adhesive power of starch pastes. He takes 9 gm. of air-dried potato starch, makes it into 200 gm. of mucilage with water of known temperature, and sinks a 22 mm. disc in the paste. After 24 hours the weight required to withdraw the disc from its fixed depth in the cold mucilage is determined. A similar method is used by O. Saare.<sup>42</sup> A. Bintz and T. Marx<sup>53</sup> find Saare's method applicable to rice but not to potato and wheat starches. H. Capenberg<sup>54</sup> compares the time to pull a sphere, under a pull of a known force, through a given thickness of paste. These methods give results which are seriously affected by very slight variations in the method of making the paste.<sup>33</sup> Sprockhoff<sup>34</sup> criticises these methods and adopts viscosity as a criterion of quality, using 0.4-1.0 per cent. solutions. Schulz and Parlow<sup>35</sup> also use the Sprockhoff viscometer to judge the quality of the starch and have examined starch from frozen potatoes and starch which has been frozen, to determine the effect of this treatment on the tenacity of the pastes made from the two starches. Reference, however, should be made to the chapter on Physical Properties, in which the rigidity of starch pastes is discussed.

**The Alkali-Labile Value.**—A technique which may prove of value in routine and factory examination of starch, modified starch and dextrin has been elaborated by T. C. Taylor and his co-workers.<sup>38-40</sup> It depends on the fact that these substances contain a portion termed 'alkali-labile' which is readily acted upon by alkali. Various pre-treatments of starch bring about changes in the amount of this alkali-labile portion, and Taylor's method, whilst giving empiric values, is precise, semi-micro and readily duplicated, providing the technique laid down is followed rigidly. The method detects changes which cannot be determined by viscosity tests, iodine reaction, specific rotation, or

initial reducing value determinations. Tests should be done in duplicate at least and are carried out as follows <sup>40</sup> :—

50.0 mg.  $\mp$  0.1 mg. of starch are weighed into a pyrex test tube, 8"  $\times$  1", and 10 ml. of 0.1 N NaOH added. The tube, loosely stoppered, is floated on a boiling water-bath for 1 hour, after which it is cooled for 30 seconds under running cold water, 10 ml. of 0.1 N HCl being added immediately it is removed from the cold water and the contents of the tube well mixed by thorough shaking. The mixture is transferred to a 250 ml. Erlenmeyer flask, the tube being washed twice with 10 ml. distilled water and the washings added to the flask.

Two drops of nitrazine yellow solution are added and the liquid is neutralised with 0.1 N NaOH. Five ml. of the alkali are then added, followed immediately by 5.0 ml. of 0.025 M standard iodine solution; the last three operations should be carried out within 3 minutes. The flask is kept at 25-30° C. for 45  $\mp$  1 minute in the dark, and then 5.0 ml. of conc. HCl added, mixed well by shaking and immediately titrated with 0.025 M thiosulphate solution. If the back titration is less than 3 ml. the resulting alkali-labile value will be low and 7 ml. instead of 5.0 ml. of iodine solution should be added for the oxidation of a second sample, so that a reading of at least 3 ml. is obtained in the back titration with thiosulphate. One hundred times the number of milligrams of iodine consumed divided by the weight of the sample in mgs. gives the alkali-labile value.

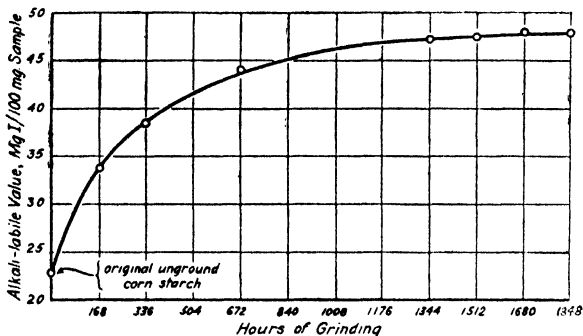
Pastes may be precipitated with acetone-free dry methyl alcohol after which the alkali-labile value of the carbohydrate material may be determined. If the paste contains no electrolyte the addition of a few drops of 0.1 N BaCl<sub>2</sub> greatly assists the precipitation; free chlorine, hypochlorous acid, peroxides, borax or sulphur dioxide should be absent, as these absorb iodine. Fig. 28 shows the connection between the alkali-labile value and time of grinding a starch.

If the long chains of glucose units forming the starch molecule are parallel and co-ordinate links exist between the H of the OH groups of some chains and the O bridges of others as has been suggested (see Chap. 8, Part I), the free aldehyde groups at the end of the chain, although primarily chemically free, might be protected by a dovetail-like fitting end to end of the bundles of parallel-bound chains. Disassociation of the co-ordinately-linked chains from one another would make the aldehyde groups available whilst hydrolytic scission of glucosidic linkages would give shorter chains and consequently new aldehydic groups. Taylor and Keresztesy <sup>40</sup> think the great increase in alkali-labile value in

making soluble starch by dry-grinding (see Fig. 65), or by the Lintner acid-process, is due to the terminal aldehyde exposed by disassociation of the chains, whereas the slower hydrolytic breakdown of the glucosidic links is responsible for the fairly steady rate of fall ultimately attained.

A good grade, commercial air-dry maize starch gives an alkali-labile value of about 22, tapioca starch 14, a thin-boiling starch 60, and a yellow dextrin 20. The method should be of value in examining oxidised and acid-treated soluble starches.

M. Samec and B. Škerl<sup>48</sup> have studied the dependence of alkali-labile value on the length of boiling and temperature to differentiate between erythro- and amylo-substances. Native and



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FIG. 65.

partially attacked starch gave quite different results. The fractionation methods previously employed by these workers, e.g. pressure cooking, electro-dialysis, coagulation by freezing or by ageing, caused no structural changes as judged by alkali-labile value. They consider that a large number of starches can be characterised by this value.

T. J. Schoch and C. C. Jensen<sup>60</sup> have devised a simplified alkalimetric method to estimate the relative hydrolytic degradation of starch. They consider that Taylor's alkali-labile value is liable to variations at the hands of different workers, especially where the starch has been precipitated by solvents, as the latter are tenaciously retained even on prolonged drying.

Schoch and Jensen digest 0.5 gm. of starch (calculated on a dry basis) with sodium hydroxide. The starch, which should pass a 60-mesh sieve, is introduced into an eight-ounce flask and shaken with 10 ml. of water until suspended, 25 ml. of 0.4 N sodium are added, taking care to agitate the contents of the flask

so as to obtain uniform gelatinisation of the starch, followed by 65 ml. of hot distilled water. The flask is closed with a bung carrying a bunsen valve and immediately placed in a vigorously boiling water-bath. After heating for exactly 60 minutes the flask is placed in cold water and 50 to 75 ml. of cold distilled water are added to the contents of the flask to halt the decomposition. The liquid is then titrated against thymol blue to a yellow end-point with 0.2 N sulphuric acid. With highly coloured compounds the titration is carried out to a pH value of 8, using a glass electrode. The alkali number is calculated as (ml. of acid to titrate blank — ml. of acid to titrate sample)  $\times$  normality of acid  $\times 10 \div$  weight of sample on dry basis.

Close adherence to the above procedure is recommended. Should the starch product contain added acid or alkali, sufficient to affect the alkali number, 1 gm. of the product is gelatinised and titrated with alkali or acid against thymol blue, correcting the alkali number for this titre. With cold-swelling products the sample should be introduced into a perfectly dry digestion flask and wetted with 1.2 ml. of benzene; 25 ml. of 0.4 N sodium hydroxide solution are then added followed by 75 ml. of hot water. In this way complete dispersion without the formation of lumps is obtained.

Commercial corn and wheat starches have consistently higher alkali numbers than those of the common tuber starches. Schoch and Jensen find the following values: tapioca, 5.9-6.9; potato, 5.7-6.9; rice, 6.7-7.5; wheat, 9.7-11.5; maize, 9.8-12.1; maize (specially prepared by non-aqueous steeping), 10.6, 11.4.

By leaching at temperatures just below the gelatinising point raw corn starch gave a minor fraction of soluble carbohydrate possessing a high alkali number (35.9). The insoluble residue had an alkali number, 8.8, and the raw starch, 11.2.

By fractionation with cold water a typical acid modified thin-boiling starch was found to be heterogeneous in character. The initial thin-boiling starch gave a value of 44.1, the cold water soluble fraction, 50.6, and the insoluble residue 36.9 for the alkali number.

With increasing degree of acid conversion the alkali number rises progressively in thin-boiling starches, e.g. 20-fluidity, 14.5; 40-fluidity, 15.0; 60-fluidity, 15.7; 75-fluidity, 20.7; 90-fluidity, 41.5; white dextrin, 25 per cent. cold water soluble, 56.3; white dextrin, 65 per cent. cold water soluble, 62.6; Lintner's soluble starch, 66.4.

British gums and yellow dextrans show alkali numbers only a little higher than that of raw corn starch, e.g. British gum,

17.5 per cent. soluble, 16.5; British gum, 80 per cent. soluble, 15.6; gum, 85 per cent. soluble, 16.3; dextrin, acetic acid conversion, 98 per cent. soluble, 16.4; yellow dextrin, nitric acid conversion, 85 per cent. soluble, 19.9; yellow dextrin, hydrochloric acid conversion, 97 per cent. soluble, 23.0. The alkali numbers bear no relation to viscosity or solubility. With oxidised starches the alkali number is generally lower than that of the raw corn starch. Alkali lability, however determined, must not be construed as a quantitative evaluation of aldehyde content, but merely as an empirical index of hydrolysis.

**The Examination of Modified Starches.**—With these products it is generally desired to characterise them either for use or for matching purposes. Oxidised starches are invariably whiter in colour than acid-treated starches, and a microscopical examination will at once determine the raw material used. In use the most important property is the viscosity, but in endeavouring to characterise a starch for matching purposes a number of drawbacks are encountered in viscometric determinations. Some soluble starches made by acid treatment are not washed free from acid or the acid is incompletely neutralised, and this leads to a rapid fall in the initial viscosity on heating the solution. Solutions of soluble starch are also sensitive to the presence of impurities and electrolytes and to mechanical treatment. Highly modified starch swells before dissolving and must be heated to complete the dispersion, and such heating brings about further modification.

In spite of these drawbacks, viscometric methods are employed for the examination of these products, together with the reducing value by the method of W. A. Richardson, Higginbotham and Farrow.<sup>45</sup> With potato, maize, and sago starches which have been modified by hot acid treatment followed by neutralisation, these workers find that the degree of modification is accurately measured by the reducing value (R) which does not, however, indicate the degree of modification of oxidised starches or those treated in the granular state with acids. Starches in these two classes are washed free from reagent after modification, and such washing removes the water-soluble reducing substances.

Interesting results in the examination of soluble starches have been obtained by Richardson,<sup>46</sup> using the following technique. A suspension of starch in cold water is poured into sufficient boiling water to produce a 2.5 per cent. solution which is heated for 1 minute and then cooled. After passing through a homogeniser the concentration is determined by the dichromate method.<sup>45</sup> To 10 ml. of this solution is added 15 ml. of a 50 per cent. calcium thiocyanate solution, a control solution of 30 gm.

of this salt in 100 ml. of water also being prepared. Ten millilitres of each solution are used in separate U-tube viscometers (cf. Higginbotham and Richardson <sup>47</sup>), and  $n$ , the viscosity of the starch solution relative to the control, is determined at 25° C.  $\mp$  5° C., and if  $c$  is the concentration of the starch in gm. 100/ml., the 'thiocyanate viscosity' (TV) is given by the quantity  $(\log n)/c$ . Where  $c$  is between 0.6 and 1.0 per cent. TV is independent of  $c$ , and so long as  $c$  is known accurately it is unnecessary to adjust it to a given value. TV runs parallel to R for acid modified neutralised starches, and, like this value, may be used as a satisfactory index of the degree of modification. TV is preferable to R as the modification index for oxidised and acid-treated, washed starches (*vide supra*).

With unmodified or lightly modified starches TV is very sensitive to mechanical treatment, but this effect diminishes with increase in the degree of modification until, when R exceeds 40, homogenisation is superfluous. With oxidised starches, however, R may be lower than for an acid-treated, neutralised starch of the same degree of modification and may not, therefore, need homogenisation even when R is less than 40.

M. I. Knyaginichev <sup>59</sup> uses 30 or 50 per cent. sodium salicylate solutions containing 0.2 gm. of starch per 100 ml. for the examination of starches. He finds the viscosities for the different starches and modified products are unaffected by time and often differ more than the corresponding aqueous solutions. Incidentally, this worker finds that the larger granules give more viscous pastes than do the smaller granules.

By the judicious use of the microscope and the determination of the reducing value R, the thiocyanate viscosity and the alkali-labile value the previous history and degree of modification of a sample of soluble starch may be determined sufficiently accurately to enable it to be matched for factory production.

#### REFERENCES

1. K. FREUDENBERG, *J. Soc. Chim. Ind.*, 1931, **50**, 288T.
2. H. N. HAWORTH, *ibid.*, 1934, **53**, 1059.
3. G. BLUNCK, *Zeit. Nahr. Genussm.*, 1915, **29**, 246.
4. E. UNNA, *ibid.*, 1918, **36**, 49.
5. F. BENGEL, *ibid.*, 1915, **29**, 247.
6. A. P. SCHULZ and G. S. STEINHOFF, *Zeit. Spiritusind.*, 1932, **55**, 162.
7. W. H. SYMONS, *Pharm. J.*, 1882, **13**, 237.
8. K. BAUMANN, *Zeit. Nahr. Genussm.*, 1899, **2**, 27.
9. G. EMBREY, *Analyst.*, 1900, **25**, 315.
10. W. LENZ, *Zeit. öffentl. Chem.*, 1910, **15**, 224.

11. K. V. GIRI and P. N. BHARGAVA, *J. Indian Inst. Sci.*, 1936, **19A**, 53 ;  
K. V. GIRI, *Science*, 1935, **81**, 343.
12. W. HARRISON, *J. Soc. Dyers and Col.*, 1916, **32**, 40.
13. O. A. SJOSTROM, *Ind. Eng. Chem.*, 1936, **28**, 72.
14. O. SAARE, *Zeit. Spiritusind.*, 1884, **7**, 550.
15. H. S. MCTAGART, *Can. Chem. Met.*, 1934, **18**, 8.
16. T. ZEREWITINOFF, *Zeit. anal. Chem.*, 1911, **50**, 680.
17. J. F. HOFFMANN and J. H. SCHULZE, *Woch. Brauerei*, 1903, **20**, 217.
18. S. MAQUENNE, *Compt. rend.*, 1905, **141**, 609.
19. H. TRYLLER, *Chem. Zeit.*, 1920, **44**, 833.
20. T. C. TAYLOR and J. M. NELSON, *J. Amer. Chem. Soc.*, 1920, **42**,  
1726.
21. T. C. TAYLOR and L. LEHRMANN, *ibid.*, 1926, **48**, 1739.
22. H. TRYLLER, *Zeit. Spiritusind.*, 1934, **57**, 19.
23. W. F. A. ERMEN, *J. Soc. Chem. Ind.*, 1907, **26**, 501.
24. G. SCHUTZ and L. WEIN, *Chem. Zeit.*, 1915, **39**, 143.
25. G. BRUCKNOR and B. THOMAS, *Zeit. Getreid. Mühl. Bäcker.*, 1938,  
**25**, 34.
26. J. G. A. GRIFFITHS, *Analyst*, 1937, **62**, 510.
27. SPROCKHOFF, *Zeit. Spiritusind.*, 1929, **52**, 27.
28. T. H. FAIRBROTHER and R. J. WOOD, *Ind. Chem.*, 1930, **6**, 442.
29. BURTON and PITT, *Can. J. Res.*, **1**, 2, 155.
30. J. MAYRHOFER, *Oesterr. Chem. Ztg.*, 1935, **38**, 178.
31. C. SCHÉELE, J. AFZELIUS and K. LEANDER, *Zeit. Spiritusind.*, 1937,  
**60**, 163.
32. W. EKHARD, *ibid.*, 1929, **52**, 70.
33. SPROCKHOFF, *ibid.*, 1929, **52**, 358.
34. — *ibid.*, 1929, **52**, 341 ; *Chem.-Ztg.*, 1930, **54**, 411.
35. SCHULZ and PARLOW, *ibid.*, 1930, **53**, 135, 186.
36. E. T. REICHART, 'The Differentiation and Specificity of Starches  
in Relation to Genera, Species, etc.,' *Carnegie Inst. Publ.* **178**, 1913.
37. S. WOODRUFF and others, *Ind. Eng. Chem.*, 1938, **30**, 1409 ; *J. Agr.*  
*Res.*, 1936, **52**, 233 ; *Univ. Illinois Agr. Exp. Sta. Bull.*, **445**, 1938 ;  
*J. Agr. Res.*, 1933, **46**, 1108.
38. T. C. TAYLOR and SALZMANN, *J. Amer. Chem. Soc.*, 1933, **55**, 264.
39. T. C. TAYLOR, H. H. FLETCHER and M. H. ADAMS, *Ind. Eng. Chem.*  
(*Anal. Ed.*), 1935, **7**, 321.
40. T. C. TAYLOR and J. C. KERESZTESY, *Ind. Eng. Chem.*, 1936, **28**, 502.
41. WIEDMER, *T.I.B.A.*, 1936, **14**, 103, 167.
42. O. SAARE, *Zeit. Spiritusind.*, 1903, **26**, 436 ; *Chem.-Ztg.*, 1929, **53**,  
975.
43. E. VOGT, *Zeit. Unters. Nahr.-Genussm.*, 1921, **42**, 145.
44. T. E. WALLIS, *Pharm. J.*, 1922, **109**, 82.
45. W. A. RICHARDSON, HIGGINBOTHAM and FARROW, *J. Text. Inst.*,  
1936, **27**, 131T.
46. W. A. RICHARDSON, *Chem. and Ind.*, 1939, **58**, 468.
47. HIGGINBOTHAM and W. A. RICHARDSON, *J. Soc. Chem. Ind.*, 1938,  
**57**, 234.
48. M. SAMEC and B. ŠKERL, *Kolloidchem. Beih.*, 1937, **47**, 91.
49. N. I. OZOLIN, *Trudy Odesskogo Inst. Tekhnol. Zerna i. muki im.*  
*I.V. Stalina*, 1938, 81.
50. C. SCHWEIZER, *Mitt. Geb. Lebensm. Hyg.*, 1925, **16**, 95 ; 1929, **20**, 119.
51. H. KÜHL, *Zeit. ges. Getreiden.*, 1930, **17**, 122 ; *Chem. Zentr.*, 1930,  
ii, 1299.



52. E. BERLINER, *Mühlenlab.*, 1939, **9**, 13 and 87.
53. A. BINTZ and T. MARX, *Chemische Ind.*, 1909, **32**, 167.
54. H. CAPPENBERG, *Chem.-Ztg.*, 1910, **34**, 218.
55. V. VILIKOVSKI, *Chem. listy*, 1911, **5**, 412.
56. O. HOYER, *Chem. Zentralbl.*, 1911, **2**, 305.
57. W. NEUWOHNER, *Zeit. Tierernähr. Futtermitt.*, 1939, **3**, 1.
58. KLAUSS, *Vorratspflege Lebensmitt.*, 1938, **1**, Nos. 6 and 7.
59. M. I. KNYAGINICHEV, *Colloid J. (U.S.S.R.)*, 1939, **5**, 899.
60. T. J. SCHOCH and C. C. JENSEN, *Ind. Eng. Chem. (Anal. Ed.)*, 1940, **12**, 531.
61. F. ROBERTSON DODD, *Analyst*, 1939, **64**, 735.
62. — *ibid.*, 1939, **64**, 877.
63. KAVCIC, *Koll. Beih.*, 1930, **30**, 406.
64. WINTON and WINTON, 'The Structure and Composition of Foods,' Vol. II. Chapman & Hall, London, 1935.
65. I. A. VESELOVSKII, *Amer. Potato J.*, 1940, **17**, 330.
66. C. R. JONES, *Cereal Chem.*, 1940, **17**, 135.
67. G. STEINHOFF, *Zeit. Untersleb.*, 1938, **75**, 39.
68. J. KÖNIG and F. BARTSCHAT, *Zeit. Unter. Nahr. Genussm.*, 1923, **46**, 321; Abstr. in *Analyst*, 1924, **49**, 187.
69. R. STROHECKER, *ibid.*, 1924, **47**, 90; Abstr. in *Analyst*, 1924, **49**, 282.
70. M. WAGENAAR, *Zeit. Unters. Lebensm.*, 1937, **54**, 357.
71. L. PICKENS and D. T. ENGLIS, *Food Res.*, 1940, **5**, 563.

## ADDITIONAL REFERENCES

- F. PUGH, *Microscope*, 1938, **2**, 239. (Qualitative and quantitative microscopical examination of starches.)
- GASTINE, *Compt. rend.*, 1906, **142**, 1207; *J. Soc. Chem. Ind.*, 1906, **25**, 655. (Detection of rice flour in wheat flour.)
- W. N. JONES, *Ann. Bot. [N.S.]*, 1939, **3**, 505. (Preparation of double stained slides of starch and plastids.)
- L. ROSENTHALER, *Pharm. Zentr.*, 1925, **66**, 631. (Starches heated with alcoholic caustic potash, diluted with waters titrated give characteristic numbers.)
- W. KRÖNER, *Zeit. ges. Getreidew.*, 1939, **26**, 162. (Colour, odour, gloss, moisture, ash, acidity, etc., of potato starch discussed.)
- C. H. BUTCHERS, *Food*, 1934, **3**, 244. (Microscopy of starch discussed.)
- S. CAMILLA, *Ann. Chim. Applic.*, 1938, **28**, 541. (Characteristics of starch granules originating in the dried bulb in powdered saffron described.)
- C. GRIEBEL, *Z. Unters. Lebensm.*, 1927, **54**, 477. (Notes some hazel nuts contain starch contrary to previous assumptions to the contrary.)

## CHAPTER 2

## THE DETERMINATION OF STARCH

MANY methods are in use for the determination of starch, but most of them are applicable to a limited type of work only. They may be roughly classified under the following headings:—

**1. Non-hydrolytic Methods.**—In these the starch is dispersed in a solvent, and then (*a*) recovered and weighed, or (*b*) precipitated from the solvent in the form of a derivative, or (*c*) determined polarimetrically.

**2. Hydrolytic Methods.**—In these the starch is hydrolysed to reducing sugar and the sugar determined. The inversion may be carried out by means of (*a*) acid, (*b*) enzymes, or (*c*) enzymes, followed by acid treatment.

**Non-hydrolytic Methods (*a*).**—Various acids, such as hydrochloric and trichloroacetic,<sup>2</sup> salts such as calcium chloride,<sup>35, 116</sup> potassium thiocyanate, zinc chloride,<sup>1</sup> and magnesium chloride,<sup>82</sup> caustic alkalies, glycerol and formamide have all been used to disperse starch, which can then be recovered without undergoing appreciable hydrolysis, although its physical properties may have been radically altered.

One of the most reliable methods is that of O. Rask,<sup>3</sup> which has been tentatively adopted in America by the Association of Official Agricultural Chemists.<sup>4</sup> The starch is dispersed in cold concentrated hydrochloric acid to give a filterable solution from which the starch is recovered by precipitation with alcohol. The recovered starch has lost its identity, as seen under the microscope, and is water-soluble, but still gives the iodine reaction, and does not reduce Fehling's solution, but A. R. Ling and F. E. Salt<sup>5</sup> consider that some hydrolysis occurs. C. W. Herd and D. W. Kent-Jones<sup>6</sup> do not think that the method is entirely satisfactory, and to overcome its defects have modified it, reducing the manipulative difficulties to a minimum when dealing with flours, wheats, brans, sharps, and all classes of mill stocks. One gram of the material is well mixed with 1 gm. of acid-washed sand and covered with ethyl ether in a centrifuge tube, well stirred for about 1 minute, after which it is centrifuged and the liquid poured off. This is repeated twice more, so reducing the filtration difficulties, due to impurities, which would arise later in the determination. To the residue is added 2.5 ml. water and 0.25 ml. N/1 caustic soda solution, which is thoroughly stirred in. Fifteen

minutes later 5 ml. of pure methyl alcohol are added and mixed in, followed by 5 ml. of dilute methyl alcohol (5 ml. alcohol, 2.5 ml. water), and after mixing and centrifuging the alcoholic layer is removed. The residue is washed twice with 10 ml. of the diluted alcohol, and finally given three washings with water.

The residue is mixed to a thick paste with a few ml. of water, taking care that no lumps are formed. A total of 20 ml. of water is employed to transfer the paste to a 100 ml. flask, to which 20 ml. of concentrated hydrochloric acid are then added, and the total volume is made up to 100 ml. with Rask's acid, using this first to rinse out the centrifuge tube. After shaking the flask, the contents are filtered through a Gooch crucible having a layer of acid-washed sand superimposed on the asbestos; 50 ml. of the filtrate are pipetted into a 200 ml. beaker containing 110-115 ml. of 96 per cent. alcohol, and not until the pipette has drained is the liquid in the beaker thoroughly stirred. A flocculent precipitate is formed; after it has partially settled the contents of the beaker are centrifuged for 10 minutes and the residue washed four times with 70 per cent. (by volume) alcohol and twice with 96 per cent. alcohol to remove the last trace of acid, care being taken that the residue and alcohol are thoroughly mixed each time.

It is essential that the time between the addition of the acid to the sample and the precipitation of the starch with the alcohol does not exceed 35 minutes, otherwise hydrolysis of the starch may materially affect the results. The final residue is transferred to a tared Gooch crucible, using 96 per cent. alcohol, washed with ethyl ether and dried in the oven at 40° C. for 10 to 15 minutes; this is followed by heating to 130° C. until the weight is constant.

For 'sharps' or bran, these workers recommend starting with 2-4 gm. of material, increasing the methyl alcohol and caustic soda mixture in proportion, and retaining the crude fibre on glass before making up the volume to 100 ml.

With commercial starches the above method was found to give results nearer to 100 per cent. than methods using malt or barley diastase, but the results on commercial flours and wheat offals were lower.

L. Jones <sup>7</sup> and O. S. Rask <sup>8</sup> find that variations occur between the figures obtained by Rask's method and those obtained by diastatic methods. F. E. Denny <sup>9</sup> suggests that the material be gelatinised with water, extracted with four successive portions of acids, precipitating the starch in these with alcohol, and then acting on the starch present with takadiastase (see below). In this way he proposes to overcome the incomplete extraction of

starch by the above method and at the same time eliminate the inclusion of non-starch fractions in the alcoholic precipitate.

W. H. Krug and H. W. Wiley<sup>10</sup> use salicylic or lactic acid under pressure to disperse the starch, but their method gives high results due to the degradation of pentosans and hemicelluloses, whilst P. Biourge<sup>2</sup> uses a 3 per cent. solution of trichloroacetic acid.

Other workers have used alkali to disperse the starch, for example, J. Mayrhofer<sup>11</sup> and M. Piettre<sup>12</sup> treat foodstuffs with alcoholic caustic potash, which dissolves fats, sugars, and albumins. After filtering, the residue is treated with aqueous caustic potash, the solution acidified, and the starch precipitated with alcohol, washed and dried. G. Baumert and H. Bode<sup>13-14</sup> use caustic soda to dissolve the starch, precipitate it with alcohol, dissolve the precipitate in hydrochloric acid and re-precipitate with alcohol, after which the dried residue is ignited and the loss on ignition is taken as the weight of starch present. P. Behrend and H. Wolfs<sup>15</sup> state that this method gives accurate results.

**Non-hydrolytic Methods (b).**—The formation of additive products between starch and iodine (see pp. 128, 131) was used by A. Kaiser<sup>16</sup> as a method for determining starch, and later Th. von Fellenberg<sup>17-18</sup> used calcium chloride solution to dissolve starch, which was then precipitated by the addition of iodine solution. The calcium chloride acts as a salting-out agent for the starch-iodine complex, and this is decomposed with alcohol to give starch. J. J. Chinoy and F. W. Edwards,<sup>19</sup> other workers,<sup>20-21</sup> and H. Weiss<sup>22</sup> have used similar methods. J. C. Small<sup>23</sup> uses ammonium sulphate to salt out the starch iodide from aqueous dispersions and to remove the dextrin-iodine complexes present. W. Whale,<sup>68</sup> using an iodometric method for food products, points out that the presence of dextrin introduces an error not easily overcome, and in such cases advocates one or other of the hydrolytic methods. He has successfully applied the volumetric iodide method to the determination of starch in cocoa and sweetened chocolate.<sup>80</sup>

F. E. Denny<sup>24-25</sup> determined the amount of starch, first by precipitation with iodine, which was then titrated, and later by hydrolysing the starch to glucose by means of takadiastase. J. T. Sullivan<sup>26</sup> has applied the method to the estimation of starch in woody plants, and hydrolyses the starch-iodide precipitate to glucose with acid. Although his results are lower than those obtained by other methods, he considers them to be accurate. Tian<sup>27</sup> has attempted to eliminate errors due to the indefinite composition of starch iodide. Using dilute aqueous solutions

of starch, an excess of iodide solution is added and the colour matched against standards, red light being used for the comparisons, as this light is absorbed only by the starch iodide and not by the excess iodide present (see also p. 134).

P. Biourge<sup>2</sup> dissolves the starch in 3 per cent. trichloroacetic acid, and after adding iodine solution, the starch-iodine complex is salted out with magnesium sulphate. G. Rankoff<sup>84</sup> determines starch in potato meal by extracting it with calcium chloride, precipitating with I/KI solution saturated with sodium sulphate, washing, heating with 25 per cent. sulphuric acid to remove iodine and determining the starch by oxidation with potassium permanganate.

A. von Asboth<sup>28</sup> has suggested a method based on the formation of a sparingly soluble barium salt of starch, but other workers consider the method unreliable.<sup>29</sup>

**Non-hydrolytic Methods (c).**—A. Baudry<sup>30-31</sup> refluxes the sample with benzoic or salicylic acid and examines the filtrate polarimetrically, the polarimeter being graduated for reading the starch-content directly. A modification of this method has been used by L. Pellet.<sup>32</sup> Effront<sup>33</sup> uses hydrochloric acid as the solubilising agent, and after polarisation makes allowance for the small amount of glucose present, which he determines by titrating with Fehling's solution. D. Crispo<sup>34</sup> uses caustic potash solution for polarising, and C. Mannich and K. Lenz<sup>35</sup> boil the starch with a concentrated solution of calcium chloride, which is N/500 with respect to acetic acid, and thus obtain a clear solution suitable for direct polarimetric observation.

C. J. Lintner<sup>36</sup> triturates 5 gm. of the sample with 20 ml. water, then mixes with 40 ml. concentrated hydrochloric acid, and after standing 30 minutes transfers to a flask, using hydrochloric acid of sp. gr. 1.125. Ten ml. of a 4 per cent. phosphotungstic acid solution are added and the volume made up to 200 ml. with hydrochloric acid (sp. gr. 1.125). After filtering the solution, the rotation is determined in a 200 mm. tube with sodium light. Lintner found  $[\alpha]_D^{20} = 200.3$  for barley starch. The amount of starch in a sample can be found from the formula

$$\text{Percentage} = \frac{4000 \times \text{observed rotation}}{L \times [\alpha]_D^{20}}$$

where  $L$  = length of tube in decimetres and  $[\alpha]_D^{20}$  = specific rotatory power of barley starch under above conditions.

O. Wenglein<sup>37</sup> used sulphuric acid instead of hydrochloric acid and obtained for barley starch a specific rotatory power,

$[\alpha]_D = 191.7$ . Lintner<sup>38-39</sup> has also used this method, but thinks that the sulphuric acid used by Wenglein may cause decomposition of the starch and suggests using a weaker acid of sp. gr. 1.4.

Although M. Canet and O. Durieux<sup>40</sup> found Lintner's method to be satisfactory with starch and amylaceous materials, they suggest the specific rotatory power  $[\alpha]_D^{20} = 202$  be used in the above formula. The specific rotatory powers of the more important starches have been determined by J. König and co-workers,<sup>41</sup> using Lintner's hydrochloric acid method and Ewers' method (see below). Their results show that the starch probably undergoes hydrolysis during the precipitation of the solution by Ewers' procedure, and that noticeable errors would be introduced into the determination by this method if the average value of  $[\alpha]_D^{20}$  was taken, as the rotations for different starches vary more widely than by Lintner's method. With the latter method, the value  $[\alpha]_D^{20} = 202$  is sufficiently accurate for ordinary work. The two methods have also been compared by S. Hals and S. Heggenhangen.<sup>42</sup>

E. Ewers<sup>43</sup> treated the starch with glacial acetic acid, hydrochloric acid and hot water, and used potassium ferrocyanide to clear the solution, allowance being made for the rotation due to soluble carbohydrates. Later<sup>44-46</sup> he used dilute hydrochloric acid (1.124 per cent. by weight) with which the starch was heated, clarified with sodium molybdate or phosphotungstic acid, filtered and polarised. The specific rotation for barley starch was found to be 181.5 by this method. A similar method was employed by him for estimating starch in potatoes.<sup>45</sup> M. I. Knyaginichev and Y. K. Palilova<sup>46</sup> have found that starch from legumes have a lower specific rotation ( $[\alpha]_D^{20} = 192.7$ ) than that from wheat starch, and consider that the value of the specific rotation runs parallel with the degree of evolutionary development. They found, for example, that the starch from primitive varieties of scaly grain wheat has a lower specific rotation ( $[\alpha]_D^{20} = 199.8$ ) than that from cultivated varieties with naked grain ( $[\alpha]_D^{20} = 204.0$ ). The significance of this in botanical and horticultural investigation will be appreciated. J. Kavčič<sup>46</sup> has also found that the starch from four different varieties of *Solanum tuberosum* (potato) showed different values for optical rotation as well as mean diameter of grains, ash, and nine other different properties of the starches. The values of these properties were consistent within any one variety but differed from the corresponding values for other varieties.

When examining substances containing optically active constituents in addition to the starch, resort can be made to thorough

washing with cold water, alcohol and ether, to remove the interfering substances.

**Hydrolytic Methods (a).**—As previously stated, the amount of glucose obtained by acid hydrolysis of starch multiplied by the factor 0.90 should indicate the amount of starch present, but W. A. Noyes and co-workers<sup>47</sup> consider that a complete recovery cannot be obtained unless the factor 0.93 is used. This is a completely empirical figure, but it has been adopted by a number of workers, as losses certainly do occur which may be due to the presence in the hydrolysate of disaccharides having a lower reducing power than glucose. Once it has been formed, the glucose undergoes no change on heating with acid, unless the concentration of the latter is abnormally high or the duration of the heating is excessive.<sup>48</sup> Acid-hydrolysis methods are limited to starch determinations in materials which are free from other cellulosic materials which may also yield glucose on treatment with boiling acid. Cellulose is less readily attacked by dilute acid than starch at about 60–80° C. for a short time, and G. S. Fraps<sup>49</sup> uses 0.02 N acid to separate the starch from the insoluble matter, completing the hydrolysis with stronger acid in the usual way and correcting the results for the pentosans present.

V. Jahn<sup>83</sup> estimates the amount of starch in sausages, meat pastes or mayonnaise in the following manner: 20 gm. of the material are digested for several hours on the water-bath with 50 ml. of 8 per cent. alcoholic potash, and, after filtering, the residue is washed with 96 per cent. alcohol, water being added to make a total weight of 25 gm. The mixture is treated with 0.5 N hydrochloric acid until neutral to phenolphthalein, after which it is heated with 25 ml. dilute hydrochloric acid (80 ml. of 25 per cent. acid diluted to 1 litre) at 100° C. for 15 min. to invert the starch. After cooling 6 ml. of a 4 per cent. phosphotungstic acid solution are added, the whole diluted to 100 ml., clarified with kieselguhr, and filtered. The filtrate is examined in the polarimeter and the reading on the sugar scale  $\times 0.475$  represents the percentage of pure starch present.

Ling<sup>50</sup> considers that none of the polarimetric methods in which acid is used as a converting agent gives reliable results, at any rate, for starch in cereals, because certain other substances pass into solution.

**Hydrolytic Methods (b).**—As the products of enzyme action on starch consist of a mixture of sugars and dextrans, numerous methods have been elaborated that embrace both enzymatic and chemical or physical treatments. Enzymatic methods are most useful where other carbohydrate material, capable of hydrolysis to glucose with acid, is present besides the starch.

Several methods of an empirical kind which yield products giving definite values for the reducing power or the rotation of polarised light must be carried out under strictly controlled conditions. In other methods the amount of each end-product present in the mixture is found by different ways, and the amount of each product present calculated by the use of simultaneous equations. A third group of methods comprises those in which a preliminary enzymic reaction is used to separate the starch from other bodies present; the separated degradation products are hydrolysed to glucose, which is estimated and the amount of starch deduced.

*Barley and Malt Diastase Methods.*—So many diastatic methods have been proposed and employed that only a few can be mentioned here.

Both  $\alpha$ - and  $\beta$ -amylase are present in malt, the former causing liquefaction of the starch and the latter, the so-called saccharogenic enzyme, converting the amylose to maltose (see p. 437). E. Waldeschmidt-Leitz, M. Reichel and A. Purr<sup>51</sup> have shown that, contrary to previous belief, both enzymes are present in varying amounts in ungerminated barley, and G. Nordh and E. Ohlsson<sup>52</sup> have found that both enzymes possess saccharogenic and dextrinogenic activity. Such observations throw doubt on the accuracy of the results obtained by the method of Ling, Nanji, and Harper,<sup>53</sup> in which the precipitated, undried diastase from ungerminated barley<sup>54</sup> is used on the assumption that only the amylose is attacked, and that the ratio of amylose to amylopectin is 2-1. As previously stated (see p. 40), the accuracy of this ratio is disputed by several workers,<sup>55-57</sup> although H. Lüers and F. Wieninger<sup>74</sup> found the method gives concordant results.

Simultaneously with the barley or wheat conversion, Ling and his co-workers make a blank estimation, using high-grade potato starch, and determine the amount of maltose present, either iodometrically or by Fehling's method, expressing the result as a percentage of the starch. The percentage of starch in the barley or wheat is expressed as  $100m/M$ , in which  $m$  is the maltose obtained from 100 pts. of dry cereal and  $M$  is the maltose present in the potato-starch experiment expressed in 100 pts. of the dry starch. The accuracy of the method depends on the amylose-maltose figure for potato starch and the extent to which this is applicable to other starches and cereal products. If this relationship holds the method is reliable and precise.

In a number of methods germinated barley is used, and maltose, together with a small amount of dextrans of varying constitution, is produced. Ling<sup>58</sup> has used malts with a diastatic activity of



20-100, as measured by his scale, under strictly controlled conditions, and has obtained maltose corresponding to 80-87.5 per cent. of the weight of starch. In determining the amount of starch in cereal products the amount of maltose produced is compared with that theoretically obtainable by the same malt. Ling and Price<sup>75-76</sup> propose to avoid limiting the method to the use of malt of diastatic power of 80 Lintner by means of a simple formula.

H. T. Brown<sup>59-60</sup> extracts the malt or barley for 9 or 3 hours, respectively, with alcohol to remove sugars and certain nitrogenous compounds, boils the sample under examination with water, and digests it at 57° C. with an active malt extract. After 1 hour the liquid is boiled, cooled, and filtered, and the maltose-content determined. The starch equivalent to this maltose-content is calculated on the assumption that 84.4 pts. of maltose correspond to 100 pts. of starch. This assumption is justified only if the malt from which the active extract is prepared has a diastatic power of 80 Lintner, but malts with a lower diastatic activity give less, and highly active malts give more, maltose than the above figure.

**Takadiastase.**—Takadiastase preparations, which contain many different enzymes,<sup>61</sup> were introduced as a quantitative reagent for determining starch by W. A. Davis and A. J. Daish<sup>62</sup> in 1914. I. D. Collins<sup>63</sup> pointed out that, at proper pH value and correct time, a high concentration of the enzyme gives complete hydrolysis to glucose, basing her conclusions on the fact that takadiastase, and also acid hydrolysis, gave a recovery of 93 per cent. of the dry weight of starch; if the factor 0.93 is used, she suggests that a recovery of nearly 100 per cent. is obtained. Denny<sup>25</sup> and O. Lehmann<sup>64</sup> report complete recovery of starch by this method. The use of takadiastase is widespread among workers on plant products, and thus deserves attention, but it should be remembered that these preparations also contain enzymes which act on materials other than starch, so that its use should be examined critically before trying any new unproven departure from previous work.

**Hydrolytic Methods (c).**—Methods embracing the acid hydrolysis of the products obtained by enzyme action have found wide favour in America. Maerker<sup>70</sup> extracts 3 gm. of the finely-ground material with ether, after which it is boiled with 100 ml. of water, cooled to 65° C. and treated for about 2 hours with 10 ml. of a 10 per cent. infusion of malt. It is then heated on a boiling water-bath for 30 minutes. On cooling to 65° C. another 10 ml. of malt infusion are added, and after half an hour the contents of the flask are boiled, cooled, made up to 250 ml. and

filtered. Two hundred millilitres of the filtrate are heated on a boiling water-bath for  $2\frac{1}{2}$  hours with 15 ml. of 25 per cent. hydrochloric acid. After cooling and neutralising, the dextrose present is estimated by one of the standard methods, the conversion factor 0.90 being used to calculate the weight of starch present in the sample. A correction is made for the reducing power of the malt extract, which is determined separately.

R. P. Walton and M. R. Coe<sup>65</sup> have worked out a method in which the insoluble non-starchy material present in the products of diastatic hydrolysis are removed by filtration, the pectin being precipitated by 60 per cent. alcohol, which does not precipitate the dextrins present. This method has been adopted by the Association of Official Agricultural Chemists.<sup>66</sup> The precautions to be taken in this work are contained in the preliminary papers by these workers.<sup>71-73</sup> Hartmann and Hillig<sup>67</sup> suggest that starch products containing much protein matter should be digested overnight with pepsin, a process which would destroy the proteins that occlude starch and at times render results unreliable. P. Fleury and G. Boyeldieu<sup>81</sup> determine starch in bread prepared for diabetic patients by hydrolysing with dilute sulphuric acid and precipitating the proteins by the addition of an acid solution of mercuric sulphate which they claim to be better for this purpose than lead acetate. The dextrose remaining is then determined by polarimetric or reduction methods. A. Hock<sup>69</sup> uses diastase followed by acid treatment, but his chief modification is connected with the adequate removal of fats, protein and water-soluble substances that may interfere.

Herd and Kent-Jones,<sup>6</sup> surveying the field of enzymes for use in the determination of starch, point out that the difficulties arising from the various methods proposed for the determination of starch in natural materials may be summarised as follows:—

1. Acid hydrolysis: presence of other hydrolysable carbohydrates.
2. Hydrolysis by prepared diastase: variable hydrolytic powers and the unknown action on various components of the starch.
3. Hydrolysis by malt diastase: variable hydrolytic action on hemicelluloses.
4. Hydrolysis by barley diastase: as 3; and in addition, it is also necessary to assume that the ratio amylose/maltose for other starches is the same as for potato starch.

A survey of methods for the determination of starch has been carried out by M. P. Etheridge.<sup>117</sup>

The investigation included the current official American methods of hydrolysis with hydrochloric acid and with diastase

and acid, and the Hopkins modification<sup>116</sup> of the Mannich-Lenz calcium chloride method.<sup>35</sup> Pure corn, wheat and potato starches were used, their moisture, ash and protein-contents being determined and a rough estimate of the starch obtained by difference. For the determination of the moisture, the results obtained by drying for 24 hours in a Freas oven at 105-106° C. compared favourably with those given by drying *in vacuo* for a shorter period. Natural materials, such as cake flour, whole wheat flour and whole Lima beans, were also used. When the calcium chloride method was applied to these, the samples were washed on filter paper, instead of being centrifuged with alcohol, as recommended by Hopkins. Contrary to statements in the literature, this worker found that all the starch in the reputed pure and commercial starches was not obtained either by hydrolysis with hydrochloric acid or by diastase treatment followed by acid hydrolysis. The results were even lower than the 96 to 97 per cent. of the total starch found by Noyes *et al.*<sup>47</sup> On the other hand, with natural materials the diastase and hydrochloric acid method (a) gave fairly concordant results comparing better with those obtained by the calcium chloride method (b). Thus the following mean percentages were found: cake flour, (a) 72.87, (b) 75.90; whole wheat flour, (a) 66.95, (b) 67.29; rice bran, (a) 6.84, (b) 8.22; corn meal, (a) 62.44, (b) 63.76; whole rice, (a) 71.75, (b) 77.31; Lima beans, (a) 45.56, (b) 42.54. In most instances the calcium chloride method was very satisfactory with the pure starches. The Hopkins modification seemed to be the most promising single method and, by careful control of heating and the use of accelerated filtration, can be advantageously applied to natural materials. Other chemicals were tried as dispersing agents in an effort to prevent filtration difficulties. The use of calcium nitrate and sodium salicylate showed possibilities, although the dispersions were still difficult to filter. On the other hand, sulphosalicylic and formic acids gave promising results, and the dispersions could be readily filtered.

R. T. Balch<sup>118</sup> finds that using sodium hypochlorite as a dispersing and solubilising agent filtration difficulties are overcome, and after filtration a clear liquid is obtained which can be examined in the polariscope without difficulty. The residue with root starches contains no starch, but with grain starches only 93-94 per cent. of starch is removed. Balch considers this may be due to the residue retaining the so-called amylohemcellulose stated to occur in grain but not root starches and which is convertible by the diastatic or acid hydrolysis methods, so that it is included in the determination of starch by the usual methods.

A comprehensive résumé of methods for determining starch is given by Herd and Kent-Jones,<sup>6</sup> R. Kutscha,<sup>77</sup> O. Wolff,<sup>78</sup> K. Alpers,<sup>79</sup> and additional information has been classified for convenience under products in Table 10.

TABLE 10  
DETERMINATION OF STARCH IN VARIOUS MATERIALS

<i>Product.</i>	<i>Type of Method Used.</i>	<i>Reference.</i>
Adhesives	Various methods discussed.	Nagel, <i>Zeit. Spiritusind.</i> , 1920, <b>43</b> , 129.
Barley	A number of methods reviewed, Lintner's preferred. Polarimetric method. Ewers' method. Effect on pentosans evaluated.	Ref. 77. C. J. Lintner, <i>Zeit. gesam. Brauw.</i> , 1911, <b>34</b> , 301 G. Fertman and V. Rudzevich, <i>Spirto-Vodochnaya Prom.</i> , 1939, No. 6, 37; <i>Khim. Referat Zhur.</i> , 1939, No. 11, 66.
Beet leaves	Iodine coloration.	G. Rowland, <i>Pub. inst. belge amélioration betterave</i> , 1939, <b>7</b> , 463.
Cocoa	Ewers' method satisfactory. Volumetric iodide method (see p. 397). Lintner's cold polarimetric method. Sulphuric acid hydrolysis then sugar estimated by Fehling's method.  Takadiastase followed by polarimetric or copper reduction estimation of sugar formed.	W. Greifenhagen, <i>Biochem. Zeit.</i> , 1911, <b>35</b> , 194. W. Whale. <sup>68, 80</sup> G. Savini, <i>Chem. Zentralbl.</i> , 1923, <b>4</b> , 804. P. Trojanowsky, <i>Arch. Pharm.</i> , 1887, <b>210</b> , 30; W. L. Dubois, <i>U.S. Dept. Agric. Bur. Chem.</i> , 1909, BULL. <b>122</b> , 214; BULL. <b>132</b> , 136. C. Revis and H. R. Burnett, <i>Analyst</i> , 1915, <b>40</b> , 429.
Cassava flour	Ewers' method. Specific rotation of cassava starch, $[\alpha]_D = 183.1$ .	Riechelmann, <i>Zeit. offentl. Chem.</i> , 1921, <b>27</b> , 5.
Cattle foods	Saccharification with malt then estimate sugar by Fehling's solution.	P. L. Hibbard, <i>J. Amer. Chem. Soc.</i> , 1895, <b>17</b> , 64.
Cereals	Saccharification with malt then estimate sugar by Fehling's solution.	C. O'Sullivan, <i>J. Chem. Soc.</i> , 1884, <b>45</b> , 1.

TABLE 10—(Continued)

Product	Type of Method Used.	Reference.
Cinnamon	Ewers' method (see under cocoa).	Greifenhagen (see Cocoa).
Confectionery (peach and apricot kernels)	Polarimetric method.	J. Grossfeld, <i>Zeit. unters. Lebensm.</i> , 1927, <b>58</b> , 156.
Faeces	Extracts with $\text{CaCl}_2$ , ppt. with alcohol, hydrolyses and glucose determined by Kolt-hoff's iodometric method.	J. Terrier and J. Deshussies, <i>Mitt. Lebensm. Hyg.</i> , 1940, <b>31</b> , 249.
Farinas (various)	von Fellenberg's method gives low results. Ewers' method preferred for baked products.	J. Terrier, <i>ibid.</i> , 1940, <b>31</b> , 305.
Flour	Ewers' method.	J. Gerum, <i>Zeit. unters. Nahr.- u. Genussm.</i> , 1919, <b>37</b> , 145.
Fodder	Ewers' method and Lintner's method (see Cattle foods).	A. Scholl, <i>ibid.</i> , 1909, <b>18</b> , 157.
Gluten	Polarimetric method.	P. Fleury and G. Boyeldieu, <i>J. Pharm. Chem.</i> , 1928, <b>7</b> , 207, 248.
Grain	Modified Ewers' method.  Specific gravity method. Table used to calculate starch-content.  Uses petroleum in Ershov's method. Limited in scope.  Comparison of methods. Polarimetric methods considered most suitable.	K. Musolin, <i>Spirto-Vodochnaya Prom.</i> , 1937, <b>14</b> , Nos. 10-11, 59; <i>Chem. Zentr.</i> , 1938, <b>II</b> , 973. V. Ershov, <i>Brodil'naya Prom.</i> , 1935, <b>12</b> , No. 6, 50; <i>Chem. Zentr.</i> , 1937, <b>I</b> , 2486. R. Goldfarb, <i>Spirto-Vodochnaya Prom.</i> , 1937, <b>14</b> , No. 8, 34; <i>Chem. Zentr.</i> , 1938, <b>II</b> , 202. M. V. Jonescu and H. Slusanschi, <i>Anuar. Inst. tercetari Agron. Român.</i> , 1937, <b>9</b> , 160; <i>Chem. Zentr.</i> , 1938, <b>II</b> , 3028.
Grain meal	Precipitation of starch as barium starch.	A. von Asboth, <i>Chem. Ztg.</i> , 1889, <b>13</b> , 591, 611.
Grains, spent	Iodine coloration or precipitation with iodine.	H. Weiss, <i>Zeit. gesam. Brauw.</i> , 1922, <b>45</b> , 122.

TABLE 10—(Continued)

<i>Product.</i>	<i>Type of Method Used.</i>	<i>Reference.</i>
Green leaves	Iodine method.  Extract with HCl. Hydrolyse with salivary amylase. Estimate sugar by oxidation with ferricyanide and titration with ceric sulphate.	J. J. Chinoy, <i>Analyst</i> , 1938, <b>63</b> , 876. W. Z. Hassid, R. M. McCready and R. S. Rosenfels, <i>Ind. Eng. Chem. (Anal. Ed.)</i> , 1940, <b>12</b> , 142.
Grist	Successive hydrolysis by malt and acid and the sugar estimated.	R. Chrzaszcz, <i>Zeit. unters. Nahr.- u. Genussm.</i> , 1924, <b>48</b> , 306.
Marmalades	Iodine colorimetric method.	C. Griebel and M. Nothnagel, <i>ibid.</i> , 1925, <b>49</b> , 352.
Margarine	Hydrolyses with H <sub>2</sub> SO <sub>4</sub> , pot. ferrocyanide and zinc acetate and sugar estimated by Fehling's solution.	M. van Aerde, <i>Chem. Zentralbl.</i> , 1923, <b>4</b> , 890.
Meat products	Iodine colorimetric method.  Dissolves in KOH, ppt. starch with alcohol, hydrolyses and estimate sugar with Fehling's solution. (Factor—0.9.)	G. Ambuehl and H. Weiss, <i>Mitt. Geb. Lebensm.</i> , 1922, <b>13</b> , 170. T. M. Price, <i>U.S. Dept. Agric. Bur. Anim. Chem.</i> , 1912, Circular 203, 6.
(Sausages, etc.)	Rapidly polarimetric method.  Acid hydrolysis and polarimetric determination of sugar.	O. Braadlie and A. Moen, <i>Tids. Kjemi Bergvesen</i> , 1940, <b>20</b> , 17. V. Jahn. <sup>83</sup>
Oats, porridge	See Toasted wheat flakes. Various methods reviewed.	H. Frankenbach, <i>Papier-Fabr.</i> , 1922, <b>20</b> , 1173.
Paper	Hydrolyses with HCl and estimates of sugar with Fehling's solution. Spectrophotometric curves of iodine coloration.  See also	V. Voorhees and O. Kamm, <i>Paper</i> , 1919, <b>24</b> , 1091. L. E. Simerl and B. L. Browning, <i>Ind. Eng. Chem. (Anal. Ed.)</i> , 1939, <b>11</b> , 125. H. A. Bromley, <i>Paper</i> , 1915, <b>16</b> , 13; O. Kamm and H. Tendick, <i>ibid.</i> , 1919, <b>24</b> , 1091.

TABLE 10—(Continued)

<i>Product.</i>	<i>Type of Method Used.</i>	<i>Reference.</i>
Pectin juices	Colorimetric iodine method.	H. Eckart, <i>Chem. Zentralbl.</i> , 1925, <b>2</b> , 2106. G. Perrier, <i>Ann. falsif.</i> , 1924, <b>17</b> , 208.
Pepper	Lintner's or Ewers' method. Polarimetric method.	A. Scholl (see Fodder) and Greifenhagen (see Cocoa), E. von Raumer, <i>Zeit. angew. Chem.</i> , 1893, <b>6</b> , 453.
Plant materials (see also Green leaves and Vegetable foods)	Saccharification with salivary amylase and sugar estimated by oxidation with ferricyanide combined with cerate titration.	W. Z. Hassid, R. M. McCready and K. J. Rosenfels, <i>Ind. Eng. Chem. (Anal. Ed.)</i> , 1940, <b>12</b> , 142.
Potatoes	Approximate-starch content from S.G.	Rathsack, 'Der Speisewert der Kartoffel,' Berlin, 1935, 47; Sprockhoff, <i>Z. Spiritusind.</i> , <b>53</b> , 35 (see also Addit. Refs., p. 416).
	Diastatic hydrolysis and sugar determination preferred to S.G. or polarimetric methods.	S. Ostanin, <i>Spirto-Vodochnaya Prom.</i> , 1937, No. 2, 33; <i>Khim. Referat Zhur.</i> , 1938, No. 6, 137.
Freshly dug sweet potato	Approximate analysis. Starch and moisture-content fairly constant. Estimates moisture and calculates starch-content.	W. D. Kimbrough, <i>Amer. Soc. Hort. Sci.</i> , 1940, <b>37</b> , 846.
	Polarimetric method.	R. Zima, <i>Chem. Listy</i> , 1940, <b>34</b> , 81.
	Ewers' method.	G. Behr, <i>J. Landw.</i> , 1939, <b>87</b> , 103.
Frozen potatoes	Apparent increase in starch-content on thawing compared with unfrozen potatoes.	A. Antonov, <i>Spirto-Vodochnaya Prom.</i> , 1939, <b>6</b> , 36; <i>Khim. Referat Zhur.</i> , 1939, No. 11, 70.
	Ewers' method preferred.	A. Scholl (see Fodder).
Potato products	Iodine precipitation followed by oxidation (see p. 398).	G. Rankoff. <sup>24</sup>
Rice	Lintner's method.	H. Schreib, <i>Zeit. angew. Chem.</i> , 1888, <b>1</b> , 694.
	Precipitation as barium starch.	A. von Asboth. <sup>25</sup>

TABLE 10—(Continued)

<i>Product.</i>	<i>Type of Method Used.</i>	<i>Reference.</i>
Sardine paste	Starch rendered accessible with cuprammonium solution.	F. Kaulfersch, <i>Zeit. Unters. Nahr- u. Genussm.</i> , 1920, <b>39</b> , 344.
Soda crackers	(See Toasted wheat flakes.)	
Toasted wheat flakes	Modified Mannich-Lenz polarimetric method better than the Lintner-Schwarz method.	C. G. Hopkins, <i>J. Assoc. Off. Agric. Chem.</i> , 1940, <b>23</b> , 489; <i>ibid.</i> , 1939, <b>22</b> , 525.
Vegetable foods	Takadiastase followed by acid hydrolysis, then colorimetric determination of sugar by picrate method.	V. C. Myers and H. M. Croll, <i>J. Biol. Chem.</i> , 1921, <b>46</b> , 537; M. R. Coe and G. L. Bidwell, <i>J. Assoc. Offic. Agric. Chem.</i> , 1923, <b>7</b> , 297; W. Thomas, <i>J. Amer. Chem. Soc.</i> , 1924, <b>46</b> , 1670.
	Hydrolyses with diastase and estimates of sugar with interferometer.	O. Wolff, <i>Zeit. angew. Chem.</i> , 1924, <b>37</b> , 206.
	Sample first heated with strong $\text{NH}_3$ solution, then diastatic method employed.	F. Tempus, <i>J. Soc. Chem. Ind.</i> , 1923, <b>42</b> , 992.

In order that readers may gain some idea of the composition of various starch-bearing materials the following table has been compiled from different sources. It must naturally be assumed that the figures given in the latest work are the most reliable and based on up-to-date analytical methods, but the original papers should be consulted in special cases to ascertain the method used, bearing in mind the comments made in the preceding pages.



TABLE II—ANALYSES OF STARCH-BEARING MATERIALS

Material.	Water.	Starch.	Fibre.	Protein.	Fat.	Ash.	Sugar.	Gluten.	Carbo- hydrates not Fibre.	Reference.
Acorns, whole . . . . .	13.0	70	3.0 small	— below 1.0	2.0 small	5.8 small	10 2.8-4.1	—	—	87.
Arrowroot . . . . .	13.47	81-85	—	1.56	0.26	1.23	—	—	—	} 119.
Arrowroot, tubers . . . . .	62.9	25.30	—	—	—	—	—	—	—	
Avocado seed.	51.86	27.54	3.76	2.38	1.0	1.24	—	3.30	—	
Mexican variety. Undried basis . . . . .	9.08	57.21	9.08	5.72	2.07	2.98	—	7.31	—	
Dry weight basis . . . . .	50.56	20.60	3.65	2.45	1.1	1.39	—	2.21	—	
Fuerte variety. Undried basis . . . . .	59.87	59.87	7.28	4.95	2.21	2.70	—	4.47	—	
Dry weight basis . . . . .	6.47	62.09	3.81	11.52	2.67	2.87	7.02	—	—	
Barley . . . . .	10.95	—	3.85	11.0	2.25	2.50	—	—	69.5	} Wiley, quoted by Kent-Jones 88. Warrington, quoted by Kent-Jones 88.
American . . . . .	14.3	—	4.5	10.6	2.1	2.5	—	—	66.0	
English . . . . .	14.3	—	5.0	12.0	2.4	2.6	—	—	63.7	92.
feeding . . . . .	14.0	—	4.5	11.0	1.5	2.5	—	—	66.5	88.
" average . . . . .	14.3	—	3.9	9.4	2.1	2.5	—	—	67.8	
German . . . . .	13.06	—	7.28	11.46	1.93	2.32	1.34	—	—	
long-eared . . . . .	12.7	63.51	0.8	7.4	1.2	1.2	—	—	—	
Pearl . . . . .	88.82	7.28	1.46	1.40	0.1	0.94	—	—	—	
Beans . . . . .	14.4	—	4.2	20.6	1.1.2	3.35	—	—	—	
haricot . . . . .	15.7	—	5.35	26.7	1.2	3.95	—	—	48.3	
Buckwheat flour . . . . .	14.0	—	0.6	7.1	1.2	2.89	—	—	55.93	94.
Bahruah millet . . . . .	12.9	68.60	5.31	7.54	3.37	2.89	—	—	75.9	90.
Canavatia sp. seeds . . . . .	13.78	44.84	8.58	23.45	3.54	3.58	2.23	—	—	93.
Canna tubers (dried) . . . . .	67.83	—	3.39	3.6	0.78	7.45	—	—	84.78	M. H. French 100.
Cassava . . . . .	63.7	25.19- 26.5	0.71	0.81	0.58	0.44	1.07	—	29.63	93.
Mambo Leo, East Africa . . . . .	—	—	—	2.46	0.68	—	—	—	102.	
" flour . . . . .	12.9	—	1.91	2.18	1.48	1.91	—	—	80.24	
" . . . . .	12-14.8	—	—	1.0-1.9	0.86	0.9-2.4	—	—	—	
" meal . . . . .	9.22	—	8.14	2.01	0.49	1.91	—	—	78.2	96.
" starch . . . . .	12.16	81-89	4.94	26.82	1.10	0.8-1.0	—	—	—	
Cowpeas . . . . .	10.88	—	3.12	28.81	3.01	2.98	—	—	52.35	93.
" . . . . .	10.20	39.20	3.12	Trace	nil	0.14	—	—	50.32	114.
" starch . . . . .	12.02	87.30	0.01	42.99	1.12	3.05	—	—	59.42	114.
Gram, black, husked . . . . .	13.41	—	0.01	21.7	0.96	4.23	—	—	57.70	} 93.
" green . . . . .	12.98	—	3.33	10.2	—	0.42	—	—	—	
Ground durum, flour . . . . .	13.5	—	—	13.6	—	0.61	1.77	11.1	—	88.
" . . . . .	14.4	—	—	—	—	—	2.75	11.94	—	

TABLE II—Continued

Material.	Water.	Starch.	Fibre.	Protein.	Fat.	Ash.	Sugar.	Gluten.	Carbo- hydrates not Fibre.	Reference.
Guinea corn	9.38	—	1.31	7.57	3.92	2.89	—	—	74.93	93.
Lentils	14.0	55.3	3.4	25.5	1.9	5.0	—	—	52.2	90.
"	15.0	68.2	—	33.3	—	2.94	—	—	—	97.
Maize	12.81	—	1.2	7.2	3.99	1.04	—	—	73.76	93.
"	12.34	64.66	1.86	14.27	3.58	1.35	1.94	—	—	Beil via Jago 89.
"	9.34	66.91	1.41	10.8	5.34	1.54	2.18	—	—	Richardson via Jago 89.
" embryo	—	—	2.9	21.7	29.6	11.1	—	—	34.7	} 88.
" endosperm.	—	—	0.6	12.2	1.5	0.7	—	—	85.0	
" flour, average	12.5	66.78	0.9-1.8	7.0-9.5	1.5-3.5	0.6-1.3	—	—	74.1	
" hull	—	—	16.4	6.6	1.6	1.3	—	—	—	
" starch	12.15	84.87	—	0.2-0.5	0.02-0.1	0.1-0.5	—	—	—	
" whole	9.14	54.72	1.7	12.6	4.3	1.5-1.7	—	—	—	
Oats	6.92	56.91	1.29	14.27	7.87	2.22	6.07	—	—	Richardson via Jago 89.
" English	11.86	49.78	13.53	14.67	5.14	2.66	2.36	—	—	Beil via Jago 89.
" feeding, Canadian	10.0	61.1	7.5	12.75	5.4	3.17	—	—	—	} Dyer via Kent-Jones 88.
" feeding, English	9.97	53.3	6.8	8.65	3.32	2.57	—	—	—	
" grain	15.97	63.0	11.9	14.3	5.86	3.78	—	60.23	—	} Berry via 88.
" husk	13.4	—	8.96	9.46	5.33	2.62	—	52.20	—	
" kernel.	6.77	—	33.45	2.45	1.27	3.86	—	63.47	—	} Hutchinson via 88.
" meal.	13.4	—	1.33	12.34	7.73	1.83	—	65.9	—	
" average	7.2	—	3.5	14.2	7.3	1.9	—	66.0	—	} Hutchinson via 88.
" rolled.	8.5	—	1.5	14.0	8.0	1.8	—	64.8	—	
Potatoes	7.2	—	3.5	15.4	7.2	1.9	—	21.01	—	99.
"	74.98	—	0.69	2.08	0.15	1.09	—	2.5	—	
"	76	16.23	—	2.0	—	1.0	—	22.85	—	Moore and Partridge 103.
"	74.0	—	—	2.0	0.15	1.0	—	15.98	—	Leach 104.
"	66.1	—	0.37	1.43	—	0.44	—	30.53	—	
"	80.6	—	0.68	2.81	—	1.18	—	14.05	—	
"	75.37	—	0.28	1.14	—	0.78	—	20.37	—	
"	82.15	—	0.85	2.98	0.02	1.16	—	17.36	—	} Winton 105.
" average	78.89	—	0.56	1.14	0.18	0.95	—	13.3	—	
"	67.8	—	0.28	1.1	0.10	0.5-1.9	—	26.5	—	
"	84.0	—	0.90	1.1	0.02	—	—	—	—	
" average	78.3	—	0.4	2.2	0.1	1.0	—	18.4	—	

TABLE II—Continued

Material.	Water.	Starch.	Fibre.	Protein.	Fat.	Ash.	Sugar.	Gluten.	Carbo- hydrates not Fibre.	Reference.
Potatoes	71.99- 82.18 Average	—	0.5-0.76 Average	1.37- 2.2 Average	0.025- 0.185 Average	0.87- 1.185 Average	—	—	14.39- 24.58 Average	Schradler 106.
"	77.41	—	—	1.66	0.056	1.01	—	—	19.12	} Carpentier 107. Singh and Mather 108.
"	75.9	20.8	—	1.3	0.11	0.72	—	—	22.85	
"	79.3	18.4	0.33	2.0	0.10	0.64	—	—	19.19	} Mangold 109. Headdon 110.
"	80.8	13.4	0.49- 0.76	2.37	—	1.3	—	—	14.6-	
"	72-82.2	—	0.42- 0.56	1.42- 2.2	0.02- 0.18	0.87- 1.18	—	—	24.6	
"	79.18- 81.3 Average	12.88- 16.13	0.51	2.31- 2.77 Average	0.07- 0.12	0.94- 1.10	—	—	13.32- 16.56 Average	} Headdon 110.
"	79.98	—	0.50	2.63	0.09	1.03	—	—	15.6	
"	72.41- 81.85 Average	14.56 11.35- 21.81	—	0.87- 3.19 Average	—	1.02- 0.63	—	—	15.88	} Goldthwaite 111.
"	78.78	—	—	2.74	—	1.02	—	—	15.62	
"	77.23	16.02	—	2.02	—	0.95	—	—	19.79	} Rath sack 112. Metzger 113.
"	73.55- 80.31 Average	13.6- 17.68	—	1.787- 3.1 Average	—	0.87- 1.29	—	—	22.56 Average	
"	77.12	15.43	1.20- 2.58	2.31	—	1.073	—	—	19.49	} Leach 104. See also 101. Hutchinson via 88. 99. Bull. Dept. Agric., U.S.A., 1916. Bull. Dept. Agric., U.S.A., 1916. 93. 79.43 79.74 79.14
"	71.8- 81 Average	12.3- 20.3	—	—	—	—	—	—	—	
"	75.68- 79.96 Average	13.19- 18.03	—	2.13- 2.52 Average	—	0.88- —	—	—	15.95- 30.53 Average	} Bull. Dept. Agric., U.S.A., 1916. Bull. Dept. Agric., U.S.A., 1916. 93. 79.43 79.74 79.14
"	75.8	18.27	—	2.1	Trace	—	—	—	0.5-1.5 Average	
Rice	10.14	70-80	—	6.9	0.5	0.4-2.0	—	—	79.5	} Leach 104. See also 101. Hutchinson via 88. 99. Bull. Dept. Agric., U.S.A., 1916. Bull. Dept. Agric., U.S.A., 1916. 93. 79.43 79.74 79.14
" flaked	11.7	—	—	7.9	0.5	0.4	—	—	79.5	
" husked, average	11.68	—	0.7	7.71	1.19	0.93	—	—	77.79	} Bull. Dept. Agric., U.S.A., 1916. Bull. Dept. Agric., U.S.A., 1916. 93. 79.43 79.74 79.14
" Burma	12.38	—	0.85	7.24	1.52	1.13	—	—	76.88	
" Carolina	12.15	77-66	Trace	9.34	0.59	0.38	—	0.38	75.15	} Bull. Dept. Agric., U.S.A., 1916. Bull. Dept. Agric., U.S.A., 1916. 93. 79.43 79.74 79.14
" Honduras	12.32	—	0.99	8.57	1.79	1.18	—	—	78.14	
" polished	13.24	—	0.33	6.31	0.38	0.60	—	—	79.43	} Bull. Dept. Agric., U.S.A., 1916. Bull. Dept. Agric., U.S.A., 1916. 93. 79.43 79.74 79.14
" average	12.9	—	0.25	6.47	0.46	0.49	—	—	79.74	
" Burma	12.82	—	0.29	6.61	0.22	0.32	—	—	79.74	} Bull. Dept. Agric., U.S.A., 1916. Bull. Dept. Agric., U.S.A., 1916. 93. 79.43 79.74 79.14
" Honduras	11.89	—	0.3	8.06	0.25	0.36	—	—	79.14	

TABLE 11—Continued

Material.	Water.	Starch.	Fibre.	Protein.	Fat.	Ash.	Sugar.	Glucon.	Carbo- hydrates and Fibre.	Reference.
Rice, skinned, average	13.02	—	0.68	6.0	2.24	1.44	—	—	75.71	99.
" " Burmas	13.36	—	0.29	6.50	0.3	0.49	—	—	79.03	} Bull. Dept. Agric., U.S.A., 1916.
" " Honduras	12.5	—	0.30	7.88	0.28	0.47	—	—	78.57	
" " unhusked, average from dif- ferent sources	12.55	—	7.84	6.35	2.14	5.93	—	—	65.19	99.
" unhusked, Honduras	11.57	—	8.67	7.48	1.58	3.4	—	—	65.6	} Bull. Dept. Agric., U.S.A., 1916.
" " "	11.43	—	7.93	6.48	1.74	5.81	—	—	66.19	
Rye . . . . .	18.82	61.87	3.23	14.87	1.43	1.85	—	4.30	—	Bell via 89.
" " " " "	18.85	61.87	1.47	11.6	1.83	2.00	—	7.57	—	Richardson via 89.
" German	12.64	—	3.74	3.74	—	0.4	—	—	—	} Morrison, 1926, via 88.
" " flours	4.5	—	—	7.4	—	1.32	—	—	—	
" " "	9.82	—	—	6.72	—	0.64	—	—	—	
" " "	13.35	—	—	14.04	—	2.07	—	—	—	
" " "	12.17	81.87	0.1-0.4	0.1-0.4	—	0.1-0.7	—	—	—	Lane and Eyron.
" " flour	11.7	—	0.13	0.13	0.13	0.35	—	—	87.56	99.
Sweet potato	81.01	15.99	0.17	1.40	0.22	1.21	—	—	—	93.
Taro	12.7	—	4.87	0.88	0.23	0.85	—	—	—	99.
Taro, Hachae (dry weight basis)	7.75	77.91	1.42	2.0	0.56	1.55	—	0.52	—	} Very complete analysis given 115.
" " "	9.45	67.88	1.9	11.03	2.30	1.84	—	3.5	—	
Wheat	13.7	—	—	10.03	—	0.39	10.01	1.37	—	
" Barusso (Bahia)	15.06	—	—	12.03	—	—	13.0	1.65	—	} 88.
" " "	10.3	—	—	10.2	—	—	—	—	—	
" " Manitoba	15.2	—	—	15.0	—	—	—	2.38	—	
" " " "	12.7	—	—	12.8	—	—	—	1.85	—	
" " " " average	14.08	65.86	2.93	11.59	1.56	1.74	—	2.24	—	
" " " " winter	12.08	63.71	3.03	15.53	1.48	1.60	—	2.57	—	
" " " " flour, Australian	12.13	—	—	9.06	—	0.37	9.4	1.52	—	
" " " " "	13.8	—	—	10.89	—	—	—	2.32	—	
" " " " English	12.42	—	—	7.81	—	0.50	—	1.0	—	
" " " " "	15.47	—	—	10.03	—	0.33	8.15	1.63	—	
" " " " Manitoba	14.4	—	—	10.2	—	0.44	10.48	1.34	—	} 88.
" " " " "	15.79	—	—	13.6	—	0.37	10.85	1.94	—	
" " " " Russian	14.49	—	—	8.89	—	0.45	14.16	1.18	—	
" " " " "	15.87	—	—	11.17	—	0.34	9.75	1.66	—	
" " " " "	13.7	—	—	7.75	—	0.44	8.06	1.09	—	
" " " " Yeoman	16.35	—	—	11.12	—	0.46	11.56	1.97	—	

## REFERENCES

1. A. LECLERC, *J. pharm. chim.*, 1890, **21**, 641.
2. P. BOURGE, *Zeit. ges. Brauw.*, 1908, **31**, 277.
3. O. RASK, *J. Assoc. Off. Agric. Chem.*, 1927, **10**, 108 and 473.
4. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 'Official and Tentative Methods of Analysis,' 1930.
5. A. R. LING and F. E. SALT, *J. Inst. Brewing*, 1931, **37**, 595.
6. C. W. HERD and D. W. KENT-JONES, *J. Soc. Chem. Ind.*, 1931, **50**, 15T.
7. L. JONES, *J. Assoc. Off. Agric. Chem.*, 1932, **15**, 582.
8. O. S. RASK, *ibid.*, 1927, **10**, 473.
9. F. E. DENNY, *Contrib. Boyce Thompson Inst.*, 1934, **6**, 381.
10. W. H. KRUG and H. W. WILEY, *J. Amer. Chem. Soc.*, 1898, **20**, 266.
11. J. MAYRHOFER, *Forsch. Ber. Lebensm.*, 1896, **3**, 141 and 429.
12. M. PIETRE, *Seventh Int. Congr. Appl. Chem.*, 1909, p. 291.
13. G. BAUMERT and H. BODE, *Zeit. angew. Chem.*, 1900, **13**, 1074 and 1111.
14. G. BAUMERT, *Zeit. Nahr. Genussm.*, 1909, **18**, 167.
15. P. BEHREND and H. WOLFS, *Zeit. angew. Chem.*, 1901, **14**, 461.
16. A. KAISER, *Chem.-Ztg.*, 1902, **26**, 180.
17. TH. VON FELLEBERG, *Mitt. Lebensm. Hyg.*, 1916, **7**, 369.
18. — *ibid.*, 1917, **8**, 55 ; 1928, **19**, 51.
19. J. J. CHINOY, F. W. EDWARDS and H. R. NANJI, *Analyst*, 1934, **59**, 673.
20. H. ECKART, *Chem. Zell. Gewebe*, 1925, **12**, 243.
21. W. S. LONG, *Trans. Kansas Acad. Sci.*, 1916, **28**, 172.
22. H. WEISS, *Zeit. ges. Brauw.*, 1922, **45**, 122.
23. J. C. SMALL, *J. Amer. Chem. Soc.*, 1919, **41**, 107, 113.
24. F. E. DENNY, *J. Assoc. Off. Agric. Chem.*, 1922, **6**, 175.
25. — *Contrib. Boyce Thompson Inst.*, 1934, **6**, 129.
26. J. T. SULLIVAN, *Ind. Eng. Chem. (Anal. Ed.)*, 1935, **7**, 311.
27. TIAN, *Bull. Soc. Chim. France*, 1923, **33**, 898.
28. A. VON ASBOTH, *Repert. Anal. Chem.*, 1887, **7**, 299 ; *Chem.-Ztg.*, 1887, **11**, 785 ; 1889, **13**, 591, 611.
29. C. MONHEIM, *Zeit. angew. Chem.*, 1888, **1**, 126 and 401.
30. A. BAUDRY, *Zeit. Spiritusind.*, 1892, **15**, 41.
31. — *Dingler's Polytechn. J.*, 1892, **285**, 238.
32. L. PELLET and MÉTILLON, *Bull. Assoc. Chim. suc. distil.*, 1906, **24**, 1720.
33. J. EFFRONT, *J. Soc. Chem. Ind.*, 1896, **15**, 923.
34. D. CRISPO, *Ann. chim. anal.*, 1899, **4**, 290.
35. C. MANNICH and K. LENZ, *Zeit. Nahr. Genussm.*, 1920, **40**, 1 ; *Can. J. Res.*, 1934, **11**, 751.
36. C. J. LINTNER, *Zeit. ges. Brauw.*, 1907, **30**, 109.
37. O. WENGLER, *ibid.*, 1908, **31**, 53.
38. C. J. LINTNER, *Zeit. Nahr. Genussm.*, 1908, **16**, 509.
39. — *Zeit. angew. Chem.*, 1912, **25**, 1177.
40. M. CANET and O. DURIEUX, *Bull. Soc. chim. Belg.*, 1907, **21**, 329.
41. J. KÖNIG, W. GREIFENHAGEN, and A. SCHOLL, *Zeit. Nahr. Genussm.*, 1911, **22**, 714.
42. S. HALS and S. HEGGENHANGEN, *Landsw. Vers. Stat.*, 1917, **90**, 391.
43. E. EWERS, *Zeit. öffentl. Chem.*, 1905, **11**, 407 ; **14**, 8 and 150.
44. — *ibid.*, 1910, **15**, 8.

45. E. EWERS, *Zeit. ges. Brauw.*, 1915, **21**, 232.
46. — *ibid.*, 1908, **31**, 250.
47. W. A. NOYES, *et al.*, *J. Amer. Chem. Soc.*, 1904, **26**, 266.
48. W. A. DAVIS and A. J. DAISH, *J. Agric. Sci.*, 1913, **5**, 437; *ibid.*, 1914, **6**, 152.
49. G. S. FRAPS, *J. Assoc. Offic. Agric. Chem.*, 1932, **15**, 304.
50. A. R. LING, *J. Soc. Chem. Ind.*, 1923, **42**, 48T.
51. E. WALDESCHMIDT-LEITZ, M. REICHEL, and A. PURR, *Naturwiss.*, 1932, **20**, 254.
52. G. NORDH and E. OHLSSON, *Zeit. physiol. Chem.*, 1932, **204**, 89.
53. A. R. LING, D. R. NANJI, and W. J. HARPER, *J. Inst. Brewing*, 1924, **30**, 838.
54. A. R. LING and D. R. NANJI, *Biochem. J.*, 1923, **17**, 593.
55. G. A. VAN KLINKENBERG, *Zeit. physiol. Chem.*, 1932, **212**, 173.
56. T. C. TAYLOR and H. A. IDDLES, *Ind. Eng. Chem.*, 1926, **18**, 713.
57. T. C. TAYLOR and R. P. WALTON, *J. Amer. Chem. Soc.*, 1929, **51**, 3431.
58. A. R. LING, *J. Inst. Brewing*, 1922, **28**, 828 and 851.
59. H. T. BROWN, *Trans. Guinness Res. Labs.*, 1903, **1**, 79.
60. H. T. BROWN and HERON, *Chem. Soc. Trans.*, 1879, **35**, 601.
61. S. NISHIMURA, *Chem. Zell. Gewebe*, 1925, **12**, 202.
62. W. A. DAVIS and A. J. DAISH, *J. Agric. Sci.*, 1914, **6**, 152.
63. I. D. COLLINS, *Science*, 1927, **66**, 430.
64. O. LEHMANN, *Planta*, 1931, **13**, 575.
65. R. P. WALTON and M. R. COE, *J. Agric. Res.*, 1923, **23**, 995.
66. M. R. COE, *J. Assoc. Offic. Agric. Chem.*, 1926, **9**, 147.
67. HARTMANN and HILLIG, *ibid.*, 1926, **9**, 482.
68. W. WHALE, *Analyst*, 1938, **63**, 328, 421.
69. A. HOCK, *Biochem. Zeit.*, 1937, 294, 336.
70. M. MAERKER, *Chem.-Ztg.*, 1885, **9**, 319.
71. R. M. COE, *J. Assoc. Offic. Agric. Chem.*, 1923-24, **7**, 341.
72. — *ibid.*, 1924-25, **8**, 358.
73. G. P. WALTON and R. M. COE, *ibid.*, 1923-24, **7**, 995.
74. H. LÜERS and F. WIENINGER, *Zeit. ges. Brauw.*, 1925, **48**, 35.
75. A. R. LING, E. H. CALLOW, and W. J. PRICE, *J. Soc. Chem. Ind.*, 1923, **42**, 48.
76. A. R. LING and W. J. PRICE, *J. Inst. Brew.*, 1923, **29**, 732.
77. R. KUTSCHA, *Woch. Brauer.*, 1917, **34**, 277, 290, 294, 304, 313, 323, 332, 339, 350, 359, 368, 375, 381, 391, 398 and 406.
78. O. WOLFF, *Zeit. Spiritusind.*, 1924, **47**, 178.
79. K. ALPERS and H. ZIEGENSPECK, *Zeit. Unters. Nahr.-Genussm.*, 1923, **45**, 163.
80. W. WHALE, *Analyst*, 1939, **64**, 588.
81. P. FLEURY and G. BOYELDIEU, *Ann. Falsif.*, 1928, **21**, 124.
82. L. W. JIRAK, *Zeit. Spiritusind.*, 1935, **58**, 81.
83. V. JAHN, *Zeit. Unters. Lebensm.*, 1927, **53**, 262.
84. G. RANKOFF, *ibid.*, 1927, **53**, 138.
85. M. I. KNYAGINICHEV and V. K. PALILOVA, *Biokhimiya*, 1939, **4**, 423.
86. J. KAVČIČ, *Kolloidchem. Beih.*, 1930, **30**, 406.
87. S. SUGIZAKI, *J. Agric. Chem. Soc., Japan*, 1939, **16**, 1173.
88. D. W. KENT-JONES, 'Modern Cereal Chemistry,' 3rd ed., The Northern Publ. Co. Ltd., Liverpool, 1939.

89. W. JAGO and W. C. JAGO, 'The Technology of Breadmaking,' Simpkin, Marshall, Hamilton, Kent & Co. Ltd., 1911.
90. R. HUTCHINSON, 'Food and the Principles of Dietetics,' Arnold, London, 1927.
91. C. B. MORISON, *Baking Technol.*, No. 8, 232.
92. T. B. WOOD, 'Farm Crops,' 1925, I, 248, Gresham Publ. Co. Ltd., London.
93. JOACHIM, *Trop. Agric.*, 1938, 90, 3.
94. A. W. MARSDEN, *J. Roy. Agric. Soc.*, 1941, 66, 98.
95. F. W. FRIEZE, *Zeit. Unters. Leb.*, 1938, 75, 566.
96. ANON, *Farming*, S. Africa, 1939, 14, 404.
97. J. CARLES, *Compt. rend.*, 1940, 210, 111.
98. R. A. BERRY, 'Farm Crops,' 1925, 1, 188. Gresham Publ. Co. Ltd., London.
99. ANON, *Bull. Imperial Inst.*, 1917.
100. M. H. FRENCH, *Ann. Rep. Dept. Vet. Sci. Animal Husbandry, Tanganyika Territ.*, 1938. Pt. 2, 37; Publ. 1939.
101. V. SADASIVAN and A. SREENIVASAN, *Indian J. Agric. Sci.*, 1938, 8, 807. (Analyses of different varieties of rice.)
102. W. D. RAYMOND, W. JOJO and Z. NICODEMUS, *East Afric. Agric. J.*, 1941, 6, No. 3, 154.
103. MOORE and PARTRIDGE, 'Aids to the Analysis of Foods and Drugs,' 1934.
104. LEACH, 'Food Inspection and Analysis,' Chapman & Hall, London, 1920, 282.
105. WINTON and WINTON, 'The Structure and Composition of Foods,' Vol. II, Chapman & Hall, London, 1935.
106. SCHRADER, *Arch. Tierernahr. Tierzucht*, 1933, 9, 524; *Nutr. Abst. and Reviews*, 1933, 3, 955.
107. BARKER, D. S. I. R., Food Invest. Board Rept., 1931, 78, H.M.S.O.
108. SINGH and MATHER, *Ann. Appl. Biol.*, 1937, 24, 469.
109. MANGOLD, via *Chem. Abst.*, 1936, 30, 6840.
110. HEADDON, *Colo. Expt. Stat. Bull.*, 1924, 291,
111. GOLDTHWAITE, *ibid.*, 1925, 296.
112. RATHSACK, 'Der Speisewert der Kartoffel,' Berlin, 1935, 47.
113. METZGER *et al.*, *Proc. Am. Soc. Hort. Sci.*, 1937, 35, 635.
114. J. L. GAVIN and M. H. QUESKI, *Ind. Eng. Chem.*, 1941, 33, 640.
115. G. J. LEY, J. H. PAYNE and D. W. EDWARDS, *Hawaii Agric. Exp. Stat., Ann. Rept.*, 1937, 49.
116. C. G. HOPKINS, *Canad. J. Res.*, 1934, 11, 751.
117. M. P. ETHERIDGE, *J. Assoc. Off. Agr. Chem.*, 1941, 113.
118. R. T. BALCH, *Ind. Eng. Chem. (Anal. Ed.)*, 1941, 13, 246.
119. L. S. WEATHERBY and D. G. SORBER, *Ind. Eng. Chem.*, 1931, 23, 1421.

#### ADDITIONAL REFERENCES

- E. MUNSEY, *J. Assoc. Offic. Agric. Chem.*, 1937, 20, 360. (Determination of starch in flour.)
- G. STEINHOFF, *Textilber. (Eng. Ed.)*, 1935, 16, 73. (Specifications for potato starch.)
- H. LUEHRIG, *Pharm. Zentr.*, 1921, 62, 141. (Ewers' method gives the most consistent results.)

- C. FAULENBACH, *Zeit. physiol. Chem.*, 1882, **83**, 510. (Enzymatic, followed by acid hydrolysis, then sugar estimated with Fehling's solution.)
- O. REINKE, *Zeit. anal. Chem.*, 1890, **29**, 472. (Similar method to Faulenbach's.)
- C. J. LINTNER and G. DÜLL, *Zeit. angew. Chem.*, 1891, **4**, 537. (Suggests factor of 0.94 to convert dextrose figure to starch.)
- E. HORTON, *J. Agr. Sci.*, 1921, **11**, 240. (Concludes use of taka-diastase in estimating starch requires control tests with each set of analyses.)
- A. DUERING, *Zeit. Unters. Nahr.-Genussm.*, 1924, **47**, 248. (Close agreement obtained between Ewers' method and Mayrhofer's gravimetric method.)
- A. LASERDA, *Rev. soc. brasilquím.*, 1938, **7**, 27. (Determination of starch in flour.)
- J. STRAUB and A. MIDDELBECK, *Chem. Weekblad.*, 1938, **35**, 743. (Chemistry of alkaline copper estimation of sugars discussed.)
- M. V. JONESCU and L. GAAL, *An. Inst. cerc. agron. Rom.*, 1936, **8**, 453. (Methods of estimating glucose compared.)
- V. DORFMAN, *Spirtovod. Prom.*, 1937, **14**, No. 3, 25. (Modified Maerker method for starch determination.)
- ANON, *Jokhels'son Voprosy Pitaniya*, 1937, **6**, No. 2, 99. (Determination of wheat and potato starches in sausage products.)
- C. G. HOPKINS, *J. Assoc. Offic. Agric. Chem.*, 1939, **22**, 523. (Compares methods of Chinoy (ref. 19), Mannich-Lenz (ref. 35) and Lintner-Schwarz (*Zeit. Brauw.*, 1913, Nos. 8 and 9), but draws no conclusions. Chooses modification of last method.)
- SCHWONKE, *Zeit. Spiritusind.*, 1929, **52**, 198. (Sampling of damaged tubers.)
- B. LAMPE and W. KILP, *ibid.*, 1929, **52**, 199. (Sampling of damaged tubers.)
- E. W. STONE, *J. Amer. Chem. Soc.*, 1897, **19**, 183, 347. (Estimation of sugars and starch in foodstuffs described.)
- F. W. TRAPHAGEN and W. M. COBLEIGH, *ibid.*, 1899, **21**, 369. (Fehling's solution used and cuprous oxide dissolved in ferrous sulphate and sulphuric acid and titrated with potassium permanganate.)
- W. C. McVEY, *J. Assoc. Offic. Agr. Chem.*, 1941, **24**, 928. (Quicker and slightly more accurate method than *Assoc. Offic. Agr. Chem.*, 'Method of Analysis,' 1940, 378, for starch in, meat products.)



## CHAPTER 3

## THE ANALYSIS OF DEXTRIN

A FACTORY may make dextrans either for sale or to be used as the raw material for manufacturing a product in some other part of the factory, but in either case the manufacture and the characteristics of the dextrans must be kept as near to standard as possible. This can be done only by means of constant checking at every stage of the process, from receiving the raw starch to packing the re-moistened dextrin produced from it. A purchased dextrin should be examined against a sample of standard dextrin known by previous experience to be suitable for the purpose in mind, and once a good source of supply is found, it is often preferable to deal exclusively with that firm.

If a sample of dextrin is received for matching purposes all the information possible should be obtained from the chemical and physical tests, but where a standard process is being worked, it is only necessary to carry out a few tests at the various stages of the process to determine the end-point of a particular operation. The tests in this case would include determinations of moisture in the raw starch, which should not exceed certain maxima, varying with the particular starch used, the iodine reaction and the viscosity of the dextrin in the roaster, the amount of sugar after roasting, and the amount of water present after re-moistening the dextrin. The following details are set forth merely as a groundwork for the examination of a dextrin for any particular purpose, and are not intended to cover every case which is likely to arise.

The analytical and physical examination of starch and dextrin has been dealt with by F. L. P. Krizkovsky.<sup>11</sup> W. Hönsch<sup>12</sup> states that the figure for the specific gravity of dextrin given in Beilstein's 'Handbuch der Organischen Chemie,' 1893, 1, 1088, 3rd edition, is a misquotation, and finds that fine white dextrin has  $D_{20}^{20} = 1.593$ , fine yellow 1.527, superior yellow, thick-boiling 1.561, and thin-boiling 1.542. The specific gravity appears to vary with the method of preparation and the starch used, but a mean value of 1.556 may be assumed. In practice, however, the specific gravity of dextrin is rarely, if ever, required.

**Appearance.**—Dextrans which have been made by an acid treatment are invariably lighter in colour than those made by merely roasting the raw starch, which produces products analogous to the dextrans known as British gums. The 'acid dextrans' are

white, cream, yellow or buff powders, and those made by heat alone are brownish. A further modification is 'crystal gum,' which is a dextrin that has been dissolved, the solution treated with activated charcoal, filtered, and evaporated to dryness. This type of product resembles gum-arabic in appearance, and constitutes a special grade for use in very high-class work which can stand the extra price that its use entails. Some dextrins, made by one of the wet processes, appear as syrupy liquids, or as amorphous masses which have a flaky appearance due to their solution having been evaporated after the conversion on a drum- or a band-dryer.

The coloured varieties of dextrin are almost completely soluble in cold water, and invariably soluble in hot water, viscous pastes to slightly syrupy liquids being obtained according to the concentration and the degree of conversion. These solutions, on standing, sometimes show 'setback,' i.e. they thicken and develop cloudiness. In dilute solutions they may show a clear supernatant layer of liquid. Such dextrins are termed 'unstable,' and their use may cause trouble, especially when employed in adhesives for use on machines where the workability has been adjusted to an optimum value by means of careful formulation. Should such an adhesive increase in viscosity on standing, the other properties necessary for satisfactory running of the machine are thrown out of balance, and a poor performance on the part of the adhesive results. The cloudiness in this case is probably caused by the reversion of unconverted amylose, present as soluble starch, due to the conversion being carried out too rapidly.

The finer the quality of the starch used for the manufacture of the dextrin the better will be the appearance of the finished product. Thus good potato or tapioca starch will give a lustrous dextrin, and the lustre is greatly impaired if the starch used is inferior. The use of maize or wheat starch results in the production of a matt-looking dextrin, irrespective of the quality of the starch employed.

The acid used as the catalyst has some effect on the appearance of the dextrin made with its aid, and in general nitric acid imparts a yellow colour, hydrochloric acid a reddish-yellow tinge, whilst sulphuric acid, which is sometimes used, imparts a brownish tinge. The colour of the dextrin gives an approximate idea of the degree of conversion, but very little more information can be obtained without the use of a microscope.

Although the dextrin under examination may be soluble in water, its dissolution can be impeded by the use of glycerol or glycol, or by suspension in alcohol. The effect of roasting upon the shape and structure of the starch grain can therefore be

examined. The granules of the dextrin, if not too strongly converted, appear very similar to those of the starch from which it is made, but by adjusting the amount of glycerol and water it can be caused to disintegrate slowly. As mentioned on page 377, the disintegration consists in the peeling off of layers, which float away and dissolve, and in this respect dextrins differ from soluble starches and can thus be differentiated from them. The dextrin so examined should be lightly stained with iodine to bring it into contrast with the rest of the field (see Photomicrograph No. 13).

An interesting example of the value of this method came to the notice of the author and is described on p. 372.

With dextrins in solution, or dried from solution, very little information as to the starch used can be obtained by the microscope, but in these cases the odour of the heated solution of the particular dextrin may assist, maize and potato products being readily identified.

The presence of black specks and dirt should be looked for, as their presence in excessive amounts renders a dextrin unsuitable for certain types of work, for example, in paper-surfacing. Tactile examination gives some information, as potato dextrins do not cling to the hand if dry, and are harsher to the touch than the dextrins made from cereals or tapioca, which do cling to the hand.

**Smell and Flavour.**—Most types of dextrin have a characteristic odour, and after a little practice it is possible to distinguish one from another. The odour of dextrin solutions is sometimes very marked, and if a solution cannot be made, a simple test is to moisten the palm of one hand with saliva, place a little dextrin on it, and rub vigorously with a finger until it is quite warm; on cupping the hand over the nose, the characteristic smell of the dextrin is generally quite distinct. The characteristic odour of potato dextrin has been described as resembling that of cucumbers, but the odour of any dextrin is liable to be modified on ageing, the brown varieties of dextrin having the strongest odour, which is somewhat earthy with a faint suggestion of charring. The nearer a dextrin approaches a neutral conversion, i.e. the smaller the amount of acid used as a catalyst, the nearer the smell approaches an 'earthy' odour. By placing and manipulating a small amount of the dextrin on the tip of the tongue, some idea of the acid used in its manufacture, and of the solubility and sugar-content, may be obtained by experienced workers, although the author has found even these to err occasionally.

**Moisture Estimation.**—The moisture present in a sample may be estimated by direct heating to constant weight in an

oven. A temperature of  $105^{\circ}$  C. for four hours is generally sufficient to drive off practically all the moisture present in a 5 gm. sample. When a dextrin in liquid form is being examined it is preferable to mix it with a known weight of ignited sand, so that the sand is present in great excess, and then to dry very gently at first, gradually bringing the temperature up to  $105^{\circ}$  C., at which it is maintained until the loss in weight is constant. While cooling, the hot dish containing the dextrin should be kept in a desiccator over concentrated sulphuric acid, as dry dextrin rapidly absorbs moisture from the air.

It is very difficult to remove all the moisture from dextrin. If a sample of dextrin be taken straight from the roaster, moistened with a known weight of water and then heated to expel this moisture in the usual manner, there is generally a difference of 1 or 2 per cent. between the calculated moisture-content and that found by heating. L. Maquenne has examined this point and records that by drying at  $120^{\circ}$  C. for one hour, followed by two hours' drying at  $100^{\circ}$  C. in a current of dried air, the amount of moisture found was 1 per cent. higher than by the usual methods (see p. 382).

From this it may be considered that methods in which the amount of moisture present originally is found by difference are preferable to the method of direct drying. Such methods are quicker to carry out, but the accuracy is limited by the facts that several operations are involved which may result in an accumulative error, and one is dependent on the accuracy of at least two instruments, i.e. the thermometer and the hydrometer.

The method of Brix is a hydrometric one, in which the reading on the hydrometer is multiplied by a factor to give the amount of solid matter taken originally to make the solution. E. Preuss<sup>9</sup> has modified this method, and dissolves from 4.5 to 20 per cent. of the dextrin in warm water, cools the solution to  $17.5^{\circ}$  C., and makes the volume up to 100 ml. The specific gravities of solutions of various strengths between the above two concentrations were found to lie between 1.018 and 1.0799, and Preuss gives a table showing the connection between the specific gravity thus found and the moisture-content. For ordinary control or sampling analysis, the method of direct heating is generally employed and sufficiently accurate.

Powdered dextrins usually contain between 8.0 to 14.5 per cent. of moisture, the higher figure being typical of the less converted products. Most yellow dextrins left in contact with the air in a thin layer for several days take up about 10 per cent. moisture in the first week, and at the end of a further week only

an additional 1 to 1.5 per cent. These figures were obtained on one sample of a yellow potato dextrin: it was found that after the first fortnight there was a very slow uptake of moisture, which amounted to only about 0.8 per cent. after six weeks.

**Coloration with Iodine.**—Practically all dextrans give some coloration with dilute solutions of iodine, ranging from a deep blue, similar to that given by starch and persisting for some time, to a pale reddish-brown that rapidly fades until the solution is colourless. The reaction affords some measure of the degree of conversion, and for factory control, empirical standards applicable to the products of the factory must be drawn up. A small spoon with a round bowl and holding about 0.1 gm. of dextrin is very useful, this amount of dextrin being put into a test-tube of about 20 ml. capacity and containing about 15 ml. of water.<sup>1</sup> To the solution is added N/50 iodine solution in potassium iodide, drop by drop, and after shaking, the colour is examined against that of the standard sample. It must be borne in mind when making this test on dextrin from the roasters that the colour of the solution tends to contain a little more red, which is fugitive, than when the same sample has been re-moistened. It would appear that some sort of oxidation process takes place after the cooling and during the time of re-moistening, which has the effect of slightly altering the colour given with iodine. The number of drops of iodine added, the colour produced, and also the approximate time of fading should be noticed, as all give information concerning the progress of the conversion. The fading of the colour is probably due to the iodine present being reduced by the aldehyde groups of the sugar formed in the roasting, and therefore a very rough idea as to the formation of sugar may be obtained from the rate at which the colour disappears. A 'heavy cooking' white dextrin will give a deep blue coloration with two drops of the solution, and when held to the light will show a deeper blue vertical zone at the centre of the tube. As the conversion proceeds this zone disappears, the blue colour becoming more uniform and darker. The time taken by the colour of both the solution and the deposit to fade is also much quicker, and less deposit is observed.

Some typical results obtained with the iodine solution on dextrin from the roasters are given below:—

'Heavy cooking' white dextrin: 1 drop; deep blue; fades very slowly.

'Medium cooking' white dextrin: 2 drops; deep blue; fades more quickly and shows less deposit than above.

*Canary dextrin, medium conversion* : 4 drops ; violet ; no deposit.  
*Yellow dextrin* : 5-6 drops ; red-violet ; fades in about 10-15 minutes.

For factory control O. Saare<sup>1</sup> heats 1 gm. of the dextrin in 5 ml. of water until dissolved, dilutes the solution to 100 ml. with cold water, and adds one drop of N/10 iodine. He notes the coloration produced by the falling drop, after which the tubes are shaken and the colour of the homogeneous solution observed.

Saare's results showing the colours obtained and the amount of soluble dextrans present in various samples are appended :—

<i>Falling Drop</i>	<i>After Shaking</i>	<i>Percentage of Soluble Dextrin</i>
Blue . . . . .	Blue . . . . .	nil
Blue . . . . .	Pale blue . . . . .	6.1
Blue-violet . . . . .	Pale blue . . . . .	15.2
Violet . . . . .	Violet . . . . .	39.2
Violet-red . . . . .	Pale violet . . . . .	49.5
Reddish-brown . . . . .	Colourless . . . . .	62.5

It must be borne in mind that in the absence of a rational method of measuring the colour values, the opinion of two different observers on the same colour may vary considerably. The highly converted dextrans that are completely soluble in cold water show very little differences towards N/10 iodine solution, but the use of a N/50 iodine solution allows of a better differentiation, and the rates of fading also give useful indications. With the most highly converted dextrans, however, scarcely any differences are noted and the method ceases to be of value.

**Viscosity and Stability.**—The determination of the viscosity of the solution made by dissolving a known amount of dextrin in water is extremely valuable in factory control. Such determinations can be carried out in a few minutes. For white dextrans a 50 per cent. solution can be employed, but for well-converted dextrans a 66.6 per cent. or even a 75 per cent. solution can be taken.

The dextrin is taken from the roaster and rapidly weighed, added to a known weight of water, and heated to 80° C. until completely dissolved. It is then cooled to 50° C. and the viscosity determined with a viscometer of the pipette type, i.e. one in which the time for the liquid surface to pass between two marks on the top and bottom tubes, respectively, of a pipette is measured. The pipette is enclosed in a hot-water jacket maintained at 50° C. If the type of dextrin to be matched contains a known moisture-content, the anhydrous sample from the roaster is weighed out and sufficient water added to ensure the final solution containing 50 or 66.6 per

cent. of the re-moistened dextrin. For example, assuming that the dextrin to be matched contains 10 per cent. moisture, 90 gm. of the sample from the roaster is dissolved in 110 gm. water, giving 200 gm. of dextrin solution containing 100 gm. of re-moistened dextrin containing 10 per cent. moisture. The viscosity is determined, and if required the conversion continued until the viscosity of a sample determined in this manner is the same, or just a little greater, than that of the sample being matched, when the roaster is discharged.

To determine stability, the solution used for the viscosity determination is set aside for 24 hours and then examined to see if any appreciable increase in the viscosity has taken place and if any cloudiness can be observed in the solution made from a yellow dextrin.<sup>1</sup> In yellow dextrans that have been 'burnt,' i.e. have had insufficient acid added as catalyst and have therefore required a higher temperature or longer time for conversion, a distinct cloudiness is often observed which is sometimes so marked as to make the solution look like a yellowish paste. Such dextrans may have the same solubility as the standard and the same viscosity when first made, but this may increase on standing. A good dextrin should show scarcely any appreciable increase in viscosity and have practically the same appearance as when set aside the previous day.

If the solution be kept for several days a large number of fine cracks may appear on the surface, denoting the presence of an excessive amount of sugar (see p. 288) which may render the dextrin unsuitable for use in adhesives.

**Mineral Matter.**—A good dextrin should be free from sand, grit, or any other extraneous matter. A determination of the ash will give an indication of the presence of any excessive amounts of the first two substances, as the ash-content of a dextrin is very close to that of the starch from which it is made (after making allowance for the difference in moisture-content between the two substances). Thus potato dextrans have an ash-content of about 0.5 per cent., maize dextrans about 0.1-0.2 per cent., and tapioca dextrans 0.2-0.6, or even a little more. In each case about 60 per cent. of the ash is soluble in hot water or dilute acid.

For determining ash-content, 10 gm. of the dextrin are incinerated in the muffle furnace at a fairly low temperature, and before the operation is complete the contents of the dish are moistened with one or two drops of ammonium nitrate solution and re-ignited. Calcium is often present in the ash and also sulphates, chlorides, and phosphates.

Some dextrans met with in commerce are of the prepared type,

and contain certain ingredients so that they give an adhesive by mere solution in water. The so-called 'arable gums' are of this type. Such dextrans contain soda ash and sometimes borax, and others contain agents which, by virtue of a bleaching effect, lighten the colour of the solution. One process for attaining this end is to spray the dextrin in the re-moistening plant with a solution of sulphur dioxide and, at a later stage, borax solution or one containing sodium bisulphite or persulphate. The smell and colour of light-coloured dextrans are reduced by this processing, but with dark-coloured dextrans the reduction in the intensity of colour is not worth the extra process; in any case, the colour of the dextrin invariably returns when alkali is added to the solution of a dextrin that has been bleached by reducing agents.

The presence of these extraneous compounds may be suspected from the ash-content, and generally an examination of the ash will give indications of the previous history of the sample.

**Acidity.**—In making a dextrin by the torrification process, a certain amount of the acid catalyst is lost in the preliminary drying process, and a still further loss occurs in the course of the roasting, whereby in general the higher the temperature of the conversion, the less is the acidity of the finished product.

To determine the acidity, 10 gm. dextrin are dissolved in 100 ml. water and titrated with N/10 caustic soda, using phenolphthalein as indicator (see, however, p. 386). The figure for the titration should not exceed 5 ml. of the soda solution. The following figures were obtained from three samples of tapioca dextrans :—

*Lightly converted dextrin* : practically neutral.

*Moderately converted dextrin* : 0.7 ml. N/10 NaOH.

*Well-converted dextrin* : 1.6 ml. N/10 NaOH.

The first dextrin had little acid added at the start and a high temperature was needed to bring about the required conversion; the second dextrin had more acid and needed a somewhat lower temperature, whilst the third received still more acid, and was converted at a relative low temperature; thus the time of roasting has some effect on the final acidity factor. Sometimes the acidity may be due to the presence of sulphur dioxide, and according to H. Tryller, it depends largely on the conditions of titration (see p. 386). Little information of practical value is obtained by determining the acidity, and it certainly gives no indication whether the dextrin has been made by the acid process or not.

**Solubility in Cold Water.**<sup>2</sup>—Twenty grams of the dextrin are well shaken with 100 ml. of water and when all the large lumps



have been dispersed it is diluted with another 100 ml. of water, shaken for a further ten minutes, and then rapidly filtered through a dry filter paper. Finally, the density of the filtrate is taken with the Brix hydrometer, the reading of which multiplied by 10 gives the percentage of dextrin soluble in cold water.

The percentage of dextrin soluble in hot water need rarely be determined, as most dextrans are completely soluble.

**Determination of Starch in Dextrin.**—The solubility and physical behaviour of dextrin may be strongly influenced by the presence of unchanged or soluble starch, so that a method of determining starch is required. M. C. Lamb and A. Harvey<sup>13</sup> determine the amount of ash and sugar in a cold-water extract of a dextrin and by difference obtain the weight of dextrin. The insoluble matter they return as starch. F. W. Babington, A. Tingle, and C. E. Watson<sup>14</sup> consider that imperfect separation of the dextrin from the starch, slow filtration, and the amount of starch which passes into solution, constitute serious disadvantages of the method. They determine the starch indirectly by precipitating it with a half-saturated solution of barium hydroxide and estimating the dextrin in the filtrate, making allowances for the ash-content of the soluble material.

Caesar and Cushing<sup>21</sup> have made a series of maize and cassava dextrans, withdrawing samples at intervals and testing them for solubility, alkali-labile value (see p. 387) and the barium hydroxide value by the method of Babington, Tingle and Watson. These values were plotted against time of conversion. The results vary within certain limits and with some products vary linearly with the concentration of barium hydroxide. This tends to show that the barium hydroxide method for determining dextrin is arbitrary. It appears to be primarily an index of the relative size of the amylaceous micelles. These workers suggest that the alkali-labile value curve probably affords the most satisfactory approach to the classification of a starch or a dextrin.

The method of J. J. Chinoy, F. W. Edwards, H. R. Nanji,<sup>15</sup> makes use of the fact that 'starch iodide' is quantitatively precipitated by 95 per cent. alcohol and certain coagulants. F. W. Edwards and co-workers<sup>16</sup> elaborated on this method as follows: One gram of the material is gelatinised with 0.7 per cent. potassium hydroxide solution, cooled and diluted to 200 ml. Ten ml. of this solution are neutralised to phenolphthalein with dilute acetic acid and 1 ml. of 0.1 N iodine solution is added, followed by 40 ml. of a reagent made by mixing 4 ml. of 10 per cent. potassium acetate solution to a 100 ml. of 50 per cent. (by volume) alcohol. Ten minutes later the supernatant liquor is

decanted through a tared alundum crucible, the residue in the beaker is washed several times by decantation with 50 per cent. alcohol, and twice with 95 per cent. alcohol, transferred to the crucible using more 95 per cent. alcohol, dried and re-weighed. The washing liquid at each decantation is, of course, poured into the crucible. Using this method, the weight of starch iodide found multiplied by 0.8865 gives the amount of starch present.

**Determination of Dextrin in Presence of Starch.**—One gram is pasted with 5 ml. of cold water and after adding 100 ml. of hot water the solution is gently boiled for 30 minutes. It is then transferred to a 200 ml. flask, cooled, and made up to 200 ml. with the cold-water washings of the beaker.

Twenty ml. of the solution are transferred to a graduated 100 ml.-graduated flask, 2 ml. of 0.1 N iodine solution added, and the liquid made up to the mark with the potassium acetate/alcohol reagent mentioned above. After shaking the flask and standing for 5 minutes the suspension is filtered. Fifty ml. of the filtrate are evaporated to 3-4 ml., cooled, and the dextrin precipitated by the addition of 100 ml. of 95 per cent. alcohol. After standing overnight the suspension is filtered through a tared crucible of medium size, washed with 95 per cent. alcohol, dried and re-weighed.

**Determination of Sugars.**—The amount of sugar present in a dextrin may be determined directly, using Fehling's solution with methylene blue as internal indicator. Although some dextrans have a slight reducing action, for general purposes it may be ignored. The sugar found is returned as dextrose, although some of the reducing matter present may be maltose, especially in long 'roasts' or in roasts carried out at high temperatures. The cupric reducing powers of certain dextrans have been determined by G. W. Rolfe<sup>3</sup>; some of his figures are as follows:—

*White potato dextrin* : 0.0778.

*Medium-white potato dextrin* : 0.0342.

*Light-canary dextrin* : 0.0673.

*Well-converted tapioca dextrin* : 0.0374.

*Fairly-well converted maize dextrin* : 0.0802.

This worker found no connection between the cupric reducing power and the specific rotation of 13 commercial samples of dextrin. O. Philipp<sup>20</sup> finds that the amount of dextrose formed depends on the amount of acid used (see p. 240). Rolfe<sup>3</sup> considers that a figure greater than 2 per cent. for this value indicates the use of acid as the catalyst, and this the author has confirmed.

One of the most widely adopted methods of estimating sugars

is due to Lane and Eynon<sup>17</sup> and is carried out as follows: Add to a known amount of the Fehling's solution (Soxhlet's modification), say 10 or 25 ml. contained in a 400 ml. flask, enough of the solution to reduce nearly all the Fehling's solution and boil gently for two minutes, add one or two drops of a 1 per cent. methylene-blue solution and run in the solution under examination from a burette a few drops at a time; not more than 1 ml. or less than 0.5 ml. should be necessary. The end-point is reached when the boiling solution in the flask goes green and then suddenly changes to orange. With a fresh portion of the solution the determination is repeated until the end-point is reached, a high degree of accuracy being attainable. The strength of the sugar solution should be such that not less than 15 ml. or more than 50 ml. of it is required for the volume of the Fehling's solution used (10 ml. or 25 ml.). The final titration should be carried out as quickly as is compatible with accuracy, and the liquid should be gently boiling all the time to exclude air, which causes the colour of the indicator to return. For this reason the determination is carried out in a flask and not in a dish as directed in some literature. By reference to the tables worked out by Lane and Eynon the amount of sugar present can be found. The accuracy of this method has been checked by H. T. S. Britton and L. Phillips,<sup>22</sup> using a potentiometric titration method.

Another indicator which may be used is that of Ling, which is prepared by dissolving 1.5 gm. ammonium thiocyanate and 1.0 gm. ferrous ammonium sulphate in 10 ml. water at about 40° C., cooling the solution, and adding 5 ml. concentrated hydrochloric acid. Should this solution show a reddish tinge, a little zinc dust may be added to decolorise it. This indicator is used externally. The end-point is reached when no red coloration is produced on mixing the two solutions. The external indicator, however, is not so satisfactory as the methylene-blue indicator. Stieglitz and Horne<sup>18</sup> determine the end-point electrometrically and get results closely agreeing with those given by the above method.

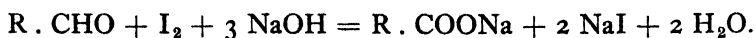
The method of G. M. Kline, S. Slater and S. F. Acree<sup>4-5</sup> for the determination of aldose sugars may be used to estimate the amount of dextrose in dextrin when the former is present to the extent of 4 per cent. or less, and is stated to be accurate to 0.05 per cent. The method depends upon the fact that alkali added slowly to an aldose sugar solution containing iodine tends to react with the sugar in preference to forming the iodate, which does not appear until the oxidation of sugar is complete. H. S. Miller<sup>6</sup> has pointed out that ketose sugars are negligibly affected and F. A.

Cajori <sup>7</sup> notes that in this reaction maltose is oxidised more slowly than glucose.

The test is carried out as follows: To 2 gm. dextrin, if necessary previously neutralised with a predetermined amount of alkali in the absence of an indicator, are added 11 ml. N/10 iodine followed drop by drop by 16.5 ml. N/10 NaOH. After two minutes the solution is acidified with N/10 sulphuric or hydrochloric acid and the liberated iodine titrated with N/10 sodium thiosulphate using starch indicator. If the excess iodine does not lie between the limits of 1.5-3.0 ml. of N/10 thiosulphate solution the process is repeated with different quantities of iodine and sodium hydroxide solution, which must, however, always be present in the ratio 2 : 3. The iodine and sodium hydroxide solutions may be added in portions of 4 ml. and 6 ml. respectively, shaking after each addition, and the temperature should be approximately 25° C. For pure dextrose solutions 8 minutes is the best length of time to leave the solution before acidification; less time than this tends to give incomplete oxidation and a longer time tends to produce over-oxidation.

If the iodine liberated requires more than 3 ml. of 0.1 N thiosulphate solution too much iodine has been added, resulting in over-oxidation, but if less than 1 ml. is required insufficient has been added. After a preliminary trial the amount of iodine solution to be taken for a second time can be estimated by adding 2 ml. to the previous amount used less the number of ml. of thiosulphate solution required.

After titrating the iodine, the excess acid present may be titrated against phenolphthalein, and a figure thus obtained for the amount of sodium hydroxide used in the reaction. This figure should act as a check on the value for the amount of iodine used, as will be seen from the equation:



Each ml. of 0.1 N iodine solution used represents 0.009 gm. of dextrose.

When adding the alkali the liquid should be well shaken. The best indication of the end-point is a rapid jump in the amount of iodate present, as a small amount of iodine (detectable with starch) can exist in solution with unoxidised sugar. An iodate-content equivalent to 2 ml. or more of 0.1 N thiosulphate solution shows that the end-point has been reached, whereas a low iodate-content shows that insufficient iodine has been added.

Braun and Bleyer <sup>8</sup> consider that it is very doubtful whether many dextrans do contain dextrose and that any reducing power

shown by solutions of these substances are due to the dextrans themselves; therefore the figures obtained by the usual Fehling's method are quite empirical, whilst those obtained by the above method are stoichiometric in terms of oxidation of the CHO-groups. Actually, the figures obtained by both methods are in close agreement.

H. S. Miller<sup>6</sup> has shown that lactose is oxidised more slowly than dextrose, and points out that if sodium carbonate is used as the alkali the oxidation of maltose is appreciably slower than that of dextrose. The above method therefore offers a good means of determining the various sugars, sometimes in the presence of one another.

F. W. Edwards and co-workers<sup>16</sup> find that the method of C. L. Hinton and T. Macara<sup>19</sup> gives reliable results. In this method 10 ml. of a 1 per cent. solution of the dextrin are diluted to 50 ml., after which 1 ml. of 0.1 N potassium hydroxide solution, 2 ml. potassium iodide solution (10 per cent.) and 10 ml. of Chloramine T solution (7.1 gm./l.) are added in that order. At the end of 90 minutes the solution is acidified with sulphuric acid and the liberated iodine titrated with 0.02 N sodium thiosulphate. A blank determination should also be carried out without the dextrin. Every ml. of 0.02 N sodium thiosulphate used represents 1.8002 mg. of dextrose or, in the case of dextrans produced with enzymes, 3.148 mg. of maltose.

**Detection of Dextrin in Presence of Glue.**—Occasionally a dextrin is used to adulterate an animal or fish glue, or it may be added for some particular and more honest purpose. Therefore, a method for its examination and estimation may be briefly noticed. When a glue contains added dextrin the addition may often be detected by a microscopical examination, providing the sample has not been heated too long and the dextrinisation of the starch has not been carried too far. The iodine test may or may not give an indication of the presence of dextrin, depending on the amount of conversion and the colour of the glue, which may mask the reaction if too dark.

J. Alexander<sup>10</sup> has evolved a method which overcomes these difficulties and allows of the determination of the amount of dextrin added: 0.6 gm. of glue are soaked in 10 ml. of dilute hydrochloric acid (5 pts. of conc. hydrochloric acid to 100 pts. water) and the mixture hydrolysed by heating at 60° C. After hydrolysis the solution is made up to 50 ml., neutralised, and the dextrose estimated with Benedict's solution. The figure obtained for the amount of dextrose present multiplied by 0.9 gives the amount of dextrin present.

**Chlorine.**—The presence of chlorides or of hypochlorous acid is sometimes observed and for many purposes may be undesirable. The presence of chlorine may be accidental, as when the starch has been insufficiently washed after manufacture, or hypochlorite may have been deliberately added during the re-moistening of the dextrin in order to bleach and reduce the odour. It may be estimated volumetrically in neutral solution, using silver nitrate with potassium chromate as indicator.

Another source of chlorides in dextrin and starch which is, however, rare, is that due to spoilage or contamination with seawater. In the very few cases of this kind that have come to the notice of the author, there were other obvious indications of the source of spoilage, but the presence of abnormally high amounts of chloride in these cases confirmed that contamination had taken place during transport by sea and was not due to rain, wet storage, or transport conditions after landing.

The amount of chlorine found may possibly indicate more or less at what point of manufacture it entered the dextrin, i.e. via the starch, due to insufficient washing, or as a deliberate addition, or as contamination.

## REFERENCES

1. O. SAARE, *Zeit. Spiritusind.*, 1900, **23**, 53.
2. F. LIPPMANN, *ibid.*, 1902, **25**, 237, 249, 269, 291, 304, 316.
3. G. W. ROLFE, *Eighth Int. Congr. Appl. Chem.*, 1912, **13**, 237.
4. G. M. KLINE and S. F. ACREE, *Ind. Eng. Chem. (Anal. Ed.)*, 1930, **2**, 413.
5. S. SLATER and S. F. ACREE, *ibid.*, 1930, **2**, 274.
6. H. S. MILLER, *ibid.*, 1937, **9**, 37.
7. F. A. CAJORI, *J. Biol. Chem.*, 1922, **54**, 617.
8. BRAUN and BLEYER, *Zeit. anal. Chem.*, 1929, **76**, 32.
9. E. PREUSS, *Zeit. Spiritusind.*, 1925, **48**, 326.
10. J. ALEXANDER, *Ind. Eng. Chem. (Anal. Ed.)*, 1933, **5**, 200.
11. F. L. P. KRIZKOVSKY, *Mell. Textilber.*, 1925, **9**, 594, 766.
12. W. HÖNSCH, *Chem.-Ztg.*, 1934, **58**, 76.
13. M. C. LAMB and A. HARVEY, *J. Soc. Dyers Col.*, 1918, **34**, 10.
14. F. W. BABINGTON, A. TINGLE, and C. E. WATSON, *J. Soc. Chem. Ind.*, 1918, **37**, 257T.
15. J. J. CHINYOY, F. W. EDWARDS, and H. R. NANJI, *Analyst*, 1934, **59** 673.
16. F. W. EDWARDS, H. R. NANJI, and W. R. CHANMUGAM, *ibid.*, 1938, **63**, 697.
17. J. H. LANE and L. EYNON, *J. Soc. Chem. Ind.*, 1923, **42**, 32T, 143T, 463T; 1925, **44**, 150T; 1927, **46**, 434T; 1931, **50**, 85T; see also J. FITELSON, *J. Assoc. Offic. Agric. Chem.*, 1932, **15**, 624.
18. E. R. STIEGLITZ and L. C. HORNE, *Proc. Queensland Soc. Sugar Tech.*, 1936, 101.
19. C. L. HINTON and T. MACARA, *Analyst*, 1927, **52**, 668.

20. O. PHILIPP, *Zeit. Chem.*, 1867, **10**, 400.
21. G. V. CAESAR and M. I. CUSHING, *Ind. Eng. Chem.*, 1939, **31**, 921.
22. H. T. S. BRITTON and L. PHILLIPS, *Analyst*, 1940, **65**, 18.

## ADDITIONAL REFERENCES

- V. KONN, *Chem. Obzor.*, 1936, **11**, 68. (Estimation of borax in starch preparations.)
- ALLEN, 'Commercial Analysis, Organic,' 5th edit., vol. 1, Blakiston, 1923. (Analysis of dextrin.)
- T. ZEREWITINOFF, *Zeit. anal. Chem.*, 1911, **50**, 680. (Methyl magnesium iodide used to determine moisture.)
- J. F. HOFFMANN and J. H. SCHULZE, *Woch. Brau.*, 1903, **20**, 217. (Sample distilled with toluene/turpentine mixture at 150° C. and water condensed and measured.)
- O. KAMM and F. H. TENDICK, *Paper*, 1919, **24**, 1091. (Estimation of dextrin and soluble starch in paper.)
- F. T. VAN DER VOORST, *Chem. Weekbl.*, 1940, **37**, 180, 220. (Determination of sugars in dextrin.)
- A. JONESCU and E. SPIRESCU, *Bull. Soc. Chim. România*, 1924, **6**, 101. (Ferricyanide method for determining dextrose.)

## PART V

## AMYLASES AND THEIR ACTION ON STARCH

## CHAPTER I

## GENERAL FEATURES AND NOMENCLATURE OF AMYLASES

**Occurrence.**—The enzymes capable of hydrolysing starch to simpler products are known generally as ‘Amylases’ and they occur very widespread in nature. The conversion of starch into sugar by saliva was noted in 1831,<sup>1</sup> although the significance of the reaction was not then fully grasped.

Since that time amylases have been found to occur in yeasts, the small intestine, liver and pancreas of the human body<sup>2</sup> and of animals, in the muscle,<sup>107–110</sup> in dogs’ intestines,<sup>3</sup> in the saliva of humans, rats<sup>4</sup> and rabbits,<sup>5</sup> in the seeds of many cereals and the tubers of plants, in maple sap,<sup>6</sup> cabbage,<sup>7</sup> in wheat,<sup>8</sup> in *Corynebacterium diphtheriae*,<sup>33</sup> in a large number of plants<sup>9</sup> and leaves,<sup>10</sup> such as the mature green leaves of the onion (but not the leek), the snowdrop and the dock,<sup>106</sup> in the eggs of cephalopods,<sup>11</sup> in the organs of silkworms and larvæ of butterflies<sup>12</sup> and in human seminal plasma.<sup>13</sup> Certain moulds and fungi produce so much amylase that they constitute an excellent source for its preparation for industrial uses (see p. 460). This list is neither exhaustive nor significant from the point of view of importance but is given merely to show the ubiquitous nature of amylases in the animal and vegetable kingdoms.

**Composition.**—The purification of amylases is difficult and it is doubtful whether any has been prepared in a state approaching purity. The impurities often appear to have a protective action on the amylases so that the more pure they are obtained the more readily they appear to be inactivated (see below, p. 442). It is doubtful whether any analytical figures obtained on the various preparations have any real significance at all.

In some cases broad generalisations as to their composition may be drawn from the action of various compounds on their activity or by the changes in activity brought about by various physical means, e.g. in some cases the amylases are inactivated by heat which might bring about coagulation of protein matter, but with certain amylases heat has a much less marked effect



on the activity. H. C. Sherman, M. L. Caldwell, and S. E. Doebbeling,<sup>14</sup> for example, prepared a highly active  $\beta$ -amylase preparation which contained 16 per cent. nitrogen and behaved like a typical protein. Denaturing of the protein destroyed its activity. In this instance it is reasonable to suppose that the amylase is either a protein, or that it is a body loosely combined or adsorbed on to a protein without which it cannot exert its full function. E. Příbam<sup>15</sup> considered malt diastase to consist of two substances, a carbohydrate which only reduces after hydrolysis and a polypeptide containing 7-8 per cent. N. M. W. Beijerinck,<sup>16</sup> in 1908, however, found 'amylase' incapable of replacing either carbohydrate or nitrogenous matter in nutrient media for yeasts and bacteria. H. C. Sherman and A. O. Gettler<sup>35</sup> examined samples of pancreatic and malt amylases and found all of the eight forms of nitrogen distinguishable by Van Slyke's method. Bokorny<sup>36</sup> included takadiastase and malt diastase for total and alkylamide nitrogen estimations, and he considers<sup>37</sup> that the action of pepsin—HCl shows it to be a protein, while T. B. Osborne<sup>205</sup> considers that malt amylase is a combination of an unknown substance with albumin, the activity corresponding roughly to the amount of the latter.<sup>206</sup> K. Mohs<sup>38</sup> goes as far as to consider that the amyloclastic and saccharogenic differ only in that they are composed of the same protein but differing only in their state of 'colloidal distension'. H. Haehn,<sup>39</sup> however, points out that the comparative stability of potato amylase in the presence of proteolytic enzymes indicates that it is not an ordinary protein compound. R. Fricke and P. Kaja,<sup>207-208</sup> furthermore, have obtained malt amylases with enhanced amylolytic activity by electro-osmosis. They gave no protein reactions except Millon's test, but showed a strongly positive carbohydrate test and migrated to the cathode chamber. The important effect of associated protein matter on the activity of amylases is discussed below (see p. 443), and R. A. Kehoe<sup>146</sup> believes ptyalin to be protein in nature, its activity depending on its combination with certain salts or metals.

There are indications that some amylases contain a mineral content. The loss of activity of diastase on electrolysis is ascribed by F. Maignon<sup>204</sup> to dissociation of the mineral-organic constituent of the amylase. It is well known that salt-free salivary amylase is inactive. In dialysed saliva a precipitate appears simultaneously with a loss of diastatic activity. On addition of sodium chloride the precipitate redissolves and the activity returns. H. Ninomiya<sup>40</sup> found that the filtrate from the dialysed saliva was inactive but the precipitate can be activated, the greatest activity being shown

in 0.05 M NaCl solution, and at  $pH$  6.8 with a range of activity from  $pH$  4-10. The iso-electric point of salivary amylase is  $pH$  4.0 and Ninomiya thus considers it to be in the nature of a globulin. Purified takadiastase is not activated by NaCl and gives no precipitate on dialysis. L. Michaelis<sup>41</sup> considers malt diastase to be amphoteric. L. E. Rozenfeld, A. A. Rukhelman and A. A. Zhuravskaya<sup>42</sup> from the reactions of amylase with hydroxylamine and aniline conclude it contains an active aldo group. The possibility of the co-existence of this together with an amphoteric group is not excluded by these workers. E. Rona<sup>43</sup> finds that the activity of amylase is not affected by the presence of substances which react with the aldehydic group. H. Friedenthal<sup>203</sup> has suggested that pancreatic amylase may be a nucleoprotein.

K. V. Giri<sup>44</sup> concludes that the purified amylase of sweet potato is not a protein and has shown, with J. G. Shrikhande,<sup>45</sup> that the enzyme is activated by anions, the effect decreasing in the order  $F'$ ,  $Cl'$ ,  $SO_4''$ ,  $NO_3'$ .

There is no doubt that enzymes are colloidal in nature and this may account for the effect of the  $pH$  value and temperature on the activity.

**Effect of Temperature and  $pH$  Value on Activity.**—The effect of  $pH$  value, temperature and the influence of metallic salts and certain organic compounds (see below) may give us some guide to the constitution of the amylases as more data is collected. The fact that amylases exhibit an apparent optimum temperature is interesting as it would appear to be the net result of speeding up the reaction and simultaneous deactivation or partial deactivation of the enzyme by heat. D. H. Cook<sup>46</sup> finds that below the deactivating temperature the rate of hydrolysis of starch by pancreatic or malt amylase is roughly doubled for every  $10^\circ C.$  increase in temperature. P. Kolbach and G. W. Haase,<sup>47</sup> investigating the starch degradation in cooked mashes, found that for malt amylase the optimum temperature varied with the  $pH$  value as follows:  $55-60^\circ C.$  at  $pH$  6.6;  $60-65^\circ C.$  at  $pH$  6.0;  $55-60^\circ C.$  at  $pH$  5.1;  $50-55^\circ C.$  at  $pH$  4.5, and  $45-50^\circ C.$  at  $pH$  4.2 for the liquefying action, but the optimal zones are rather broad and not well defined. Similarly, for the saccharification the values for temperature and  $pH$  optimum were, respectively,  $20-40^\circ C.$  at  $pH$  4.4-5;  $50^\circ C.$  at  $pH$  4.4-5;  $60-70^\circ C.$  at  $pH$  5.7-6.2.

L. Corvaisier<sup>48</sup> (see also<sup>49</sup>) considers that the mineral content of the ash of various starches affects the action on them of diastase owing to the changes in  $pH$  value it brings about in unbuffered

solutions. If buffered it has no effect. At 60-70° C. the hydrolysis is at a maximum in a pH range of 4.5-6.8 and very slight at pH 3 or pH 7.6, at 45° C. at pH 4.7 falling off rapidly at lower pH but more slowly at higher pH values until pH 8, then decreasing rapidly. Various animal amylases were found by J. Munk<sup>60</sup> to show marked differences in optimum pH values. Many workers have investigated the effect of pH value or acidity on amylase action.<sup>51-62</sup> Sherman and Thomas<sup>63</sup> investigated the action of twenty acids and salts and found the optimum pH value for malt amylase to lie between 4.2-4.6 and to be uninfluenced by the acid used.<sup>64</sup> The buffer used exerts some influence on the optimum pH value; according to G. A. Ballou and J. M. Luck<sup>65</sup> takadiastase has an optimum pH of 5.1 at 30° C. when the salts of aliphatic acids are used as buffers. Using phthalate and citrate buffers the pH optimum is at 5.4. The effect of the buffer to give changes in the optimum pH on pancreatic diastase has also been confirmed by other workers.<sup>66-68</sup> G. L. Funke<sup>69</sup> puts the optimum pH value of takadiastase at between 3.5 and 5.5. Hahn<sup>70</sup> has shown that the maximum flocculation occurs at pH 3.63 with malt amylase. Further papers on the effect of pH value on diastatic activity are given in references,<sup>64, 71-72</sup> and the effect of pH value on the maltase component present in takadiastase has been dealt with by A. Compton.<sup>105</sup>

C. S. Hanes and M. Cattle<sup>104</sup> suggested the following as being very close to the optimum conditions:  $\beta$ -malt, pH 4.8, 0.015 N acetate buffer;  $\alpha$ -malt, pH 5.3, 0.015 N acetate buffer; *Aspergillus* amylase, pH 4.7, 0.015 N acetate buffer; pancreatic and salivary amylases, pH 6.8, 0.005 N phosphate buffer. With the two animal amylases 30 mgm. of sodium chloride per 100 ml. reaction mixture (containing 0.2 per cent. starch) was added, this concentration having been found to produce maximum activation.

**Nomenclature.**—Two current systems exist for naming the particular amylases and both systems are descriptive of the degradation products rather than of the specific constitutional features of the enzymes. Kuhn<sup>17</sup> found that malt amylase acted on starch to give products showing rising (dextro-rotatory) mutarotation, but with pancreatic amylase and that from *Aspergillus oryzae* a falling mutarotation was observed. Accordingly he classified the two types of amylase in  $\alpha$ - (falling) and  $\beta$ - (increasing) according as the products showed these behaviours. It is now clear, however, that  $\beta$ -mutarotation does not, by itself, constitute a definition of the specificity of the  $\beta$ -malt enzyme. The other system makes use of the terms dextrinogenic ( $\alpha$ -)

and saccharogenic ( $\beta$ -) amylase. The latter was suggested by Ohlsson <sup>78</sup> as the sole reducing product formed by the action of this enzyme on starch is maltose. It is true that dextrans are also formed, but as this worker pointed out these dextrans must be regarded as the residue of the starch molecule after the maltose has been split off and not as the primary fission product of the starch molecule.

E. Ohlsson <sup>18</sup> later noted that the isolated components of malt amylase, namely the dextrinogenic malt enzyme and the saccharogenic malt enzyme, belonged, respectively, to the  $\alpha$ - and  $\beta$ -types of Kuhn's classification, the preponderating effect of the saccharogenic enzyme on the mutarotational behaviour of the products leading previously to the false conclusion that malt amylase was solely of the  $\beta$ -type. Other workers, have confirmed the dual nature of malt extract. Holmbergh, <sup>19</sup> for example, separated them by differential adsorption on rice starch (see p. 459), Waldschmidt-Leitz and Reichel <sup>20</sup> by adsorption on alumina C<sub>v</sub> and Freeman and Hopkins <sup>21</sup> reported concordant results when acting on different starch products and glycogen with the two enzymes. The work of the last two workers precludes the possibility that these phenomena are due in either case to mutarotation of unattacked substrate or to those products present in but a slightly degraded form.

T. Chrzaszcz and J. Janicki <sup>91</sup> find that 'sisto-amylases' are present in germinated and ungerminated cereals as well as in animals. These bodies are presumed to inactivate animal and plant amylases by adsorbing them. The activity is restored by treatment with peptone which they consider acts as an 'eluto-amylase'. This restoration of activity by various means is dealt with below (Inhibition and Activation).

R. Willstätter and M. Rohdewald <sup>92</sup> use the term 'iso-dynamic' to describe enzymes which act on the same substrate. Iso-dynamic amylases include saccharogenic and dextrinogenic amylases and the 'lyo-' and 'desmo-' amylases which are distinguished primarily by solubility. The so-called 'complement' of amylase is dealt with below.

R. Kuhn found there are two different types of amylase, the  $\alpha$ - and  $\beta$ -type, which produce  $\alpha$ - and  $\beta$ -maltose, respectively. This, he considered, would indicate either that starch consists of two distinct compounds built up from  $\alpha$ - and  $\beta$ -maltose units, respectively, or that both  $\alpha$ - and  $\beta$ -forms of glucosidic linkage exist in the starch molecule and on hydrolysis the chain breaks down at one or the other linkage, depending on the type of amylase used. Kuhn's hypothesis is now of historical interest only.

G. E. Glock<sup>24</sup> considers starch to be homogeneous, though different varieties or even portions of the same starch appear to differ in their resistance to enzyme action. Samec<sup>25</sup> ascribes this fact to differences in the residual valencies of the molecules of the various fractions, which enable them to combine with varying amounts of the enzyme.

van Klinkenberg<sup>26</sup> holds that starch consists of two fractions, the  $\alpha$ - and the  $\beta$ -fraction, which are attacked by  $\alpha$ - and  $\beta$ -amylases, respectively. He supports his suggestion by the fact that when soluble starch is hydrolysed to completion by  $\beta$ -amylase the reducing power of the products corresponds to 64 per cent. of the theoretical maltose, but with  $\alpha$ -amylase this value falls to 36 per cent., but a very slow rise continues. This, he considers, is due to the slow conversion of  $\beta$ - into  $\alpha$ -starch, which is then hydrolysed. His theory was intended not only to explain the mutarotation of the products by each enzyme but also the progress of the degradation. He assumes  $\alpha$ -starch to contain exclusively  $\alpha$ -linkages, but advances no conception of the type of linkage in the  $\beta$ -starch. It seems inevitable to assume from this theory that alternating  $\alpha$ - and  $\beta$ -linkages occur since the hydrolysis of  $\beta$ -linkages in  $\beta$ -starch is assumed to liberate maltose, but as pancreatic and *Aspergillus* amylases readily degrade at least 70 per cent. of the starch giving  $\alpha$ -mutarotating products they would be acting upon part at least of the postulated  $\beta$ -starch fraction. Thus the Klinkenberg's hypothesis does not circumvent the inherent difficulties of Kuhn's simpler hypothesis. G. G. Freeman and R. H. Hopkins<sup>27</sup> also have shown that  $\alpha$ -amylase attacks the  $\beta$ -fraction as well as the  $\alpha$ -fraction, hydrolysing it completely to maltose (see also C. S. Hanes<sup>28</sup>). The  $\beta$ -amylase is without effect on the  $\alpha$ -fraction, which is attacked incompletely by the  $\alpha$ -amylase. These workers find that malt  $\alpha$ -amylase produces dextrans and maltose but that with  $\beta$ -amylase only maltose and starch appear to be present at the same time. This seems to confirm the theory of Ohlsson, that starch is broken down by  $\alpha$ -amylase to dextrin and maltose, but that with  $\beta$ -amylase successive maltose groups are detached. Freeman and Hopkins<sup>29</sup> also found that  $\alpha$ -amylase from malt and from pancreas give  $\alpha$ -dextrans and then  $\alpha$ -maltose, whilst the  $\beta$ -amylases from malt and from ungerminated barley give  $\beta$ -maltose. They have also confirmed that the mutarotation is due to the maltoses formed. J. Blom, A. Bak and B. Braae<sup>30</sup> also consider that  $\alpha$ -amylase produces maltose and is responsible for the liquefying action, whereas the action of  $\beta$ -amylase is purely saccharogenic. Later, they<sup>31</sup> formed the opinion that  $\alpha$ -amylase acts on potato starch

to give dextrans but that maltose makes its appearance when the reducing power of the medium reaches 7 per cent.

One anomalous fact has not so far been explained, viz. why the action of  $\alpha$ - and  $\beta$ -amylases on the same material (some 60 per cent. of the starch) produces  $\alpha$ - and  $\beta$ -maltose, respectively.

The terminology of the products of hydrolysis by means of  $\beta$ -amylase can be confusing. A starch solution is hydrolysed by  $\beta$ -amylase until some 50-60 per cent. is converted into maltose and a portion remains which is *relatively* resistant to further action by the enzyme. The resistant portion is referred to variously as erythrogranulose,<sup>190</sup>  $\alpha$ -amylodextrin,<sup>191</sup>  $\alpha$ -starch<sup>192</sup> and  $\beta$ -dextrin.<sup>193</sup> Erythrogranulose and  $\alpha$ -amylodextrin are the two most commonly used names, although the former is a misnomer, since the material gives a blue colour with iodine.

**The Nature and Significance of the Mutarotating Products.**—It may be taken as established that the mutarotation in the case of  $\beta$ -amylase is due to the liberation of  $\beta$ -maltose, and Freeman and Hopkins<sup>21</sup> have shown that the rate of mutarotation agrees closely with that of pure maltose under similar conditions. With  $\alpha$ -amylases, however, the crediting of the mutarotation solely to the maltose is misleading in that we might assume from this that the reducing products are entirely maltose. Freeman and Hopkins have refuted this and shown that reducing dextrans account for the bulk of the reducing power and but little maltose is present, hence the  $\alpha$ -mutarotation is due to groups in the dextrin molecules. With pancreatic amylase, however, they showed that the mutarotation in the early stages is due to the liberation of the  $\alpha$ -forms of reducing dextrans with some  $\alpha$ -maltose while  $\alpha$ -maltose contributes chiefly to the mutarotation in the later stages.

Thus there is a variation of the proportion of the mutarotating groups attached to the dextrans and to maltose with the amylase used and the particular stage of degradation, but in special cases the principal mutarotating products are reducing dextrans.

In the case of the  $\alpha$ -type, such as pancreatic, *Aspergillus* and  $\alpha$ -malt amylases, the preliminary degradation of the starch into dextrin molecules takes place. The liberation of  $\alpha$ -mutarotating products therefore appears related to this most common type of degradation. \*

For some time the only known example of Kuhn's  $\beta$ -amylase type appeared to be the  $\beta$ -amylase of malt and barley which liberates  $\beta$ -maltose by the cleavage, however, of an  $\alpha$ -glucosidic linkage (as it cannot now be admitted that  $\beta$ -linkages exist in starch). It would therefore appear that it is the enzyme and the

mode of fission that determines the fission product and not the configuration of the product. Ugrumov<sup>74</sup> considered, with others, that the appearance of active  $\beta$ -amylase alone in dormant cereal grains depended on the differential inactivation of the dextrinogenic component during maturation of the seed. Giri<sup>75-77</sup> has shown, however, that the amylase of the sweet-potato tuber, *Ipomea batatas*, induces a closely similar degradation of starch. It appears to contain no  $\alpha$ -component and it would appear that this enzyme exists in a state of isolation in the tuber and is closely similar to  $\beta$ -malt amylase.

**Amylase Action on Starch.**—Native starch is scarcely affected by amylases, and if all the granules were intact it is probable that no action at all would occur with certain starches, e.g. potato. In the plant tissue the erosion of the starch appears confined to localised areas on the surface of the granule, and during the late stages of endosperm depletion in germinating barley numerous empty sacs may be seen. As noted by Brown and Heron<sup>22</sup> these sacs are usually perforated with small holes through which entrance of the enzyme and removal of the fission products of hydrolysis has apparently taken place. These workers consider that these sacs consist of 'starch cellulose,' and Ling and Mehta<sup>23</sup> mention that starch granules are apparently enclosed in a thin membrane that appears to differ decidedly in properties from the starch substance it encloses. The recently postulated 'oxygen bridges' between the crystals of starch in the granule may, however, give an alternative explanation as to why native starch does not swell in cold water and remains unaffected by amylase but does not seem capable of explaining away the sacs left in the plant as mentioned above. Sande-Bakhuyzen<sup>31</sup> considers it to be a less hydrated form of starch (see also Schoen<sup>32</sup>).

Starch paste which has been stirred at high speed (see p. 506) or autoclaved at 120° C. for 30 minutes are suitable substrates for studying amylase action on starch, but solutions of the so-called soluble starch are most widely used for this purpose. The criteria of starch degradation by the liquefying, dextrinogenic and saccharogenic amylases are discussed in Part V, Chapter 7, on the Methods of Determining the Activity of Amylase Preparations.

By the use of diastatic methods Ivanoff, Kurgatnikov and Kirsanova<sup>172</sup> reported that fine differences were shown by individual starches. The variations in the digestibility of starches from different sources have been noted by W. Stone,<sup>173</sup> J. O'Sullivan,<sup>174</sup> Y. Nagao,<sup>175</sup> H. C. Sherman and co-workers,<sup>180</sup> K. Amberger,<sup>177</sup> A. J. Hermano and O. S. Rask,<sup>178</sup> G. Kashiwaya<sup>179</sup> and others.<sup>218</sup> Nikolaev<sup>217</sup> finds that different varieties of potato

starch are saccharified at different rates and to different degrees by diastase, and in such cases the starch pastes had different viscosities. Most investigators report potato starch to be more rapidly digested than cereal starches.

Besides acting on pasted starch  $\alpha$ - and  $\beta$ -amylases will act on starch in which the structure has been destroyed by, for example, milling in a ball<sup>181</sup> or rod mill.<sup>176</sup> The effect of pre-treatment temperature on the extent of digestion by diastase has been discussed by E. Wiegel,<sup>182</sup> whilst C. E. Mangels,<sup>183</sup> J. S. Andrews and C. H. Bailey,<sup>184</sup> S. R. Snider and D. A. Coleman,<sup>185</sup> H. R. Sallans and J. A. Anderson,<sup>186</sup> S. Redfern and W. R. Johnston<sup>187</sup> and others have investigated the effect of the substrate on the course of amylolysis. V. D. Martin and J. M. Newton<sup>188</sup> have shown that the temperature at which the starch substrate is prepared affects the rate as well as the degree of digestibility by  $\beta$ -amylase from soybeans. Using soybean amylase the following temperatures of gelatinisation were found to give the maximum rates of digestion of the various starches: potato, 70° C.; tapioca, 80° C.; wheat and corn, 90° C.; rice, 80° C. or above. The yield of maltose was plotted against the temperature of pre-treatment and characteristic curves obtained which differed in their extrapolated intercept on the temperature axis, in their slope and in their value for maximum digestion. These curves are striking enough to suggest the application of this method to industrial modification of starches.<sup>189</sup> Newton and co-workers<sup>189</sup> find that the intercept on the digestion curve, i.e. the point at which digestion by soybean  $\beta$ -amylase commences, closely corresponds with the point at which the polarisation cross begins to disappear and the granules will commence to take up Congo red. They also found that the so-called 'chlorinated,' i.e. oxidised with hypochlorite, maize starches were not attacked by malt extract or by soybean  $\beta$ -amylase if more than 0.1 equivalent of chlorine per glucose unit had been employed. They therefore consider that oxidation leads to alteration of some of the glucose units of starch to structures which are not attacked by  $\beta$ -amylase under their conditions. Dextrinised and acid-hydrolysed starches were also examined, and whereas increased dextrinisation increased the susceptibility at low pre-treatment temperatures and decreased digestion at high pre-treatment temperatures, slight acid hydrolysis generally increases digestibility at high pre-treatment temperatures with but little effect at low pre-treatment temperatures. All this work shows how very many factors can influence the course of amylase action and the necessity of stating all conditions in order to obtain correlation of results with those of other workers.



Discrepancies in the literature may be due, in view of Martin and Newton's work, to differences in the method of preparing the starch substrates. E. D. Day<sup>219</sup> is another worker who has studied the effect of cooking different starches on their digestibility by amylase.

**Inhibition and Activation of Amylases.**—There is a parallelism between amylolysis and most other enzyme reactions in that it comes to a halt before reaching the theoretical end-point. Many workers conclude that the fission products of the reaction inhibit the enzyme's action. This is considered by these workers to be accountable if one assumes that the enzyme has an affinity for the fission products, which increase in amount as the reaction proceeds. In the case of starch hydrolysis, however, other causes must exist as some of the bodies produced have no affinity for the enzyme (see below<sup>87</sup>).

Holmbergh<sup>79</sup> has shown that if the fission products are separated from the reaction mass, by dialysis or by precipitating them as osazones, the reaction starts again. Effront and also H. van Laer<sup>80</sup> have shown that the amylase can be recovered unchanged at the end of the reaction, and Glimm and Sommer<sup>81</sup> claimed a 100 per cent. recovery. E. R. Moritz and T. A. Glendinning<sup>82</sup> have pointed out that the addition of more starch to the mixture after the reaction has ceased causes it to restart. Brown and Heron<sup>83</sup> and also Kjeldahl<sup>84</sup> considered that the fission products did not halt the reaction, but in the view of Wohl Glimm<sup>85</sup> even small concentrations cause inhibition, and no reversion of these products takes place. Lüers and Wasmund<sup>86</sup> and K. Sjöberg and E. Eriksson<sup>87</sup> have also worked on this subject. Glucose, maltose, dextrin, galactose and mannose have an inhibiting effect, whereas sucrose and fructose are inert. The latter workers consider that pancreatic amylase is inhibited by maltose to a greater extent than is malt amylase and that the action of germinating barley extract on amylose, amylopectin and soluble starch is inhibited to the same extent in each case by the addition of maltose.

R. Kuhn<sup>88</sup> considers that the configuration of the added sugar has a decided influence on the inhibiting effect exercised by the sugar. In general he found that hydrolysis with malt was little influenced by  $\alpha$ -glucose but strongly by  $\beta$ -glucose,  $\alpha$ - $\beta$ -maltose and  $\beta$ -maltose. Pancreatic amylase is equally, but only slightly, inhibited by both forms of glucose, whilst  $\alpha$ - $\beta$ -maltose and  $\beta$ -maltose also inhibit equally and more strongly. With takadiastase  $\alpha$ - and  $\beta$ -glucose are equally retarding, but 'equilibrium' maltose has a greater effect than the  $\beta$ -form,

In further experiments Kuhn obtained parallel results when using amylose and amylopectin instead of starch. K. Sjöberg has shown in these cases that the final equilibrium is attained even although the reaction has been slowed down. He further points out that using dialysed malt extract on 1 per cent. solution of soluble starch a 60 per cent. saccharification is attained before the reaction halts and this is not affected by removal of the reaction products by dialysis. Therefore there must be some cause for the halting of the reaction other than the affinity of the enzyme for the fission products.

Inactivation of barley-malt diastase has been brought about by shaking, as shown by Holmbergh,<sup>89</sup> who gives convenient methods for preparing large amounts of pure ( $\alpha$ - and  $\beta$ -) malt amylases.<sup>90</sup> L. Hollander<sup>93</sup> finds that rat-liver amylase preparations vary greatly in their ability to digest starch to a blue-violet end-point. Incubation of the aqueous liver suspensions was found to increase the maltose-forming ability and this worker considers that two amylases are present together with an unstable inhibitory substance which can be precipitated by acetic acid.

C. Wunderly<sup>94</sup> discusses whether one enzyme reaction can be influenced by another taking place in the same medium. Casein inhibits the action of salivary amylase on starch, but if trypsin is added the reaction commences. By the addition of more trypsin an activity was shown by the salivary amylase which was greater than that of the control, and the *pH* optimum for the amylolysis appears to depend upon the *pH* optimum of the trypsin and not that of the amylase. T. Chrzaszcz and J. Janicki<sup>95</sup> find that rennin increases the diastatic activity of ungerminated grain. They find<sup>96</sup> that active papain also increases the amylolytic power of barley and other grains with the exception of millet, which becomes less active. They consider the effect on barley is either due to the production of more amylase or to the setting free of amylase bound in an inactive form to protein. K. Myrbäck and B. Örtenblad<sup>97</sup> consider the  $\beta$ -amylase in barley is partly bound to an insoluble protein and is inactive in this form. By the action of proteolytic enzymes, e.g. papain, the amylase is freed and thus activated. Y. P. Barmenkov<sup>209</sup> finds free amylase to be electropositive (see also ref.<sup>213</sup>), but in the presence of electro-negative mucin the whole complex may be predominantly electronegative. With amylases which are activated by sodium chloride he considers that the latter breaks up the mucin-amylase process, giving an electropositive NaCl-amylase complex which can exert its fully amylolytic action.

Hydrogen sulphide has been considered by Chrzaszcz and co-workers to activate amylase, but K. Myrbäck and Örténblad<sup>97</sup> suggest that the amylase had been inactivated in the first place by traces of copper impurities which are removed by the hydrogen sulphide giving the active enzyme. J. Janicki<sup>98</sup> supports this view but maintains that ascorbic acid plays an important rôle in inhibiting barley amylase. C. S. Hanes<sup>99</sup> has also studied the reversible inhibition of  $\beta$ -malt amylase by ascorbic acid and the allied compounds reductone and dihydroxy maleic acid. He considers the inhibition is due to the dienol grouping common to these compounds and finds the inhibition is reversed (*a*) by oxidative destruction of the dienol group, and (*b*) by the presence of reducing substances such as hydrogen cyanide, sodium thio-sulphate, cysteine, thiosalicylic and thiolacetic acids. Methylene blue and potassium ferricyanide reverse the inhibiting effect of ascorbic acid. Ferrous sulphate has but little effect on inhibition, but traces of copper increase the inhibition.

J. Janicki<sup>98</sup> also ascribed the inhibition of amylase in the presence of ascorbic acid and its re-activation by other substances to oxido-reduction processes. He considers the inhibition to be associated with the dehydrogenation of the ascorbic acid. It is reversible and can be prevented by hydrogen sulphide and other reducing substances. The dehydrogenation of ascorbic acid and therefore the inhibition of amylase is strongly accelerated by traces of copper, so that it is important to use carefully purified water. This dehydrogenation process also takes place in starch-buffer solutions. If the re-oxidation is accelerated by the addition of water containing traces of copper then the reversibility of the activation brought about by the hydrogen sulphide can be demonstrated. Ascorbic acid also inhibits  $\alpha$ -amylase. This inhibition results from an oxidation of ascorbic acid and can be prevented by hydrogen sulphide. The disappearance of the  $\alpha$ -amylase at the end of the ripening period of barley cannot be ascribed entirely to the oxidation of ascorbic acid since hydrogen sulphide does not, in this case, restore the dextrinogenic and liquefying power of the amylase. R. Weidenhagen and P. Lu<sup>100</sup> find the inhibition by ascorbic acid is abolished by thiol and S—S compounds, amino-acids and citrate.

The mode of inactivation of  $\beta$ -amylase in the barley grain is discussed by K. Myrbäck and S. Myrbäck<sup>101</sup> and by Chrzaszcz and Janicki.<sup>102</sup> The former workers consider that the action of hydrogen sulphide or cyanide is to activate the barley proteases, which in turn liberate the amylase in an active form (*vide supra*, p. 443), but Chrzaszcz and Janicki consider proteolysis

is of no importance in the activating cycle and point out that no parallelism is found between the rate of activation and the rate of proteolysis. K. Myrbäck and S. Myrbäck consider that this argument does not refute their postulate, but the demonstration of such a parallelism would be the only convincing proof of their hypothesis. As we have seen above, it appears that proteolysis may play some part, for instance, in liberating the amylase in a soluble, active form, but another more direct activation would appear to take place as well. All workers agree that only the  $\beta$ -amylase of barley is activated. According to K. V. Giri and A. Sreenivasan<sup>103</sup> and Chrzaszcz and Janicki,<sup>104</sup> in the early stages of the ripening of the grain, rice, barley, wheat, rye and oats,  $\alpha$ -amylase is present but becomes inactive as the ripening proceeds and then regains its activity during sprouting.

Many of these workers have studied the nature of inactive amylase in barley and similar materials and great variations appear to exist in barleys grown under different conditions.<sup>111, 112</sup> The distribution of amylase in the barley kernel has been studied by K. Linderström-Lang and C. Engel<sup>113</sup> and others,<sup>114</sup> while several workers<sup>115-116</sup> have dealt with the fate of the amylases during the ripening and germination.

**Amylase-Complement, or Co-enzyme, and Organic Activators.**—Pringsheim and co-workers, using pancreatic and salivary amylases, found certain substances, 'amylase-complement,' increased the activity. Certain yeast preparations,<sup>120</sup> egg-white, blood- and serum-albumin which have been digested with pepsin, were found to be good activators.<sup>121-123</sup> The egg-white complement is heat-labile but that from yeast is fully resistant to heat. O. Holmbergh<sup>79</sup> activated pancreatic amylase with living yeast and obtained complete saccharification, but dead yeast extract was without effect. Bakers' or brewers' yeast treated for a short time with toluene retained its maltase action. As the maltase and the pancreatic amylase possess the same optimum  $pH$  the presence of the former in the yeast will affect the results. In the case of malt amylase no complementary reaction is observed although the maltase in this case would not be operative owing to the malt amylase having its optimum  $pH$  value on the acid side.

However, K. Sjöberg,<sup>124</sup> R. Kuhn,<sup>17</sup> J. Blom, Bak and Braae have found the complement from yeast greatly increased the hydrolysis of starch by malt. Amylose, amylopectin, soluble and ordinary starch were found to be more vigorously acted upon by malt with the yeast complement present, although Sjöberg<sup>124</sup> found one of Pringsheim's complement preparations to be inactive. L. de Hoop and J. A. van Laer<sup>125</sup> consider it

possible to get an almost quantitative yield of maltose if an amylase rich in co-enzyme or complement is used.

R. Weidenhagen and A. Wolf<sup>126, 127</sup> dispute the existence of amylase-complements, but Pringsheim, H. Borchardt and H. Hupfel<sup>128</sup> maintain their previous claims. They have further showed that glutathione, which is contained in autolysed yeast, acts as an amylolytic activator.<sup>129-131</sup> E. W. Rockwood<sup>132</sup> has studied the stimulating effect of glycine and aspartic acid on the action of ptyalin and introduces the term 'auxo-amylase' to describe amylase-complements or co-enzymes<sup>141</sup> (see also p. 437). The action of pancreatin is speeded up by sodium taurocholate and glycocholate,<sup>134, 167</sup> and other workers have noted the stimulating influence on amylases of amino-acids,<sup>133, 135, 140-142, 147-149</sup> certain albumins,<sup>136, 137</sup> and bile<sup>138, 145, 171</sup>—in which the activator is thermostabile, dialysable and alcohol soluble, but which G. Buglia<sup>139</sup> considers merely lowers the surface tension—and asparagine or aspartic acid<sup>143</sup> whose effects are interchangeable rather than additive in admixture. Lysine has no effect on the amyloclastic activity of purified pancreatic amylase but favourably influences its saccharogenic action.<sup>149</sup> Tryptophane has also been shown to have a similar effect on this amylase and also on salivary amylase.<sup>150</sup> Sherman and his co-workers<sup>149</sup> consider pancreatic amylase to be a protein and suggests that lysine in the enzyme molecule is not split off until the stage of amyloclastic action has been past.

**Activation by Inorganic Compounds.**—Many workers have shown that if salivary and pancreatic or liver amylase is dialysed sodium chloride is removed and the enzymatic activity is lost.<sup>151-160</sup> O. Holmbergh<sup>79</sup> has studied liver amylase in detail and considers, in opposition to Kendell and Sherman,<sup>155</sup> Böhne<sup>161</sup> and H. Roger,<sup>163</sup> that the usual buffers are equally good activators and that the phosphate ion has no especially beneficial influence. Willstätter and co-workers<sup>167</sup> consider that the phosphate ion has merely brought the mixture to a very suitable *pH* value and thus apparently produced a good effect. Among the salts usually employed the chlorides are particularly beneficial for saccharification and the optimum concentrations are NaCl and KCl, 0.008-0.5 N; CaCl<sub>2</sub>, 0.008-0.04. O. Kellner and co-workers<sup>162</sup> noted, however, a retarding effect in the case of the amylolytic action of Koji extract in the presence of salt. The iodide ion has an inhibiting effect on the saccharification by hog-liver amylase, but accelerates dextrin formation, and in the presence of potassium iodide malt amylase shows a decreased, but salivary and pancreatic amylases, an increased saccharifying power.<sup>164</sup>

As mentioned above, pancreatic amylase loses its effectiveness on dialysis but regains it on the addition of salt.<sup>165</sup> L. Michaelis and H. Peckstein<sup>166</sup> find that the chloride ion activates this amylase to the greatest extent and this holds good not only at the optimum  $pH$  value but also, to a greater extent, in weakly alkaline media such as are found in intestinal conditions. Ammonium and magnesium chlorides are nearly as effective as sodium chloride at the optimum  $pH$ . Bile acids inhibit at the optimum  $pH$  even in the presence of chloride. The activating effect of organic amino-acids noted by Sherman and co-workers<sup>143, 147-150</sup> is ascribed by H. Haehn<sup>168, 169</sup> and U. Olsson<sup>170</sup> chiefly to the protective action they exert towards inactivation by temperature.

**Amylase Poisons.**—Mercuric chloride,<sup>149</sup> copper sulphate, picric acid and formaldehyde<sup>195</sup> were found by Sherman and co-workers to act as poisons on amylase. The observation that pancreatic amylase is inhibited in the presence of liver amylase may prove to be of physiological interest.<sup>196</sup> Malt amylase is poisoned by a number of metallic salts and other compounds, and U. Olsson<sup>197, 198</sup> finds that the poisoning effect takes a certain amount of time to develop so that there is a progressive decrease in the activity of the amylase in the presence of the poison with time.

The following table shows the action of various poisons with time :—

TABLE 12

Poison.	Gram-Mols. of Poison per Gm. Dry Weight of Enzyme.	Time in Minutes.	Percentage Decrease in Activity of Enzyme.
Alanine . . .	$2.8 \times 10^{-2}$	30	100
Ammonium mol- ybdate . . .	$3.04 \times 10^{-3}$	15, 180, 540, 1320, 3120	6, 6, 22, 38, 67
Hydroxylamine . .	$7.98 \times 10^{-4}$	30, 960, 1380, 2580	8, 65, 67, 83
Iodine . . .	$1.39 \times 10^{-7}$	10, 80, 1260	48, 56, 60
Leucine . . .	$5.48 \times 10^{-3}$	1560	5
Phenylhydrazine.	$4.81 \times 10^{-4}$	10, 40, 360	72, 77, 90
Potassium cyanide	$2.14 \times 10^{-4}$	10, 1200	33, 58
Semicarbazide . .	$4.45 \times 10^{-5}$	10, 240, 1920	3, 3, 31
Sodium fluoride . .	$3.0 \times 10^{-3}$	300	3
„ sulphite . . .	$9.03 \times 10^{-4}$	600	64
„ tungstate . . .	$6.03 \times 10^{-4}$	10, 300, 1380	13, 72, 100

According to Holmbergh malt amylase is inhibited by potassium iodide.<sup>164</sup> The inactivation by phosphotungstic and picric acids is probably due to the formation of an insoluble complex between

the acid and the basic group in the molecule of the enzyme.<sup>199</sup> Alkaloids<sup>200, 201</sup> and ethyl alcohol up to 50 per cent. on the malt extract have also been found to act as organic poisons. R. S. Potter,<sup>210</sup> contrary to previous reports, finds that the presence of iron even in concentrations of 850 parts per million is without effect on malt amylase. He notes a relationship between the toxicity of a metal and its position in the electrochemical series, metals low in this series appearing the most toxic. The injurious effect of the hot-water extract of spent grains on malt amylase he ascribes to the tannins extracted from the husk. The inactivating effect of gall-nut or apple-tannins on diastatic action had been noted previously by G. Warcollier<sup>212</sup> and others.<sup>214</sup>

The following table shows the percentage decrease in activity with certain quick-acting poisons, chiefly metallic salts :—

TABLE 13

Poison.	Concentration.	Percentage Decrease in Activity.
Formaldehyde . . . .	$6.87 \times 10^{-3}$	50
Iron chloride . . . .	$6.16 \times 10^{-3}$	11
Mercuric chloride . . .	$6.04 \times 10^{-8}$	61
Mercurous chloride . . .	$2.13 \times 10^{-7}$	50
„ nitrate . . . .	$1.78 \times 10^{-7}$	50
Uranyl sulphate . . . .	$6.44 \times 10^{-4}$	57
Zinc sulphate . . . .	$8.8 \times 10^{-2}$	13

J. A. Smorodinzew and F. E. A. Iliin<sup>215</sup> find salivary amylase is seriously affected by the following salts in the given concentrations: antimony chloride, 0.01-0.00004 N; tartar emetic, 0.18-0.0006 N; potassium and sodium monohydrogen arsenites and sodium monohydrogen arsenate, 0.1-0.0125 N. Pre-treatment of the starch with 0.1 N aluminium chloride renders it electro-positive and resistant to amylases, but pre-treatment with sodium chloride is claimed by G. P. Barmenkov<sup>216</sup> to render it somewhat more susceptible to amylase attack.

The action of light in activating or inhibiting amylase action has been studied by few workers. A. E. Navez and B. T. Rubenstein<sup>202</sup> find that ordinary and plane polarised light show no differences in their action. The speed of hydrolysis was found to be increased in the presence of some fluorescent substances, especially fluorescein. These workers think that a loose enzyme/dyestuff complex is formed which is light sensitive and acts in the same manner as hydrogen peroxide does when

exposed to light and oxidises any inhibiting agent present. Whatever may be the mechanism of the reaction the spectral region lying between the absorption and fluorescence bands is especially active. It may be noted that H. Tappeiner<sup>21</sup> found that eosin in sunlight retarded the saccharogenic activity of amylase and that this effect is shown by those compounds only whose light absorption lies in the green or blue portion of the spectrum.

## REFERENCES

1. E. F. LEUCHS, *Ann. Phys. Chem.*, 1831, **22**, 623.
2. W. BUSCH, *Arch. path. Anat. Physiol.*, 1858, **14**, 140.
3. H. P. PIERCE, E. S. NASSET and J. R. MURLIN, *J. Biol. Chem.*, 1935, **108**, 239.
4. E. W. COHN and M. H. BROOKS, *ibid.*, 1936, **114**, 139.
5. I. M. THOMAS, *Nature*, 1936, **138**, 1015.
6. E. BOIS and A. NADEAU, *Naturaliste canadien*, 1935, **62B**, 106.
7. B. A. RUBIN and V. E. TRUPP, *Compt. rend., U.R.S.S.*, 1935, **3**, 299.
8. P. S. UGRUMOV, *Biochem. Zeit.*, 1935, **282**, 74.
9. C. KOSMANN, *Bull. Soc. chim. Paris*, 1937, **27**, 251.
10. L. BRASSE, *Compt. rend.*, 1884, **99**, 878.
11. T. KAMACHI, *Z. physiol. Chem.*, 1936, **238**, 91.
12. K. YAMAFUGI, I. HIRAIWA and S. GOTO, *Biochem. Zeit.*, 1936, **286**, 229; **287**, 23.
13. M. GOLDBLATT, *Biochem. J.*, 1935, **29**, 1346.
14. H. C. SHERMAN, M. L. CALDWELL and S. E. DOEBBELING, *J. Biol. Chem.*, 1934, **104**, 501.
15. E. PRIBAM, *Biochem. Zeit.*, 1912, **44**, 293.
16. M. W. BEIJERINCK, via 'Nature of Enzyme Action,' W. M. BAYLISS, 4th ed., p. 36, London, 1919.
17. R. KUHN, *Liebig's Ann.*, 1925, **443**, 1.
18. E. OHLSSON, *Hoppe-Seyl. Z. physiol. Chem.*, 1930, **189**, 17.
19. O. HOLMBERG, *Biochem. Zeit.*, 1933, **266**, 203.
20. E. WALDSCHMIDT-LEITZ and M. REICHEL, *Hoppe-Seyl. Z. physiol. Chem.*, 1934, **223**, 76.
21. G. G. FREEMAN and R. H. HOPKINS, *Biochem. J.*, 1936, **30**, 451.
22. H. T. BROWN and J. HERON, *J. Chem. Soc.*, 1879, **35**, 596.
23. A. R. LING, *J. Soc. Chem. Ind.*, 1927, 279T.
24. G. E. GLOCK, *Biochem. J.*, 1936, **30**, 1386.
25. M. SAMEC, *Z. physiol. Chem.*, 1935, **236**, 103.
26. VAN KLINKENBERG, *Proc. Acad. Sci. Amsterdam*, 1931, **34**, 893; *Z. physiol. Chem.*, 1932, **209**, 253; *ibid.*, 1932, **212**, 173; *Ergbn. Fermentforsch.*, 1934, **3**, 73.
27. G. G. FREEMAN and R. H. HOPKINS, *Biochem. J.*, 1936, **30**, 446.
28. C. S. HANES, *Canad. J. Res.*, 1935, **13B**, 185.
29. G. G. FREEMAN and R. H. HOPKINS, *Biochem. J.*, 1936, **30**, 442, 445.
30. J. BLOM, A. BAK and B. BRAAE, *Z. physiol. Chem.*, 1937, **250**, 104.
31. H. L. SANDE-BAKHUYZEN, *Proc. Soc. exp. Biol., N.Y.*, 1925, **23**, 187.
32. M. SCHOEN, *Bull. Soc. chim. biol. Paris*, 1930, **12**, 1033.
33. F. DI CHIARA, *Boll. sez. ital. Soc. intern. microbiol.*, 1939, **11**, 205.
34. W. L. DOYLE, *Nature*, 1939, **144**, 867.



35. H. C. SHERMAN and A. O. GETTLER, *J. Amer. Chem. Soc.*, 1913, **35**, 1790.
36. Th. BOKORNY, *Biochem. Zeit.*, 1919, **100**, 100.
37. — *Allgemeine Brauer- u. Hopfen-Zeitung*, 1915, **55**, 431.
38. K. MOHS, *Brewers' J.*, 1920, **46**, 516.
39. H. HAEHN, *Zeit. Spiritusind.*, 1919, **42**, 241.
40. N. NINOMIYA, *J. Biochem., Japan*, 1940, **31**, 69. (In German.)
41. L. MICHAELIS, *Biochem. Zeit.*, 1909, **17**, 231.
42. L. E. ROZENFELD, A. A. RUKHELMAN and A. A. ZHURAVSKAYA, *Biochem. J., Ukraine*, 1939, **13**, 557. (In English.)
43. E. RONA, *Biochem. Zeit.*, 1920, **109**, 279.
44. K. V. GIRI, *ibid.*, 1934, **275**, 106.
45. — and J. G. SHRIKHANDE, *J. Ind. Chem. Soc.*, 1935, **12**, 273.
46. D. H. COOK, *J. Biol. Chem.*, 1925, **65**, 135.
47. P. KOLBACH and G. W. HAASE, *Wochschr. Brau.*, 1939, **56**, 105, 113, 124, 134 and 140.
48. L. CORVAISER, *Bull. soc. sci. Bretagne*, 1938, **15** (extra facsimile), 1 (Publ. 1939).
49. ZWINKKER, *Rec. trav. bot. neerland.*, 1921, **18**, 47.
50. J. MUNK, *Chem. Centralbl.*, 1876, 822.
51. T. DEFRESNE, *Compt. rend.*, 1879, **89**, 1070.
52. W. DETMER, *Hoppe-Seyl. Z. physiol. Chem.*, 1882, **7**, 1.
53. J. R. DUGGAN, *Amer. Chem. J.*, 1885-86, **7**, 306; 1886, **8**, 211.
54. O. JOHN, *Virchow's Arch. path. Anat. Physiol.*, 1890, **112**, 271.
55. R. W. WOOD, *Amer. Chem. J.*, 1892, **15**, 663.
56. J. EFFRONT, *Moniteur sci. d. docteur Quesneville, Paris*, 1895, **46**, 541 and 711.
57. P. GRUETZNER and M. WACHSMANN, *Pflüger's Arch. gesam. Physiol.*, 1902, **91**, 195.
58. H. BIERRY and E. F. TERROINE, *Compt. rend.*, 1905, **141**, 146.
59. C. QUINAN, *J. Biol. Chem.*, 1909, **6**, 53.
60. A. FERNBACH and M. SCHOEN, *Compt. rend.*, 1910, **151**, 894.
61. A. ZIMMERMAN, *J. Ind. Eng. Chem.*, 1911, **3**, 823.
62. F. ANDO, *Int. Congr. Appl. Chem.*, 8th, 1912, **14**, 13.
63. H. C. SHERMAN and F. W. THOMAS, *J. Amer. Chem. Soc.*, 1915, **37**, 623.
64. — and J. A. WALKER, *ibid.*, 1917, **39**, 1476.
65. G. A. BALLOU and J. M. LUCK, *J. Biol. Chem.*, 1940, **135**, 111.
66. A. HAHN and R. MICHALIK, *Zeit. Biol.*, 1921, **73**, 10.
67. — and H. MEYER, *ibid.*, 1922, **76**, 227.
68. T. CHRZASZCZ, *Biochem. Zeit.*, 1925, **160**, 155.
69. G. L. FUNKE, *Akad. Wetensch. Amsterdam*, 1922, **31**, 12.
70. A. HAHN, *Zeit. Biol.*, 1922, **74**, 217.
71. W. BIEDERMANN and A. RUEHA, *Fermentforschung*, 1922, **5**, 56.
72. T. CHRZASZCZ, *Biochem. Zeit.*, 1924, **150**, 60.
73. E. OHLSSON, *C.R. lab. Carlsberg*, 1926, **16**, No. 7.
74. P. S. UGRUMOV, *Biochem. Zeit.*, 1935, **282**, 74.
75. K. V. GIRI, *ibid.*, 1934, **275**, 106.
76. — *J. Indian Chem. Soc.*, 1934, **11**, No. 5, 339.
77. — *J. Indian Inst. Sci.*, 1934, **17A**, (Part II), 127.
78. J. BLOM, A. BAK and B. BRAAE, *Zeit. physiol. Chem.*, 1938, **252**, 261.
79. O. HOLMBERGH, *Hoppe-Seyl. Z. physiol. Chem.*, 1924, **134**, 68-96.
80. H. VAN LAER, *Bull. soc. chim. Belg.*, 1912, **26**, 223.
81. E. GLIMM and W. SOMMER, *Biochem. Zeit.*, 1927, **188**, 290.

82. E. R. MORITZ and T. A. GLENDINNING, *J. Chem. Soc.*, 1892, **61**, 689.
83. H. T. BROWN, and J. HERON, *Liebig's Ann. Chem.*, 1879, **199**, 127.
84. J. KJELDAHL, *Dingler's Polytechn. J.*, 1880, **235**, 457.
85. A. WOHL and E. GLIMM, *Biochem. Zeit.*, 1910, **27**, 349.
86. H. LÜERS and W. WASMUND, *Fermentforsch.*, 1921, **5**, 169.
87. K. SJÖBERG and E. ERIKSSON, *Hoppe-Seyl. Z. physiol. Chem.*, 1924, **139**, 118.
88. R. KUHN, *Liebig's Ann. Chem.*, 1925, **443**, 1.
89. O. HOLMBERGH, *Svensk. Kem. Tid.*, 1937, **49**, 252.
90. — *ibid.*, p. 258.
91. T. CHRZASZCZ and J. JANICKI, *Biochem. J.*, 1934, **28**, 296.
92. R. WILLSTÄTTER and M. ROHDEWALD, *Z. physiol. Chem.*, 1934, **229**, 255.
93. L. HOLLANDER, *Science*, 1934, **78**, 17.
94. C. WUNDERLY, *Helv. Chim. Acta*, 1940, **23**, 414.
95. T. CHRZASZCZ and J. JANICKI, *Biochem. Zeit.*, 1935, **278**, 112.
96. — *ibid.*, 1934, **272**, 402.
97. K. MYRBÄCK and B. ÖRTENBLAD, *Enzymologia*, 1939, **7**, 176.
98. J. JANICKI, *ibid.*, 1939, **7**, 182.
99. C. S. HANES, *Biochem. J.*, 1935, **29**, 2588.
100. R. WEIDENHAGEN and P. LU, *Z. Wirtschaftsgruppe Zuckerind.*, 1936, **86**, 240.
101. K. MYRBÄCK and S. MYRBÄCK, *Biochem. Zeit.*, 1936, **285**, 282.
102. T. CHRZASZCZ and J. JANICKI, *ibid.*, 1935, **281**, 408; 1936, **285**, 47; *Biochem. J.*, 1936, **30**, 342.
103. K. V. GIRI and A. SREENIVASAN, *Nature*, 1936, **138**, 406.
104. T. CHRZASZCZ and J. JANICKI, *Biochem. J.*, 1936, **30**, 1298; *Biochem. Zeit.*, 1936, **286**, 13.
105. A. COMPTON, *Roy. Soc. Proc.*, 1915, **88B**, 408; *J. Soc. Chem. Ind.*, 1914, 977.
106. R. E. CHAPMAN, *Biochem. J.*, 1924, **18**, 1388.
107. R. WILLSTÄTTER and M. ROHDEWALD, *Compt. rend. trav. lab. Carlsberg*, 1938, **22**, 553.
108. H. LEHRMANN, *Nature*, 1938, **141**, 470.
109. E. M. MYSTKOWSKI, *Enzymologia*, 1937, **2**, 151.
110. A. M. WALKER and F. G. YOUNG, *Biochem. J.*, 1938, **32**, 94.
111. T. CHRZASZCZ and J. JANICKI, *Enzymologia*, 1937, **4**, 79.
112. K. MYRBÄCK and B. ÖRTENBLAD, *ibid.*, 1938, **2**, 305.
113. K. LINDERSTRÖM-LANG and C. ENGEL, *ibid.*, 1938, **3**, 138.
114. A. KIESEL and S. MICHLIN, *Biokhimiya*, 1937, **2**, 734; *Chem. Zentr.*, 1938, II, 1061.
115. E. OHLSSON and N. THÖRN, *Compt. rend. trav. lab. Carlsberg, Sér. Chim.*, 1938, **22**, 398.
116. K. V. GIRI and A. SREENIVASAN, *Biochem. Zeit.*, 1938, **296**, 428.
117. H. PRINGSHEIM and K. SCHMALZ, *ibid.*, 1923, **142**, 108.
118. — and A. BEISER, *ibid.*, 1924, **148**, 336.
119. — and J. LEIBOWITZ, *Ber.*, 1925, **58**, 1262.
120. — and W. FUCHS, *ibid.*, 1923, **56**, 1762.
121. — and G. OTTO, *Biochem. Zeit.*, 1926, **173**, 399.
122. — and M. WINTER, *ibid.*, 1926, **177**, 406.
123. — J. BONDIN and E. THILO, *ibid.*, 1928, **197**, 143.
124. K. SJÖBERG, *ibid.*, 1925, **159**, 468.
125. L. DE HOOP and J. A. VAN LAER, *ibid.*, 1925, **155**, 235.

126. R. WEIDENHAGEN and A. WOLF, *Z. Ver. deutsch. Zuckerind., Tech. Teil*, 1930, **80**, 264, 866.
127. — *ibid.*, 1930, **80**, 644.
128. H. PRINGSHEIM, H. BORCHARDT and H. HUPFER, *ibid.*, 1931, **81**, 633.
129. — *Biochem. Zeit.*, 1931, **238**, 476.
130. — *Naturwiss.*, 1932, **20**, 64.
131. — *Biochem. Zeit.*, 1932, **250**, 109.
132. E. W. ROCKWOOD, *J. Amer. Chem. Soc.*, 1924, **46**, 1641.
133. M. L. CALDWELL, *J. Biol. Chem.*, 1924, **59**, 661.
134. S. MARTIN and D. WILLIAMS, *J. Roy. Soc. London*, 1890, **48**, 160.
135. J. EFFRONT, *Moniteur scient. du docteur Quesneville*, 1904, **60**, 561.
136. — *Compt. rend.*, 1892, **115**, 1324.
137. W. SCHNEIDEWIND, D. MEYER and F. MÜNTER, *Landwirtsch. Jahrb.*, 1906, **35**, 911.
138. J. WOHLGEMUTH, *Biochem. Zeit.*, 1909, **21**, 447; 1911, **33**, 303.
139. G. BUGLIA, *ibid.*, 1910, **25**, 239.
140. E. F. TERROINE and J. WEILL, *Compt. rend. Soc. biol.*, 1912, **72**, 542.
141. E. W. ROCKWOOD, *J. Amer. Chem. Soc.*, 1917, **39**, 2745.
142. K. UJIHARA, *Chemical Abstracts, Easton*, 1918, **12**, 1971.
143. H. C. SHERMAN and F. WALKER, *J. Amer. Chem. Soc.*, 1919, **41**, 1866.
144. — *Year-book of Carnegie Inst.*, 1919, **18**, 328.
145. G. J. TEMMINCK, *Nederl. tijds. geneeskunde*, 1920, **64**, 1B, 1157.
146. R. A. KEHOE, *J. lab. clinical med.*, 1921-22, **7**, 736.
147. H. C. SHERMAN and F. WALKER, *J. Amer. Chem. Soc.*, 1921, **43**, 2461.
148. — and M. L. CALDWELL, *ibid.*, 1921, **43**, 2469.
149. — *ibid.*, 1922, **44**, 2923, 2926; 1923, **45**, 1960.
150. — and N. M. NAYLOR, *ibid.*, 1922, **44**, 2957; *ibid.*, 1925, **47**, 1702.
151. H. M. VERNON, *J. physiol.*, 1901-02, **27**, 174.
152. E. GUYENOT, *Compt. rend. Soc. biol.*, 1907, **63**, 768.
153. L. PRETI, *Biochem. Zeit.*, 1907, **4**, 1.
154. B. BRUNACCI, *Bull. sci. mediche, Bologna*, 1909, **80**, 243.
155. E. C. KENDALL and H. C. SHERMAN, *J. Amer. Chem. Soc.*, 1910, **32**, 1087.
156. E. STARKENSTEIN, *Biochem. Zeit.*, 1910, **24**, 210; *ibid.*, 1912, **47**, 300.
157. I. BANG, *ibid.*, 1911, **32**, 417.
158. M. LISBONNE, *Compt. rend. Soc. biol.*, 1911, **70**, 62, 132, 207.
159. H. BIERRY, *ibid.*, 1922, **87**, 1111.
160. T. KOGA, *Biochem. Zeit.*, 1923, **141**, 410.
161. C. BÖHNE, *Fermentforschg.*, 1922, **6**, 200.
162. O. KELLNER, Y. MORI and M. NAGAOKA, *Hoppe-Seyl. Z. physiol. Chem.*, 1890, **14**, 297.
163. H. ROGER, *Compt. rend. Soc. biol.*, 1908, **65**, 374 and 388.
164. O. HOLMBERGH, *Biochem. Zeit.*, 1924, **145**, 244.
165. H. BIERRY and J. GIAJA, *Compt. rend.*, 1906, **143**, 300.
166. L. MICHAELIS and H. PECKSTEIN, *Biochem. Zeit.*, 1914, **59**, 77.
167. R. WILLSTÄTTER, E. WALDSCHMIDT-LEITZ and A. R. F. HESSE, *Hoppe-Seyl. Z. physiol. Chem.*, 1923, **126**, 141, 157.
168. H. HÄHN, *Biochem. Zeit.*, 1923, **135**, 587.
169. — and H. SCHWEIGART, *ibid.*, 1923, **143**, 516.
170. U. OLSSON, *diss.* (Stockholm, 1925).
171. D. MINAMI, *Biochem. Zeit.*, 1912, **39**, 339.

172. N. N. IVANOFF, M. M. KURGATNIKOV and V. A. KIRSAKOVA, *Enzymologia*, 1937, **4**, 163.
173. W. C. STONE, *U.S.D.A. Off. Exp. Station Bull. No. 34*, 1896.
174. J. O'SULLIVAN, *J. Chem. Soc.*, 1904, **85**, 616.
175. Y. NAGAO, *Z. Exp. Pathol. Ther.*, 1911, **9**, 227.
176. O. E. STAMBERG and C. H. BAILEY, *Cer. Chem.*, 1939, **16**, 319.
177. K. AMBERGER, *Z. Untersuch. Nahr. Genuss.*, 1921, **42**, 181.
178. A. J. HERMANO and O. S. RASK, *Cer. Chem.*, 1926, **3**, 361.
179. G. KASHIWAYA, *J. Chem. Soc. Japan*, 1930, **51**, 719.
180. H. C. SHERMAN, F. WALKER and M. L. CALDWELL, *J. Amer. Chem. Soc.*, 1919, **41**, 1123.
181. M. J. BLISH, *Proc. 23rd Ann. meeting Amer. Assoc. Cer. Chem.*, 1937, **13**.
182. E. WIEGEL, *Kolloid-Zeit.*, 1933, **62**, 310.
183. C. E. MANGELS, *Cer. Chem.*, 1926, **3**, 316.
184. J. S. ANDREWS and C. H. BAILEY, *ibid.*, 1934, **11**, 551.
185. S. R. SNIDER and D. A. COLEMAN, *ibid.*, 1937, **14**, 1.
186. H. R. SALLANS and J. A. ANDERSON, *ibid.*, 1937, **14**, 708.
187. S. REDFERN and W. R. JOHNSTON, *ibid.*, 1938, **15**, 327.
188. V. D. MARTIN and J. M. NEWTON, *ibid.*, 1938, **15**, 456.
189. J. M. NEWTON, F. F. FARLEY and N. M. NAYLOR, *ibid.*, 1940, **17**, 342.
190. H. P. WIJSMAN, (1889), quoted by G. H. KLINKENBERG, *Z. Physiol. Chem.*, 1932, **209**, 253.
191. J. L. BAKER, *J. Chem. Soc.*, 1902, **81**, 1177.
192. G. H. KLINKENBERG, *Z. Physiol. Chem.*, 1932, **212**, 173.
193. K. MYRBÄCK, *Skand. Arch. Physiol.*, 1937, **77**, 58.
194. C. S. HANES and M. CATTLE, *Proc. Roy. Soc.*, 1938, Series B, **125**, 387.
195. H. C. SHERMAN and M. WAYMAN, *J. Amer. Chem. Soc.*, 1922, **44**, 2923.
196. A. MIEKELEY, *Ber.*, 1930, **63**, 1957; 1932, **65**, 69.
197. U. OLSSON, *Hoppe-Seyl. Z. physiol. Chem.*, 1921, **117**, 91.
198. — *ibid.*, 1923, **126**, 29.
199. K. MYRBÄCK, *ibid.*, 1926, **159**, 1.
200. F. GOEBEL, *Biochem. Zentralbl.*, 1905, **4**, 1356. (In Russian.)
201. H. W. BYWATERS and A. D. WALLER, *J. physiol.*, 1910, **40**, 45.
202. A. E. NAVEZ and B. T. RUBENSTEIN, *J. Biol. Chem.*, 1932, **95**, 645.
203. H. FRIEDENTHAL, *Arch. Anatom. Physiol.*, 1900; *Physiol. Abt.*, 1900, 181.
204. F. MAIGNON, *Compt. rend.*, 1924, **178**, 420; *ibid.*, 1925, **181**, 51.
205. T. B. OSBORNE, *J. Amer. Chem. Soc.*, 1895, **17**, 587.
206. — and G. F. CAMPBELL, *ibid.*, 1896, **18**, 536.
207. R. FRICKE and P. KAJA, *Ber.*, 1924, **57**, 310, 313.
208. R. FRICKE, *ibid.*, 1924, **57**, 765.
209. Y. P. BARMENKOV, *Biokhimiya*, 1939, **4**, 160.
210. R. S. POTTER, *J. Soc. Chem. Ind.*, 1940, **59**, 45.
211. H. TAPPEINER, *Ber.*, 1903, **36**, 3035.
212. G. WARCOLLIER, *Compt. rend.*, 1905, **141**, 405.
213. M. LISBONNE and E. VULQUIN, *Compt. rend. Soc. biol.*, 1912, **72**, 936.
214. Z. HATTA, *Mitt. Mediz. Fakultät Kaiserlichen Univ. Tokyo*, 1935, **14**, 511.
215. J. A. SMORODINZEW and F. E. A. ILIIN, *Biochem. Zeit.*, 1923, **141**, 297.

216. G. P. BARMENKOV, *Biokhimiya*, 1938, **3**, 740.  
 217. NIKOLAEV, *Bull. Acad. Sci., U.R.S.S. Biol. Ser.*, 1939, p. 899.  
 218. S. FORD, *J. Soc. Chem. Ind.*, 1904, **23**, 414.  
 219. E. D. DAY, *U.S. Dept. Agric. Off. Exp. Sta. Bull.* 202, 1908.

## ADDITIONAL REFERENCES

- H. C. SHERMAN and M. D. SCHLESINGER, *J. Amer. Chem. Soc.*, 1913, **35**, 1784. (Suggest terms 'amylolytic' and 'saccharogenic' to describe 'liquefying' and 'saccharifying' respectively.)  
 T. PANZER, *Hoppe-Seyl. Z. physiol. Chem.*, 1913, **84**, 161; **85**, 97, 292; **86**, 322, 401; **87**, 115. (The effect of various treatments of malt amylase with gaseous ammonia and hydrogen chloride.)  
 C. B. DAVIS, *J. Ind. Eng. Chem.*, 1915, **7**, 115. (Claims preparation of enzyme from malt acting on hemicellulose to give starch. Results not confirmed by S. Born, *ibid.*, 1915, **7**, 722.)  
 H. C. SHERMAN and M. D. SCHLESINGER, *J. Amer. Chem. Soc.*, 1915, **37**, 1305. (Properties of pancreatic and malt amylase preparations compared. Considers they are similar but not identical.)  
 J. L. BAKER and H. F. E. HULTON, *J. Chem. Soc.*, 1922, **121**, 1929. (Consider insoluble amylase of barley is associated with the alcohol soluble group of proteins.)  
 E. OHLSSON, *Compt. rend. Soc. biol.*, 1922, **87**, 1183. (Clearly distinguishes between 'dextrinogenic' and 'saccharogenic' amylases in malt.)  
 K. SJÖBERG, *Biochem. Zeit.*, 1922, **133**, 218. (Determines amylase activity in various parts of many plants at various times and also in algae grown in various media.)  
 H. C. SHERMAN, *Proc. Nat. Acad. Sci.*, 1923, **9**, 81. (Investigation of pancreatic and malt amylases.)  
 J. MROTSCHKOVSKY, *Hyg. Rundsch.*, 1891, **1**, 324. (Phenol and iodoform do not influence, but salicylic acid and mercuric chloride destroy, amylolytic action.)  
 M. ARTHUS and A. HUBER, *Compt. rend.*, 1892, **115**, 839. (NaF destroys living organisms but not amylases.)  
 A. R. LING and G. McLAREN, *J. Inst. Brewing*, 1908, **14**, 160. (Inactivating effect of copper noted.)  
 C. GERBER, *Compt. rend. Soc. biol.*, 1911, **70**, 139, 391, 547, 724, 726, 728, 822; 1911, **71**, 41, 208, 247. (Effect of many metallic salts of organic and inorganic acids, alkaloids and nitrogen compounds on saccharification noted.)  
 L. MICHAELIS and H. PECKSTEIN, *Biochem. Zeit.*, 1914, **59**, 77. (Investigation of salivary amylase.)  
 H. C. SHERMAN, A. W. THOMAS and M. E. BALDWIN, *J. Amer. Chem. Soc.*, 1919, **14**, 231. (Use Clark cell and rocking electrode to determine limiting and optimal pH values for malt, pancreatic and takadiastases.)  
 W. WEDEMAN, *Zeit. Fleisch.-Milchhyg.*, 1924, **35**, 301. (Milk diastase.)  
 D. H. COOK, *J. Biol. Chem.*, 1925, **65**, 135. (Temperature coefficients and killing temperatures of pancreatic and malt amylases.)  
 R. WEITZ and R. LECOQ, *Compt. rend. Soc. biol.*, 1924, **91**, 926. (Barley and salivary amylase have same optimum temperature.)

## CHAPTER 2

PREPARATION OF ENZYMES USED IN THE  
STARCH INDUSTRY

A VERY extensive literature concerning the action of enzymes on starch has developed following research on the structure of starch and on enzyme action, and, although few definitive conclusions have as yet been reached, great use is made commercially of the hydrolytic power of certain enzymes on starch.

A very brief summary of the methods of preparation of the various enzymes *used commercially* is presented below for those who are interested in the foodstuffs, adhesive, textile or other starch-using industries.

Table 14 summarises the chief points connected with the enzymes used commercially for the hydrolysis of starch, and we may now consider briefly the preparation of these substances. They may be classed into :—

1. Enzymes from cereals, e.g. malt diastase.
2. Enzymes from fungi or moulds.
3. Enzymes from animal juices.
4. Bacterial enzymes.

**1. Enzymes from Malt.**—Amylase from malt consists of a mixture of separate enzymes which induce distinctive types of starch break-down. The saccharogenic component produces considerable maltose from starch without destroying the iodine coloration properties, whereas the dextrinogenic component progressively destroys the iodine coloration with the liberation of but relatively few reducing groups (see pp. 479 and 480). Ungerminated barley grain forms the most convenient source of saccharogenic amylase. This is the only enzyme existing in an *active* state in the dormant grain, its occurrence being confined to the mealy endosperm. At the onset of germination the dextrinogenic amylase makes its appearance in the aleurone layer, and later in the scutellar epithelium, and from thence diffuses into the endosperm, upon which the starch granules start to corrode.

In addition to these components there appears to exist in ungerminated grain, according to E. Waldschmidt-Leitz and K Mayer,<sup>38</sup> another amylase, designated amylophosphatase, which causes liquefaction of the starch. It is separated by

TABLE 14

<i>Preparation.</i>	<i>Appearance.</i>	<i>Opt. pH.</i>	<i>Opt. Temp.</i>	<i>Catalysis.</i>	<i>Killing Temp.</i>	<i>Poisons.</i>	<i>Strength used Commercially.</i>	<i>Advantages.</i>
<i>Milk Extract.</i> Clenbumol. <sup>23</sup> Brimal. <sup>23</sup> Farinol. <sup>23</sup> Balmal. <sup>23</sup> Diastafor.	Thick syrup to thin liquid.	4.5-14. <sup>26</sup> 5-0. 6-5 <sup>23</sup>	For liquefaction 60-65° C. Saccharification, 50-55° (50-63° C. used commercially).	0.5 per cent. NaCl stabilises and accelerates action. <sup>25</sup> Small amounts of H <sub>2</sub> SO <sub>4</sub> or other acids KH <sub>2</sub> PO <sub>4</sub> .	80° C.	Salicylic acid. Zinc chloride. Formaldehyde. Ammonia. Copper salts.	5-8 lb./100 gall.	Price. Ease of control.
<i>Fungus or Mould Preparations.</i> Polyzymes and Laund-Aid Taka-diastase.	Thin liquids or pale yellow powder.	5-4 4-8 <sup>14</sup>	55-57° C. <sup>23</sup>	Sulphates. NaCl. Phosphates. <sup>24</sup> Aluminium salts. <sup>24</sup>	82° C.		1 lb. to 2 lb./100 gall.	Dry preparations stable to storage. Resistant to antiseptics.
<i>Animal Enzymes.</i> Degomma. Novo FermaSol.	Pale buff powder.	4-6. 6-8 <sup>23</sup> Limits 4-10-0	46° C.	0.25 per cent. NaCl and 0.004 per cent. Ca(OH) <sub>2</sub> .	55° C. (70° C.)		2.5 lb./100 gall.	Attacks protein as well as starch. No oxydases, hence no risk of yellow stains on fabric. Fairly stable to storage.
<i>Bacterial.</i> Rapidase. Biolase. Supercelastase. Rhenania.	Thin liquid.	7-7.5	70° C.	0.5 per cent. NaCl. 0.025 per cent. Na <sub>2</sub> CO <sub>3</sub> .	93° C.		5-10 lb./100 gall. (Strength employed is dependent on temperature and time of operation.)	Can use at higher temps. and hence get better penetration. Attacks proteins as well as starch. Stores indefinitely. Resistant to alkalies.

adsorption, together with the saccharogenic amylase, on to alumina  $C_r$ , after which the adsorbate is removed by elution and the saccharogenic component removed by selective adsorption by kaolin under appropriate conditions.

After barley has been allowed to sprout under controlled conditions of temperature, humidity, etc., it is carefully dried. During the germination three enzymes are generated. One, cytase, is destroyed during the drying process, another is proteolytic, but the third, which, as mentioned above, is really a mixture, is the most important from our point of view and is called 'diastase'. W. Windisch is of the opinion that the high diastatic value of American malts is due to the smallness of the grains used in that country, as he found that the diastatic activity of the extract from small grains was  $1\frac{1}{2}$ -2 times that obtained from large grains. To obtain the highest yield of diastase, the grains should be dried at the lowest temperature as is practicable. After drying they are ground and macerated with water and, after standing for an hour or so, the liquid is separated and evaporated, under vacuum at a low temperature, to a syrupy consistency. The syrupy consistency is due chiefly to the presence of sugars and dextrans which act as preservatives for the solution. According to W. Windisch and W. Jetter,<sup>1</sup> the diastase-content of cereals decreases in the order: rye, wheat, barley, oats, corn, rice, and potatoes, the last containing enough diastase to liquefy all the starch present in the tuber. Another interesting observation is that of H. von Euler,<sup>2</sup> who found that if barley was allowed to germinate in  $N/10$  disodium phosphate the diastatic extract prepared from it was 2.3 times as active amylolytically as the extract prepared from the same barley germinated in water. The malt extract prepared as above is very widely used in the textile, adhesive, and foodstuff trades, but special preparations with enhanced activity have been made by a number of workers. For example, a preparation producing 10,000 times its weight of maltose from a 2 per cent. starch paste in 30 minutes at  $40^\circ C.$  at a dilution of 1 in 9,000,000, was prepared by H. C. Sherman and co-workers,<sup>3-4</sup> who dialysed a malt extract of sp. gr. 1.27 against water for 24 hours, separated the solid matter by centrifuging, and after cooling the clear liquid in ice, dissolved 45 gm. of ammonium sulphate in 100 ml. of liquid. The precipitate formed was separated by centrifuging, then dissolved in 500-600 ml. of water, and the solution dialysed for 20 hours. The dialysate was concentrated by freezing, fractionally precipitated first by the addition of an equal volume of 99.8 per cent. alcohol, and next by bringing the alcohol-content to 65 per cent. by volume



by adding more alcohol, and finally adding ether. The first precipitate was separated and discarded, and the final precipitate was collected and carefully dried below  $15^{\circ}$  C. in a partial vacuum over sulphuric acid, the desiccator being kept in the dark during the drying operation.

The material so obtained appears to be similar in many respects to that discussed by R. Kuhn<sup>7</sup> and O. Holmbergh,<sup>8</sup> but differs in others. M. L. Caldwell and S. F. Doebbeling<sup>10</sup> find two types of product present in barley-malt extract, both of which are deactivated by heating in solution, give positive protein reactions, but are free from carbohydrates. Although they appear to act differently on starch, they both have their maximum effect at pH values between 4.3 and 4.6. Other optimum values have been given by other workers,<sup>12-18</sup> but, owing to different conditions under which they were obtained, such as differing buffer solutions, they may not be comparable. The substances prepared by Sherman and his co-workers have a very high activity, judged by the weight of maltose formed, but only a negligible activity if judged by the weight of starch hydrolysed to products giving no blue iodine reaction, as determined by a modified Wohlgemuth's method.<sup>11</sup> R. Fricke and P. Kaja<sup>53, 54</sup> have obtained highly active malt amylase preparations by an electro-osmosis technique.

H. Lüers and E. Sellner<sup>5</sup> claim to have prepared a malt amylase having an activity of more than twice that of any preparation previously made from malt, to obtain which they employed the method of selective adsorption which has been used by several investigators in this field. These workers point out the importance of keeping all the solutions cold throughout the operations. Aluminium hydroxide adsorbs many enzymes, almost quantitatively,<sup>6</sup> from solution, with the exception of amylase, which is not so strongly adsorbed as are other enzymes. According to M. A. Rakusin,<sup>19</sup> the portion of the amylase that is adsorbed does not give Ostromlevsky's reaction, whereas the unadsorbed fraction does. The adsorption method has been further studied by H. Kraut and E. Wenzel,<sup>20</sup> who used pancreas extract and plotted the equilibrium curves for adsorbed and unadsorbed enzyme at different concentrations, and in the presence of different amounts of adsorbents. The preparation of pure  $\beta$ -amylase<sup>36</sup> from sweet potato 'malt' has been described by K. V. Giri.<sup>37</sup>

**Preparation of Individual Malt Enzymes.**—When it is desired to prepare the component enzymes of malt comparatively free from contamination by one another three methods are open to us. The principles they embrace are briefly :—

- (a) Differential adsorption, which has already been mentioned.
- (b) Differential diffusion.
- (c) Differential inactivation.

(a) Waldschmidt-Leitz, M. Reichel and A. Purr<sup>39</sup> adjust the malt solution to  $pH$  3.8 and adsorb the saccharogenic amylase (with a little of the dextrinogenic amylase) on to alumina  $C_r$ , leaving a solution rich in dextrinogenic amylase. O. Holmbergh<sup>40</sup> prepares the latter by adsorbing it from a 40 per cent. aqueous alcoholic solution of the mixed amylases, adjusted to a  $pH$  value of between 4.4 and 7.6, on to native potato or rice-starch granules at a low temperature. On washing the starch granules with a 1 per cent. solution of maltose in 50 per cent. aqueous alcohol the amylase is removed in a reasonably pure form.

(b) H. P. Wijsman<sup>41</sup> and later G. A. van Klinkenberg<sup>27</sup> placed drops of malt extract on a gelatine-starch gel and 3 or 4 days later stained the gels with iodine. There was a colourless central zone up to the limits of diffusion of the dextrinogenic amylase surrounded by a wider violet band showing the extent of diffusion of the saccharogenic amylase. This technique has been used by Giri (see p. 375) to distinguish between samples of pasted starch. By repeating the above experiment under the same conditions, omitting the iodine staining and removing small blocks of the gel where the outer zone would have been made visible if stained by iodine, and bringing these blocks into contact with a new gel, it was found that only mauve diffusion zones were obtained on staining. This showed that the saccharogenic amylase had been separated entirely by diffusion. Using this technique K. V. Giri,<sup>42</sup> without the confirmation furnished by the secondary diffusion, concluded that *aspergillus* and pancreatic enzymes were mixtures.

(c) While Wijsman's work foreshadowed the later methods of differential inactivation by weak acids and heating, respectively, E. O. Ohlsson<sup>12</sup> provided reliable methods for preparing relatively pure preparations of the component enzymes of malt. The saccharogenic enzyme is rapidly inactivated at elevated temperatures, but is not deactivated by acid. The malt extract at 0° C. is therefore adjusted to a  $pH$  value of 3.3 with hydrochloric acid, whereupon the dextrinogenic activity falls to 1-2 per cent. of its original value, whereas the drop in saccharogenic activity is only of the order of 20-30 per cent. The dextrinogenic enzyme is unusually thermostable so that a malt extract of  $pH$  value 6.7 retains 60 per cent. of its dextrinogenic activity if heated

to 70° C. for 10 minutes, whereas the saccharogenic activity has fallen to 5-7 per cent. of its original value.

E. Waldschmidt-Leitz<sup>43</sup> and T. Sabalitschka and R. Weidlich<sup>44</sup> think that these treatments may substantially alter the properties of the enzymes present in malt, so that the same enzyme could show one of two sets of properties according to the method of treatment, but other work has shown beyond doubt that two amylolytic enzymes do exist in malt extract.

**2. Enzymes from Moulds or Fungi.**—Certain species of the yellow-green mould *Aspergillus oryzae* produce a mixture of enzymes, including diastase, invertase, maltase, amidase, and protease, the amounts of which vary with the different species employed. To obtain a preponderance of any one enzyme, it is therefore necessary to select the desired species and to isolate it in the pure state by repeated subculturing. The pure culture so obtained is immunised against certain antiseptics that are met with in the textile industry, by subculturing it on a semi-solid nutrient media containing increasing amounts of the selected preservatives, e.g. sodium fluoride and salicylate,<sup>31</sup> until a strain is finally obtained which possesses a marked resistance to the action of these compounds. This strain is then used as the starting-material for preparing batches of the enzyme preparation on a large scale. A mass of sterilised nutrient material consisting of wheat, bran, corn, or rye, and containing certain salts in large rotating drums kept at 30-40° C. is inoculated with the culture, and after this has spread over the mass and the enzymes have formed, they are separated by extraction with water, the solution filtered, or separated by centrifuging,<sup>30</sup> standardised to a given strength, preservative added, and the preparation is ready for marketing.

To obtain powdered preparations of high activity the enzymes are precipitated with alcohol, purified by several treatments, and dried at a low temperature. Sherman and Tanberg<sup>21</sup> have obtained very active preparations from commercial takadiastase in the same manner as they prepared the active malt diastase described above. K. Oshima<sup>28</sup> finds both amylase and protease are obtained simultaneously by the action of *Aspergillus oryzae* on both natural and synthetic culture media. The optimal quantity appears after two days' growth and both enzymes are extra-cellular after sporulation.

Different species of *Aspergillus* show variation in activity of enzyme. T. Nagatomo<sup>29</sup> has noted a deviation of the optimum pH value for the amylases from various strains of the fungus. In general the optimum activity is shown between limits of pH value 4.8-5.2 and most stable towards heat at pH 6.4. L. A.



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FIG. 66.—Equipment used in microbiological enzyme production.

[Facing p. 460.]



Underkofler and co-workers<sup>46</sup> have examined the saccharification of starchy grain mashes for the alcoholic fermentation industry and find two strains of *Aspergillus oryzae* are eminently suitable. They did not separate the enzyme but used the mouldy bran in the same manner as malt and obtained about 12 per cent. higher alcohol yields than with good barley malt. They describe the preparation of the mouldy bran on a pilot-plant scale.

The commercial preparation of amylolytic enzymes from *Aspergillus oryzae* was first introduced by Takamine<sup>47</sup> and further descriptions of the preparation and properties of the enzyme from this source are given by L. Wallerstein,<sup>30</sup> Oshima and Church,<sup>49</sup> H. Leopold and H. G. Germann<sup>57</sup> and by Harada.<sup>50</sup>

**3. Enzymes from Animal Juices.**—The source from which enzymes are obtained in the animal world are the pancreatic glands of horses or of cows. The liquids expressed from these glands are rich in starch-converting enzymes which are precipitated from the liquor by salting out. The precipitated enzymes are dried and mixed with neutral salts, which serve to adjust the activity to a desired standard strength, so that the product shall always be uniform in activity when it comes on the market. According to J. Larsen and C. F. Poe<sup>55</sup> aqueous acetamide was found to be the most selective solvent for extracting the amylase from pancreas.

Sherman and Schlesinger<sup>22</sup> prepare an extract of high activity as follows: 20 gm. pancreatin powder are macerated with 200 ml. of 50 per cent. alcohol, and the suspension filtered after 20 minutes. The active constituent is precipitated as an oily mass by pouring the solution into 1400 ml. of a mixture of 20 per cent. alcohol and 80 per cent. ether, and after decanting the liquid, the precipitate is re-dissolved in a little water, re-precipitated by pouring into five times its volume of absolute alcohol, and the flocculent precipitate obtained dissolved in 200-250 ml. of 50 per cent. alcohol containing 5 gm. maltose. This solution is dialysed three times against ten times its volume of 50 per cent. alcohol for 15, 9, and 40 hours, respectively, for the three portions. The three dialysates are mixed, filtered, and poured into an equal volume of a 1:1 alcohol-ether mixture. The precipitate which forms is filtered off and dried under vacuum. The temperature at which the above operations are carried out should be around 10-15° C. throughout, and should never exceed 20° C.

Sherman and Schlesinger found that such preparations, used at a concentration of one in a million on a 1 per cent. starch solution, completely digested the starch to the stage of giving no iodine reaction in 96 hours. In this time the enzyme had produced over

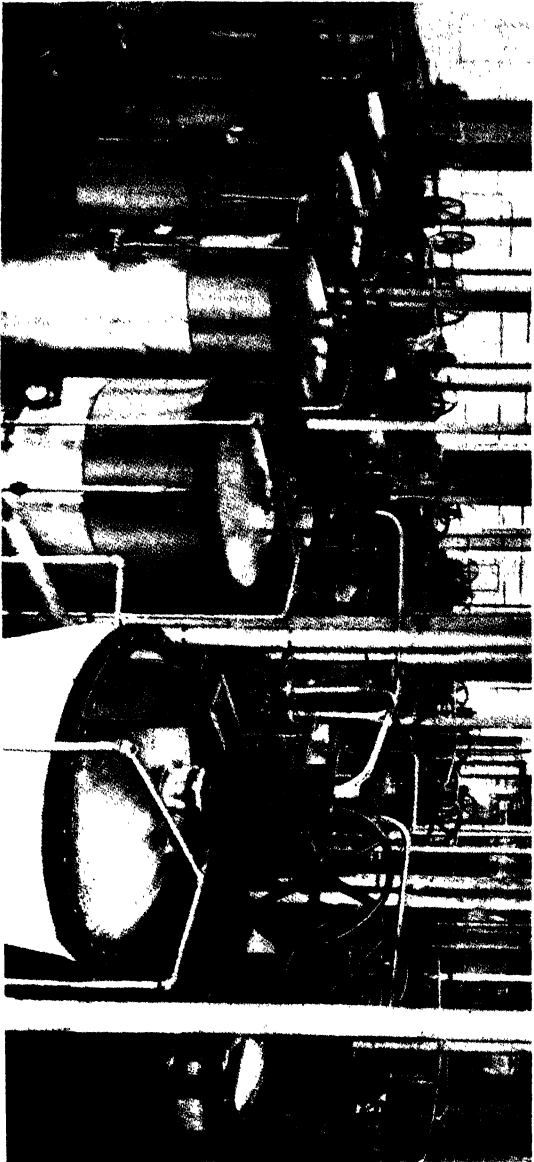
500,000 times its own weight of reducing sugar, and its activity on the Lintner Scale was of the order of 5000-6000. Later it was found <sup>23</sup> that only about 5 per cent. of the amylolytic activity was recovered in the final product, and that the precipitate formed in the inner solution during dialysis had a high proteolytic and little or no amylolytic activity.

**4. Bacterial Enzymes.**—Boidin and Effront <sup>48, 51, 52</sup> may be credited with the first commercial method for preparing amylolytic enzymes by the use of bacteria. The enzymes are of the liquefying type, characterised by maximum activity in alkaline or neutral solutions. Certain micro-organisms of the *B. subtilis* or *mesentericus* species when allowed to act on a sterilised nutrient medium of wort produce powerful diastatic and proteolytic enzymes, and owing to the rapidity of action of preparations made in this way, they have been marketed under the name of Rapidase (see Table 14). This preparation is notable for the high temperatures at which it can work, which in textile work means better penetration. It has been noticed that this preparation does not appear to contain any albuminoid or protein matter that is heat-sensitive, whereas other enzyme preparations from other sources appear to contain such constituents, and this may explain why it is active at temperatures at which other enzymes show diminution, or even destruction, of their activity. Table 14 shows the salient features of this preparation.

The bacteria is allowed to grow over large surfaces in thin layers so as to produce a thin film. For maximum liquefying power Boidin and Effront found successive subculturing in a culture medium relatively rich in nitrogenous material was best. Strong aeration at the start followed by a slight lowering of the oxygen supply after full development was beneficial and for most strains of the bacteria used temperatures between 25-30° C. are best.

The culture medium consists of hydrolysed soybean cake or peanut cake, together with hydrolysed starchy material and phosphates, potassium, magnesium and calcium salts. The media is sterilised by heating under pressure at 125-130° C., filtered, cooled and inoculated. The media on trays is kept in a culture apparatus (Fig. 67) some 8 feet in height and 6 feet in diameter <sup>48</sup> under a continuous internal pressure, so that air-borne contamination cannot take place and each vessel has a capacity of some 500-1000 gallons of media. The most scrupulous cleanliness is observed throughout the whole process.

After about one week the liquid is cooled and passed directly to a high-speed centrifuge where the bacteria are removed, after



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*[Facing p. 462.]*





which antiseptics are added to the liquid, which may then be stored. According to the composition, pH value and aeration of the media, a large measure of control over the ratio of amylolytic enzymes to proteolytic enzymes is obtained. The liquid can be concentrated *in vacuo* without further processing or, for some purposes, purification by means of precipitation by ammonium sulphate, alcohol, acetone, etc., is carried out. Such processes enable a thousandfold increase in enzymatic activity to be obtained.

Another method of preparation<sup>45</sup> is to take the crude enzymic liquid from the mass obtained by the fermentation of the amylaceous substance with *B. mesentericus* and extract the fatty matter present with a fat solvent. The colloidal matter is precipitated, either by adjustment of the pH of the solution or by the addition of heavy metal salts, and the precipitate removed, together with the micro-organisms from the solution by centrifuging. The amylase and protease can then be separated, if required, by the use of various adsorbents. A. Janke and B. Schaefer<sup>56</sup> list 37 species of bacteria that split starch. The most active, according to these workers, were *B. mesentericus*, *B. megathericum*, *B. vulgatus*, *B. mycoides*, *B. subtilis*, *B. ovalecticus*, *B. macerans*, *B. albus*, *Sarcina aeritronuxa* and *Sarcina lutea*. The optimum temperature was 40-50° C. in 1/300 molar sodium chloride solution.

## REFERENCES

1. W. WINDISCH and W. JETTER, *Zeit. Spiritusind.*, 1907, **30**, 541, 552.
2. H. VON EULER and B. UGLAS, *Archiv. kemi. mîner. geol.*, 1908, No. 30; 1910, No. 3.
3. H. C. SHERMAN, A. W. THOMAS, and M. L. CALDWELL, *J. Amer. Chem. Soc.*, 1924, **46**, 1712.
4. H. C. SHERMAN, E. C. KENDALL, and E. D. CLARK, *J. Biol. Chem.*, 1934, **104**, 501.
5. H. LÜERS and E. SELLNER, *Woch. Brauerei*, 1925, **42**, 97, 103 and 110.
6. R. WILLSTÄTTER, E. WALDSCHMIDT-LEITZ, and A. R. F. HESSE, *Zeit. physiol. Chem.*, 1925, **142**, 14.
7. R. KUHN, *Ann. Chem.*, 1925, **448**, 1.
8. O. HOLMBERGH, *Biochem. Zeit.*, 1933, **258**, 134; 1933, **266**, 203.
9. H. C. SHERMAN and A. W. THOMAS, *J. Amer. Chem. Soc.*, 1915, **37**, 623.
10. M. L. CALDWELL and S. F. DOEBBELING, *J. Biol. Chem.*, 1935, **110**, 739.
11. J. WOHLGEMUTH, *Biochem. Zeit.*, 1908, **9**, 1.
12. E. C. OHLSSON, *Compt. rend. trav. lab. Carlsberg*, 1926, **12**, No. 7.
13. — *Zeit. physiol. Chem.*, 1930, **189**, 17.
14. H. C. SHERMAN, A. W. THOMAS, and M. E. BALDWIN, *J. Amer. Chem. Soc.*, 1919, **41**, 231.
15. H. C. SHERMAN and H. H. BOYNTON, *ibid.*, 1930, **52**, 1669.

16. S. PRONIN, *Biochem. Zeit.*, 1932, **249**, 7.
17. H. PRINGSHEIM and J. LEIBOWITZ, *ibid.*, 1925, **161**, 436.
18. M. SAMEC and E. WALDSCHMIDT-LEITZ, *Zeit. physiol. Chem.*, 1931, **203**, 16.
19. M. A. RAKUSIN, *J. Russ. Phys. Chem. Soc.*, 1916, **48**, 321 and 465.
20. H. KRAUT and E. WENZEL, *Zeit. physiol. Chem.*, 1924, **133**, 1.
21. H. C. SHERMAN and A. P. TANBERG, *J. Amer. Chem. Soc.*, 1916, **38**, 1638.
22. H. C. SHERMAN and M. D. SCHLESINGER, *ibid.*, 1912, **34**, 1104; 1913, **35**, 1617; 1915, **37**, 643.
23. H. C. SHERMAN, I. D. GARARD, and V. K. LA MER, *ibid.*, 1920, **42**, 1900.
24. L. ADLER, *Biochem. Zeit.*, 1916, **77**, 146.
25. J. T. GROLL, *Nederl. tijds.*, 1921, **65**, **2B**, 2541.
26. W. M. SCOTT, *Ind. Eng. Chem.*, 1940, **32**, 784.
27. G. A. VAN KLINKENBERG, *Proc. Acad. Sci. Amsterdam*, 1931, **34**, 893; *Z. physiol. Chem.*, 1932, **209**, 253; *ibid.*, 1932, **212**, 173; *Ergbn. Fermentforsch.*, 1934, **3**, 73.
28. K. OSHIMA, *J. Coll. Agric. Hokkaido Imp. Univ.*, 1928, **19**, 135.
29. T. NAGATOMI, *J. Agric. Chem. Soc. Japan*, 1939, **15**, 753.
30. L. WALLERSTEIN, *Ind. Eng. Chem.*, 1939, **31**, 1218.
31. TAKAMINE FERMENT CO., U.S.P. 1,680,926; 14/8/1928. Appl. 9/1/1923.
32. A. F. MUSGRAVE, *Sci. Amer. Suppl.*, 1916, **82**, 107.
33. B. S. HILLMAN, *Text. Col.*, 1932, **54**, 457, 490.
34. J. EFFRONT, *Compt. rend.*, 1892, **115**, 1324.
35. L. MICHAELIS and H. PECKSTEIN, *Biochem. Zeit.*, 1914, **59**, 77.
36. K. V. GIRI, *J. Indian Chem. Soc.*, 1938, **15**, 249.
37. — *ibid.*, 1934, **11**, 339.
38. E. WALDSCHMIDT-LEITZ and K. MAYER, *Hoppe-Seyl. Z. physiol. Chem.*, 1935, **236**, 168.
39. E. WALDSCHMIDT-LEITZ, M. REICHEL and A. PURR, *Naturwiss.*, 1932, **20**, 254.
40. O. HOLMBERGH, *Ark. Kemi Min. Geol.*, 1935, **11A**, No. 20, 1. (See also Ref. 8.)
41. H. P. WIJSMAN, 'Die Diastase beschouwd als mengsel, etc.', diss. Amsterdam, 1889, *Rec. Trav. Pays-Bas*, 1890, **9**, 1.
42. K. V. GIRI, *J. Ind. Inst. Sci.*, 1934, **17A**, (Part II), 127.
43. E. WALDSCHMIDT-LEITZ, 'Enzyme Actions and Properties,' transl. R. P. WALTON, p. 204, New York, 1929.
44. T. SABALITSCHKA and R. WEIDLICH, *Biochem. Zeit.*, 1929, **210**, 414.
45. A. R. BOIDIN and J. A. EFFRONT, E.P. 315,877, 18/7/29; Fr. 21/7/28.
46. L. A. UNDERKOFER, E. L. FULMER and L. SCHOENE, *Ind. Eng. Chem.*, 1939, **31**, 734.
47. J. TAKAMINE, *J. Ind. Eng. Chem.*, 1914, **6**, 824.
48. A. R. BOIDIN and J. A. EFFRONT, U.S.P. 1,227,374, May 22, 1917.
49. K. OSHIMA and M. B. CHURCH, *Ind. Eng. Chem.*, 1923, **15**, 67.
50. T. HARADA, *ibid.*, 1931, **23**, 1424.
51. A. R. BOIDIN and J. EFFRONT, U.S.P. 1,227,525, May 22, 1917.
52. J. EFFRONT, 'Biochemical Catalysts in Life and Industry,' 1st ed., 1917.
53. R. FRICKE and P. KAJA, *Ber.*, 1924, **57**, 310.
54. R. FRICKE, *ibid.*, 1924, **57**, 765.

55. J. LARSEN and C. F. POE, *J. Biol. Chem.*, 1940, **132**, 129.  
56. A. JANKE and B. SCHAEFER, *Zentr. Bakt. Parasitenk.*, 1940, **II**, **102**, 241.  
57. H. LEOPOLD and H. G. GERMANN, *ibid.*, 1940, **II**, **102**, 65.

## ADDITIONAL REFERENCES

- J. E. EVANS, *J. Soc. Dyers and Col.*, 1933, **49**, 250. (General.)  
ANON, *T.I.B.A.*, 1937, **15**, 13. (Large-scale preparation of enzymes.)  
N. BOURGUIGNON, *ibid.*, 1937, **15**, 85. (Separation and determination.)  
J. PORZKY, *Zeit. ges. Textile-Ind.*, 1936, **39**, 198. (Optimum conditions for enzymatic activity.)  
ANON, *Tinctoria*, 1933, **32**, 487. (General.)  
Y. K. SHIH, *Lingnan Sci. J.*, 1937, **16**, 27. (*A. oryzae* and *A. flavis* produce large amounts of amylase, 26 species examined.)  
E. V. KOLOBKOVA, *Arch. sci. biol. (U.S.S.R.)*, 1936, **43**, 101. ( $\beta$ -amylase situated in part of plants above ground and  $\alpha$ -amylase in parts below ground.)  
A. TYCHOWSKI, *Biochem. Zeit.*, 1937, **291**, 138. ( $\beta$ - and  $\alpha$ - $\beta$ -amylases from different sources.)

## CHAPTER 3

THE ACTION OF  $\beta$ -AMYLASE ON STARCH

WHEREAS among the  $\alpha$ - or dextrinogenic enzymes from different sources there appears to be slight variations in properties or in their action on starch,  $\beta$ -amylase from wheat, barley or other cereals appears to be a definite entity existing in the one form only.<sup>1</sup> It was at one time considered that, as  $\beta$ -amylase existed only in dormant cereal grains, the  $\alpha$ -amylase was differentially inactivated during maturation.<sup>2</sup> A number of workers have studied the preparation and properties of amylase concentrates from a variety of cereals such as germinated wheat and rye,<sup>53, 55</sup> germinated maize,<sup>56</sup> germinated oats<sup>54</sup> and soybeans.<sup>57-60</sup> There has been some discussion as to whether two enzymes were present in soybeans or not, and the results of G. L. Teller<sup>61</sup> indicate that although two amylases are probably present the soybean amylase is a better sugar producer than any other amylase studied with the exception of that in the sweet potato. Recently Giri<sup>2-5</sup> has shown that the amylase of the sweet potato tuber, *Ipomea batatas*, resembles  $\beta$ -amylase very closely (see p. 440).

**Soluble Starch.**—A number of workers have followed the action of  $\beta$ -amylase on starch pastes and soluble starch by means of the *optical rotation* of the medium. H. T. Brown and G. H. Morris<sup>6</sup> claimed there was no agreement between the increase in reducing power and the loss in optical rotation, but Brown and Heron, using different conditions, later obtained a good correlation. H. van Euler and K. Helleberg<sup>7</sup> showed that during hydrolysis of the starch maltose is liberated in the low rotatory form, and R. Kuhn,<sup>8, 9</sup> acting on soluble starch and on amylose with amylases from different sources, arrived at his system of nomenclature from a consideration of the optical properties shown by the various products (see p. 436).

E. Olhsson<sup>10</sup> was probably the first to recognise clearly that malt saccharogenic amylase is a  $\beta$ -amylase and the malt dextrinogenic amylase is an  $\alpha$ -amylase. Kuhn acted on electro dialysed soluble starch with both untreated and dialysed malt extract. From his results it would appear that maltose can be split off in the low rotation form so that a higher degree of fission is recorded if polarimetric methods instead of reducing methods are used to follow the enzymatic action. Olhsson<sup>11</sup> and G. G.

Freeman and R. H. Hopkins<sup>12</sup> have confirmed these results on all essential points.

By following the *reducing power* of the reaction mixtures later workers showed that, in the  $\beta$ -amylase action on starches, reduction products were formed right at the beginning but that the iodine coloration is only slightly affected. The reaction proceeds relatively quickly until the reducing power reaches a value corresponding to 50-55 per cent. of the theoretical maltose. From this point greater resistance to fission is shown and the reducing value slowly rises to its final level. Table 15 shows the final hydrolysis limits obtained by various workers on different substrates.

The differences observed may be due to variations in the substrate or its temperature of preparation (see p. 441), or to differences in preparing the enzymes. The limit of production of maltose between 60-70 per cent. was found by Martin, Naylor and Hixon<sup>47</sup> to hold also for corn, wheat, rice and tapioca starches.

Wheat, buckwheat, arrowroot,<sup>19, 25</sup> maize and apple,<sup>21, 26</sup> barley, rice and potato starches all appear to be degraded similarly whether a paste from a solubilised form or the native starch is used, and the limit of hydrolysis does not, according to Hanes<sup>21</sup> and Klinkenberg,<sup>27</sup> appear to be appreciably influenced by variations in the enzyme concentration.

A. Tychowski,<sup>24</sup> using different raw starches in the absence of added buffer salts, found the saccharification limit to be independent of the enzyme concentration, pH value of media (4.2-7.9), temperature (20-50° C.) or the presence of glucose, maltose or the other degradation products of starch.

R. H. Hopkins, G. F. Cope and J. W. Green<sup>20</sup> observed a considerable rise in the saccharification limit when living yeast was present in the reaction mixture, and they ascribe this to the effect of an amylase activator (see p. 445) which they considered may have diffused into solution from the yeast. O. Meyerhoff,<sup>28</sup> however, using an amylase activator from oats, was unable to obtain this increased effect.

A number of investigators report a relatively sharp cessation of hydrolysis at 60-61 per cent. fission, whereas in other cases the reducing power has been found to rise very slowly beyond this point until a 64-67 per cent. fission has been attained. C. S. Hanes<sup>29</sup> reports 59-60 per cent. as the limit when using 0.5-1.0 per cent. solutions of soluble starch, but with solutions containing 0.2 per cent. values up to 66 per cent. were obtained. The concentration of substrate would, therefore, appear to have a definite influence on the fission limit attained.

TABLE 15  
HYDROLYSIS OF STARCH BY  $\beta$ -AMYLASE

<i>Substrate.</i>	<i>Enzyme Used.</i>	<i>Limits of Hydrolysis.</i>	<i>Observer.</i>
Lintner soluble starch	Barley extract	60-65	Baker <sup>13</sup>
Amylose (Grużewska)	Dialysed malt extract	59-61	Sjöberg <sup>14</sup>
	Dialysed barley extract	39	Sjöberg and Eriksson <sup>15</sup>
Amylopectin ( , , )	Dialysed barley extract	40	Sjöberg and Eriksson <sup>15</sup>
Potato amylopectin	Alcohol precipitated barley extract	67	Ling and Nanji <sup>16</sup>
Potato starch autoclaved at 130° C. for 10 hours	Barley extract	65	Syniewski <sup>17</sup> Pollak and Tychowski <sup>18</sup>
Autoclaved soluble starch	Barley extract	66	
Lintner soluble starch	Wheat and barley $\beta$ -amylase	64	Klinkenberg <sup>19</sup>
	Alcohol precipitated barley extract	64-65	Hopkins, Cope and Green <sup>20</sup>
	Alcohol precipitated barley extract	61	Hanes <sup>21</sup>
Lintner soluble starch and Fouard starch	$\beta$ -amylase prepared by Klinkenberg's method	66	Samec <sup>22</sup>
Soluble starch	$\beta$ -amylase from barley prepared by Blom's method	60-61	Blom, Bak and Braae <sup>23</sup>
Potato starch autoclaved as above <sup>17</sup>	$\beta$ -amylase from—		
	barley	62.6	Tychowski <sup>24</sup>
	wheat	68.6	"
	rye	62.16	"
Autoclaved wheat starch	barley	65.4	"
	wheat	67.2	"
	rye	64.6	"
Autoclaved rye starch	barley	63.0	"
	wheat	64.7	"
	rye	62.5	"
Autoclaved barley starch	barley	65.7	"
	wheat	67.1	"
	rye	61.5	"
Autoclaved maize starch	barley	59.1	"
Autoclaved rice starch	barley	58.7	"

Blom and co-workers<sup>30</sup> report that at pH 3.4 hydrolysis ceases at 53 per cent. conversion, but at pH 4.6 and 6.8 the normal limit of 60-61 per cent. is attained. This result may be due to

the presence of amylophosphatase (see p. 22), and it is tempting to suggest that some  $\alpha$ -amylase may have been present, as this is practically inactivated at pH 3.4 and could thus exert no beneficial effect which it might hitherto have exerted.

The *iodine coloration* has also been used to study the effect of  $\beta$ -amylase on starch pastes. Until C. S. Hanes and M. Cattle<sup>31</sup> accurately measured the extinction coefficients of the coloured solutions obtained by adding iodine to portions of the reaction mixture at various stages of the hydrolysis, very few quantitative observations on the iodine coloration had been made, with the exception of the work of Samec<sup>22, 32</sup> and Blom, Bak and Braae.<sup>23, 30</sup> From the beginning of the process the extinction coefficient (E) value decreased over the whole spectral range, the curves showing a general similarity in form to that given by undegraded starch and no shift in the position of the maximum absorption peak takes place.

The extinction coefficient of maximum absorption is approximately proportional to the concentration of the residual dextrin. Actually the residual dextrin gives, after 30 per cent. hydrolysis has occurred, a more intense coloration with iodine than the undegraded starch of equal concentration. During the first phase of hydrolysis the decrease in intensity is smaller than the percentage of maltose formed. Samec and Blinc<sup>33</sup> have confirmed this and find that the iodine coloration of peptised starch is more intense than that of the raw starch.

The only products of  $\beta$ -amylase action on starch are the practically non-reducing, amyloid dextrans which give a blue-violet iodine coloration whilst retaining, in a marked manner, the ability to form molecular aggregates, and maltose (see Baker,<sup>13</sup> Syniewski,<sup>17</sup> Hanes,<sup>21</sup> Freeman and Hopkins,<sup>34</sup> Blom and co-workers<sup>30</sup>).

Thus hydrolysis to the extent of about 60 per cent., retention of the capacity to give a blue-violet or violet colour with iodine, due to the presence of the resistant  $\alpha$ -amylo-dextrin or erythrogranulose and the production of maltose as the sole reducing compound formed is characteristic of  $\beta$ -amylase and differentiates it from other amylases.<sup>1</sup>

**1.  $\alpha$ -amylo-dextrin.**—The name given to the material left at the end of the degradation of starch by  $\beta$ -amylase stresses its starch-like nature. A certain variability in the properties of this product as obtained by various workers may be ascribed to the method of preparing the  $\beta$ -amylase. Using the enzyme prepared by precipitation with alcohol, which also induces concurrent dehydration, an  $\alpha$ -amylo-dextrin with a high rotation



( $[\alpha]_D^{20} + 221^\circ$ ) and containing practically all the phosphorus originally present in the starch is obtained.<sup>18</sup> Using an untreated barley extract or a freshly precipitated amylase which has only had a short contact time with the alcohol an  $\alpha$ -amylo-dextrin with no phosphorus and a low rotation ( $[\alpha]_D^{20} + 193^\circ$ ) is obtained.

These results are readily explained if the presence of amylo-phosphatase (see p. 22) in the latter preparation is assumed and that the longer alcoholic treatment in the former case destroys its properties. This would lead to an aggregated, high phosphorus-containing, high rotational but low reducing form of  $\alpha$ -amylo-dextrin from the action of alcohol-treated enzyme preparation. Where no destruction of amylophosphatase had taken place, as in the case of the untreated barley extract, the disaggregated phosphorus-free, lowly rotating product showing a fairly high reducing power should be obtained, as is the case.

Should the presence of amylophosphatase be proved in such cases it further follows that the slow increase in reducing power from 60 per cent. to 66 per cent. apparent conversion may be due to the transformation of the aldehydic end unit of the  $\alpha$ -amylo-dextrin into a reducing form and not to the slow liberation of further maltose.

Samec<sup>22</sup> obtained a low rotating form of  $\alpha$ -amylo-dextrin which gave  $R_M$  16 by the hypiodate method, which corresponds to a chain-length of some 12 glucose units, which is in agreement with end-group assay results.

The  $\alpha$ -amylo-dextrin, if separated, can be shown to be highly but not completely resistant to  $\beta$ -amylase. By autoclaving, a technique devised by Hopkins and co-workers (see Hanes<sup>35</sup>), further degradation can be brought about, and if the resistant material left after this treatment is successively separated, autoclaved and treated with enzyme a yield of only 3 per cent. of resistant material on the original starch is attained after six such successive cycles.

The  $\alpha$ -amylo-dextrin is not resistant to  $\alpha$ -amylase, the iodine coloration being destroyed, and Klinkenberg<sup>19, 25</sup> and Hanes<sup>21</sup> report that  $\beta$ -amylase will further degrade the products of this reaction and that this effect is to be noted from the beginning of the  $\alpha$ -amylase action.<sup>21</sup>

**2. Amyloamylose.**—The degradation of amyloamylose with  $\beta$ -amylase resembles the degradation of soluble starch which can be attained by periodical resort to autoclaving. It should be noted that autoclaving starch is the first step in the process for preparing amyloamylose. This step is followed by electro-

dialysis in which a gelatinous material, consisting of impure erythroamylose, migrates to the positive membrane. By repeated autoclaving followed by electro-dialysis this impure erythroamylose gives the pure material, which has a red iodine coloration, after some five or six cycles.

It has been variously reported that amylose and amylopectin are hydrolysed at very different rates by amylases. Ling and Nanji<sup>16</sup> reported amylose to be completely hydrolysed by  $\beta$ -amylase or by malt diastase, i.e.  $\alpha$ - and  $\beta$ -amylase, and concluded that the amylopectin was not attacked. Van Klinkenberg, elaborating on the resultant definition of amylopectin suggested by the work of Ling and Nanji, proposed the view that selective hydrolysis of a preformed fraction in the starch is responsible for the halt in the degradation by  $\beta$ -amylase at 60-65 per cent. conversion.

Hirst and co-workers<sup>36</sup> were unable to confirm Ling and Nanji's results, finding amylose and amylopectin were hydrolysed to the same extent as starch, and Hanes<sup>39</sup> obtained the same result with amylose and amylopectin prepared by milling starch.

Samec and Waldschmidt-Leitz<sup>37</sup> and, later, Freeman and Hopkins,<sup>38</sup> Samec<sup>22</sup> and Hanes<sup>26</sup> found that the amyloamylose of Samec and Meyer is degraded almost to completion by  $\beta$ -amylase. It may be mentioned here that the discrepancy between the apparent degree of fission given by the polarimetric and the reduction methods noted for soluble starch (see p. 466) is also found in the case of amyloamylose. As determined by optical methods the apparent degree of fission passes through a maximum.

Amyloamylose is hydrolysed at a much slower rate than soluble starch, probably because of its higher mean molecular weight, and when about 60-70 per cent. fission is reached the reaction slows down but reducing values corresponding to 95-98 per cent. of theoretical maltose are gradually reached. In this reaction the blue colour given with iodine persists up to  $R_M$  80-90 per cent. and from then on changes through violet to pink.

**3. Erythro Bodies.**—In contradistinction to amyloamylose erythroamylose resists further action of  $\beta$ -amylase after some 40-56 per cent. conversion has occurred.<sup>12, 22</sup> These values are slightly lower than the usual limits found for soluble starch but probably not for autoclaved starch paste under similar conditions. Soluble starch and Fouard starch probably contain both amylo and erythro bodies and behave in a manner intermediate between that of amyloamylose and erythroamylose when

treated with  $\beta$ -amylase. The elucidation of the relationship between starch, amyloamylose and erythroamylose by the end-group assay method will be of great interest in explaining the effect of chain-length or degree or type of association of the macromolecules on the results obtained by the action of amylases on these products.

**4.  $\alpha$ - and  $\beta$ -Glucosides.**—Blom and Braae<sup>40</sup> have compared the action of amylases with the glucosidases. None of the glucosides are split by  $\alpha$ -amylase, and  $\beta$ -amylase does not affect the  $\alpha$ -glucosides but splits  $\beta$ -glucosides. R. Weidenhagen and A. Wolf<sup>41</sup> consider, on the experience gained on the hydrolysis of the oligosaccharides, that a chain of glucose units connected by  $\alpha$ -bonds would be attacked by  $\alpha$ -glucosidases at all points along its length where there are intact  $\alpha$ -bonds and yet starch cannot be hydrolysed by  $\alpha$ -glucosidase.

**The Mode of Action of  $\beta$ -amylase.**—As maltose is the sole reducing agent formed by  $\beta$ -amylase action on starch an end-wise mode of attack must be assumed.<sup>42</sup> In view of the properties of the amyloid dextrin which remains, the maltose must be split off from the non-aldehydic end of the chain-molecule of starch, as any other type of attack would inevitably produce some glucose which, however, has so far not been detected.

Ohlsson<sup>10</sup> found by osmotic pressure data that, during the partial conversion of the substrate into maltose, there was no increase in the number of non-dialysable fragments present. This supports the theory of end-wise fission of maltose groups from the starch chain-molecule as also does the hydrolysis of a dextrinic acid, from malto-dextrin, by malt diastase to a dextrinic acid of shorter chain-length.<sup>43</sup> Myrbäck<sup>44</sup> found that starch whose reducing groups had been converted into —COOH groups by prolonged action of iodine and caustic soda was split by  $\alpha$ - and  $\beta$ -amylases in the same manner as the original starch. Örtenblad and Myrbäck<sup>45</sup> confirmed this with wheat starch dextrin and takadiastase, and from the above observations it would appear that the aldehydic end of the chain remains intact throughout the fission process.

The first glucosidic bond in the chain-molecule of starch cannot be severed by  $\beta$ -amylase since glucose is never found. As maltose is the sole reducing product formed it follows that the fission must occur between the second and third glucose units in the chain. One can assume with Hanes<sup>46</sup> that the enzyme contains two active groups of which one attaches itself to some atomic grouping in the first glucose unit, whilst the second active group attaches itself to the second glucosidic bond. According to

Hanes <sup>46</sup> the distance between the active groups would be the length of a maltose molecule, i.e. 10 Å.

Another explanation would be that, for some unknown reason, the second glucoside linkage was particularly unstable or had become so by virtue of the loose combination with the enzyme. Concurrent with the fission of the maltose, reversion to the  $\beta$ -form must take place by Walden Inversion and there are, at present, no chemical grounds for considering the formation of  $\beta$ -maltose in this manner improbable.

Blom, Bak and Braae <sup>30</sup> consider the chain-length of the starch molecule to be 30 glucose units, and in that case the degree of fission of 53 per cent., which is observed at pH 3.4, would correspond with the splitting off of 8 maltose fragments. Conversion to the extent of 60 per cent. and 66 per cent. would correspond to the splitting off of 9 and 10 maltose units, respectively.

The reason for the cessation of action at this point may be due to structural alterations brought about by the enzyme when nearing the sixteenth or eighteenth glucose unit, whereby the enzyme can produce no effect (Hanes). K. Myrbäck <sup>48</sup> has suggested that both the fatty acids and the phosphorus in starch might stop the enzyme action. Samec <sup>49</sup> concluded that the phosphorus in potato starch blocks the action of the enzyme. Since Schoch <sup>50</sup> has been able to show that the fatty acids are readily removable from corn starch by extraction with solvents it is doubtful if the fatty acids are chemically linked to the starch and therefore it is unlikely that they will exert an inhibiting action. Even if the fatty acids were so linked Taylor and Sherman <sup>51</sup> concluded that lipase-free amylase could still attack the linkages between the fatty acids and the starch molecule. With regard to the inhibition by phosphorus, Pringsheim and Ginsberg <sup>52</sup> have reported that they obtained complete hydrolysis of starch without liberating any free phosphoric acid. V. D. Martin, N. M. Naylor and R. M. Hixon <sup>47</sup> have also concluded that neither fatty acids nor the phosphorus appear to be the groups inhibiting the action of  $\beta$ -amylase at 60-70 per cent. conversion of starch to maltose. O. E. Stamberg <sup>62</sup> thinks the phosphorus may retard  $\beta$ -amylase action but not completely block it, whilst K. H. Meyer, Bernfeld and Press <sup>63</sup> think that action stops when the point of attachment of side chains is reached. Another explanation is that aggregation of the macromolecules in 'bundles' takes place, such that the 'bundles' consist not of whole molecules but of their enzyme-resisting portion, the 'straggling' or 'overhanging' portions of the molecule being readily attacked by the enzyme. This theory

is somewhat similar to that suggested by G. V. Caesar on chemical grounds (see p. 388, also p. 486).

A further factor in favour of the latter explanation is the restoration of 'splittability' on autoclaving. The last 12 glucose units at the aldehydic end of the molecule take part in the formation of these aggregates. An investigation of the chain-length of amyloamylose will be of interest in that it would show if it was composed of macromolecules containing 30 glucose units, which appears to be the case with all other fractions and undegraded starch. If this is found to be the case it will be clear that the macromolecules can exist in a form which is almost free of the structure which causes the action of  $\beta$ -amylase to cease when about 60 per cent. conversion has been reached. Hanes suggests that the amylases which bring about liquefaction first split the bonds between the macromolecules and also that phosphoric acid is concurrently liberated, the  $\beta$ -amylase then shortening the chain until this process is halted for some unknown reason at 53-60 per cent. conversion. In view of the relative simplicity of the action of  $\beta$ -amylase on starch further study of this action appears to offer the best prospects of elucidating the mechanism of amylolytic action.

## REFERENCES

1. C. S. HANES, *New Phytologist*, 1937, **36**, 192.
2. P. S. UGRUMOV, *Biochem. Zeit.*, 1935, **282**, 74.
3. K. V. GIRI, *ibid.*, 1934, **275**, 106.
4. — *J. Indian Inst. Sci.*, 1934, **17A**, (Part II), 127.
5. — *J. Indian Chem. Soc.*, 1934, **11**, No. 5, 339.
6. H. T. BROWN and G. H. MORRIS, *J. Chem. Soc.*, 1890, **57**, 505.
7. H. VAN EULER and K. HELLEBERG, *Hoppe-Seyl. Z. physiol. Chem.*, 1924, **139**, 24.
8. R. KUHN, *Ber.*, 1924, **57**, 1965.
9. — *Liebig's Ann. Chem.*, 1925, **443**, 1.
10. E. OHLSSON, *Compt. rend. trav. lab. Carlsberg*, 1926, **16**, No. 7.
11. — *Hoppe-Seyl. Z. physiol. Chem.*, 1930, **189**, 17.
12. G. G. FREEMAN and R. H. HOPKINS, *Biochem. J.*, 1936, **30**, 451.
13. J. L. BAKER, *J. Chem. Soc.*, 1902, **81**, 1177.
14. K. SJÖBERG, *Hoppe-Seyl. Z. physiol. Chem.*, 1923, **131**, 116.
15. — and E. ERIKSSON, *ibid.*, 1924, **139**, 118.
16. A. R. LING and D. R. NANJI, *J. Chem. Soc.*, 1923, **123**, 2666; 1925, **127**, 636 and 629.
17. V. SYNIEWSKI, *Liebig's Ann. Chem.*, 1925, **441**, 285.
18. F. POLLAK and A. TYCHOWSKI, *Biochem. Zeit.*, 1929, **214**, 216.
19. G. A. VAN KLINCKENBERG, *Hoppe-Seyl. Z. physiol. Chem.*, 1932, **209**, 253.
20. R. H. HOPKINS, G. F. COPE and J. W. GREEN, *J. Inst. Brew.*, 1933, **39**, 487.
21. C. S. HANES, *Canad. J. Res.*, 1935, **13B**, 185.

22. M. SAMEC, *Hoppe-Seyl. Z. physiol. Chem.*, 1936, **286**, 103.
23. J. BLOM, A. BAK and B. BRAAE, *ibid.*, 1937, **250**, 103, 104.
24. A. TYCHOWSKI, *Biochem. Zeit.*, 1937, **291**, 138.
25. G. A. VAN KLINKENBERG, *Hoppe-Seyl. Z. physiol. Chem.*, 1932, **212**, 173.
26. C. S. HANES, *Biochem. J.*, 1936, **30**, 168.
27. G. A. VAN KLINKENBERG, *Proc. Acad. Sci. Amsterdam*, 1931, **34**, 893.
28. O. MEYERHOFF, *Biochem. Zeit.*, 1927, **183**, 176.
29. C. S. HANES, *New Phytol.*, 1937, **36**, 193.
30. J. BLOM, A. BAK and B. BRAAE, *Hoppe-Seyl. Z. physiol. Chem.*, 1936, **241**, 273.
31. C. S. HANES and M. CATTLE, *Proc. Roy. Soc.*, 1938, **125**, 387.
32. M. SAMEC, *Hoppe-Seyl. Z. physiol. Chem.*, 1937, **248**, 117.
33. — and M. BLINC, *Kolloidchem. Beih.*, 1939, **49**, 73.
34. G. G. FREEMAN and R. H. HOPKINS, *Biochem. J.*, 1936, **30**, 443.
35. C. S. HANES, *New Phytol.*, 1937, **36**, 198.
36. E. L. HIRST, M. M. T. PLANT and M. D. WILKINSON, *J. Chem. Soc.*, 1932, 279.
37. M. SAMEC and E. WALDSCHMIDT-LEITZ, *Hoppe-Seyl. Z. physiol. Chem.*, 1931, **203**, 16.
38. G. G. FREEMAN and R. H. HOPKINS, *Biochem. J.*, 1936, **30**, 446.
39. C. S. HANES, *New Phytol.*, 1937, **36**, 199.
40. J. BLOM and B. BRAAE, *Enzymologia*, 1937, **4**, 53.
41. R. WEIDENHAGEN and A. WOLF, *Z. Ver. dtsh. Zuckerind., Tech. Teil.*, 1930, **80**, 935.
42. C. S. HANES, *New Phytol.*, 1937, **36**, 202.
43. H. T. BROWN and J. H. MILLAR, *J. Chem. Soc.*, 1899, **75**, 286.
44. K. MYRBÄCK, *Biochem. Zeit.*, 1936, **285**, 290.
45. B. ÖRTENBLAD and K. MYRBÄCK, *Svensk. kem. Tidskr.*, 1938, **50**, 168.
46. C. S. HANES, *New Phytol.*, 1937, **36**, 228.
47. V. D. MARTIN, N. M. NAYLOR and R. M. HIXON, *Cereal Chem.*, 1939, **16**, 565.
48. K. MYRBÄCK, *Current Sci.*, 1937, **6**, 47.
49. M. SAMEC, *Biochem. Zeit.*, 1927, **187**, 120.
50. T. J. SCHOCH, *J. Amer. Chem. Soc.*, 1938, **60**, 2824.
51. T. C. TAYLOR and R. T. SHERMAN, *ibid.*, 1933, **55**, 258.
52. H. PRINGSHEIM and S. GINSBERG, *Bull. soc. chim. biol.*, 1935, **17**, 1599.
53. N. M. NAYLOR, M. SPENCE and M. HOUSE, *J. Amer. Chem. Soc.*, 1925, **47**, 3037.
54. — and V. L. DAWSON, *Iowa State Coll. J. Sci.*, 1936, **10**, 267.
55. M. CREIGHTON and N. M. NAYLOR, *ibid.*, 1933, **7**, 253.
56. H. J. BULBROOK, Thesis, Iowa State Coll. Libr., 1928.
57. J. M. NEWTON and N. M. NAYLOR, *Cereal Chem.*, 1939, **16**, 71.
58. G. ORESTANO, *Arch. farmacol. sper.*, 1933, **56**, 383.
59. — and C. ZUMMO, *Boll. soc. ital. biol. sper.*, 1930, **5**, 246.
60. — and C. ARTOM, *Bull. soc. chim. biol.*, 1931, **13**, 516.
61. G. L. TELLER, *J. Biol. Chem.*, 1936, **114**, 425.
62. O. E. STAMBERG, *Cereal Chem.*, 1940, **17**, 372.
63. K. H. MEYER, P. BERNFELD and J. PRESS, *Helv. Chim. Acta*, 1940, **23**, 1465.

#### ADDITIONAL REFERENCE

- G. A. BALLOU and J. M. LUCK, *J. Biol. Chem.*, 1941, **139**, 233. (Influence of buffers on activity of  $\beta$ -amylase over pH 3.8-6.2.)

## CHAPTER 4

THE ACTION OF  $\alpha$ -AMYLASES ON STARCH

W. SYNIEWSKI<sup>1</sup> and others (*vide supra*) showed that malt extract contained an enzyme which was unaffected by moderate heating whereas the saccharifying enzyme was heat labile. This enzyme is now known as  $\alpha$ -malt amylase and it readily destroys the iodine coloration capacity of starch. This amylase belongs to a class of dextrinogenic amylases which are characterised by the negative mutarotation of the products of fission. Into this class the amylases obtained from the pancreas, saliva, mould enzymes of *Aspergillus oryzae* and amylases from the livers of various animals also fall. Whilst some workers have considered that these amylases are practically pure dextrinogenases, Giesberger<sup>2</sup> obtained a violet ring besides a broad colourless diffusion band, using Wijsman's diffusion method (see p. 459) with the first three types, which would indicate the presence of small amounts of  $\beta$ -amylase. The presence of maltase may also occur (*vide infra*). This might well be the cause of the widely different conclusions reached by different workers on the subject of the beginning of 'inhibition.' On the other hand, as far as mutarotation is concerned, no considerable disturbance appears to be occasioned.

Kuhn found, with pancreatic amylase, that on stopping the action when it had reached 53 per cent. conversion the reducing power remained constant but the rotation became definitely less. From hypiodometric estimation of the maltose present and from the optical rotation that should be shown by this amount of maltose, it was found that a solution of similar strength, containing  $\alpha$ -maltose, should have a higher rotation, but that the calculated total loss in rotation was equal to the difference in rotation between  $\alpha$ -maltose and  $\alpha$ - $\beta$ -maltose in the given concentration.

O'Sullivan, in 1876, using diastase, i.e.  $\alpha$ - +  $\beta$ -amylase, found that pre-heating to 70-80° C. reduces the amount of maltose formed very appreciably, and that under certain conditions the product consists almost entirely of reducing, non-crystallisable dextrans. This is of importance in the adhesive industry (*cf.* p. 251).

It is now realised that in many of these early experiments the workers were dealing with preparations which varied in composition from isolated  $\alpha$ -malt amylase to mixtures rich in

$\beta$ -amylase. The dextrins produced in these cases underwent further hydrolysis when acted upon by unheated malt extract.

The dextrins produced vary in their complexity and their solubility in hot or cold aqueous alcohol of varying composition, the latter property being closely connected with the former. Amylo- and erythro-dextrins show low solubility in aqueous alcohol, low reducing power but high optical rotation and molecular weight, whereas the more highly degraded products, the achroo- and maltodextrins, show increased solubility and reducing power and lower rotation and molecular weight. All the products, amylo-, erythro-, achroo- and maltodextrins, are hygroscopic and are fermented only slowly or not at all by yeast.

Amylo- or erythro-dextrins appear to constitute the main product in the action of  $\alpha$ -malt amylase on starch up to the achrooic point (Syniewski<sup>14</sup>). The achroo- and maltodextrins appear to be formed only slowly or when  $\beta$ -amylase is present. Pollak and Tychowski<sup>3</sup> obtained an 87 per cent. yield of the amylo-type of dextrin when acting on amylose with pre-heated malt extract. Hanes<sup>4</sup> considers that the first type of dextrin is invariably formed by the action of  $\alpha$ -malt amylase on starch, and that it consists of fragments of the starch chain of about 6 glucose units in length and the second type to have a chain-length of some 4 glucose units. The hexaose of Waldschmidt-Leitz and Reichel<sup>5</sup> formed by the action of pancreatic amylase on rice starch and on erythroamylose has properties in close agreement with those demanded by a 6 unit dextrin.

When dealing with the  $\alpha$ -amylases optical rotation more readily shows differences than reducing power, but the differences disappear after soda has been added to the solution. After a certain period of time no further mutarotation takes place. Using electro-dialysed starch solutions there is a sharp inflection point at the beginning of the curve of optical rotation against time of action of pancreatic amylase. Kuhn, Ohlsson and Swaetichin<sup>6, 31</sup> also found that the optical rotation values before and after the addition of soda to the reaction mixture to lie close together when takadiastase is used and conclude that it should be classed as an  $\alpha$ -amylase in spite of the presence of maltase. The dextrinogenic amylase of E. Ohlsson also gives negative mutarotating hydrolysis products but the absolute values of rotation are much lower than those obtained by Kuhn with pancreatic amylase. There is, as yet, no conclusive evidence that enzymes of the  $\alpha$ -malt amylase type exist in isolation in any tissue.

J. Blom, A. Bak and B. Braae<sup>7</sup> consider the bacterial preparation, Superclastase, which has a starch liquefying action, belongs



to the  $\alpha$ -amylase type, and Janke and Holota suggest the same of Biolase.<sup>16</sup> H. Lüers and A. Löther<sup>8</sup> have shown that an energetic liquefying action is exhibited by this enzyme unaccompanied by saccharification. Freeman and Hopkins,<sup>9</sup> working with soluble starch and pancreatin, filtered the reaction mixtures through an iron phosphate gel which removed not only the enzyme and the turbidity produced during the hydrolysis (*vide infra*) but also the dextrans of large molecular size, so that optical measurements on the products of fission were simplified. These workers observed a falling mutarotation during the hydrolysis of amylo- and erythro-amyloses, glycogen and  $\alpha$ -amylodextrin with  $\alpha$ -malt amylase, and according to this the type of mutarotation is characteristic, not of the substrate, but of the enzyme employed.

The course of hydrolysis brought about by  $\alpha$ -amylases has been determined by these workers<sup>10</sup> and by Klinkenberg,<sup>11</sup> Holmberg<sup>12</sup> and Hanes<sup>13</sup> by measurement of the reducing values. The action of this group of enzymes is characterised by two distinct phases, a rapid initial reaction which is checked when an  $R_M$  value of 30-40 per cent. is reached and a subsequent slow reaction in which the reducing power of the reaction mixture slowly rises. Both the initial velocity and the final degree of hydrolysis are appreciably increased when the concentration of enzyme is increased. This suggests that the enzyme may be progressively deactivated, such deactivation would partially explain the checking of the initial, rapid hydrolysis and the dependence of the final limit of hydrolysis upon the initial concentration of enzyme used.

This inhibition occurs whichever type of  $\alpha$ -amylase is used, but the greater the amount of erythro-substances present the lower is the degree of saccharification produced. Table 16 shows the results obtained by various workers, using different substrates and types of  $\alpha$ -amylase.

S. J. Pronin<sup>19, 20</sup> obtained a degree of hydrolysis greater than that obtained by Freeman and Hopkins, but he used relatively large quantities of enzyme, so that in view of the possible contamination by  $\beta$ -amylase his results are not completely conclusive. It will be noted that the limit of hydrolysis in these cases fall close to those obtained by the use of diastase or mixtures of  $\alpha$ - and  $\beta$ -amylases.

Hanes<sup>21</sup> considers that with pure  $\alpha$ -malt amylase, prepared by the method of Holmberg or from certain malts by Ohlsson's method, the initial rapid phase would cease at 28-30 per cent. apparent conversion and that the hydrolysis limit would not exceed an  $R_M$  value of 50 per cent.

Although salivary and pancreatic amylases appear to induce the initial cleavage of starch into reducing dextrans, with the concomitant disappearance of the iodine coloration, the degradation appears to continue beyond the point at which the reaction is checked in the case of  $\alpha$ -malt amylase. This important difference appears to indicate a higher affinity, on the part of animal amylases, for the less complex products of degradation in relation to their affinities for the more complex

TABLE 16  
HYDROLYSIS OF STARCH AND DERIVATIVES BY  $\alpha$ -AMYLASES

<i>Substrate.</i>	<i><math>\alpha</math>-Amylase Used.</i>	<i>Limits of Hydrolysis.</i>	<i>Observer.</i>
Potato starch	Bacterial	48	J. Blom, A. Bak and B. Braae <sup>25</sup>
Lintner soluble starch	Malt	75-78.5	van Klinkenberg <sup>11</sup>
" " "	"	60	C. S. Hanes <sup>13</sup>
" " "	"	77-84	G. G. Freeman and Hopkins <sup>10</sup>
" " "	"	86.9	M. Samec <sup>16</sup>
" " "	Salivary	75.83	H. J. Vonk and I. P. Braak <sup>17</sup>
" " "	Pancreatic	54	" " "
Amylo-amylose	Malt (Klinkenberg)	93	M. Samec <sup>16</sup>
" " "	Pancreatic	101	M. Samec and E. Waldschmidt-Leitz <sup>18</sup>
Erythro-amylose	Purified pancreatic	70	" " "
" " "	" " "	70	E. Waldschmidt-Leitz and Reichel <sup>6</sup>
" " "	Malt (Klinkenberg)	80	M. Samec <sup>16</sup>
Lintner soluble starch	High concentration of pancreatic	86-88	S. J. Pronin <sup>19, 20</sup>

starting materials, than is the case with  $\alpha$ -malt amylase. One point in favour of this attractive but incompletely supported hypothesis (see pp. 443, 497, 500) is that the *achrooic*  $R_M$  values found by C. Hanes and M. Cattle<sup>22, 23</sup> for  $\alpha$ -malt amylase is 29-32 per cent. and is independent of the substrate concentration. The *achrooic*  $R_M$  values, determined spectrophotometrically for *Aspergillus*, pancreatic and salivary amylases increase with increasing substrate concentration.

The outstanding feature of  $\alpha$ -amylase action is the destruction of the iodine coloration of the solution, which undergoes a rapid

shift through blue, violet, reddish-brown, orange to yellow. The last two colour changes take place slowly making determination of the achroic point difficult. R. Kuhn and M. Samec have given the effect of  $\alpha$ -amylase action on various starch substrates and their results are given in Table 17. It will be seen that, despite the different substrates used, the *erythro-state* is reached after about a value of  $R_M = 30$  per cent. has been attained and the achroic state after a value of  $R_M = 55$  per cent., while the course of the hydrolysis, using substrates which have different initial iodine colorations, is also clearly brought out in the Table.

With  $\beta$ -amylase the increase in reducing power runs reasonably parallel with the disappearance of the colour given with iodine, but with  $\alpha$ -malt amylase these curves diverge considerably. The work of Hanes and Cattle<sup>22, 23</sup> is of great interest. By measuring the extinction coefficients of the reaction mixture after the addition of iodine solution they showed that for starch and a number of dextrans the extinction coefficients were proportional to the concentration of the starch products present. With  $\alpha$ -amylases a decrease in the extinction of the longer wavelengths occurs in the initial stages of the reaction, but later the value decreases throughout the whole spectral range, the decrease at the red end of the spectrum still being predominant, so that the absorption peak drifts towards the blue end of the spectrum. This is in sharp contrast to the extinction curve obtained for  $\beta$ -amylase, where a decrease over the whole spectral range is observed, but the peak of maximum absorption and the general form of the curves do not vary even up to  $R_M$  values of 59 per cent.

It should be noted that with  $\alpha$ -malt amylase the achroic point is reached in that range of hydrolysis in which the time reducing-value curve shows a sharp inflection point showing the beginning of the 'zone of inhibition.' At the achroic point dextrans containing 7 to 8 or more glucose units are probably absent, and thus the ideal dextrinogenic process would result in the transformation of the whole substrate into dextrans of 6-7 glucose units, with the liberation of the minimum number of reducing groups. In this case the lowest attainable achroic  $R_M$  value would, assuming the full reactivity of the aldehydic groups, be 29.7-34.6 per cent. The values given by different workers for the inhibition zone mentioned above are about 34 per cent.,<sup>24</sup> 34-38 per cent.,<sup>13</sup> 33-40 per cent.<sup>25</sup> and 36 per cent.<sup>8, 11</sup>

**The Intermediate Products of  $\alpha$ -Amylase Action.**—M. Samec and M. Blinc<sup>26</sup> are not in full agreement with C. Hanes.<sup>27</sup>

TABLE 17

Pancreatic Amylase.*				Pancreatic $\dagger$ Amylase.				$\alpha$ -Amylase.†											
Amylases.				Amylases.				Amylo-amylose.				Erythro-amylose.				Amylodextrin.			
Time (min.).	I coloration.	R.M.	K $\cdot 10^4$ .	I coloration.	R.M.	I coloration.	R.M.	Time (min.).	I coloration.	R.M.	I coloration.	R.M.	I coloration.	R.M.	I coloration.	R.M.			
75	Blue	7.5	45.3	Reddish-blue	25	Blue	1.99	0	Blue		Violet-red	9.0	Orange	0.98					
21	Blue	16	36.2	Deep red	32	Greenish			Greenish		Bluish		Orange						
40	Reddish blue	28	35.8	Reddish-brown	39	Yellow	41.4	60	Yellow		Reddish-orange	17.1	Orange	11.5					
63	Reddish-brown	40.5	25.9			Yellow	48.1	120	Yellow		Reddish-orange	18.6	Orange	15.9					
				Brown	45	Yellow	52.0	180	Yellow		Orange	25.0	Orange	16.9					
290	Colourless	56.5	12.5	Yellow-brown	49	Yellow		300	Yellow		Orange	33.1	Orange	17.5					
1800	Colourless	70	2.9	Colourless	54	Yellow		420	Yellow		Orange	35.4	Orange	17.5					
3300	Colourless	74.5	1.8																
00	—																		

\* R. Kuhn's results.

† M. Samec's results.

They<sup>16</sup> obtained an even lower achroic  $R_M$  value, e.g. 16.4 per cent., and consider that achroodextrins having appreciably higher molecular weights than correspond with molecules of 6-7 glucose units exist (Biltz,<sup>28</sup> Samec<sup>29</sup>).

During the first rapid stage of the degradation of starch with  $\alpha$ -amylase reducing dextrans, supplying the bulk of the reducing value of the mixture, are formed. It must be emphasised, in view of the frequent assumption to the contrary by a number of workers, that maltose is neither the sole, nor the principal, reducing substance formed in all stages of the hydrolysis. The quantitative investigation of the intermediate products has been undertaken by Ohlsson.<sup>30, 31</sup> By dialysing the reaction mixture when the iodine coloration was violet and the  $R_M$  value was 23 per cent. he was able to establish, by osmotic pressure measurements, that the number of slow or non-dialysable particles had greatly increased and that these relatively complex fragments contribute the bulk of the reducing power of the solution.

Freeman and Hopkins,<sup>32</sup> by fractional precipitation of two digests with a violet iodine coloration and  $R_M$  values of 20.6 and 25.6, respectively, gave 80 per cent. of dextrans having  $R_M$  values of 20.3 and 23.0 per cent. respectively, which are very close to the initial  $R_M$  values of the solutions. The remainder of the material consisted chiefly of products more complex than maltose, but a little of the latter was probably also present. The dextrans obtained by the various workers have been described above.

M. Samec and R. Rakusa<sup>33</sup> consider the fragments formed in the initial stage of  $\alpha$ -malt amylase hydrolysis are nearly uniform. Under suitable conditions a very rapid drop in viscosity, with scarcely any other change in the properties of the starch solution, is to be noted. Myrbäck,<sup>34</sup> using takadiastase on corn starch, claims to have isolated from the reaction mixture dextrans which were trisaccharides and discusses the importance of this in connection with the structure of starch.

Ross and Romijn<sup>35</sup> found that the rate of fission of 1 per cent. potato, rice wheat and soluble starch solutions decreased in that order when acted upon by pig-pancreas amylase, but using dog-pancreas amylase the order of the last two starches was reversed. In each case, however, the maximum  $R_M$  value was 50 per cent. The first amylase was the quicker acting on potato starch.

G. E. Glock<sup>36</sup> used pancreatic, malt and salivary  $\alpha$ -amylases on wheat, rice, maize and potato starches and glycogen. The use of very large quantities of enzyme showed maltase action. When used in the usual concentrations this was negligible but

with liver amylases of various animals, including humans, maltase action was never entirely absent. Thorough washing of rat liver with saline solution before preparing the amylase gave a preparation having no maltase action, and it may be that in the case of liver amylases the presence of blood is responsible for the maltase action reported. In the initial stages the action of ptyalin and pancreatic amylases appears very similar to that of  $\alpha$ -malt amylase in that reducing dextrins are produced and the iodine coloration is destroyed. Whether the initial fragmentation is similar or not is obscure, but irrespective of the initial size of the primary fission products these are further degraded very rapidly by the two animal amylases. The end of the primary or dextrinogenic stage is therefore not marked by any slackening or inhibition in the rate of hydrolysis such as is shown by  $\alpha$ -malt amylase.

The velocity with which the individual changes take place varies with the starch as follows, the starches being placed in order of decreasing rate of change:—

<i>Achroic point</i>	.	.	.	.	Glycogen; potato, maize, wheat and rice starches.
<i>Amount of—</i>					
<i>substrate hydrolysed</i>	.	.	.	.	Potato, maize, wheat and rice starches; glycogen.
<i>reducing dextrins and maltose formed</i>	.	.	.	.	Wheat, potato, maize and rice starches; glycogen.
<i>maltose formed</i>	.	.	.	.	Wheat, rice, potato and maize starches; glycogen.

Samec has suggested that as the amounts of maltose and dextrin vary from starch to starch that a constant value of the quotient—*starch hydrolysed/maltose formed*—would show that maltose is formed successively, but if it fell in value during the course of hydrolysis it would show that the dextrins were more or less completely converted into maltose.

According to Blom, Bak and Braae,<sup>37</sup> during the action of bacterial  $\alpha$ -amylase only maltose is formed at the beginning of the hydrolysis, and a maximum of 23 per cent. is reached when 40 per cent. starch fission has been effected. From this point onwards they consider that the number of molecules of reducing dextrin do not increase but that maltose is split off from these dextrins. Kitano,<sup>38, 48</sup> using crude takadiastase, claims to have obtained a trisaccharide which gives maltose and glucose on hydrolysis and, according to this worker, the achroic point is very rapidly reached with pure takadiastase and the final product is maltose which is found quantitatively.<sup>39</sup>

When using  $\alpha$ -malt amylase or salivary  $\alpha$ -amylase with a starch substrate which has not been strongly peptised a small amount of flocculent material is produced just before the achroic point is reached. If the solution is boiled and cooled the iodine coloration is found to have returned, and on the addition of fresh amylase the hydrolysis continues. V. D. Martin<sup>40</sup> noted that the material which flocculates and the precipitate formed by the addition of 60 per cent. alcohol to the filtered solution have the same reducing values. Incidentally, Martin and co-workers<sup>41</sup> conclude that neither the fat nor the phosphorus content appears to be responsible for the cessation of action of  $\beta$ -amylase at  $R_M$  60-70 per cent. fission. Broeze<sup>42</sup> considers the flocculent precipitate to be formed owing to association of molecules whereby the iodine reacting groups in the molecules are masked. As it occurs with starch which has not been thoroughly peptised we may conclude that the aggregates are already present in the solution and may possibly be protected by the fully peptised material (amylose). When the amylose has been destroyed by amylase action the iodine reaction is destroyed and the aggregated material flocculates and forms a 'haze' or a precipitate. Strong heating of the solution in an autoclave brings about further disaggregation, freeing more iodine-reacting groups, thus restoring the iodine coloration and further material is made available for amylolysis.

**The Action of  $\alpha$ -Amylase on Unpasted Starch.**—As previously mentioned (see pp. 311, 440) unpasted starches appear to be 'immune' against amylase attack except when the granules are injured by grinding. Pozerski<sup>43</sup> has shown that in this case it is the solubilised portion which is attacked and not the suspended fragments. Ross and Romijn<sup>35</sup> found that amylase from dog or pig pancreas had no action on granules of potato, wheat or soluble starch.

Weichsel,<sup>44</sup> however, claims that even unpasted starch granules are attacked by takadiastase at 37° C. and pH 4.9, wheat starch being liquefied in 24 hours, canna starch saccharified to an extent of 90 per cent. in 5 days but potato starch remained unaffected even after several weeks. If treated with a solution of a non-poisonous swelling agent at 37° C. for a few days he found diastatic action could then take place. The same effect was obtained by pre-treatment with alcohol for 24 hours or acetone for 3 days. The effect of these liquids is to cause the appearance of fissures in the granules similar to those observed by the author with intensely dried starch (see p. 46), so that the enzyme can enter the granule. On the other hand, intense

drying of potato starch over phosphorus pentoxide for several months was found by Weichsel to render the outer membrane swellable in cold water and thus initiate amylolysis from without the granule.

W. Ziese<sup>45, 46</sup> finds that pancreatic, and to a lesser extent salivary amylase will attack oxyethyl starch giving an appreciable fall in viscosity but little change in the optical rotation or reducing power. The products of the reaction do not diffuse through a cellophane membrane. This worker considers that dehydration takes place as an intermediate step in this reaction. It is of interest to note that takadiastase is practically without effect on oxyethyl starch.

**The Mode of Action of  $\alpha$ -Malt Amylase.**—The action of  $\alpha$ -malt amylase on starch is a more complicated process than the action of the  $\beta$ -amylase. During the first stage the bulk of the substrate is converted into complex reducing dextrans and the iodine coloration shows a progressive alteration, the achroic point being reached when the reducing power is about 29-32 per cent. of the theoretical maltose. The chemical and physical constants of the dextrin fraction having no iodine coloration suggest a *mean* chain-length of 6 glucose units. It would appear that  $\alpha$ -malt amylase can break up the long chains present into fragments having a chain-length of 6 glucose units, but whether this fission takes place in an endwise manner as in the case of  $\beta$ -amylase action is not deducible at present.

After the primary fission to dextrans a slow hydrolysis of the dextrans appears to take place until the reducing power is of the order of 50 per cent. of the theoretical maltose value. If the substrate for this second slow hydrolysis is assumed to be a 6-unit dextrin, produced in the first stage of the reaction, then the final reducing power indicates that only about one more linkage in each dextrin molecule is capable of being hydrolysed by the amylase. Neither the course of this secondary reaction nor the composition of the products obtained can, at present, be precisely defined. Whether or not the slow rate of the secondary reaction is due to the enzyme having only a low affinity for the reducing dextrans or to the slow breakdown of the enzyme-substrate complex are other questions at present unanswerable.

The rapidity of the first phase of the hydrolysis by  $\alpha$ -amylase suggests that relatively long portions of the chain structure enter into the enzyme-substrate complex. As suggested by Hanes in the case of  $\beta$ -amylase (see p. 472) it seems reasonable to suppose that there are two active groups in  $\alpha$ -amylase by which attachment to the long-chain molecule can be accomplished, followed



by fission at least one of the points of contact. As dextrins of 6 glucose units in length form a considerable portion of the products of hydrolysis these active centres must be some distance apart, and if fission takes place at the points of attachment then the distance between them will be in the neighbourhood of 6 glucose units. Hanes points out that one objection to this hypothesis is that it assumes an unlikely degree of structural organisation.

To overcome this Hanes<sup>47</sup> suggests that the starch chain might conceivably be in the form of a spiral of 5 complete turns and that 6 glucose units complete one full turn. Every seventh linkage, i.e. the linkages which are split, would then fall in close lateral proximity although separated along the chain by 6 glucose units. These two points being fairly close together the  $\alpha$ -malt amylase can readily attach itself by means of its active groups. Cleavage at these two points would liberate one complete turn of the spiral containing 6 glucose units. As dextrins with 6 to 7 glucose units give no iodine coloration this conception of a spiral molecule leads to the suggestion that the iodine coloration depends, in some unexplained manner, on the presence of more than one complete coil of the spiral. G. V. Caesar<sup>48</sup> and M. L. Cushing also have concluded that amylose has a helical-spring configuration which is well brought out in the Fischer-Hirschfelder atomic models they prepared.

More work on the configuration of the starch molecule and a rigid characterisation of the products of the first stage of  $\alpha$ -amylase action on starch is required before anything final can be decided on the mode of action of amylases on starch. The hypothesis of Hanes certainly shows that it is unnecessary to postulate different linkages to explain away the peculiarities of enzyme action.

#### REFERENCES

1. W. SYNIEWSKI, *Bull. Int. Acad. Pol. Sci. Lettres*, 1924, 131.
2. G. GIESBERGER, *Proc. Kon. Akad. Wetensch. Amsterdam*, 1934, **37**, 336.
3. F. POLLAK and A. TYCHOWSKI, *Biochem. Z.*, 1929, **214**, 216.
4. C. S. HANES, *New Phytologist*, 1937, **36**, 213.
5. E. WALDSCHMIDT-LEITZ and M. REICHEL, *Hoppe-Seyl. Z. physiol. Chem.*, 1934, **223**, 76.
6. E. OHLSSON and T. SWAETICHIN, *Bull. Soc. Chim. biol.*, 1929, **11**, 333.
7. J. BLUM, A. BAK and B. BRAAE, *Hoppe-Seyl. Z. physiol. Chem.*, 1937, **250**, 103.
8. H. LÜERS and A. LÖTHER, *Woch. Brauerei*, 1935, **52**, 49.
9. G. G. FREEMAN and R. H. HOPKINS, *Biochem. J.*, 1936, **30**, 443.
10. — *ibid.*, 1936, **30**, 446.

11. G. A. VAN KLINKENBERG, *Hoppe-Seyl. Z. physiol. Chem.*, 1932, **209**, 253 ; **212**, 173.
12. O. HOLMBERG, *Biochem. Z.*, 1933, **266**, 203.
13. C. S. HANES, *Canad. J. Res.*, 1935, **13B**, 185.
14. W. SYNIEWSKI, *Ann.*, 1902, **324**, 212.
15. A. JANKE and J. HOLOTA, *Woch. Brauerei*, 1939, **56**, 161.
16. M. SAMEC, *Hoppe-Seyl. Z. physiol. Chem.*, 1937, **248**, 117.
17. H. J. VONK and J. P. BRAAK, *Proc. Kon. Akad. Wetensch. Amsterdam*, 1934, **37**, 3.
18. M. SAMEC and E. WALDSCHMIDT-LEITZ, *Hoppe-Seyl. Z. physiol. Chem.*, 1931, **203**, 16.
19. S. J. PRONIN, *Bull. biol. med. exp. U.R.S.S.*, 1936, **2**, 376.
20. — *Biochimija*, 1937, **2**, 935.
21. C. S. HANES, *New Phytologist*, 1937, **36**, 206.
22. — and M. CATTLE, *ibid.*, 1937, **36**, 209.
23. — *Proc. Roy. Soc.*, 1938, **125**, Series B, 387.
24. W. SYNIEWSKI, *Ann.*, 1925, **441**, 288.
25. J. BLOM, A. BAK and B. BRAAE, *Z. physiol. Chem.*, 1936, **241**, 273.
26. M. SAMEC and M. BLINC, *Kolloidchem. Beih.*, 1939, **49**, 117.
27. C. S. HANES, *New Phytologist*, 1937, **36**, 208.
28. BILTZ, 'Kolloidchemie der Stärke,' by M. Samec, Dresden and Leipzig, 1926, p. 471.
29. M. SAMEC, *Biochem. Z.*, 1927, **187**, 120.
30. E. OHLSSON, *Compt. rend trav. lab. Carlsberg*, 1926, **16**, No. 7.
31. — *Hoppe-Seyl. Z. physiol. Chem.*, 1930, **189**, 17.
32. G. G. FREEMAN and R. H. HOPKINS, *Biochem. J.*, 1936, **30**, 443.
33. M. SAMEC and R. RAKUSA, *Z. physiol. Chem.*, 1940, **263**, 17.
34. K. MYRBÄCK, *Svensk. Brygg. Måndadsbl.*, 1938, **53**, 93 ; *Chem. Zentr.*, 1938, II, 201.
35. J. ROSS and C. ROMIJN, *Arch. néerl. Physiol. Homme Animaux*, 1934, **19**, 1.
36. G. E. GLOCK, *Biochem. J.*, 1936, **30**, 1386.
37. J. BLOM, B. BRAAE and A. BAK, *Hoppe-Seyl. Z. physiol. Chem.*, 1938, **252**, 261.
38. T. KITANO, *J. Soc. Chem. Ind. Japan (Suppl.)*, 1936, **39**, 389B.
39. — *ibid.*, 1936, **40**, 37B.
40. V. D. MARTIN, *Iowa State Coll. J. Sci.*, 1938, **13**, 81.
41. — N. M. NAYLOR and R. M. NIXON, *Cereal Chem.*, 1939, **16**, 565.
42. J. R. BROEZE, *Biochem. Z.*, 1928, **204**, 286.
43. E. POZERSKI, *Compt. rend. Soc. Biol. Filiales Associées*, 1927, **97**, 1592.
44. G. WEICHSEL, *Planta*, 1936, **26**, 28.
45. W. ZIESE, *Z. physiol. Chem.*, 1934, **229**, 213.
46. — *ibid.*, 1935, **235**, 235.
47. C. S. HANES, *New Phytologist*, 1937, **36**, 231.
48. T. KITANO, *J. Soc. Chem. Ind. Japan (Suppl.)*, 1935, **38**, 376B.
49. G. V. CAESAR and M. L. CUSHING, *J. Phys. Chem.*, 1941, **45**, 776.

## ADDITIONAL REFERENCE

- S. AKIYA, *J. Pharm. Soc. Japan*, 1938, **58**, 40 ; via *Brit. Chem. Physiol. Abst.*, 1940, **2A**, 324. (Bacterial hydrolysis of potato starch.)

## CHAPTER 5

THE ACTION OF MIXTURES OF  $\alpha$ - AND  $\beta$ -AMYLASES ON STARCH

MALT extract probably exemplifies the best-known natural mixture of  $\alpha$ - and  $\beta$ -amylases. The relative proportions and influence of each type of amylase varies according to the source and method of preparation of the malt extract. Sherman and Schlesinger<sup>1</sup> have shown that the disappearance of iodine reaction cannot be used as a criterion of starch splitting capacity of malt extracts, as the action of the  $\alpha$ -amylase is affected to a different degree to that of the  $\beta$ -amylase and by different factors.<sup>2</sup> E. Ohlsson showed the ratio of  $\alpha$ - to  $\beta$ -amylase to vary in different malt extracts and this has been confirmed by Fricke and Kaja.<sup>3</sup> That conflicting statements have appeared on the action and the higher limits of degradation of starch when acted upon by malt extracts is not surprising in view of the heterogeneous nature of these extracts.<sup>4, 5</sup> R. H. Hopkins and co-workers<sup>21</sup> point out that not only can the proportions of  $\alpha$ - and  $\beta$ -amylase vary in a given sample of malt but also that the extent to which the latter hydrolyses starch and amylose depends upon the severity of the pre-treatment given to the latter during its preparation. These findings agree very well with the suggestions of Lampitt (see also p. 441).

H. Kühl<sup>6</sup> has observed a greater formation of erythro-dextrin from rye starch than from wheat starch when these were acted upon by strong malt extracts. The achroo-dextrin stage was, however, reached in both cases. A. Tychowski<sup>7</sup> found that malt extracts prepared from oat, rye and wheat hydrolysed solutions, prepared by autoclaving, of various types of starches to the same extent. Increasing the temperature from 20° to 55° C. and increasing the enzyme concentration favourably influenced the hydrolysis but the final amount of maltose produced was unaffected.

Sherman and Baker<sup>8</sup> found potato amylose to be practically completely saccharified by malt extract, but amylopectin shows greater resistance and only a hydrolysis limit of 63 per cent. is finally attained. Lintner starch and soluble starch prepared by autoclaving lies between the two. H. Pringsheim and A. Beiser<sup>9</sup> confirmed these observations, using malt amylase in the presence of 'amylase-complement.' They found amylose to be com-

pletely saccharified, but the hydrolysis of amyloextrins could only be brought to completion in the presence of the 'amylase-complement.'

H. Lüers and W. Wasmund<sup>10</sup> found that small changes in the starch substrate cause a notable alteration in the reaction-constant. Samec and Blinc<sup>11</sup> and E. Waldschmidt-Leitz<sup>12</sup> have obtained similar results with amylo- and erythroamyloses when using malt extracts.

Freeman and Hopkins<sup>13</sup> investigated the action of artificial mixtures of  $\alpha$ - and  $\beta$ -amylases on glycogen, soluble starch and amyloextrin. In small concentrations the effect of the amylases in the mixtures appeared additive, but in higher concentrations, and especially where the proportion of  $\beta$ -amylase is increased, the degree of hydrolysis in the first stage is greater than the sum of the actions of the two components separately. This is probably due to the fact that  $\beta$ -amylase can act readily on the residue from the total action when this residue has been acted upon by the  $\alpha$ -amylase. The hydrolysis limits, using a mixture of the enzymes, is some 10-12 per cent. higher than the sum of the action of the two components separately, and Freeman and Hopkins never obtained a 100 per cent. hydrolysis. In view of the explanation of amylase action advanced by Hanes this is what might have been expected. A. Joszt and A. Kleindienst<sup>14</sup> studied the first rapid stage of saccharification and of this some 30 samples of distillery malts obtained a degree of hydrolysis corresponding to 51.7 to 56.2 per cent. of the theoretical maltose groups.

Blom, A. Bak and B. Braae obtained a hydrolysis limit of 80 per cent., using a mixture of bacterial  $\alpha$ - and  $\beta$ -barley malt amylase on potato starch. If a high proportion of an amylase-complement is present it is possible to obtain complete hydrolysis of the starch to a disaccharide (Samec and Blinc).<sup>11</sup>

H. Pringsheim and J. Leibowitz<sup>15</sup> considered that the action of a combination of  $\alpha$ - and  $\beta$ -amylases on starch should produce glucose, but P. Rona and J. Hefter<sup>16</sup> and Samec<sup>12</sup> dispute this. Samec worked with pancreatic and malt amylase mixtures on erythro- and amyloamyloses as substrate and obtained a hydrolysis limit of 85 per cent. with erythroamyloses and 101 per cent. with amyloamyloses. Ordinary starch is hydrolysed until the reducing value showing a limit of 80 per cent. has been reached.

The results of following the reaction by *the iodine coloration* are interesting. The fading of the blue colour, as well as the red, is more rapid the richer the substance is in amylose, and at the achroic point the reducing value is higher the more amylose

there was present at the start. If this is decreased in favour of amylopectin the reducing value at the achroic point falls to an extent dependent on the reduction in the percentage of amylose present at the start.<sup>8</sup>

H. von Euler and O. Svanberg<sup>17</sup> obtained a 71 per cent. saccharification at the achroic point, using their dialysed malt extract. According to Effront<sup>18</sup> no reducing dextrin is produced in the first stage of the malt hydrolysis but appears in the second and longer stage. The action of mixtures of  $\alpha$ - and  $\beta$ -amylases on starch has also been studied by H. and W. Brintzinger<sup>19</sup> and by R. Weidenhagen and A. Wolf.<sup>20</sup>

## REFERENCES

1. H. C. SHERMAN and M. D. SCHLESINGER, *J. Amer. Chem. Soc.*, 1913, **35**, 1784.
2. — and A. W. THOMAS, *ibid.*, 1915, **37**, 623.
3. R. FRICKE and P. KAJA, *Ber.*, 1924, **57**, 313.
4. K. SJÖBERG, *Hoppe-Seyl. Z. physiol. Chem.*, 1923, **131**, 116.
5. — *Ber.*, 1924, **57**, 1251.
6. H. KÜHL, *Mühle*, **70**, No. 15; *Muhlenlaboratorium*, 1933, **3**, 65.
7. A. TYCHOWSKI, *Biochem. Zeit.*, 1937, **291**, 138.
8. H. C. SHERMAN and J. C. BAKER, *J. Amer. Chem. Soc.*, 1916, **38**, 1885.
9. H. PRINGSHEIM and A. BEISER, *Biochem. Zeit.*, 1924, **148**, 336.
10. H. LÜERS and W. WASMUND, *Fermentforsch.*, 1921, **5**, 169.
11. M. SAMEC and M. BLINC, *Kolloidchem. Beih.*, 1939, **49**, 131.
12. — and E. WALDSCHMIDT-LEITZ, *Hoppe-Seyl. Z. physiol. Chem.*, 1931, **203**, 16.
13. G. G. FREEMAN and R. H. HOPKINS, *Biochem. J.*, 1936, **30**, 451.
14. A. JOSZT and A. KLEINDIENST, *Przemysl chem.*, 1930, **14**, 537.
15. H. PRINGSHEIM and J. LEIBOWITZ, *Ber.*, 1926, **59**, 991.
16. P. RONA and J. HEFTER, *Biochem. Zeit.*, 1929-30, **217**, 113.
17. H. VON EULER and O. SVANBERG, *Hoppe-Seyl. Z. physiol. Chem.*, 1921, **112**, 193.
18. J. EFFRONT, *Z. angew. Chem.*, 1930, **43**, 1033.
19. H. BRINTZINGER and W. BRINTZINGER, *Z. anorg. allg. Chem.*, 1931, **186**, 50.
20. R. WEIDENHAGEN and A. WOLF, *Z. ver. dtsh. Zuckerind., Techn. Teil*, 1930, **80**, 935.
21. R. H. HOPKINS, E. G. STOPHER and D. E. DOLBY, *J. Inst. Brewing*, 1940, **46**, 426.

## CHAPTER 6

## THE KINETICS OF AMYLASE ACTION

As amylases are catalysts and thus alter the rate of reaction it is necessary to study their influence on the velocity of reactions and the influence of various factors on their action. Any process which tends to reach an equilibrium becomes slower and slower the nearer the point of equilibrium. It might be thought that the rate of hydrolysis of starch was proportional to the number of starch molecules present and that the reaction should therefore be unimolecular. It must be kept in mind, however, that when the reaction is activated by amylase the process occurs in a heterogeneous system involving reactions at the surface of the amylase and of the starch micelle. If, then, we find a particular amylase action apparently obeying the unimolecular law it cannot be ascribed to the attainment of a homogeneous system but must be due to some other cause.

## MALT AMYLASE

(a) **Saccharogenic Action.**—1. *Measuring the Reaction Constant.*—The saccharification of starch is a progressive reaction in which fission takes place, producing maltose and residues which are then subjected to further hydrolysis. It is immaterial at this point to consider whether maltose units are progressively split from the end of a long chain (see p. 472) or whether the long chain is broken at random along its length into pieces which, in turn, undergo further fission.

Kjeldahl stated<sup>1</sup> that the relative diastatic power of two solutions is expressed by the copper reducing power produced in a given time when the enzyme solutions act on the same weight of starch at the same temperature, provided always that the reducing power is not allowed to go beyond  $R_M$  40-49. An examination of Kjeldahl's data shows that the reaction is not strictly linear as far as the reducing value of  $R_M$  40 but is sufficiently so for all practical purposes for measuring commercial diastase preparations. It is, however, very nearly rectilinear up to the reducing value of  $R_M$  29.

C. J. Lintner and F. Eckardt<sup>2</sup> confirmed Kjeldahl's results for soluble starch. A Janke and J. Holota,<sup>3</sup> however, consider that the linear course does not appear and that the reaction is

unimolecular up to reducing values of  $R_M$  30 at 30° C. and  $R_M$  45 at 50° C. Beyond these limits, or for total hydrolysis by any diastatic preparation, the reaction constants diminish and negative auto-catalysis prevails.

H. T. Brown and T. A. Glendinning<sup>4</sup> and H. Lüers and W. Wasmund,<sup>11</sup> using soluble starch, found that at the beginning of the reaction there was a marked increase in the constant with the progress of hydrolysis, and after a value of  $R_M$  30-40 had been reached the reaction followed a unimolecular course. In their early experiments Brown and Glendinning were convinced that the rate of change did not conform to the logarithmic formula for unimolecular action, but that there was a progressive increase in the value of the 'velocity coefficient' which, at first sight, appears to differentiate the mode of action of diastase from that of invertase which gives the logarithmic form characteristic of a unimolecular reaction. Following the work of A. Brown and V. Henry they re-examined their results and concluded that one fundamental law can express the rate of change in all enzyme reactions which can be studied quantitatively with sufficient accuracy. They used both change in optical rotation and cupric reducing power to follow the reaction, but owing to the comparatively small change in the former the method is the less accurate of the two.

They calculated the coefficient of velocity directly or else the results were plotted as a *time/proportion of unhydrolysed substrate* curve. If the reducing power of totally hydrolysed starch solution of unit volume is regarded as unity and  $x$  is the ratio of the reduction observed at any given time  $t$  from the start of the reaction, then if the time curve representing the course of the hydrolysis is logarithmic then  $\frac{1}{t} \log \frac{1}{(1-x)} = k$ , where  $k$  is the constant representing the coefficient of velocity of hydrolysis and  $t$  is the time elapsing from starting the reaction.

Throughout the reaction there is a steady increase in the value of  $k$  showing that the course of hydrolysis does not follow the logarithmic expression. If we assume that the normal curve for the rate of change is logarithmic but that secondary disturbing causes have caused some modification of the form, then the secondary conditions must be such as to produce a constant acceleration of the coefficient of velocity of change, i.e. in any given time interval there is more of the residual substrate hydrolysed than there should be according to the logarithmic formula. Brown and Glendinning thought at first that the intermediate products were probably more easily hydrolysed than the more

starch-like products, thus giving a constant increase, but were able to demonstrate experimentally that this was not so.

The time-curve expression of the rate of hydrolysis is approximately a straight line from the initial value up to values of  $R_M$  30-40. In this portion of the curve the amount of hydrolysis is approximately proportional to the time of reaction, the amount transformed being a linear function of the time. The curve then gradually changes over into a logarithmic form. C. Philoche<sup>5</sup> considers the unimolecular 'constant' decreases up to  $R_M$  30 and then becomes constant and V. Henry<sup>6</sup> considers that the value of  $k$  varies about a mean value.

H. C. Sherman and J. A. Walker<sup>7</sup> considered the reaction to follow a unimolecular course in the early but not in the later stages, but they used very low enzyme concentrations and recent work has shown that it needs especially limited requirements of substrate and enzyme concentration in order to reach a favourable approximation to the unimolecular course (see H. van Laer<sup>8</sup>).

H. von Euler and O. Svanberg<sup>9</sup> obtained a practically constant value of the velocity constant in the initial stages by the very careful choice of working limits. They found the constant of the secondary to be about 1/1000th that of the primary saccharification and the slowest speed of reaction was reached near the value of  $R_M$  75. Ordinary potassium and sodium salts and the phosphate-buffer had no influence on the reaction constant.

H. von Euler and K. Josephson<sup>10</sup> have compared the hydrolysis with malt of Lintner starch and glycogen, and from the reaction constants conclude that glycogen belongs to the erythroclass of compounds.

2. *Reaction Constant and Enzyme Concentration.*—Ch. Philoche found no linear relationship between the amount of sugar formed in a given time and the enzyme concentration. Sherman and Walker concluded from their work that within certain limits of enzyme concentration and up to values of  $R_M = 50$  the maltose formation was directly proportional to the enzyme concentration. Other workers<sup>9, 11</sup> have found a corresponding proportionality between the enzyme concentration and the reaction constant of the unimolecular reaction and consider that the value of  $k/\text{amylase concentration}$  is significant. For concentrations of amylase up to 0.1 per cent. a better constant is obtained if one considers the value—*initial value of  $k/\text{amylase concentration}$* . A similar consideration of higher amylase concentrations (0.1667 per cent.) shows that the production of maltose



increases at a slower rate than the increase in the proportion of the enzyme used. The saccharification rate may be increased by raising the amount of amylase but the final amount of maltose formed is fairly constant (see K. Sjöberg<sup>12</sup>).

K. H. Meyer and J. Press<sup>42</sup> have studied the influence of starch and enzyme concentrations on the degradation of Zulkowski soluble starch by  $\beta$ -amylase. They find that the reaction velocity is independent of the starch concentration at high starch concentrations and up to a degree of hydrolysis of 30-40 per cent. at lower concentrations. For a constant starch concentration the quotient  $k/\text{amylase concentration}$  is constant. At high starch concentrations it tends towards a limit, the existence of which they consider indicates the formation of an addition of the enzyme with the starch which later decomposes into enzyme and starch degradation products. Application of the law of mass action leads to the conclusion that, for a given constant starch concentration,  $k/\text{amylase concentration}$  should be constant. When values of the quotient are plotted against  $\log A$ , where  $A$  is the initial concentration of starch, an S-shaped curve is obtained. They give the dissociation constant of the starch— $\beta$ -amylase compound as 0.0008 (see below). Addition of maltose was found to lower the velocity of degradation. In similar studies of the degradation of maize and potato amyloses by  $\beta$ -amylase the value 0.0001 was obtained for the dissociation constant of the enzyme-amylose compound. In order to obtain the same reaction velocity with amylose and with soluble starch it is necessary to use an amylose concentration 1.5 times that of starch. In the degradation of amylose by  $\beta$ -amylase the curve  $\text{maltose formed}/\text{time}$  is a straight line up to a degradation of 65 per cent. and the reaction is of zero order according to these workers.<sup>43</sup>

3. *Concentration of Substrate.*—The concentration of substrate is more important in enzyme systems than in homogeneous systems. In the latter, according to the unimolecular reaction, the reaction constant holds over wide limits of substrate concentration, but in the former one often finds an inverse proportionality between the substrate concentration and the reaction constant, so that  $K_c = \text{constant}$ .

Henry and Ch. Philoche found that similar amounts of amylase in similar times produced more maltose from concentrated starch solutions than from dilute solutions, and H. van Laer<sup>8</sup> concluded that, in general, a constant of the absolute amount of maltose produced by the enzyme does not exist but, as the substrate concentration rises, a smaller increase in the amount

of maltose formed occurs than might have been expected on the basis of the increased amount of substrate. C. Wirth<sup>13</sup> found that with increased initial concentration of starch the reaction constant decreases, and Euler and Svanberg<sup>9</sup> found a linear relation throughout between the reciprocal of the substrate concentration and the reaction constant for starch solutions of 1, 2 and 4 per cent. strength, and Lüers and Wasmund have confirmed this for 0.4-6.0 per cent. starch solutions and amylase concentrations of 0.005-0.05 per cent. These workers, and also M. Schneider,<sup>14</sup> showed that with a constant value for the substrate/enzyme ratio, but at different dilutions, the reaction constant and the initial velocity first rise and then fall gradually with increasing dilution. Only the falling portion of this curve was examined by Schneider.

Vernon<sup>15</sup> has examined the influence of temperature on the reaction velocity of amylase action, and H. van Laer gives  $K_{35}/K_{25} = 1.93$ ;  $K_{45}/K_{35} = 1.31$ , whilst the results of Lüers and Wasmund are given in Table 18.

TABLE 18

Temperature, °C.	$K_0 + 10/K_0$		
	Initial Value of $k$ .	From Average Value of $k$ .	From Henry's Constant = 3.2.
20-30	2.046	1.899	1.96
30-40	1.675	1.688	1.65
40-50	1.294	1.506	1.43

4. *The Reasons for Deviations.*—As several workers<sup>4, 8</sup> have found the velocity of hydrolysis of the intermediate products does not deviate much from that of the final products this cannot be the reason for the deviation from the unimolecular reaction. H. van Laer considers that the kinetics of amylolysis depends essentially on the 'diastatic loading' of the starch and thus depends upon the ratio *substrate/enzyme*, but in view of the work already discussed this opinion does not clarify the situation.

The rectilinear form of the *time/change* curve up to  $R_M$  40 is interesting. Here, in contrast to the demands of the law of mass action, there is an apparent independence of the reaction on the amount of material already hydrolysed.

Duclaux<sup>16</sup> considers this to be specific for amylase and that the form of the second portion of the curve is due to the reaction

products. E. F. Armstrong<sup>17</sup> assumed the formation of a system between the ferment and the substrate which is subject to the customary equilibrium, i.e. depends upon the amount of ferment, substrate and water (see also H. T. Brown<sup>18, 19</sup>).

H. T. Brown and T. A. Glendinning<sup>4</sup> pointed out that at the beginning of the action the amount of enzyme relative to the amount of starch is small, and as long as this excess of substrate remains unhydrolysed the ratio *starch/starch-enzyme complex* will be large. As long as this is so the starch enzyme complex will remain nearly constant in amount and equal amounts of starch will be hydrolysed in equal time, thus accounting for the rectilinear portion of the curve. Subsequently, when the starch concentration has been greatly reduced the amount of combination, and consequent hydrolysis, will follow more closely the law of mass action. If the initial concentration of amylase is large the straight line portion of the curve should change from the start and result in a reaction of the second order accompanied by a change in the value of  $k$ .

Lüers and Wasmund have applied Fodor's method<sup>20</sup> for examining polypeptide fission to amylolysis. They assume that the starch is adsorbed on to the surface of the amylase, the amount adsorbed being conditioned by the speed of diffusion of the starch and hydrolytic products to and from the surface of the amylase and also by the ratio—*surface/substrate concentration*. If the amylase concentration is small relative to the substrate concentration the adsorption, following Freundlich's adsorption rule, is given by

$$\frac{y}{m} = Ac^{\frac{1}{n}}$$

where  $m$  is the amount of enzyme,  $y$  the amount of starch adsorbed,  $1/n$  is a true fraction,  $A$  is a constant and  $c$  the equilibrium concentration of the starch in the liquid.

It is now assumed that reaction occurs only in the adsorbed layer and further that this reaction in the adsorbed layer is unimolecular, i.e. the rate of reaction is proportional to the concentration in the adsorbed layer, i.e.  $\frac{dx}{dt} = k'y$ .

This assumption gives

$$\frac{dx}{dt} = Ak'(a - x)^{\frac{1}{n}} = k(a - x)^{\frac{1}{n}}$$

where  $a$  is the initial concentration in solution and  $x$  the amount

hydrolysed in time  $t$  and  $k = Ak'$ . Let  $a = 1$ , i.e. express  $x$  as a fraction of  $a$  then

$$k \int dt = \int \frac{1}{(1-x)^n} dx.$$

which gives

$$kt = \frac{n}{n-1} \left[ 1 - (1-x)^{\frac{n-1}{n}} \right].$$

Lüers and Wasmund obtained equations which agreed with their practical results very well, especially when the value of 4 was substituted for  $n$  which gives

$$k_4 = \frac{4}{3t} \left[ 1 - (1-x)^{\frac{3}{4}} \right].$$

For values of  $n = 2$  and 3 the following equations are obtained :—

$$n = 2 : k_2 = \frac{2}{t} \left[ 1 - (1-x)^{\frac{1}{2}} \right]$$

$$n = 3 : k_3 = \frac{3}{2t} \left[ 1 - (1-x)^{\frac{2}{3}} \right].$$

This conception of Lüers and Wasmund demands that the speed of reaction should increase with increasing number, because on account of the increasing dilution by decrease of the substrate concentration a progressively greater proportion of the starch is adsorbed and split on the surface of the amylase and, as stated above, the expression they derive agrees very well with their practical results.

L. Michaelis and M. Menten,<sup>21</sup> from their work on saccharose inversion, consider that the affinity between the enzyme and substrate determines the amount of combination between them. If the products of fission also had some affinity for the enzyme a competition would take place between starch and the dextrins and fission products of varying molecular weights for the amylase. This would retard the hydrolysis. M. Somogyi<sup>22</sup> supports this view and considers that the polysaccharides with the highest molecular weights have the greatest affinity.

L. Michaelis and M. Menten have calculated the dissociation constant  $K_m$  of the starch-amylase complex and have derived an expression analogous to that for the dissociation of an acid. The reciprocal of  $K_m$  is called the 'affinity constant.' K. Sjöberg and E. Eriksson<sup>23</sup> have found these values for amylose and amylopectin to be 2.0 and 2.5, respectively, which leads to the

conclusion that both have the same molecular weight, a conclusion with which they disagree (see, however, Meyer and Press <sup>42</sup> above).

(b) **Liquefaction.**—U. Olsson <sup>24</sup> has shown that the action of  $\alpha$ -malt amylase on starch follows the form of a unimolecular reaction. A comparison of the reaction constants for the  $\alpha$ - and  $\beta$ -amylases of malt shows that the liquefying action proceeds at a rate only 1.7 greater than the saccharifying action, and Olsson therefore considered that both reactions were catalysed by the same enzyme. He tried to prove this point by using poisons which he hoped would affect one reaction and not the other. The differences between the effects on the two reactions were so small that he considered his view confirmed.

#### PANCREATIC AMYLASE

It is interesting to note that several workers <sup>25-27</sup> have found that the relationship between the saccharifying and liquefying actions of pancreatic amylase is not altered by purification. They are, however, influenced in different degrees by various external factors and therefore their relative speeds differ with variation of enzyme concentration, <sup>28</sup> for example—

Concentration of pancreatic amylase . . . . .	1	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{3.2}$
Ratio $K_{\text{liquefaction}}/K_{\text{saccharification}}$ . . . . .	34.3	45.5	45.6	62.9	47.0

By following the reducing power V. Henry <sup>6</sup> and Ch. Philoche <sup>5</sup> showed that the course of hydrolysis followed a unimolecular reaction in the first stage, and this has been confirmed by H. C. Sherman and J. C. Baker <sup>40</sup> for soluble starch and  $\beta$ -amylose up to  $R_M$  40 and 50, respectively. The speed of reaction is definitely slower for amylopectin than amylose. R. Willstätter, E. Waldschmidt-Leitz and A. R. F. Hesse <sup>29</sup> obtained a hydrolysis limit corresponding to  $R_M$  75, using a glycerine extract of swine pancreas, and the reaction was unimolecular up to 40 per cent. fission.

It is important to know the limits of enzyme concentration between which an exact proportionality of the concentration and the speed of reaction exists, for then the relative efficiencies of different preparations may be compared.

The reaction constants are inversely proportional to the substrate concentration in the case of both pancreatic and malt amylases but, according to Kendall and Sherman, <sup>30</sup> the speeds at the beginning are independent of the starch concentration between 1 and 4 per cent., and at lower concentrations they

fall, but more slowly than the starch concentration. The speed is proportional to the enzyme concentration and, in the case of large amylase concentrations, G. Orestano<sup>31</sup> found a unimolecular course to prevail up to a degree of fission of 40-80 per cent. Pastes of native and of soluble starch were attacked equally fast.

S. J. Pronin<sup>32-33</sup> noted high reaction rate during a short initial period after which the results can be fitted into a unimolecular equation. He found that in a reaction that ran for 2-3 hours this initial period was 15 minutes. After some 50 per cent. hydrolysis had occurred the reaction constant fell strongly. In the case of small enzyme concentrations and small reaction speeds there is an interval in which both a linear and a unimolecular hydrolysis may be concurrent. A. Janke and J. Holota<sup>3</sup> consider that with commercial bacterial and fungi preparations no constancy of reaction constants can be found, because the enzyme is attacking the starch molecule in a number of locations, thus producing a very complex type of reaction. K. Oshima,<sup>41</sup> however, finds that although different species of *Aspergillus* show variation in activity of the enzyme, the velocity of reaction follows a unimolecular formula.

The increased initial speed is apparently dependent on the colloidal properties of the substrate. G. Orestano<sup>31</sup> found the liquefaction of soluble starch to follow the unimolecular course up to 75 per cent. fission. The speed varied in an unproportionate manner with the quantity of enzyme but it probably shows itself proportional to the substrate concentration.

#### SALIVARY AMYLASE

Chittenden and, later, McGuigan<sup>34</sup> noted that starch is not entirely saccharified by salivary amylase, and McGuigan considers an inhibitor, which is not glucose or maltose, must be present. O. Hammarsten<sup>35</sup> finds that granules of different starches are attacked at different speeds, but on grinding the granules to break down their structure these differences are eliminated.<sup>44</sup> This type of behaviour has already been discussed in Chap. 4, Part v, and on pages 309, 441.

When acting on solutions of Merck starch up to 1 per cent. strength for 30 minutes with a fixed amount of salivary amylase, the amount of sugar formed is the same irrespective of the ratio *substrate/enzyme* or of the concentration. When using the same amount of enzyme on 2-10 per cent. solutions, however, there

is a proportional decrease in the percentage of total starch saccharified.

H. von Euler and Svanberg<sup>9</sup> found, analogous to malt amylase, a unimolecular course for salivary amylase, the same saccharification limit and an almost equal reaction constant. C. L. Evans<sup>44</sup> and J. R. Broeze<sup>36</sup> found that the initial speed is proportional to the enzyme concentration when this is small. Assuming a 75 per cent. saccharification the reaction coefficients are proportional to the amount of enzyme in a given volume. J. A. Remesow<sup>37</sup> used a nephelometric method to follow the action of this amylase on rice starch and concluded it followed a monomolecular course.

K. Myrbäck<sup>38</sup> finds that in the presence of the nitrate ion the reaction constant is much lower than in the presence of the chloride ion. With regard to the temperature coefficient from 10-30° C. this has a value of 2, but from 30° C. upwards it falls rapidly, possibly because of the increased dissociation of the sodium chloride-ptyalin compound with increase of temperature. As already discussed (p. 446) the presence of sodium chloride is essential to the activity of this amylase and a loose combination between the salt and amylase appears to take place.

With both salivary and pancreatic amylases the termination of the dextrinogenic stage of the hydrolysis is not marked by any obvious slackening in the rate of hydrolysis such as is observed in the case of  $\alpha$ -malt amylase. C. S. Hanes<sup>39</sup> suggests that it is probable that the first two  $\alpha$ -amylases have higher affinities for short-chain dextrans (in relation to their affinities for the more complex components of the substrate) than  $\alpha$ -malt amylase. This might or might not be related to the presence of another component in the animal preparations. Such a conception would provide a basis for interpreting the general forms of the hydrolysis curves, the different but characteristic achroic  $R_M$  values, and the fact that for salivary and pancreatic  $\alpha$ -amylases this value increases with increasing substrate concentration, but conclusive evidence is not available to support this conception.

#### REFERENCES

1. J. KJELDAHL, *Compt. rend. trav. lab. Carlsberg*, 1879, 1; *Z. ges. Brauwes.*, 1880, 49.
2. C. J. LINTNER and F. ECKARDT, *ibid.*, 1889, 24, 389.
3. A. JANKE and J. HOLOTA, *Biochem. Zeit.*, 1940, 309, 194.
4. H. T. BROWN and T. A. GLENDINNING, *J. Chem. Soc.*, 1902, 81, 388.
5. CH. PHILOCHE, *J. Chim. physique*, 1908, 6, 212, 355.
6. V. HENRY, 'Lois générales et l'action des diastases,' Thesis, Paris, 1903.

7. H. C. SHERMAN and J. A. WALKER, *J. Amer. Chem. Soc.*, 1917, **39**, 1476.
8. H. VAN LAER, *Bull. Acad. roy. Belgique*, 1910, No. 7, 611; Nos. 9-10, 707; 1911, No. 2, 84; No. 3, 305; No. 4, 362; No. 11, 715; 1913, No. 4, 395.
9. H. v. EULER and O. SVANBERG, *Hoppe-Seyl. Z. physiol. Chem.*, 1921, **112**, 193.
10. — and K. JOSEPHSON, *Ber.*, 1923, **56**, 1749.
11. H. LÜERS and W. WASMUND, *Fermentforschg.*, 1921, **5**, 169.
12. K. SJÖBERG, *Hoppe-Seyl. Z. physiol. Chem.*, 1923, **131**, 116.
13. C. WIRTH, 'Untersuchungen über die Bestimmungen der diastatischen Kraft des Malzes und von Malzextrakten,' Diss., Munich, 1908.
14. M. SCHNEIDER, Diss., Munich, 1920.
15. H. M. VERNON, *J. Physiol.*, 1901, **27**, 190.
16. J. DUCLAUX, *Ann. Inst. Pasteur*, 1898, **12**, 96.
17. E. F. ARMSTRONG, *Proc. Roy. Soc. London*, 1904, **73**, 500.
18. H. T. BROWN, *J. Chem. Soc.*, 1902, **81**, 373.
19. H. T. BROWN, *ibid.*, 1902, **81**, 388.
20. A. FODOR, *Kolloid-Zeit.*, 1920, **27**, 242.
21. L. MICHAELIS and M. MENTEN, *Biochem. Zeit.*, 1913, **49**, 333.
22. M. SOMOGYI, *J. Biol. Chem.*, 1940, **134**, 301.
23. K. SJÖBERG and E. ERIKSSON, *Hoppe-Seyl. Z. physiol. Chem.*, 1924, **139**, 118.
24. U. OLSSON, *ibid.*, 1923, **126**, 29.
25. H. C. SHERMAN and M. D. SCHLESINGER, *J. Amer. Chem. Soc.*, 1911, **33**, 1195.
26. — *ibid.*, 1912, **34**, 1014.
27. — *ibid.*, 1913, **35**, 1784.
28. M. SAMEC and M. BLINC, *Kolloidchem. Beih.*, 1939, **49**, 73.
29. R. WILLSTÄTTER, E. WALDSCHMIDT-LEITZ and A. R. F. HESSE, *Hoppe-Seyl. Z. physiol. Chem.*, 1923, **126**, 141.
30. E. C. KENDALL and H. C. SHERMAN, *J. Amer. Chem. Soc.*, 1910, **32**, 1087, 1090.
31. G. ORESTANO, *Bull. Soc. Chim. biol.*, 1932, **14**, 1531.
32. S. J. PRONIN, *Biochimija*, 1937, **2**, 935.
33. — *Bull. biol. med. exp. U.R.S.S.*, 1937, **3**, 419.
34. MCGUIGAN, *J. Biol. Chem.*, 1919, **39**, 273.
35. O. HAMMARSTEN, *Uppsala lakereforen förhandl.*, 1871, **6**, 471.
36. J. R. REMEEZE, *Biochem. Zeit.*, 1928, **204**, 286.
37. J. A. REMESOW, *Arch. biol. Nauk.*, 1935, **37**, 425.
38. K. MYRBÄCK, *Hoppe-Seyl. Z. physiol. Chem.*, 1926, **159**, 1.
39. C. S. HANES, *New Phytologist*, 1937, **36**, 218.
40. H. C. SHERMAN and J. C. BAKER, *J. Amer. Chem. Soc.*, 1916, **38**, 1885.
41. K. OSHIMA, *J. Coll. Agric. Hokkaido. Imp. Univ.*, 1928, **19**, 135.
42. K. H. MEYER and J. PRESS, *Helv. Chim. Acta*, 1941, **24**, 50.
43. — and P. BERNFELD, *ibid.*, 1940, **23**, 1465.
44. C. L. EVANS, *J. Biol. Chem.*, 1919, **39**, 273.



## CHAPTER 7

METHODS OF DETERMINING THE ACTIVITY OF  
AMYLASE PREPARATIONS

THERE does not appear to be any entirely satisfactory and rapid method for determining the activity of enzyme preparations used in the various industries although there are several of great indicative value. In the textile industry complications arise from the use of the different starches<sup>25-26</sup> which may be employed for different types or even the same type of work, and which may give somewhat different results in large-scale working. The methods of preparation of different sizes, etc., and the after-treatments, such as drying, to which they have been subjected, may tend to alter the starch present so that it is not strictly comparable with untreated starch. The presence of inorganic salts in certain dressings and starches may act as poisons for a particular enzyme preparation and thus lead to waste or faulty results when applied on a large scale. These considerations are of particular importance in the textile industry.

Even in certain industries where standard methods have been adopted, two classes of error may appear. H. R. Sallans and J. A. Anderson,<sup>44</sup> when investigating the official method of the American Society of Brewing Chemists for estimating the diastatic power of malt, point out that the first class of errors embraces those inherent in any particular empirical method, such as that just mentioned, and into the second class fall those due to the use of unofficial modifications and non-observance of certain standard procedures. In the first class the chief errors are those arising from the faulty preparation of solutions and the vexed question of the determination of reducing power (*vide infra*).

Variations in the starch used, in the temperatures of extraction of the malt, and the method of sampling malt, introduce errors of varying magnitude which have been determined by the above workers. As examples of the second class of errors, departure from the standard ratio of water to malt during the extraction and disregard of Kjeldahl's law of diastatic conversion may be mentioned. Centrifugal separation of the malt extract from the insoluble matter was found to give similar results to the filtering recommended in the above official method, but if the liquid was separated by settling and decantation, as in the standard method of the Institute of Brewing; lower results were obtained. The

optimal conditions of activation, pH value, time and temperature for the extraction of the total  $\beta$ -amylase in barley and malt have been investigated by S. R. Snider<sup>107</sup> who uses a mixture of papain and cysteine to free the  $\beta$ -amylase. He claims that this mixture allows as much  $\beta$ -amylase to be extracted in 3 hours as can be extracted using papain alone in 24 hours and that the new method is more accurate. After correcting for malting losses the green malts showed considerably more diastatic power than the corresponding barleys, but on destruction of the  $\alpha$ -amylase in these malt extracts the diastatic powers of barley and malts agreed fairly closely.

T. Chrzyszcz<sup>51</sup> has repeatedly stressed that the activities of diastases should be considered separately according to whether they are liquefying, dextrinising or saccharifying in action. Windisch<sup>52</sup> considers that the liquefying and saccharifying actions do not appear simultaneously.

Anderson and Sallans<sup>104</sup> have modified the ferricyanide method (*vide infra*) but A. D. Dickson<sup>105</sup> finds that even this modification gives results which can be grouped into a high and a low class, within each of which there is very good agreement, but that there is no consistent association of low duplicate errors with either group. This worker, in a collaborative study of three malts examined by nine collaborators on each of four different days, each worker using the same soluble starch, concludes that it is necessary for each laboratory to examine its own sources of error. The results of his study have led the Malt Analysis Standardisation Committee (U.S.A.) to recommend the ferricyanide method, as well as that of the American Society of Brewing Chemists which is similar to that of the Institute of Brewing, for the determination of diastatic power.

**The Units of Amylase Activity.**—The oldest unit still in use is that due to Lintner,<sup>13</sup> who expresses the power of a diastase as 100 when 0.3 ml. of a solution containing 0.1 gm. diastase in 250 ml. water produces sufficient sugar to reduce 5 ml. of Fehling's solution when acting on 10 ml. of a 2 per cent. starch solution at ordinary temperatures for 1 hour. The reaction constant can be found from this value in the following way<sup>74</sup>: 200 mgm. of starch have been used, and supposing 75 per cent. fission has occurred then  $a = 150$  mgm., and since 5 ml. of Fehling's solution represents 0.03682 maltose,  $x = 36.82$  mg.,  $a - x = 113.18$  mg.  $\times k$ , the reaction constant is 0.00205.

Osborne<sup>54</sup> uses Lintner units, and Effront<sup>55</sup> bases his estimations of reactive power on the saccharification which occurs under certain conditions at 60° C. This is not too happy a choice, as

at 60° C. the enzyme is already affected by the heat. Consideration of the limiting value of saccharification at concentrations up to 40 per cent., i.e. within the range at which the proportionality law holds in certain limits of enzyme and substrate concentration shows that the equation for the monomolecular reaction yields values of  $k$  which are constant for practical purposes. In view of this the amylase activity has been measured by several workers by the value of the reaction constant.

Sherman and co-workers<sup>8, 57</sup> saccharify 2 gm. of soluble starch for 30 minutes at 40° C., and estimate the  $\text{Cu}_2\text{O}$  which is produced from an oxidising copper solution. A connection between the amount of  $\text{Cu}_2\text{O}$  formed and the reaction constant is readily obtained, and to convert this constant into the 'new scale' units it is divided by the quantity of enzyme used, e.g. if 0.0312 gm. of enzyme are employed, the amount of  $\text{Cu}_2\text{O}$  precipitated is 100 mgm. This is equivalent to 82.0 mgm. of maltose from which  $k = 31.2$  (*vide supra*), and therefore  $31.2/0.0312 = 1000$  units.

H. v. Euler and O. Svanberg<sup>58</sup> define the saccharifying power by the equation  $Sf = k \times g$  (maltose)/(gm. preparation) in which  $k$  is the monomolecular constant for saccharification, and  $g$  (maltose) is the number of grammes of maltose which can be obtained in a reaction measured by  $k$ , and (gm. preparation) is the amount of enzyme present. This relation characterises amylase preparations in 0.72 to 2.0 per cent. starch solutions and such preparations which give reaction constants of 0.004 and 0.08 at 37° C. in the presence of suitable neutral salts; 1000 Lintner units correspond to about 26Sf and 1000 units of the 'new scale' equal 38.5Sf.

Willstätter<sup>59</sup> takes as an amylase unit one hundred times the amount of enzyme, which gives a value of  $k = 0.01$ , therefore the constant expresses at the same time the number of the amylase units in the test. The degree of purity of a preparation is expressed by the *amylase value* or the number of amylase units in 1 gm. of the dry preparation. The Euler-Svanberg unit refers to 1 gm. of enzyme preparation, and to convert Sf into the 'amylase value' it is multiplied by 0.05333, i.e. amylase value = 0.05333Sf. Euler and Svanberg propose 37° C. as a normal working temperature for comparing amylases, and it has been found that  $k_{30}/k_{20} = 2.0$  and  $k_{40}/k_{37} = 1.2$ .

R. M. Sandstedt and co-workers<sup>95</sup> suggest that the  $\alpha$ -amylase present in malt be expressed as the number of grammes of soluble starch that, in the presence of excess  $\beta$ -amylase, are dextrinised by one gramme of the enzyme in one hour at 30° C.

Lintner soluble starch is generally used as substrate in the determination of the amylase units, but it is not an enzymatically

uniform substance, as mentioned below. Different amylases affect it in different ways, so that it may be used to compare the activities of the same type of amylase but not of different types of amylase preparations. H. v. Euler and K. Josephson<sup>60</sup> find that Lintner-starch gave more maltose at the end of the reaction than did Zulkowsky's soluble starch. The constants are not affected, however, if values within the first 40 per cent. of fissions are employed.

J. Blom, A. Bak and B. Braae<sup>61</sup> use viscosity measurements to determine activity and compute this quality by determining the time in seconds ( $t$ ) in which  $v$  gms. of amylase reduces the viscosity of a starch paste to that of a reference solution consisting of 50 per cent. saccharose in water. The unit of activity ( $V$ ) is given by the equation  $V = 1000/t.v$ . If  $t$  is the time in seconds for the iodine colour to match that of a comparison solution and  $v$  is as above, the same expression holds true. Other units are mentioned below, principally the 'liquefon.'

Methods of estimating the activity of an enzyme-preparation may be broadly classed under five main headings, and from these methods may be selected one that will suit the requirements of any particular problem. The five classes are as follows:—

- I. Viscosity methods.<sup>1</sup>
- II. Methods involving the determination of reducing power.
- III. Iodometric methods, i.e. noting the amount of enzyme required to destroy the iodine reaction.
- IV. Direct determinations on sized cloth.
- V. Miscellaneous methods.

**1. Viscosity Methods.**—T. Chrzaszcz<sup>62</sup> and J. Janicki<sup>63</sup> have examined the methods in use for determining the liquefying power of amylases, and give 15 methods, together with 18 proposed modifications of these methods. These methods are divided into 3 groups by these workers. In the first group come those in which the liquefaction of a starch paste to which the enzyme has been added either before,<sup>55, 62-67</sup> or after the starch has been pasted as in Lüers' method,<sup>68</sup> or those of A. Pollak,<sup>69</sup> U. Olsson,<sup>70</sup> S. A. Waksman,<sup>19</sup> and others.<sup>64, 72</sup> The second group embraces those methods in which the decrease in viscosity of solutions of soluble starch,<sup>64</sup> such as those of L. Fletcher and J. B. Westwood,<sup>39</sup> A. J. Hall,<sup>3</sup> and other<sup>2</sup> (*vide infra*). In group 3 come those methods such as those depending on iodine coloration or sugar determination. S. Jozsa and H. C. Gore<sup>2</sup> have followed the changes in viscosity of a remarkably uniform starch

paste as a means of determining the liquefying power of the diastase used. A. Janke and J. Holota<sup>94</sup> have determined the liquefying power of commercial  $\alpha$ -amylase preparations (biolase and superclastase) on 5 per cent. potato-starch pastes at various  $\text{pH}$  values and different temperatures and use the Höppler viscometer to follow the reactions. L. Fletcher and J. B. Westwood<sup>95</sup> suggest that erroneous values for the liquefying power of the enzyme are obtained if the amount of starch liquefied is assumed to be proportional to this power unless a restricted experimental range is used, and even then the results are not entirely satisfactory.

J. J. Willaman, E. W. Clark, and O. B. Hager<sup>27</sup> greatly modify this method, but inaccuracies still enter into the result due to the application to any diastase solution of certain empirical constants for a given preparation. Their method also does not take into consideration the rate-curves of different samples, especially at the point where little starch is left; and doubling the concentration of the active agent does not double the liquefying power, as the latter, by this method, represents an average value only for the rate of conversion and is not proportional to the actual enzyme-content.

K. Mayer<sup>99</sup> has introduced, as a substrate for the determination of the liquefying power of enzymes, starch which has been treated with iodine in amount insufficient to give a blue colour. To 100 ml. of a 1 per cent. starch paste is added 0.65 ml. of N/100 iodine and the resultant product is resistant to starch saccharifying enzymes but the action of liquefying enzymes and the iodine coloration are unaffected.

S. Jozsa and W. R. Johnson<sup>28</sup> have introduced a new unit of enzyme-activity which they term the 'liquefon,' and by its use they claim to be able to calculate accurately the diastatic activity of enzyme preparations from the measurement of the viscosity changes in a starch paste.

They define the 'liquefon' as the amount of starch-liquefying enzyme which will convert a standard starch paste at the rate of 25 mg. of dry starch per minute at zero time under the given experimental conditions. This unit has been fully discussed by these workers<sup>28</sup> elsewhere.

A starch paste (2000 ml.) is made containing 4.211 gm. of dry starch per 100 ml. paste, stirred with a high-speed stirrer for about  $1\frac{1}{2}$  minutes while hot, cooled to  $20^{\circ}\text{C}$ ., buffered to  $\text{pH}$  value 5.0-5.2 with 50 ml. of Walpole's acetate buffer, made up to the mark with distilled water, and then again stirred 30-45 seconds to obtain a uniform paste. A test portion of this paste

is mixed with 10 per cent. its weight of a solution of sodium chloride containing 25 mg. salt per 100 ml., and stirred for 1 minute in the high-speed stirrer. The time of outflow from a water-jacketed pipette at 21° C. is determined and should correspond within two seconds to the time of outflow of an 81 per cent. solution of glycerol (sp. gr. 1.2138 at 20-21° C.). A pipette is chosen with a delivery-time of 55-57 seconds for 100 ml. of water, the time of delivery of the same amount of the specified glycerol solution being between 165-190 seconds at 21° C. If the delivery-time of the starch paste is not within two seconds of that of the glycerol solution, the stirring is continued until it does, and the length of time of stirring is accurately determined.

It should be noted that a fresh starch paste should be made up each day, and that the stirring-jars should be of such dimensions that the whole of the paste is well-stirred.

A 150 gm. sample of the starch paste prepared as above is cooled to 19.5° C. and 15 ml. of the enzyme solution (see below) or sodium chloride solution (for blank tests) stirred in, the correct time of stirring being determined by running one or two blanks, as described above. After stirring, the temperature should be 21° C.  $\pm$  0.2° C., at which the paste is maintained for 59 minutes; then it is sucked into the pipette and the time of outflow of 100 ml. determined. The viscosity determination is begun before the end of 1 hour to correct for the liquefaction occurring during the determination. To check the stability of the paste, another blank should be run on 150 gm. of paste that has stood for 1 hour at 21° C. This value should not deviate by more than 3-4 per cent. from the original values.

From the outflow-time of a given mixture, Jozsa and Johnson obtain the amount of starch liquefied by use of the formula:  $S = 12.9 P - 0.065 P^2 + 0.0025 P^3$ , where S is the number of mg. starch liquefied, and P is the percentage decrease in time of outflow between the blank and the sample. The enzyme-content or activity of the solution is derived from the amount of starch liquefied by means of the empirical formula:  $\text{Log}_{10} L = 0.000565 (S - 1078)$ , where L is the number of 'liquefons' in 10 ml. of infusion, and S = mg. of starch liquefied in 1 hour. From the concentration of the infusion the number of L in 1 gm. of the preparation is calculated; it is an exact measure of the  $\alpha$ -amylase-content and of the liquefying power at zero time. The following example taken from the original paper is given in illustration:—

	<i>Outflow- time in Seconds</i>
Paste stirred with water for 60 secs. . . . .	181.5
Above paste after additional stirring (trial) . . . . .	164.5
Another sample stirred with water for 65 secs. . . . .	171.2
Glycerol solution . . . . .	170.0
Initial outflow-time . . . . .	171.2
Final outflow-time after enzyme-action is complete . . . . .	56.5
Range . . . . .	114.7
Outflow-time after 1 hour at 21° C. using sample (10 mg./10 ml.) . . . . .	91.8
Decrease in outflow-time (171.2-91.8) . . . . .	79.4
Percentage decrease $79.4 \div 114.7$ . . . . .	69.2
Milligrams of starch liquefied (by formula) . . . . .	1408.4

Now  $\text{Log}_{10} L = 0.000565 (S - 1078) = 0.1870$ ;  $L = 1.538$  and as 10 mg. of enzyme sample were used, 'liquefons' per gm. = 154. As the 'liquefon' is the amount of enzyme that will convert 25 mg. of dry starch per minute at zero time, the liquefying power of this sample at zero time is 3850 mg. of dry starch per minute.

To prepare the enzyme solution, a suitable quantity of the preparation (2-15 gm.) is weighed into a 1000 ml. flask, 25 gm. of sodium chloride added, and the contents made up to the mark. After standing 1 hour with occasional shaking the infusion is filtered, the first 100 ml. of the filtrate being rejected; 100 ml. of the remainder are then transferred to a 1000 ml. flask and made up to the mark.

To obtain good results the liquefaction in 1 hour should be between 50 and 90 per cent., and with materials of 1-3 'liquefons' per gm., an infusion of 10 gm. of enzyme material in 10 ml. is required to bring about liquefaction with the above range. Strong preparations of 1000-3000 'liquefons' per gm. require only 1 mg. per 10 ml. for this purpose.

A simple procedure and apparatus for use in factory routine-testing is described by A. J. Hall,<sup>3</sup> in which a 3 per cent. starch paste is treated with a definite quantity of a 0.4 per cent. solution of a diastase preparation at 21-22° C. for 40 minutes, and the viscosity determined. U. Olsson<sup>70</sup> measured the decrease in viscosity by measuring the rate of rise of a hollow glass sphere through the reaction mixture but considers the Höppler viscometer preferable to any other instrument.

**2. Methods Involving Determination of Reducing Power.**—Methods that are based on the amount of sugar formed by the completed diastatic action of malt on starch, e.g. in the method of E. F. Harrison and D. Gair,<sup>4</sup> are not so accurate as

could be desired, because the amount of liquefaction is not related to the amount of sugar formed, two distinct processes entering into the reaction—liquefaction by one enzyme and saccharification by another (see p. 455). Also, in some preparations the enzymes have different sensitivities to heat and other external agents and are affected to different extents. J. Kjeldahl,<sup>5</sup> however, has shown that if malt extract is allowed to act upon starch at 57-59° C. until about 40 per cent. of the starch has been converted, the reducing sugar formed is directly proportional to the diastatic power. Practically all the methods employing estimation of the amount of reducing sugar take this observation of J. Kjeldahl into account.

The reducing power ( $R_M$ ) is expressed as the percentage of the reducing power that would be shown under the same conditions if the substance could be completely transformed into anhydrous maltose ( $[\alpha]_D = 137.9^\circ$ ). One gramme of starch would yield theoretically 1.055 g. maltose. When maltose is the sole reducing product formed, as happens with the saccharogenic amylase of malt, this convention is devoid of ambiguity and provides an index of the amount of transformation of starch to maltose that has occurred. With most amylases, however, particularly in the early stages of the action, this is by no means the case and the significance of the conventionally expressed reduction value may obviously be different in such cases. Thus an  $R_M$  of 36 could indicate either 36 per cent. of the substrate has been converted to maltose, but it could equally mean that the whole of the substrate had been converted into reducing dextrin having a chain-length of 6 glucose units. Thus  $R_M$  gives an indication of the number of linkages hydrolysed but not of their relative positions in the original chain structure nor of the nature of the products containing the reducing groups. J. Blom, Bak, and Braae<sup>61</sup> suggest that the method of determination could be indicated by suffixing to the symbol  $R_M$  the initial of the worker whose method is employed, e.g. malto-dextrin,  $R_{MB}$  (or  $R_{MB}$ ) = 43 if the Bertrand method is used, or  $R_{MW}$  (or  $R_{MW}$ ) = 43 if the Willstätter method is employed. In this case the malto-dextrin would have 43 per cent. of the reducing power of anhydrous maltose. They suggest a similar means to denote the degree of degradation of starch and the method employed. The amount of maltose stoichiometrically equivalent to the starch destroyed is expressed as a percentage of the amount of maltose stoichiometrically equivalent to the whole of the starch employed, and this figure represents the degree of degradation of the starch. This figure would, of course, be affected if some other disaccharide



residue is present in starch, but convincing evidence of such an eventuality has yet to be produced.

C. J. Lintner<sup>13</sup> was the first to introduce the use of soluble starch for determining diastatic activity. Starch treated with 7.5 per cent. hydrochloric acid for 7 days at ordinary temperature, or 3 days at 40° C., and then washed free from acid, gives a solution which remain clear for days, and is devoid of the usual paste-forming properties of the untreated starch. The amount of reducing compounds present is practically negligible. Lintner adds varying amounts of diastatic solution to 10 tubes containing the same amount of starch, and after 1 hour 5 ml. of Fehling's solution is added to each, and they are heated on a water-bath. The tube in which all the copper has just been reduced is noted.

Two considerations affect the selection of a suitable method, speed and accuracy, and dissatisfaction is often expressed with methods for determining the reducing sugars left after the diastatic action. The high degree of accuracy claimed for some of the methods hardly appears warranted in the final results when the difference in the time of manipulation is considered.

The method adopted by the Institute of Brewing is that of Lane and Eynon<sup>34</sup> (see p. 428), which is satisfactory except that inexperienced workers may obtain discrepant results, but this probably applies to other methods also. S. Laufer, E. Schwarz, and L. Laufer<sup>46</sup> consider that the original Hagedorn and Jensen method (see p. 512) gives better results than the official method of the American Society of Brewing Chemists.

The amount of reducing sugar formed may be determined volumetrically or by gravimetric methods,<sup>6-7</sup> preferably by that of H. C. Sherman and his co-workers.<sup>8</sup> J. T. Flohill,<sup>9</sup> for example, allows the enzyme to act on a 2 per cent. solution of soluble starch at 20° C. for 1 hour, after which caustic soda is added to destroy the enzyme, the solution diluted and boiled with Fehling's solution. The amount of reduced copper is determined by adding potassium iodide solution plus sulphuric acid and titrating with sodium thiosulphate. The rate at which Fehling's solution or other alkaline copper solution is used up is dependent on the nature of the reducing substances and on the method employed. If the solution contains sugar alone concordant results are obtained from both alkaline copper reagents and iodometric methods, but the greater the reducing power attributable to any dextrans present the more the results obtained by the two types of method diverge. When dextrans are present, therefore, Bertrand's<sup>71</sup> or Fehling's methods are inferior to iodometric methods.<sup>73</sup>

Sherman and his co-workers,<sup>8</sup> and C. A. Brown<sup>30</sup> criticise adversely the classical Lintner<sup>13</sup> method. If pH control is available the Sykes and Mitchell<sup>31</sup> method is probably the most precise of the modified Lintner methods for estimating the saccharifying power by malt diastase, but owing to filtration difficulties it is too long for control purposes. Blish and Sandstedt<sup>32</sup> consider that the usual methods for determining the reduced copper in Fehling's solution fail to give concordant results when used by different workers, possibly owing to back-oxidation of the reduced copper, as noted by Hanes.<sup>33</sup> Of other methods based on the reduction of alkaline copper solutions, those of W. Engelhardt and M. Gertschuk<sup>77</sup> and C. Wirth<sup>78</sup> should be mentioned, while modified Lintner methods for determining the diastatic activity of malts have been described by W. Salač<sup>84</sup> and others.<sup>85-89</sup>

Timing errors are reduced to a minimum by the method of Sherman and co-workers, in which the starch is added to the malt and digested at 40° C. W. Syniewski<sup>101</sup> considers that when Lintner's soluble starch is used for the determination of diastatic activity the results are low, as he found that the first small quantity of diastase added caused no saccharification. With fresh starch solution the error is small but becomes considerable if the solution is stored for a few days before use.

C. T. Bennett and F. C. L. Bateman<sup>102</sup> are further workers who stress that the variability of the soluble starch causes inconsistencies in assessing the diastatic power of malt and malt extracts. They also recommend that attention should be paid to the freedom of the water and the laboratory atmosphere from ammonia and nitrites.

Lane and Eynon's<sup>34</sup> method is one of the standard methods used by workers in England, but H. C. Gore and H. K. Steele<sup>38</sup> consider that it is not precise enough to be used with highly active malts.

A volumetric method has also been devised<sup>10</sup> which utilises the conversion of maltose to maltobionic acid by means of iodine in the presence of caustic soda. The malt extract is allowed to act upon a 2 per cent. solution of soluble starch for 30 minutes at 20° C. ; the action is stopped by the addition of caustic soda, and the maltose present oxidised to maltobionic acid by the addition of standard iodine solution, the mixture then being back-titrated with standard sodium thiosulphate, the excess standard iodine acting as the indicator. W. Windisch and P. Kolbach<sup>11</sup> make a correction in this method for the amount of iodine absorbed by the diastatic solution and their method is widely used on the

Continent. In a further paper they<sup>12</sup> ascribe the varying results obtained by this method to variations in the  $pH$  value and in the state of dispersion of the starch. The hypiodite method is considered inadequate for highly active malts and has been adversely criticised by Kline and Acree<sup>35-36</sup> (see p. 428). Samec and Blinc<sup>74</sup> consider that a stoichiometric relationship is shown by the iodometric method of Willstätter and Schudel,<sup>75</sup> or that of F. Auerbach and E. Bodländer,<sup>76</sup> and that they may be used with every confidence.

Hagedorn and Jensen's<sup>37</sup> method for determining sugar in blood has been modified by Hanes (*v.s.*) for the exact titrimetric estimation of the reducing substances as maltose, formed by malt diastase, and for accuracy and convenience it leaves little to be desired. A further modification of the method by Blish and Sandstedt for determining reducing substances formed by the action of diastase on flour suspensions has been found to give excellent results. Larger amounts of maltose can be determined by this method than by Hanes' method; H. C. Gore and H. K. Steele<sup>38</sup> have used the reagents specified in the former for determining Lintner values and find it both rapid and accurate.

Twenty-five grams of finely ground malt are digested with 500 ml. of water at room-temperature for  $1\frac{1}{2}$  hours with occasional shaking, after which it is filtered, the first 50 ml. of filtrate being rejected. For syrups and liquid extracts multiples or fractions of the 5 per cent. infusion are prepared; 25 ml. of the filtered infusion are diluted to 250 ml. and 10 ml. of this dilute infusion transferred to a 200 ml. volumetric flask and kept at 20° C. At a noted time 100 ml. of soluble-starch solution, containing 2.2 gm. Lintner's soluble starch and buffered with Walpole's acetate buffer are delivered, the time being counted from the time the first drop enters the infusion. The liquid is kept rigidly at 20° C. for 30 minutes from the time of starting the clock, after which 20 ml. of a 0.4 N caustic soda solution are added, the first portions of the alkali being mixed with the solution as quickly as possible to stop the diastatic action. The alkali is neutralised with 20 ml. of 0.4 N hydrochloric acid and the volume made up to 200 ml., and 25 ml. of this solution are thoroughly mixed with 50 ml. of alkaline ferricyanide reagent, made by making 16.5 gm. potassium ferricyanide and 22 gm. of anhydrous sodium carbonate up to 1 litre with distilled water. The mixture is heated for exactly 15 minutes on a boiling water-bath, keeping the liquid in the flask below the level of the boiling water. After cooling to room-temperature, 125 ml. of an acetic acid reagent (200 ml. glacial acetic acid, 70 gm. potassium chloride, 20 gm.

crystalline zinc sulphate, made up to 1 litre) are added, and then 5 ml. of a 50 per cent. potassium iodide solution; the iodine liberated is titrated with 0.05 N sodium thiosulphate solution.

To obtain a blank experiment, 10 ml. of the infusion are mixed with 20 ml. of 0.4 N caustic solution, neutralised with 20 ml. of 0.4 N hydrochloric acid, and 100 ml. of the starch solution added. The solution is then made up to 200 ml. with distilled water and 25 ml. of this are mixed with 50 ml. of the alkaline ferricyanide reagent, the procedure from this point being the same as for the sample.

The degrees Lintner (L) of the sample are given by the formula  $L = 8.092 F$ , where F is the nett volume of the ferricyanide reagent consumed.

F. W. Norris and W. A. Carter<sup>47</sup> titrate the sugar solution against alkaline ferricyanide, using methylene blue as indicator, thereby eliminating the back-titration necessary in most ferricyanide methods, and obtain results which are comparable with those obtained with Lane and Eynon's method.

The amount of ferrocyanide formed by the action of the sugar on the ferricyanide is determined by F. C. Hildebrand and B. A. McClellan<sup>48</sup> by titrating with ceric sulphate solution, using 'Setopaline C' as an internal redox indicator, the results agreeing well with those obtained by the Blish and Sandstedt method. V. Jerschov<sup>49</sup> proposes to use either chemical or polarimetric methods, but his directions in the first stages are somewhat novel.

C. F. Silbernagel<sup>15</sup> considers that refined potato starch is a much better standard reagent than the soluble starch made from it, and he therefore proposes to use it for estimating both the liquefying and saccharifying powers of a malt diastase.

**3. Iodometric Methods.**—The degradation of starch when acted upon by dextrinogenic amylase may be followed by observation of the iodine reaction (see also p. 480). The quantity of enzyme necessary to reach the stage at which the blue colour turns to red (the erythro point) or alternatively the point at which the colour entirely disappears (the achroic point) in a constant time<sup>16</sup> is determined, or, as in the method of J. Blom, A. Bak, and B. Braae,<sup>61</sup> the time elapsing between the addition of the enzyme and the moment of reaching the erythro or achroic points.

When using malt extract the iodine method, as mentioned below, is unreliable. When it is realised that the amounts of dextrinogenic and saccharogenic amylases may vary over wide limits for various malt extract preparations and that the action of each is influenced to a different extent by certain unlike factors

the inherent sources of error in this method of assessing activity will be seen. The action of mixtures of  $\alpha$ - and  $\beta$ -amylases is discussed in Chapter 5, page 488.

Sherman, Kendall, and Clark<sup>8</sup> have modified the method of J. Wohlgemuth,<sup>16</sup> in which the enzyme preparation acts on a given amount of soluble starch in solution for 30 minutes at 40° C., and the amount of enzyme necessary to effect the disappearance of the blue coloration with iodine is noted. The results are expressed as the number of ml. of standard solution digested by 1 ml. of enzyme under these conditions. Dymond,<sup>22</sup> D. L. Davoll,<sup>23</sup> V. Dorfman<sup>97</sup> and Scheermesser<sup>24</sup> have employed variations of Wohlgemuth's method, but Davoll considers it unreliable owing to variations in the ratio of dextrin to maltose. When pure  $\beta$ -amylase hydrolyses amylose the iodine coloration remains blue until over 90 per cent. maltose has been formed, when the colour turns violet and then red.<sup>90, 91</sup> Giri<sup>92</sup> finds that sweet-potato amylase behaves in this way, and concludes that it is practically pure  $\beta$ -amylase. This worker<sup>93</sup> finds that the iodine colour reaction with different starches varies markedly with the kind of starch hydrolysed and the concentration of iodine used. Samec shows that the colour tone-intensity changes during amylolysis. With  $\beta$ -amylase, however, although the blue colour is retained to beyond the 90 per cent. saccharification stage the intensity of the colour with iodine diminishes as the hydrolysis proceeds, and smaller quantities of iodine are required to attain the maximum coloration of the solution. Samec and Mayer<sup>79, 80</sup> have evolved a method in which the maximum intensity of blue colour obtainable at each stage of hydrolysis is compared with the initial intensity or with a standard starch iodide solution. Every stage of the hydrolysis is then represented by characteristic numbers which can be plotted in a similar manner to the values obtained from measurements of optical rotation or reduction values.

T. Sabalitschka and R. Weidlich<sup>53</sup> follow the reaction to the erythro point by use of comparison solutions of methylene blue and safranine.

R. M. Sandstedt, E. Kneen and M. J. Blish<sup>95</sup> point out that in the Wohlgemuth method of measuring  $\alpha$ -amylase activity the presence of  $\beta$ -amylase causes some doubt as to the true end-point, owing to the variable effect and amount of this substance in malt extract. In combinations where the saccharogenic power of the  $\beta$ -amylase is more than twice that of the  $\alpha$ -component, a condition generally characteristic of malts, the saccharogenic activity of a mixture of the two amylases is the sum of

the individual activities when the components act separately. They overcome this by the addition of  $\beta$ -amylase in such an amount that its further addition does not further increase the rate of dextrinisation. When this is done they find that there is a linear relationship between the  $\alpha$ -amylase content and the time of dextrinisation. In a further paper<sup>106</sup> they give a rapid method for measuring the  $\beta$ -amylase activity of barley malts using, however, a ferricyanide method for estimating the maltose formed. A method incorporating these observations has been evolved by L. E. Ehrnst, G. J. Yakish and W. Olson.<sup>96</sup>

W. A. Johnson<sup>14</sup> digests potato-starch pastes with increasing proportions of a solution of the diastatic preparation for 10 minutes at 40° C., and notes the amount required to be added to obtain no iodine reaction at the end of the period. He finds that the results closely agree with those obtained by determining the amount of sugars formed. The absorption spectrum of starch-iodide in various stages of hydrolysis has been followed by Hanes and Cattle<sup>56</sup> by means of the spectrophotometer (see pp. 469, 480). The differentiation of starches and of some amylases by the iodine reaction has already been discussed on p. 375.

**4. Direct Determination on Textiles.**—From the point of view of factory control it is preferable to have some method of determining the activity of a desizing enzyme on the cloth upon which it is subsequently to be used. Such a method has been elaborated by Clibbens and Geake,<sup>17</sup> who determine the sizing in a piece of cloth of known weight by treating it in a desizing enzyme solution, wringing it out by hand twelve times in a stream of running water, and then drying to a constant weight. Such a procedure may lead to variable results owing to the wringing being done by hand, but it might be possible to standardise a procedure whereby the fabric was passed through a squeezing roller set to a known expression, say 80 per cent., then suspending the fabric in running water for a determined time and repeating the whole process a definite number of times. They found that 3 per cent. of the total weight of cloth should be ascribed to the waxes and non-sizing agents and gums removed from the cellulose, and allowance must be made for this in determining the weight of size removed. This correction is applied to American, Egyptian, and South American cottons, but rayon fabrics do not require it. Using this method the comparative activity of several enzymes could be determined, the results giving an indication of the most advantageous preparation for technical use.

D. H. Powers<sup>18</sup> suggests a modification of this method in which 10 gm. of cloth, dried to a constant weight, are treated with 200 ml.

of a 5 per cent. desizing solution for 30 minutes at 135° F. in a stout jar. A number of Monel metal balls are added and the whole shaken or rotated at frequent intervals. At the end of the 30 minutes the desizing solution is drained off, replaced by 200 ml. of a 0.5 per cent. soap solution, and the whole maintained at 160° F. for 15 minutes with constant agitation. The soap solution is replaced by an equal amount of water at the same temperature and the whole agitated for 15 minutes, when the cloth is drained and dried to a constant weight. The washing machine of the Society of Dyers and Colourists would be very suitable for this purpose, and the results used in the manner suggested above.

W. M. Scott<sup>98</sup> has evaluated the desizing efficiency of enzymes by two methods, one involving immersion, the other padding, which correspond, respectively, with the actual jig and padding methods used commercially.

**5. Miscellaneous Methods.**—S. A. Waksman<sup>19</sup> stains the starch in a 2 per cent. paste with Neutral Red, maintains the mixture at 40° C., and notes the time taken by the opaque solution to become clear.

A polarimetric method<sup>20-21, 41</sup> has been introduced by H. C. Gore, who determined the relationship between the reducing power and the decrease in polarisation before and after mutarotation was destroyed by the action of ammonia. The mixture of soluble starch and diastatic infusion must be held within the *pH* range of 4.5 to 5.5 to obtain correct results. During saccharification Kjeldahl's law of proportionality is followed, and the fall in polarisation observed is a little over 3° V. This author<sup>40</sup> later increased the magnitude to 11.3° V. by the use of a special type of soluble starch from which comparatively strong solutions could be prepared, and thus increased the accuracy of the method. The starch is prepared by treating it for 6 days with 1½ times its volume of 13 per cent. hydrochloric acid, after which it is well washed with distilled water, the last trace of acid neutralised with ammonia, and the product dried in warm air.

Another soluble starch has been suggested by S. R. Snider and D. A. Coleman,<sup>42</sup> but M. Rossatkevitch<sup>43</sup> considers that soluble starches of different origin can give variations in the diastatic value obtained from the same malt (cf. p. 467), clear solutions giving higher values than opalescent solutions. They point out that the diastatic value decreases with the concentration of the starch, and that the optimum *pH* value for a given malt varies also with this concentration. All these points emphasise the necessity for adhering strictly to the requirements of standard

methods, so that the results obtained by different workers may be comparable.

Rona and Eweyk<sup>81</sup> have used a *nephelometric* method for the examination of the hydrolysis of amylo-amylose with  $\beta$ -amylases, but the examination of  $\alpha$ -amylases by this method cannot be carried out owing to the partial coagulation which takes place in the solution. J. A. Remasow<sup>82</sup> tests various amylases by this method, using a rice starch which has been solubilised by heating under pressure, and found that at concentrations up to 1.0 per cent. the Kleinman and Beer law holds for dilution, but for higher concentrations a correction must be applied to the results.

O. Wolff<sup>100</sup> has followed the course of the action of diastase on starch, using the Zeiss interferometer, which he considers is far more sensitive than the refractometer for this purpose. After a simple calibration with mixtures of sugar and starch solutions the readings obtained are claimed to be proportional to the amount of diastase present. Traces of copper, a well-known poison for diastase, in the water was found by D. Schenk<sup>103</sup> to reduce the apparent diastatic power of malt extracts to a half or even to one-third of their real value.

H. B. Sreerangachar<sup>83</sup> has used a *dilatometric* method to follow the hydrolysis of starch and its components by takadiastase. For soluble starch he found a linear relationship between the falling off of the dilatometric value, the amount of sugar formed and the change in optical rotation.

#### REFERENCES

1. W. C. DAVIDSON, *Johns Hopkins Hosp. Bull.*, 1925, **30**, 281.
2. S. JOZSA and H. C. GORE, *Ind. Eng. Chem. (Anal. Ed.)*, 1930, **2**, 26.
3. A. J. HALL, *Canad. Dyer and Col. User*, 1921, **1**, 156, 161.
4. E. F. HARRISON and D. GAIR, *Pharm. J.*, 1906, **77**, 94.
5. J. KJELDAHL, *Meddelelser fra Carlsberg Laboratoriet*, 1876-82, **1**, 107.
6. E. W. ADAMS, *Colour Trades J.*, N.Y., 1920, **7**, 170.
7. R. GUYATT, *Brewers' J.*, 1909, **45**, 123.
8. H. C. SHERMAN, E. C. KENDALL, and E. D. CLARK, *J. Amer. Chem. Soc.*, 1910, **32**, 1073.
9. J. T. FLOHILL, *J. Ind. Eng. Chem.*, 1920, **12**, 677.
10. J. L. BAKER and H. F. E. HULTON, *Analyst*, 1921, **46**, 90.
11. W. WINDISCH and P. KOLBACH, *Woch. Brauerei*, 1921, **38**, 149.
12. W. WINDISCH and P. KOLBACH, W. DIETRICH and A. CASPARY, *ibid.*, 1922, **39**, 213, 219 and 225; *ibid.*, 1925, **42**, 139.
13. C. J. LINTNER, *J. prakt. Chem.*, 1886, **34**, 378, 383, 386; 1888, **36**, 481.
14. W. A. JOHNSON, *J. Amer. Chem. Soc.*, 1908, **30**, 798.
15. C. F. SILBERNAGEL, *Ind. Eng. Chem. (Anal. Ed.)*, 1930, **2**, 31.
16. J. WOHLGEMUTH, *Biochem. Zeit.*, 1908, **9**, 1.
17. D. A. CLIBBENS and A. GEAKE, *J. Test. Inst.*, 1931, **22**, 465T.



18. D. H. POWERS, *Dyer and Calico Printer*, 1932, **67**, 688.
19. S. A. WAKSMAN, *J. Amer. Chem. Soc.*, 1920, **42**, 293.
20. H. C. GORE, *J. Assoc. Official Agric. Chem.*, 1923, **7**, 364.
21. — *J. Amer. Chem. Soc.*, 1925, **47**, 281.
22. DYMOND, *Pharm. Zeitung.*, 1884, **29**, 671.
23. D. L. DAVOLL, *Chem. Centralbl.*, 1898, **2**, 135.
24. SCHEERMESSER, *Apoth. Zeit.*, 1913, **28**, 752.
25. B. TAMPE, *Woch. Brauerei*, 1922, **39**, 31.
26. A. ASTRUC and A. RENAUD, *J. Pharm. Chim.*, 1923, **27**, 333.
27. J. J. WILLAMAN, E. CLARK, and O. B. HAGER, *Biochem. Zeit.*, 1933, **258**, 94.
28. S. JOZSA and W. R. JOHNSON, *Ind. Eng. Chem. (Anal. Ed.)*, 1935, **7**, 143.
29. W. R. JOHNSON and S. JOZSA, *J. Amer. Chem. Soc.*, 1935, **57**, 701.
30. C. A. BROWN, 'Handbook of Sugar Analysis,' J. Wiley & Sons, New York, 1922.
31. SYKES and MITCHELL, *Analyst*, 1896, **21**, 122.
32. M. J. BLISH and R. M. SANDSTEDT, *Cereal Chem.*, 1933, **10**, 189.
33. C. S. HANES, *Biochem. J.*, 1929, **23**, 99.
34. J. H. LANE and L. EYNON, *J. Soc. Chem. Ind.*, 1923, **42**, 32.
35. G. M. KLINE and S. F. ACREE, *J. Res. Bur. Stands.*, 1930, **5**, 1063.
36. — *Ind. Eng. Chem. (Anal. Ed.)*, 1930, **2**, 413.
37. H. C. HAGEDORN and B. N. JENSEN, *Biochem. Zeit.*, 1923, **135**, 46 ; **137**, 92.
38. H. C. GORE and H. K. STEELE, *Ind. Eng. Chem. (Anal. Ad.)*, 1935, **7**, 324.
39. L. B. FLETCHER and J. B. WESTWOOD, *J. Inst. Brew.*, 1930, **36**, 550.
40. H. C. GORE, *Ind. Eng. Chem.*, 1928, **20**, 865.
41. — *J. Assoc. Off. Agr. Chem.*, 1924, **7**, 364.
42. S. R. SNIDER and D. A. COLEMAN, *Cereal Chem.*, 1937, **14**, 1.
43. M. ROSSATKEVITSCH, *Ann. Soc. brasseurs*, 1937, **46**, 1.
44. H. R. SALLANS and J. A. ANDERSON, *Cereal Chem.*, 1937, **14**, 708.
46. S. LAUFER, E. SCHWARZ, and L. LAUFER, *Amer. Brewer*, 1938, **71**, No. 6, 25.
47. F. W. NORRIS and W. A. CARTER, *J. Inst. Brew.*, 1935, **41**, 167.
48. F. C. HILDEBRAND and B. A. MCCLELLAN, *Cereal Chem.*, 1938, **15**, 107, 819.
49. V. JERSCHOV, *Spiritusind. (Russ.)*, 1936, **13**, 38.
50. R. GARDNER, *Analyst*, 1939, **64**, 103. (Analysis of malt extract.)
51. T. CHRZASZCZ, *Woch. Brau.*, 1913, **30**, 538.
52. W. WINDISCH, W. DIETRICH, and A. BEYER, *ibid.*, 1923, **40**, 49, 55, 59, 61, 67.
53. T. SABALITSCHKA and R. WEIDLICH, *Biochem.-Z.*, 1929, **207**, 477 ; **210**, 414.
54. T. R. OSBORNE, *J. Amer. Chem. Soc.*, 1895, **17**, 598.
55. J. EFFRONT, *Die Diastasen. Deut. Ausgabe*, 1910, §212.
56. C. S. HANES and M. CATTLE, *Proc. Roy. Soc. Lond.*, 1938, **125**, 387.
57. H. C. SHERMAN and M. D. SCHLESINGER, *J. Amer. Chem. Soc.*, 1915, **37**, 616.
58. H. v. EULER and O. SVANBERG, *Hoppe-Seyl. Z. physiol. Chem.*, 1921, **112**, 193 ; **115**, 179.
59. R. WILLSTÄTTER, E. WALDSCHMIDT-LEITZ, and A. R. F. HESSE, *ibid.*, 1923, **126**, 141.

60. H. v. EULER and K. JOSEPHSON, *Ber.*, 1923, **56**, 1749.
61. J. BLOM, A. BAK, and B. BRAAE, *Hoppe-Seyl. Z. physiol. Chem.*, 1937, **250**, 103.
62. T. CHRZASZCZ, *Biochem.-Z.*, 1931, **242**, 130.
63. — and J. JANICKI, *ibid.*, 1932, **256**, 252.
64. — and S. PIOROZEK, *Zeit. Spiritusind.*, 1910, **33**, 66.
65. — and F. TERLIKOWSKI, *Woch. Brau.*, 1912, **29**, 636.
66. C. J. LINTNER and P. SOLLIED, *Z. ges. Brauw.*, 1902, **26**, 329.
67. H. DOESEL, *Diss.* (München), 1923.
68. H. LÜERS, *Methodik der Fermente*, C. Oppenheimer, 1925.
69. A. POLLAK, *Woch. Brau.*, 1903, **20**, 595.
70. U. OLSSON, *Hoppe-Seylers Z. physiol. Chem.*, 1922, **119**, 2.
71. G. BERTRAND, *Bull. Soc. chim. ind.*, 1906, **35**, 1285.
72. W. GLINSKI, *Die Arbeit der Verdauungsdrüsen*, J. P. Pawlow, Wiesbaden, 1898.
73. K. JOSEPHSON, *Ber.*, 1923, **56**, 1758.
74. M. SAMEC and M. BLINC, *Kolloidchem. Beih.*, 1939, **49**, 75.
75. R. WILLSTÄTTER and G. SCHUDEL, *Ber.*, 1918, **51**, 780.
76. F. AUERBACH and E. BODLÄNDER, *Zeit. angew. Chem.*, 1923, **36**, 602.
77. W. ENGELHARDT and M. GERTSCHUK, *Biochem.-Zeit.*, 1925-26, **167**, 43.
78. C. WIRTH, *Z. ges. Brauw.*, 1908, **31**, 421.
79. M. SAMEC and M. MAYER, *Kolloidchem. Beih.*, 1921, **13**, 272.
80. M. SAMEC, *Hoppe-Seylers Z. physiol. Chem.*, 1935, **236**, 103.
81. P. RONA and C. VAN EWYK, *Biochem. Zeit.*, 1924, **149**, 174.
82. J. A. REMASOW, *Arch. Sci. biol.*, 1935, **37**, 425.
83. H. B. SREERANGACHER, *Proc. Indian Acad. Sci.*, 1935, **B2**, 393.
84. W. SALAČ, *Böhm. Bierbrau.*, 1938, No. 41; *Woch. Brau.*, 1938, **55**, 355.
85. V. ERSHOV, *Spirtoovaya Prom.*, 1936, **13**, No. 8, 38.
86. A. ZUBRODSKIĪ, *ibid.*, 1937, **14**, No. 3, 22.
87. T. SINYAVIN and G. VOSLOTSKIĪ, *ibid.*, 1937, **14**, No. 3, 36.
88. S. TARASYUK, *ibid.*, 1937, **14**, No. 4, 36.
89. G. FERTMAN and I. KALER, *ibid.*, 1938, **15**, No. 5, 29; *Chim. et Ind.*, 1938, **41**, 759. (Modification of Effront's method.)
90. M. SAMEC and E. WALDSCHMIDT-LEITZ, *Z. physiol. Chem.*, 1931, **203**, 16.
91. M. SAMEC, *ibid.*, 1935, **236**, 103.
92. V. K. GIRI, *J. Indian Chem. Soc.*, 1938, **15**, 249.
93. — *Science*, 1935, **81**, 343.
94. A. JANKE and J. HOLOTA, *Wochschr. Brau.*, 1939, **56**, 161.
95. R. M. SANDSTEDT, E. KNEEN and M. J. BLISH, *Cereal Chem.*, 1939, **16**, 712.
96. L. E. EHRNST, G. J. YAKISH and W. OLSON, *ibid.*, 1939, **16**, 721, 724.
97. V. DORFMAN, *Spirto-Vodochnaya Prom.*, 1938, **15**, No. 2, 19; *via Chem. Zentr.*, 1938, II, 973.
98. W. M. SCOTT, *Ind. Eng. Chem.*, 1940, **32**, 784.
99. K. MAYER, *Z. physiol. Chem.*, 1939, **262**, 29.
100. O. WOLFF, *Chem.-Zeit.*, 1915, **39**, 105.
101. W. SYNIEWSKI, *Bull. Int. Acad. Pol. Sci. Lettres*, 1924, 149.
102. C. T. BENNETT and F. C. L. BATEMAN, *Quart. J. Pharm.*, 1930, **3**, 349.
103. D. SCHENK, *Chem.-Ztg.*, 1927, **51**, 814.
104. J. A. ANDERSON and H. R. SALLANS, *Canad. J. Res.*, 1937, **16C**, 70.

105. A. D. DICKSON, *Cereal Chem.*, 1940, **17**, 645; *J. Inst. Brewing*, 1940, **46**, 440.  
106. E. KNEEN and R. M. SANDSTEDT, *ibid.*, 1941, **18**, 237.  
107. S. R. SNIDER, *Proc. Amer. Brew. Chem.*, 1940, 49; *J. Inst. Brewing*, 1941, **47**, 65.

## ADDITIONAL REFERENCES

- J. C. BLAKE, *J. Amer. Chem. Soc.*, 1917, **39**, 315; 1918, **40**, 623. (Amyolytic activity followed by iodine reaction using Dubocsq colorimeter.)  
V. ANDREEV, *Spirtovaya Prom.*, 1936, **13**, 27. (Determining saccharifying of malt. Modified Wohlgemuth method.)  
A. SOKOVYCH, *ibid.*, 1937, **14**, 35. (Modified Wohlgemuth method.)  
O. S. RASK, *J. Assoc. Off. Agr. Chem.*, 1939, **22**, 200. (Anderson and Sallan's method and the modification of this proposed by Laufer. Schwarz and Laufer give more accurate results than titration with Fehling's solution.)  
J. BLOM and A. BAK, *Z. physiol. Chem.*, 1938, **256**, 197. (Viscometric method used to determine amylase activity.)  
M. SOMOGYI, *J. Biol. Chem.*, 1938, **124**, 179. (Examines diastatic digestion products.)  
O. E. STAMBERG and C. H. BAILEY, *ibid.*, 1938, **126**, 479. (Action of wheat amylases on soluble starch.)  
V. D. MARTIN and J. M. NEWTON, *Cereal Chem.*, 1938, **15**, 456. (Rate of action of amylases on starch followed by potentiometric determination of reducing sugars formed.)  
R. KLEMEN and I. ŠKERLAK, *Z. anal. Chem.*, 1939, **116**, 169. (Determination of maltose by Bertrand's method.)  
B. A. BURKHART, *Cereal Chem.*, 1939, **16**, 652. (Potentiometric determination of reducing sugars used to follow diastatic action of malts.)  
D. KLIMOVSKIĀ and S. STASHKO, *Spirto-Vodochnaya Prom.*, 1938, **15**, No. 3, 25; via *Chem. Zentr.*, 1938, II, 2662. (Methods of determining the diastatic power of green malt compared.)  
H. C. GORE, *Ind. Eng. Chem.*, 1936, **28**, 86. (Three quantitative methods for measuring diastatic activity are contrasted.)  
I. GLAZUNOV, *Biokhimiya Khlebopecheniya*, 1938, No. 1, 51; *Khim. Referat Zhur.*, 1939, **2**, No. 3, 86. (Determination of  $\alpha$ - and  $\beta$ -amylases in flour.)  
L. H. LAMPITT, E. B. HUGHES and H. S. ROOKE, *Analyst*, 1930, **55**, 666. (Diastatic activity of honey discussed and method of measurement given.)  
P. WEINSTEIN, *Zeit. Unters. Lebensm.*, 1930, **59**, 513. (Detection of amylase in milk.)  
R. H. HOPKINS and C. B. KRAUZE, 'Biochemistry Applied to Malting and Brewing,' London, 1937.

## APPENDIX

NOTES ON THE SIGNIFICANCE OF PATENT  
REFERENCES

*Contributed by C. PAINE, B.Sc. Hons. (Lond.)*

AN adequate bibliography on a field of technology such as that relating to starch will inevitably contain many patent references, that is, references to patent specifications. Such references are a common source of misapprehension and misunderstanding in the mind of the general reader. The present notes are an attempt to set down briefly some of the main points which arise in considering patent references.

In most of the industrially important countries it is an accepted principle that a patent monopoly, that is a piece of legal property, may be granted to an inventor in return for a public technical description of his invention. A patent specification is the usual document through which an inventor may present the technical description of his invention and also attempt to define the scope of the invention which he seeks to monopolise as a piece of legal property. This dual function of a patent specification is a frequent source of misunderstanding by the general reader. Thus, whilst a patent specification once published will always remain as a piece of technical information, it may have ceased at any given time and place to signify a live piece of legal property. That is, the specification remains but the patent (granted monopoly) for the invention may be dead or may never have been granted at all. Some countries (e.g. Canada) do not publish printed specifications, but most of the important countries do so and copies are purchasable at a trivial cost (e.g. in Britain, 1s. from the Patent Office). It is obvious that such a copy is, like a textbook, physically transportable as technical information throughout the world. The patent monopoly which it may represent is, however, limited to a geographical area prescribed by the laws of the country from which the specification originates. Thus, for example, a British patent has legal force in the British Isles, Northern Ireland and the Isle of Man, but not in Eire or the British colonies or Dominions. India and Canada, for example, have their own separate patent systems. French and Dutch patents on the other hand extend their scope to all the colonies of their respective countries. It will be understood that a United States patent has no force in Britain, Germany, etc., and vice versa. The existence of a United States or other foreign specification may indicate, however, that there is a separate and corresponding British patent.

The maximum potential life of a patent as a piece of legal property varies from country to country. In Britain it is sixteen years, France fifteen years, Germany eighteen years, U.S.A. seventeen years, and so

forth. The date from which this potential life is calculated and the manner in which this date can vary is dealt with below, but first it must be made clear that the potential and actual lives of a patent are not necessarily co-terminous. Thus, in most important countries a patent once granted can only be kept in force as a piece of legal property by payment of renewal fees, usually at annual intervals from a prescribed date. If the renewal fees are not paid the patent dies, that is, its actual life as a legal instrument does not extend to the maximum potential life permissible under the laws of the country in question. A notable exception is the United States where a granted patent runs its full term of seventeen years without any payment of renewal fees being necessary.

As indicated above the maximum potential life (term of years) of a patent varies from country to country and, in addition, the starting point of this term of years also varies somewhat. In most countries this starting point is the date at which application for a patent was made in the country in question, after making due allowance for any priority the applicant may have claimed under International Convention (see below). A notable exception again is the United States, where the term of maximum potential life runs from the date of grant of the patent and not from the application date. Since the U.S. Patent Office handling is often lengthy and the interval between application and grant is correspondingly great it frequently happens that a patent monopoly may be in existence in the United States long after all corresponding patents on the same invention are dead.

Reference has been made above to International Convention. This is an international arrangement whereby countries which are parties to it permit a foreign applicant the optional privilege of retaining the application date of his home country as a basis for establishing priority for his invention provided certain conditions are fulfilled. One of the most important conditions is that he shall file his application claiming such privilege within twelve months of his home application date. In Great Britain there is a further important condition. A foreign inventor (say U.S.A.) wishing to claim 'convention priority,' i.e. his home (U.S.A.) application date, under International Convention in Britain must file a *complete* specification (complete with claims) corresponding to his foreign application within twelve months and then within a prescribed time (eighteen months from his U.S. application date) such specification becomes available for inspection by the public at the Patent Office and typescript or photostat copies (*Note: not printed*) are purchasable. Such published specifications are correctly referred to as British applications (*not patents*) with a five-figure number followed by the year of application, e.g. Brit. applic. 14235/1940 or 14235/40. At this stage they still have to undergo British Patent Office examination and are not yet accepted (printed) specifications, may never become so or may ultimately be accepted in greatly modified form, especially in respect of claims. Such circumstances (application under convention) are the only ones in which a British specification becomes

available to the public for inspection earlier in its life than the issue of a finally accepted (printed) specification.

In many countries the issue of a printed specification is followed by an interval of a few months before formal grant of a patent monopoly. During this interval interested parties, given suitable grounds, may oppose the grant of a patent. If such opposition is successful the grant may be refused entirely or, more commonly, the scope of the ultimate grant may be restricted by amendment of the specification.

What has been said so far may appear complicated and it is perhaps permissible to attempt to summarise the main points in a crude symbolism at some risk of over-simplification. An invention may be regarded as the child of the inventor's brain. The father may seek to register birth (file patent applications) in one or more countries in the hope that his progeny's credentials as a potential citizen may be then accepted (applications accepted by Patent Offices) and that the child may come of age, that is, come to full legal status (grant of patent) and thus have value as a wage earner (profit from exploitation of a protected process or as a source of royalties). As we have seen, according to country, such children may never come of age. Their credentials may be found wanting (application rejected by a Patent Office) or they may be found to be ailing and suffer mutilation or death during the process of attempting a cure (opposition by outside parties). Even after coming of age they may not live to die peacefully of old age (maximum potential term); they may die of under-nourishment (non-payment of renewal fees) or be mutilated or die of some disease diagnosed late in life (successful revocation proceedings by outside parties). In short, judging by the outward signs of birth registration (patent references) it may appear that there is a large and prosperous family in existence but this may be misleading; the death rate may have been small or large. The determination of the exact situation and the health of all concerned is usually a matter for specialised enquiry and this is one of the functions of a patent agent through whom such enquiries can be made.

How can patent references in a bibliography on starch affect a manufacturer in the field of starch technology? Let us assume that the manufacturer is British. If a given reference is to a British patent still in force or to a foreign specification corresponding to which there is a British patent still in force, it serves as a warning that he may not operate such an invention without permission (formal licence, with or without payment of royalties) from the owner of the British patent rights. If the British patent is dead or if there is no British patent corresponding to the foreign specification in question, he is perfectly free, subject to there being no other over-riding British patent rights in existence, to put the invention to technical use himself without fee or licence. If a British patent reference is more than sixteen years old the patent may be regarded as dead (maximum term) although the technical information in the specification, now free to all, may still be valuable and very much alive. A list of British patents

still in force is published annually by the Patent Office but as in certain instances the correct interpretation of the list requires some experience it is probably better to have the assistance of a patent agent in determining whether or not a particular patent is in force, especially in important or dubious cases.

A further point is important. Absence of a live British patent for a particular process indicates freedom for anyone to manufacture by such a process but the export of goods so manufactured to a foreign country where a relevant corresponding patent still remains in force would constitute an infringement of that foreign patent unless a licence had been obtained from the owner of the patent beforehand.

It can sometimes happen that a British manufacturer after using a particular process, perhaps secretly, for many years is suddenly alarmed by the appearance of a British patent granted to a competitor for the same process. The situation thus arising, however, may not be serious for either party. The patent may remain a perfectly sound and legally valuable one notwithstanding such prior use. Likewise the prior user cannot now be prevented from continuing his own use of the process free of licence or payment of royalties. He must, however, make absolutely sure of his ground, namely, that his own use of the process was in fact prior to the date of the invention now patented by his competitor. Given the date of actual first manufacture, a patent agent can speedily advise on such a point.

From what has been written above we see that the main implications of a given patent reference may be analysed by keeping in mind the following points :—

- (1) A patent specification has a dual function—
  - (a) to describe the technical nature of an invention ;
  - (b) to define that for which the grant of a legal monopoly is sought.
- (2) A monopoly granted under a given patent has—
  - (a) a limited geographical scope ;
  - (b) a fixed maximum term of years starting from a fixed date, usually the date of application for the patent or a date otherwise clearly stated on the specification (e.g. 'convention priority' date or, in U.S.A., date of grant).
- (3) A monopoly granted under a patent may, from a variety of causes, die long before the fixed maximum term.
- (4) In the absence of a live monopoly the technical information in a patent specification is free for all to use within the prescribed geographical area, also in such other parts of the world in which no live corresponding patent monopoly exists.
- (5) The existence of a live monopoly precludes others from the use of the relevant invention without a formal licence from the owner of the patent rights.
- (6) Where a foreign patent reference exists there may also be a live corresponding British patent in existence. Until this has been

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determined by expert search the exact patent position in Britain cannot be seen.

It should be emphasised that what has been written relates to normal, peace-time conditions. Circumstances were considerably altered by the outbreak of the present war and there has been new legislation to meet these circumstances. The possibility of obtaining compulsory licences under enemy-owned patents and other relevant matters are too complicated to be dealt with here and the advice of a competent patent agent should be sought where necessary.





## PHOTOMICROGRAPHS<sup>1</sup>

The following photomicrographs were in most instances reduced from a magnification of  $\times 500$  diameters to  $\times 320$  diameters. Where two photomicrographs of identical fields appear on the same page, it will be observed that the right-hand one was taken in polarised light.

The photomicrographs are intended only for use as a preliminary elimination for a particular identification. It cannot be overstressed that side-by-side microscopic comparison against freshly made slides of known materials is the only way to achieve a definite isolation.

Two methods have been used in taking the photomicrographs by polarised light: (1) the normal procedure, using a rotating, polarising condenser in the sub-stage holder, and an analyser fitted between the objective and the nose-piece; and (2) by employing two 'polaroid' screens, the apparatus being erected as for normal high-power photomicrography. A Watson 'parachromatic' sub-stage condenser with a focal length of  $\frac{3}{4}$  in. and N.A. 1.0 was used. One of the 'polaroids' was placed between the slide and the sub-stage condenser, and the second in the plane of the eye-piece.

Photomicrographs Nos. 20, 26, 28, 34, 42, 44 and 48 were taken by method (1), and the remainder by method (2).

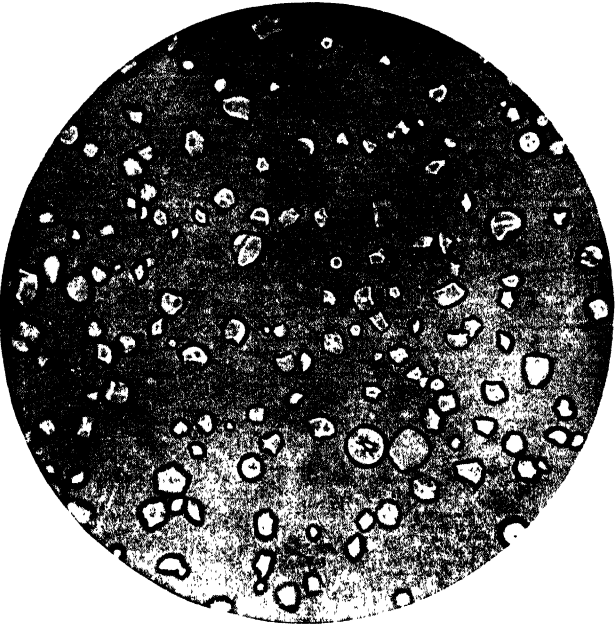
The advantage of the second method was that once a suitable field had been selected for photographing, both the normal and the polarised-light photograph could be more readily taken without losing the field, as it was not necessary to disturb the apparatus in any way. In method (1), however, although the results obtained are probably superior, the difficulty of retaining the same field while the polariser and analyser were interchanged with the normal condenser and objective, was found to be considerable. In addition, with method (1) the illumination at the magnification used was rather too weak to ensure accurate focusing on the camera screen.

The apparatus used for taking the photomicrographs consisted of a Watson 'Bactil' microscope, fitted with a triple nose-piece, a Leitz  $\times 10$  'Periplanatic' eye-piece, and a Watson 'Parachromatic' sub-stage condenser ( $F = \frac{3}{4}$  in. N.A. 1.0). For the photographs taken at 320 diameters, a Watson  $\frac{1}{8}$ -in. 'Parachromatic' objective (N.A. 0.70) was used, for those taken at higher magnifications a Leitz oil-immersion  $\frac{1}{2}$ -in. objective (N.A. 1.32) was substituted. The light-source was a 150 c.p. 'Pointolite' lamp.

The microscope was clamped rigidly in a vertical position on an optical bench. A camera bellows with considerable freedom of extension and fitted to take a  $\frac{1}{2}$  plate size ( $6\frac{1}{2} \times 4\frac{1}{2}$  in.) photographic plate, was clamped above it. A light-tight connection was made between the bellows and the microscope draw-tube. The 'Pointolite' lamp, fitted in a ventilated lamp-house complete with focusing condenser, was fixed on the optical bench about 18 in. away from the microscope. All the photomicrographs were taken on Ilford special rapid panchromatic plates (backed): 'Backed' plates must be used in order to avoid halation.

<sup>1</sup> Contributed by E. Young.





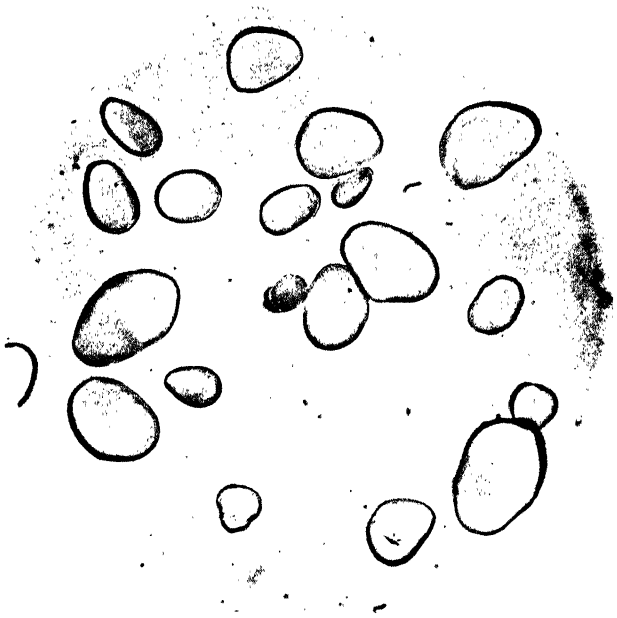
Photomicrograph No. 15.

APIO STARCH.



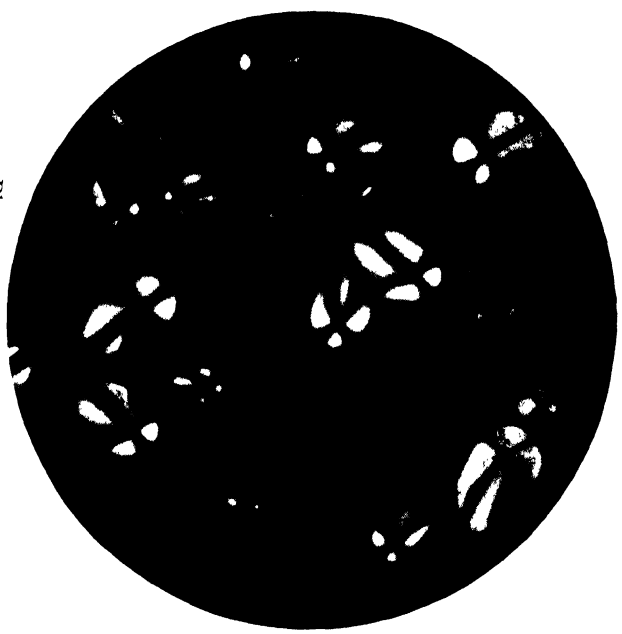
Photomicrograph No. 16.

[Facing p. 528.]

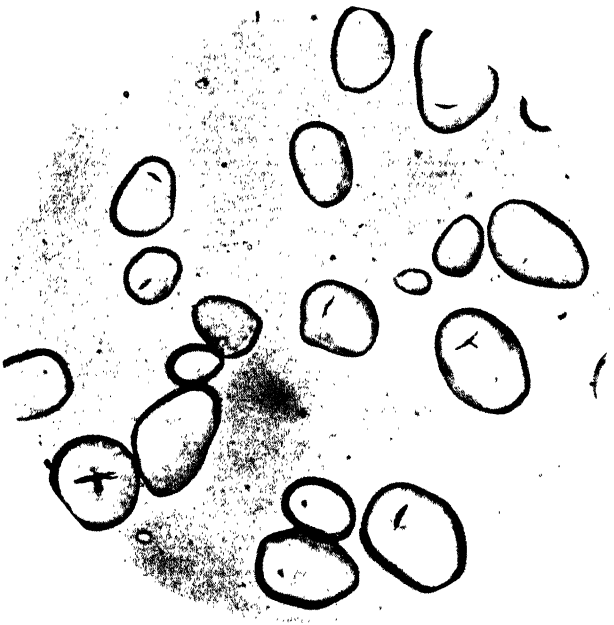


Photomicrograph No. 17.

ARROWROOT STARCH.  
[*Maranta arundinacea* L.]

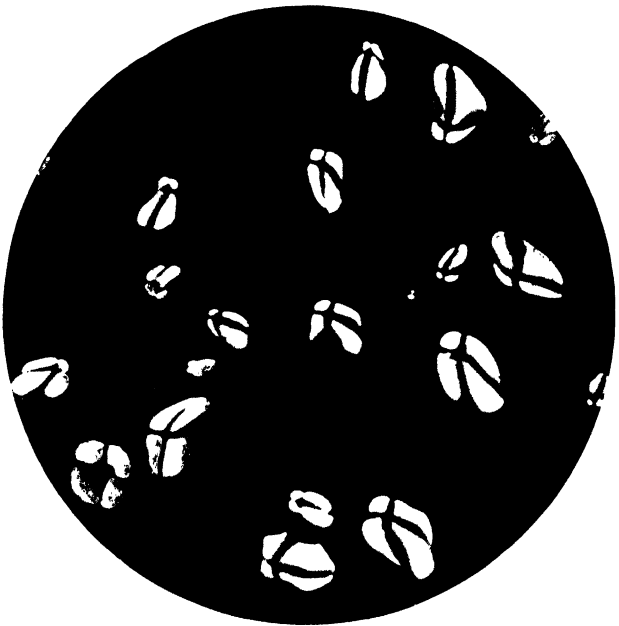


Photomicrograph No. 18.

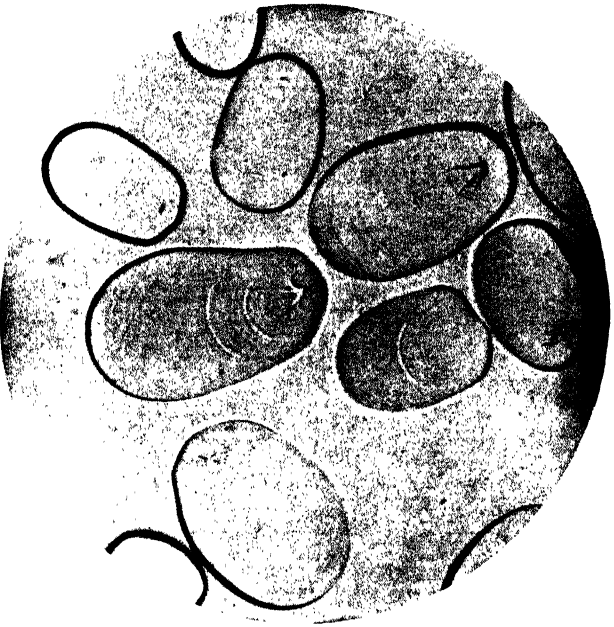


Photomicrograph No. 19.

ST. VINCENT ARROWROOT.



Photomicrograph No. 20.

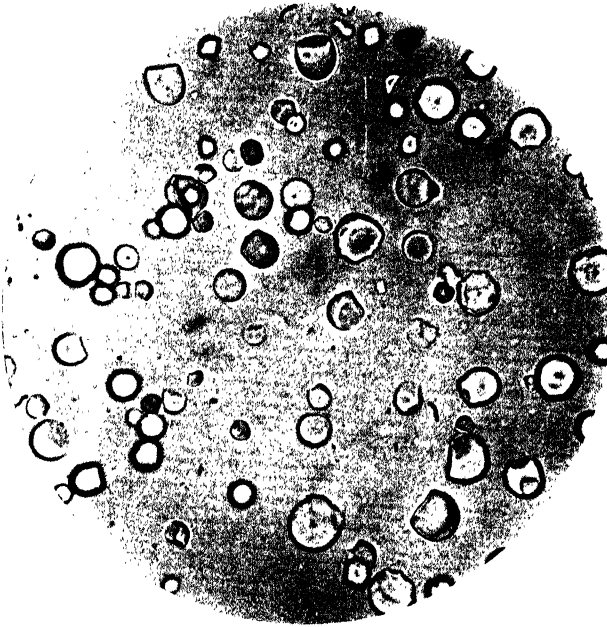


Photomicrograph No. 21.

EDIBLE CANNA STARCH.  
[*Canina edulis* Ker.]



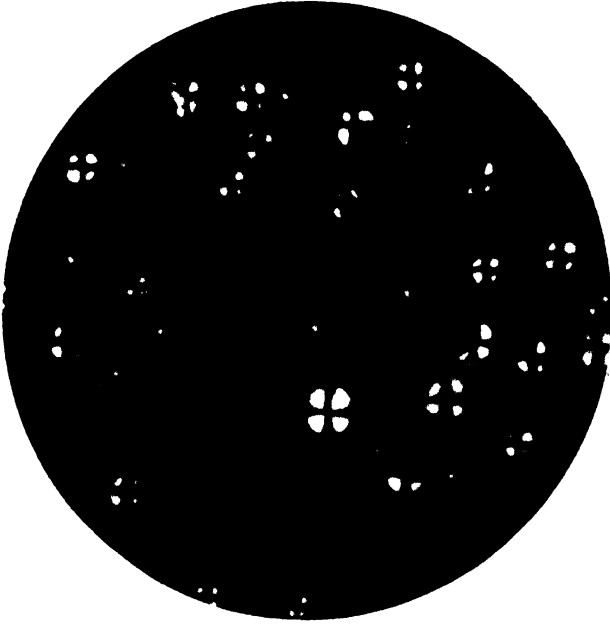
Photomicrograph No. 22.



Photomicrograph No. 23.

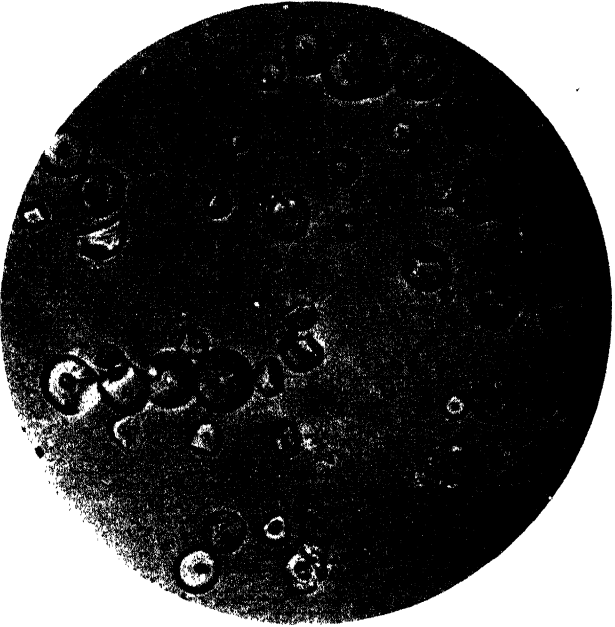
CASSAVA STARCH.

[*Manihot manihot* (L.) Cockerell.]



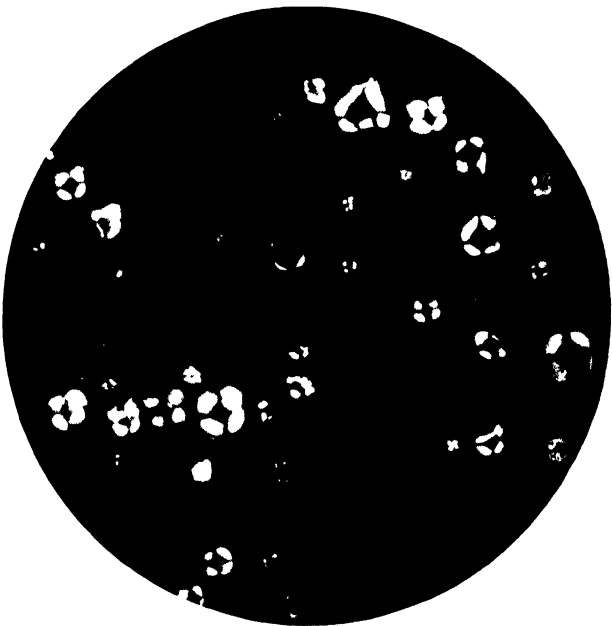
Photomicrograph No. 24.



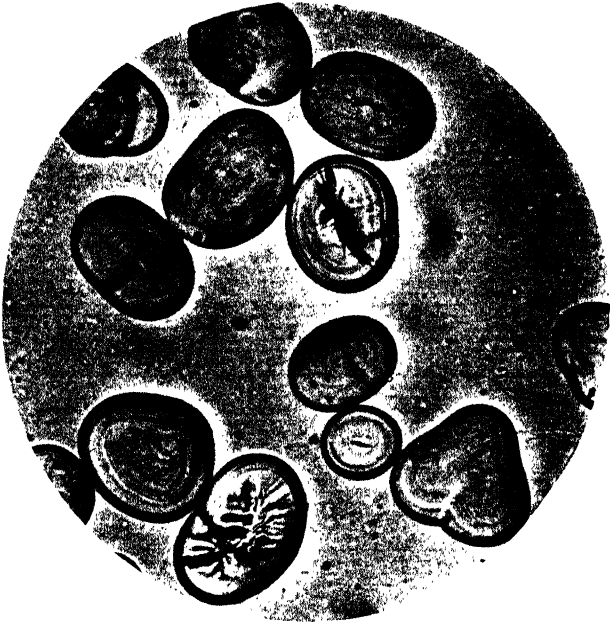


Photomicrograph No. 25.

FARINE DE MANDIOCA.

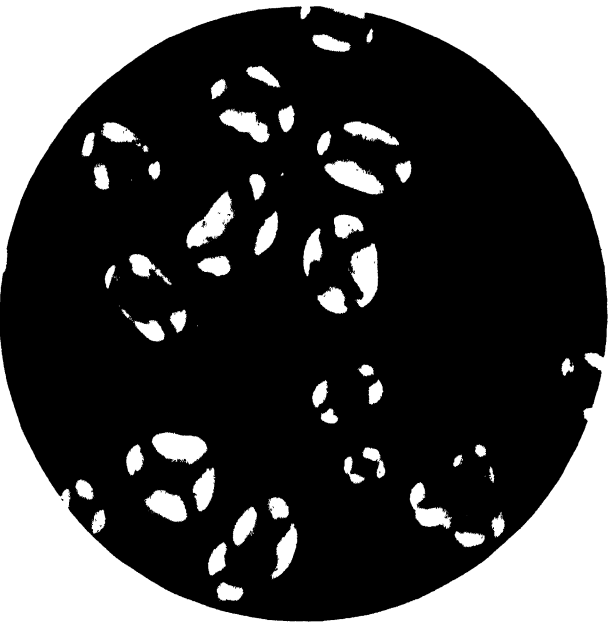


Photomicrograph No. 26.

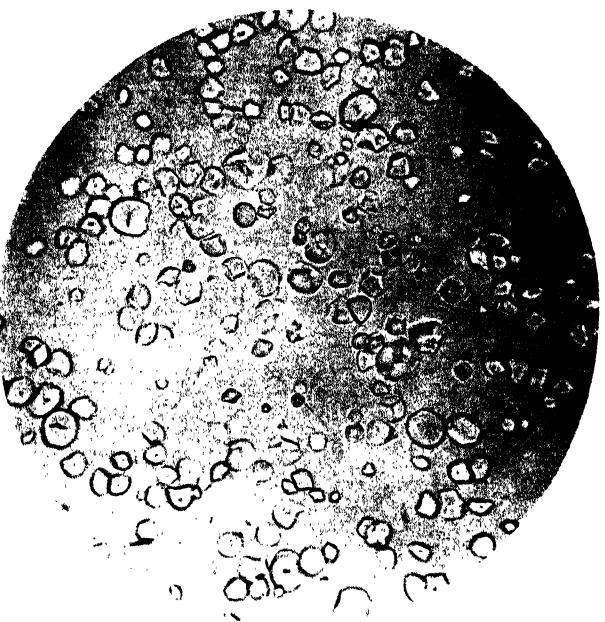


Photomicrograph No. 27.

HARICOT BEAN STARCH.

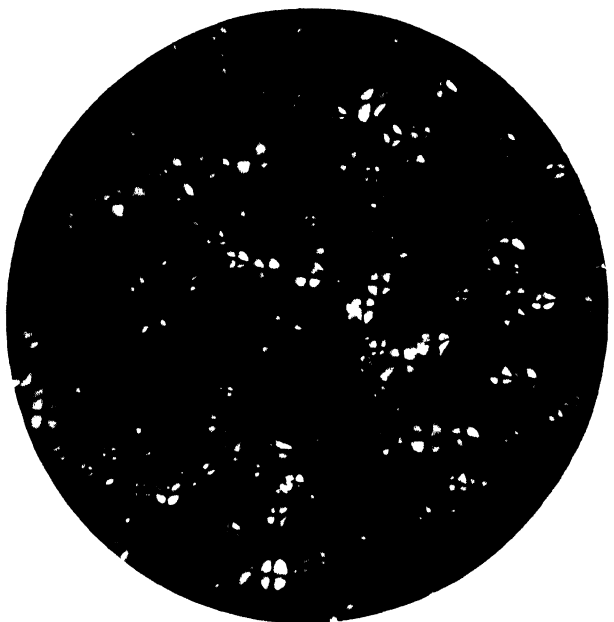


Photomicrograph No. 28.

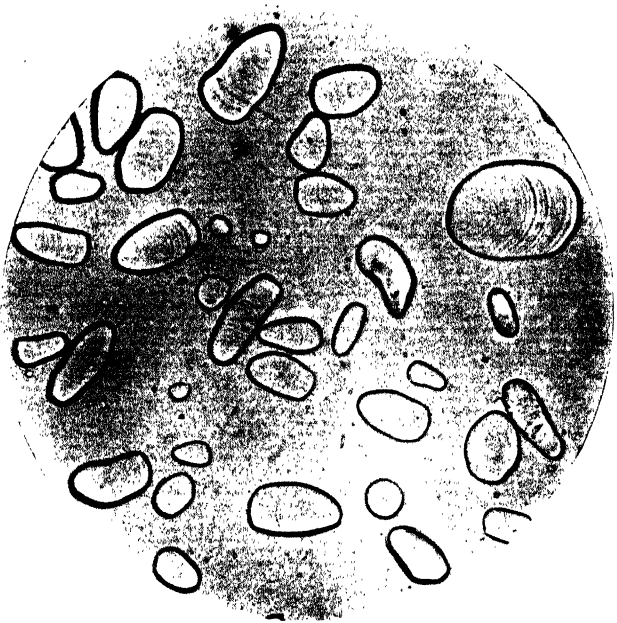


Photomicrograph No. 29.

NAME HICAMIO STARCH.  
[*Dioscorea polygonoides* H. and B.]



Photomicrograph No. 30.

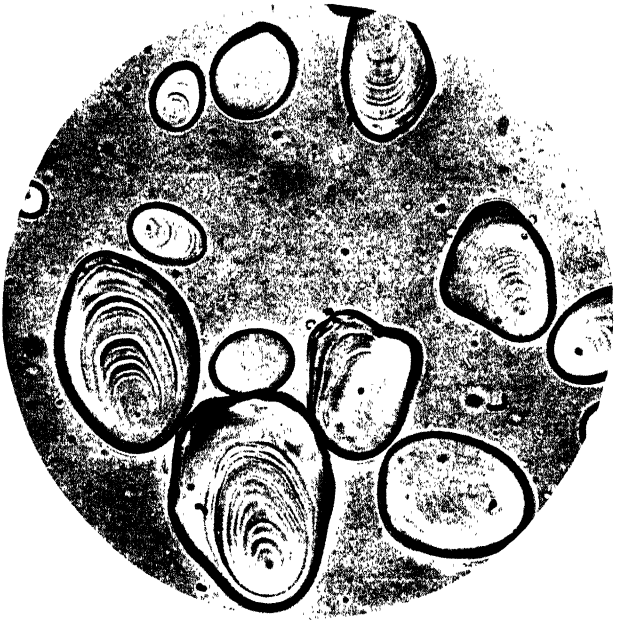


Photomicrograph No. 31.

GREEN PLANTAIN STARCH.  
[*Musa paradisiaca* L.]



Photomicrograph No. 32.

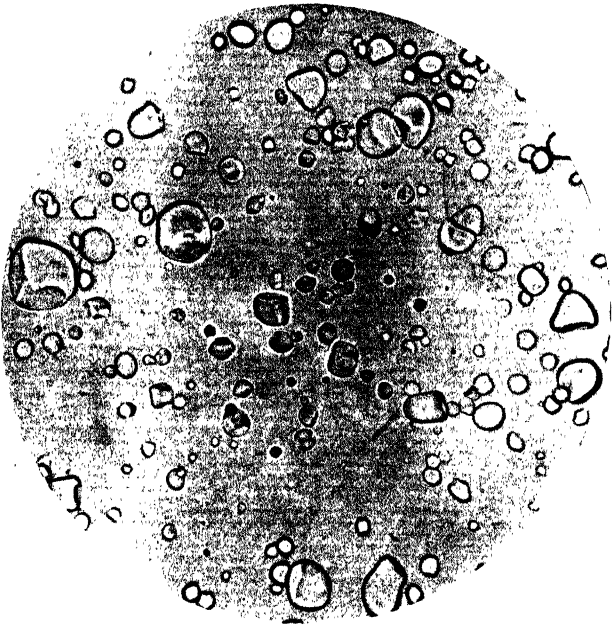


Photomicrograph No. 33.

POTATO STARCH.

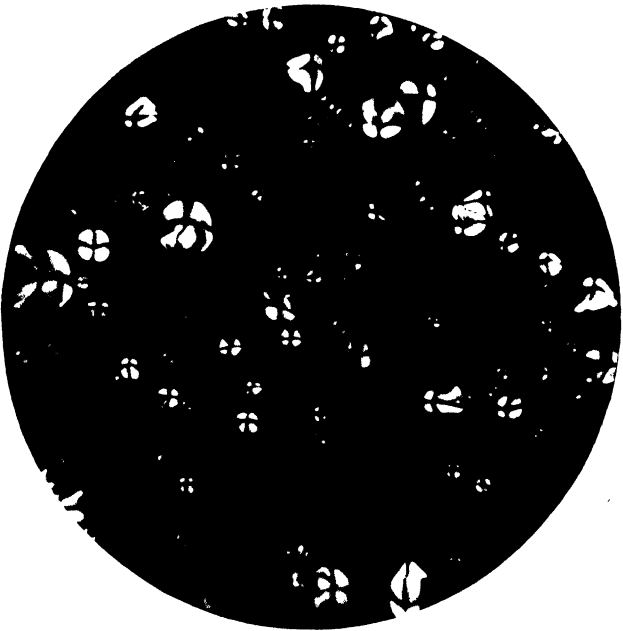


Photomicrograph No. 34.

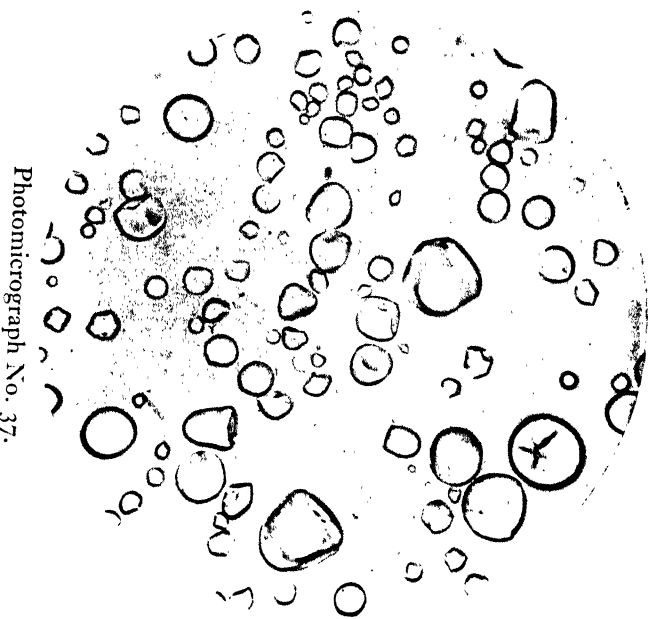


Photomicrograph No. 35.

YELLOW SWEET POTATO STARCH.  
[*Impomoea batatas* (L.) Lam.]



Photomicrograph No. 36.

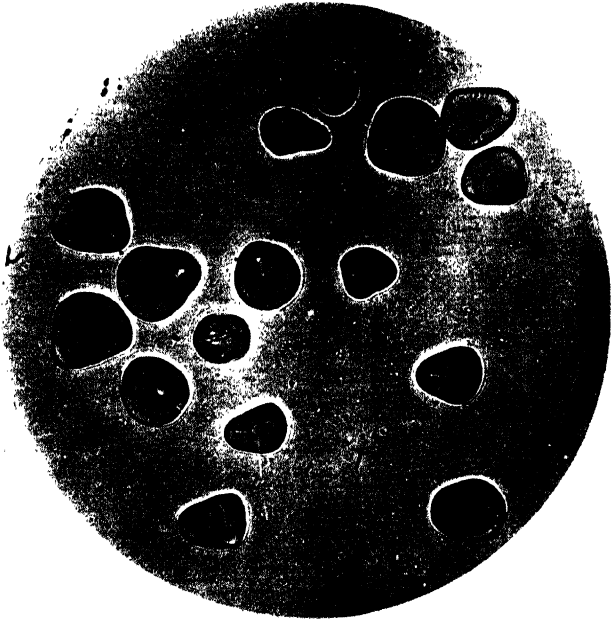


Photomicrograph No. 37.

WHITE SWEET POTATO,  
*Ipomoea batatas* (L.) Lam.]

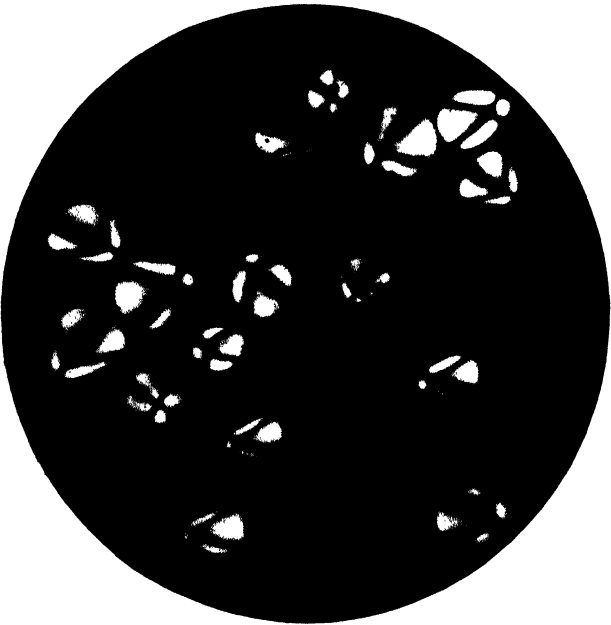


Photomicrograph No. 38.



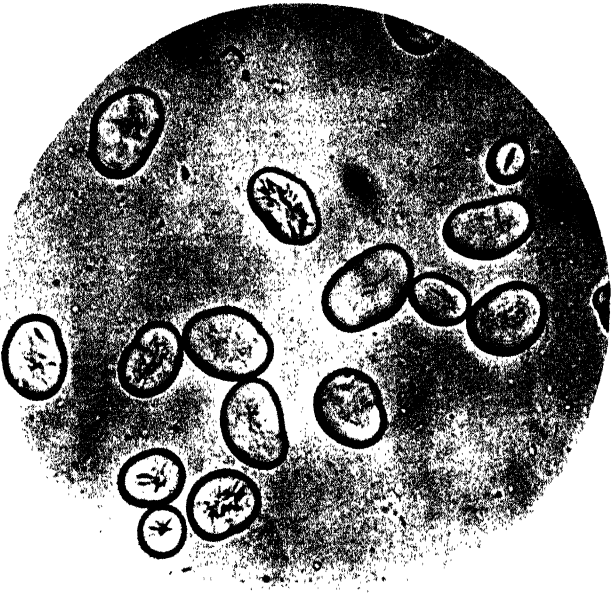
Photomicrograph No. 39.

LEREN SWEET CORN ROOT STARCH.  
[*Colathea allouis* (Aubl.) Tindl.]



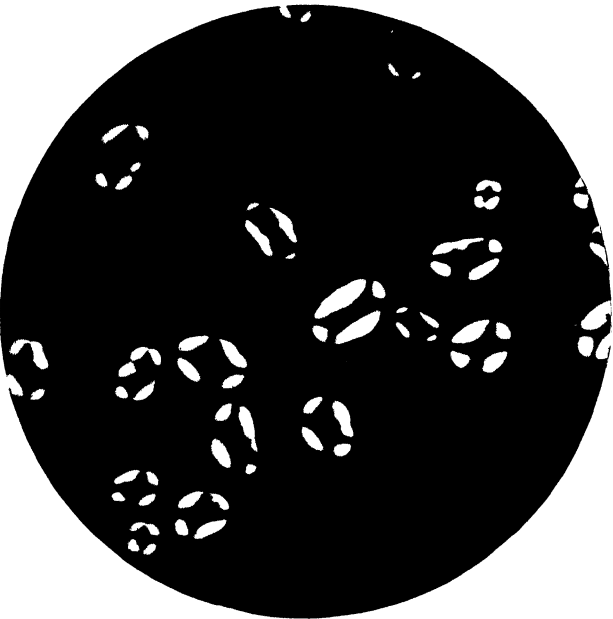
Photomicrograph No. 40.



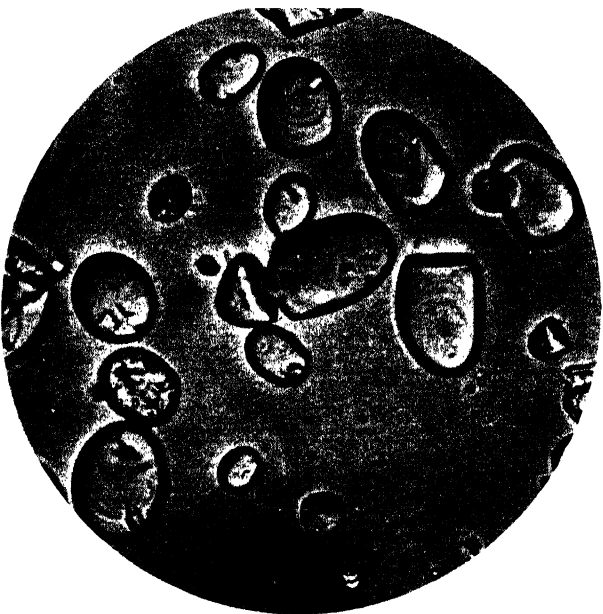


Photomicrograph No. 41.

LENTIL STARCH.  
[*Lens esculenta.*]



Photomicrograph No. 42.

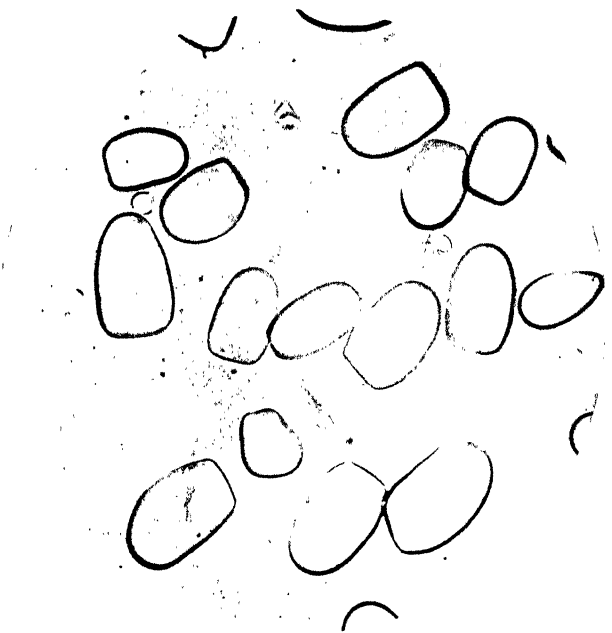


Photomicrograph No. 43.

SAGO STARCH.



Photomicrograph No. 44.

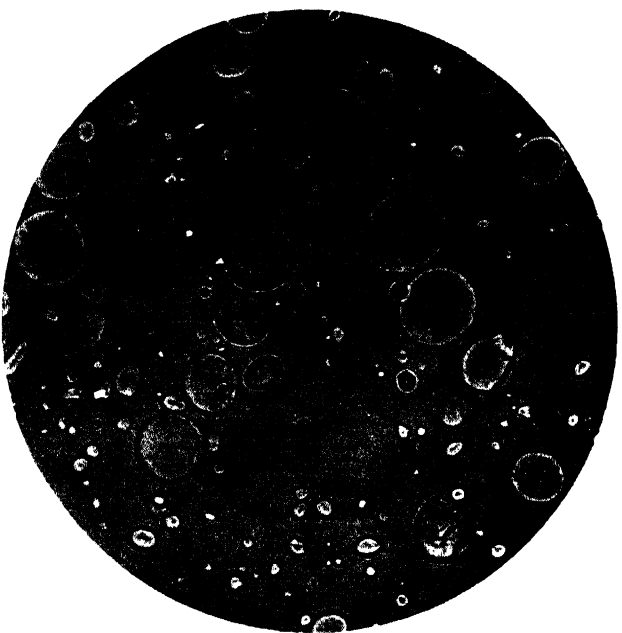


Photomicrograph No. 45.

NAME MAPLEY STARCH.  
[*Dioscorea trifida* L.f.]

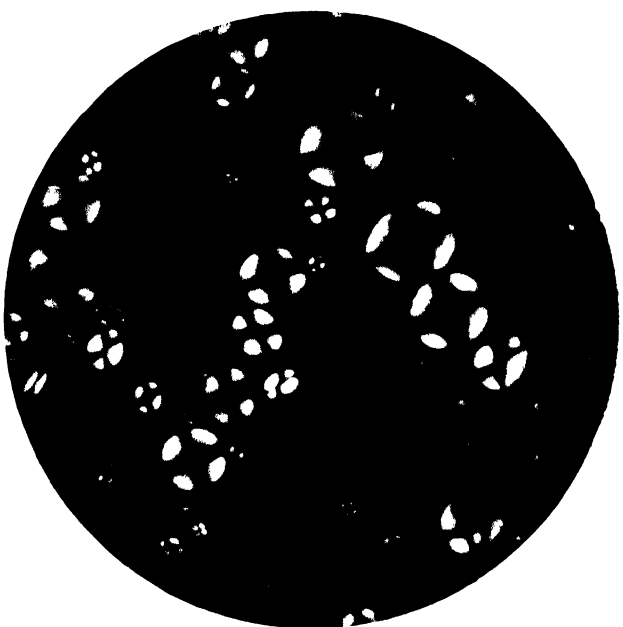


Photomicrograph No. 46.

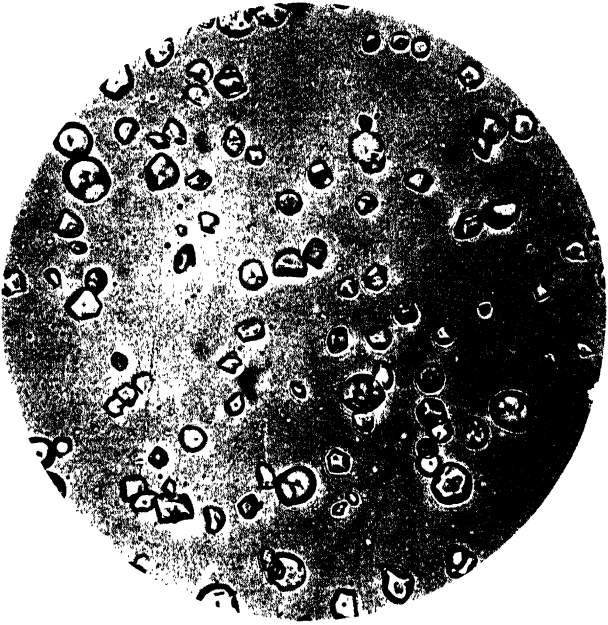


Photomicrograph No. 47.

WHEAT STARCH.



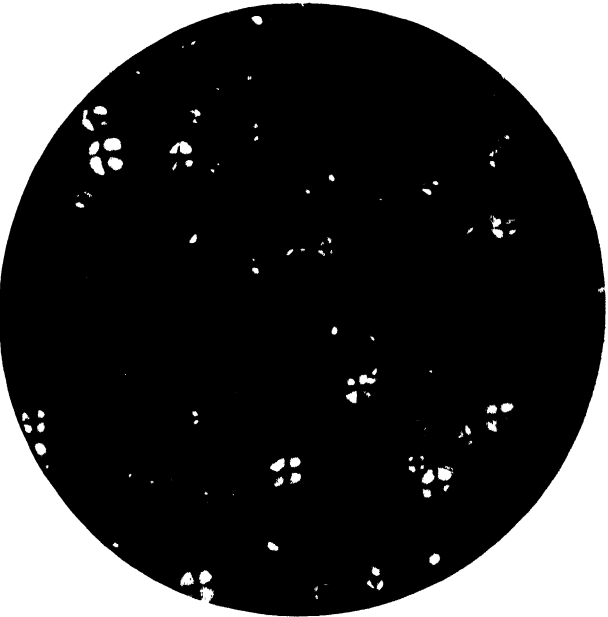
Photomicrograph No. 48.



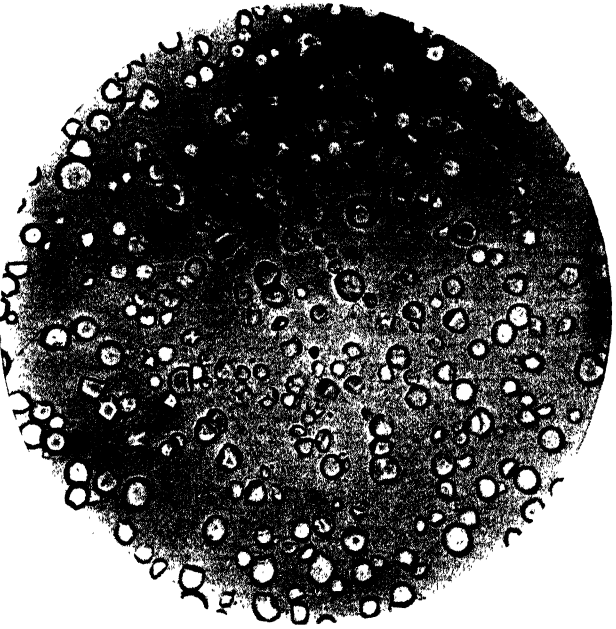
Photomicrograph No. 49.

YELLOW YAUTIA STARCH.

[*Xanthosoma sagittifolium* (L.) Schott.]



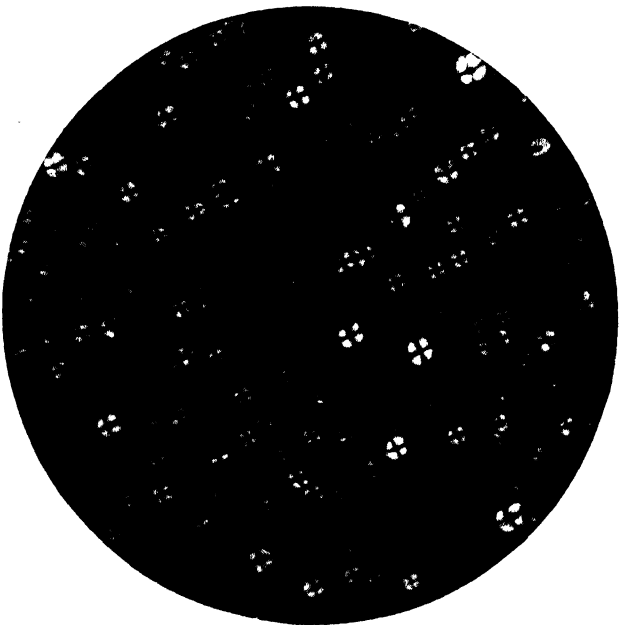
Photomicrograph No. 50.



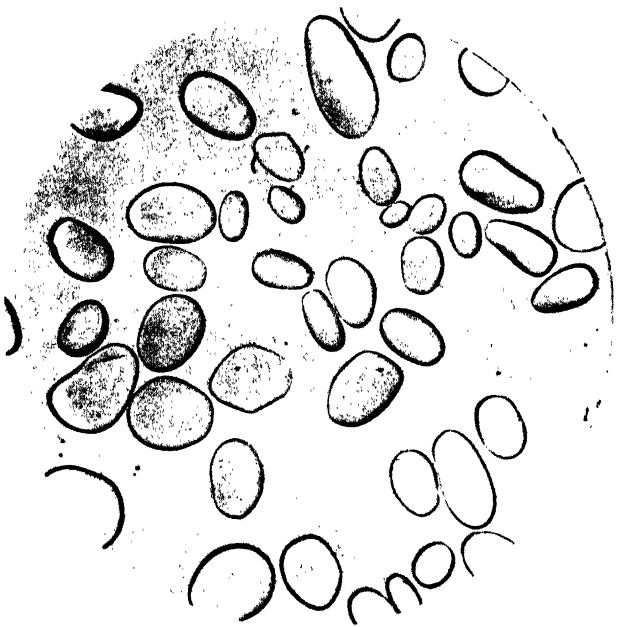
Photomicrograph No. 51.

WHITE YAUTIA STARCH.

[*Xanthosoma caracas* C. Koch and Bouche']

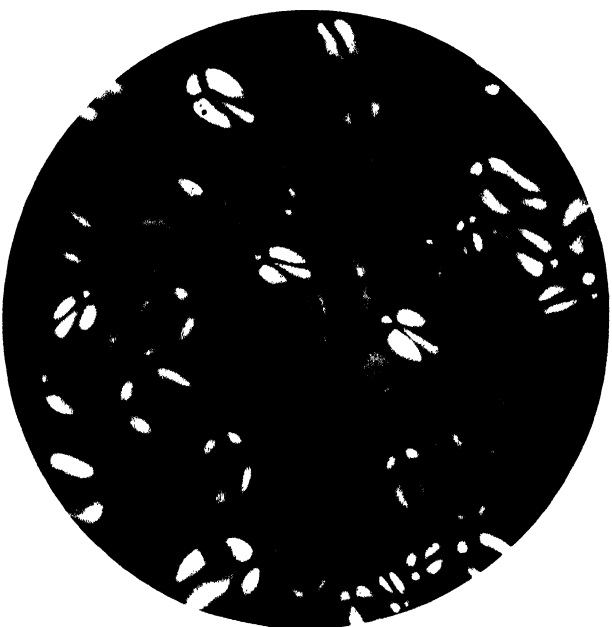


Photomicrograph No. 52.

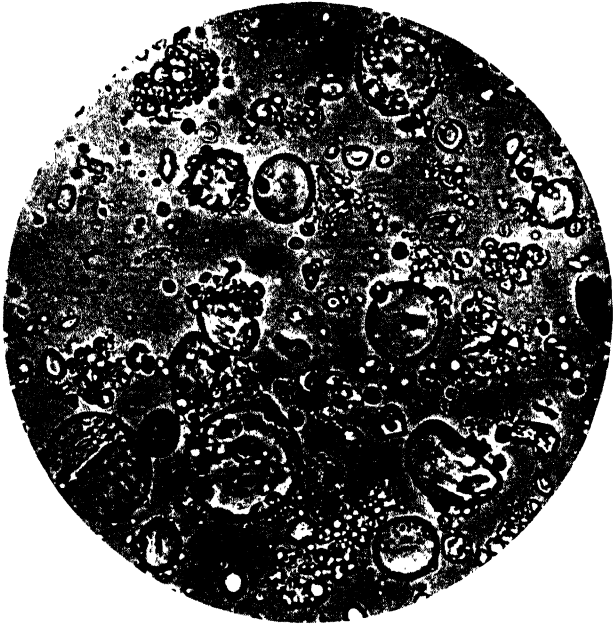


Photomicrograph No. 53.

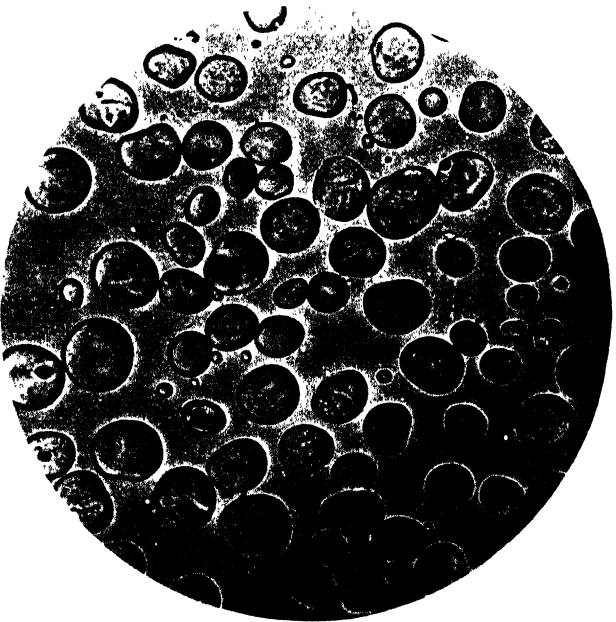
WHITE YAM STARCH  
[*Dioscorea alata* L.]



Photomicrograph No. 54.

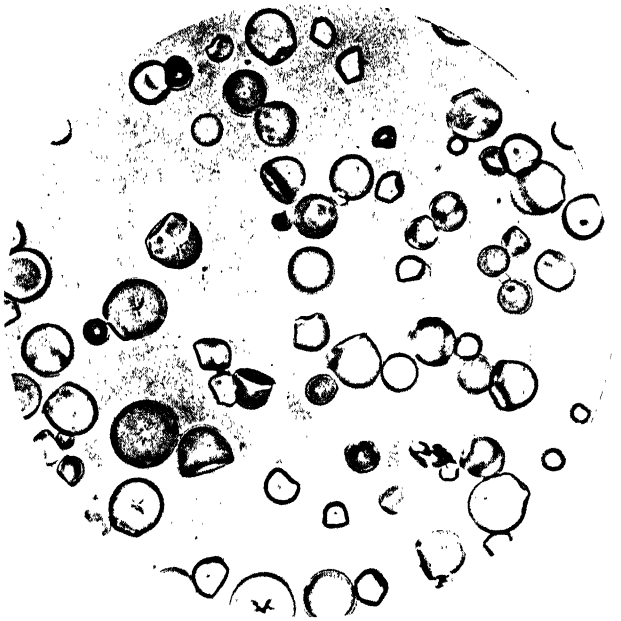


Photomicrograph No. 55.  
60 % RYE STARCH.

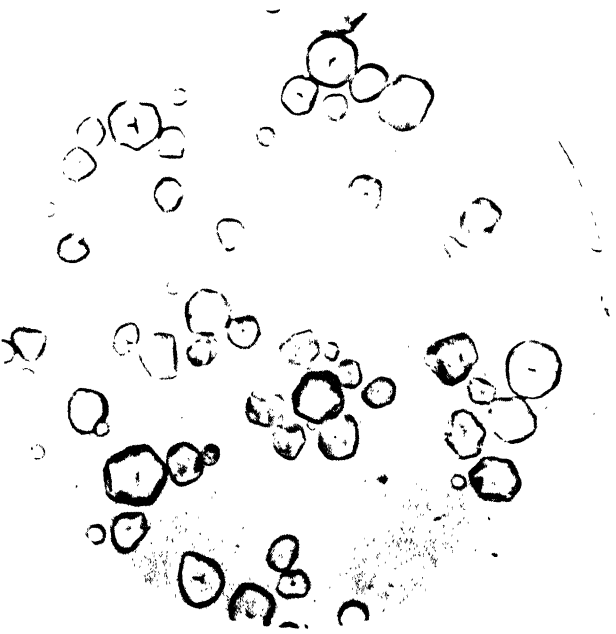


Photomicrograph No. 56.  
BARLEY STARCH.

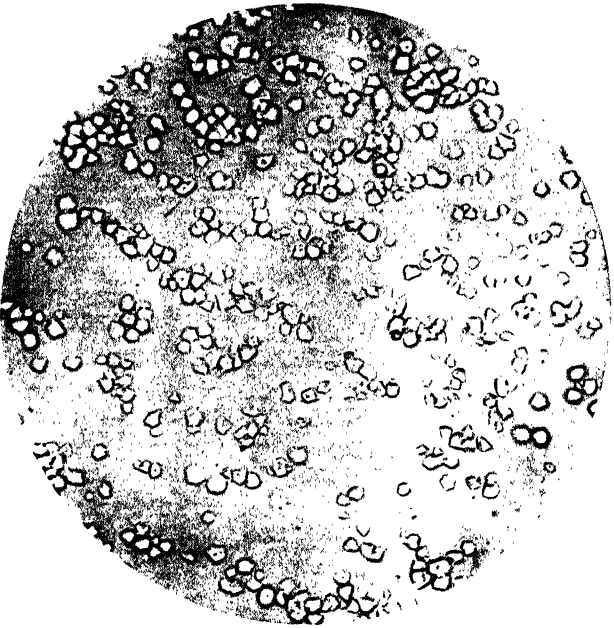




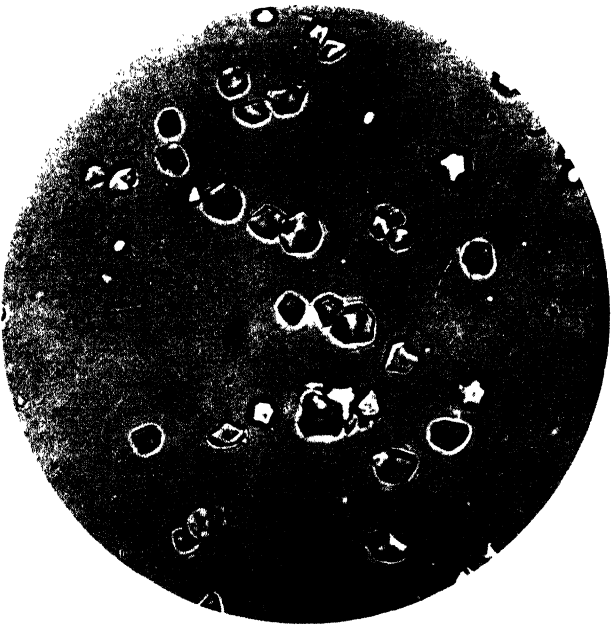
Photomicrograph No. 57.  
CASSAVA STARCH.  
(Cf. Photomicrograph No. 23.)



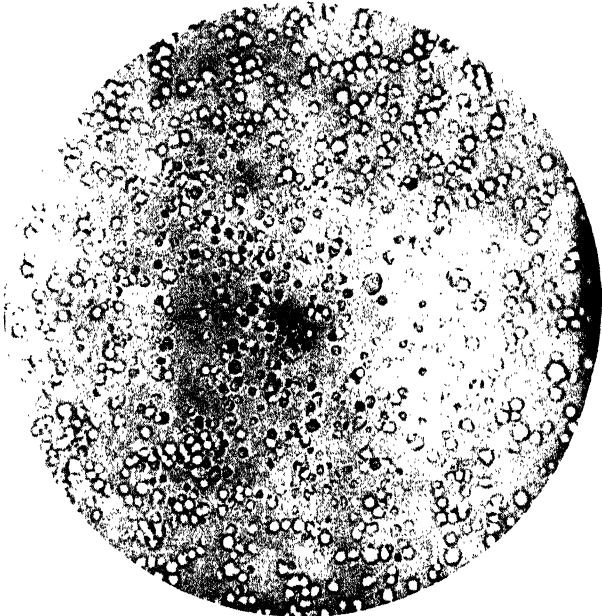
Photomicrograph No. 58.  
MAIZE STARCH.



Photomicrograph No. 59.  
RICE STARCH.  
( $\times 320$ .)



Photomicrograph No. 60.  
RICE STARCH.  
( $\times 640$ .)



Photomicrograph No. 61.  
TARO STARCH.  
[*Caladium colocasia* (L.) W. F. Wight.]  
(× 640.)

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