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THE CHEMISTRY

OF MILK

BY

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BEING VOLUME TEN OF A SERIES OF
MONOGRAPHS ON APPLIED CHEMISTRY

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

SECOND EDITION

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every worker in science or its applications knows how rapidly the contents of text-books and encyclopædias become out of date ; and those who wish to see new work published know the difficulties which abnormal taxation and high labour costs offer to the realisation of their desire.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

EVERY human being should be interested in milk since it has been the sole source of subsistence for everyone during the critical period of early infancy. Our newer knowledge of nutrition commenced with observations on the nutritive value of milk. Dairy products account for a considerable part of the food supply of nations, and part of the economic prosperity of many countries is associated with the manufacture of dairy products.

The importance of a full knowledge of the composition, behaviour and properties of milk is at once evident. We have advanced considerably in this knowledge during the past fifteen years but the information is scattered, mostly in original scientific literature and in a few specialised text-books. This book, therefore, is an attempt to gather together in a concise, ordered form, the results of all the relevant and reliable investigation on the chemistry of milk. The matter is treated under sections which deal with the important branches of the subject.

The contents will appeal to both pure and applied chemists, to physiologists, nutritionists and to members of the medical profession. Their value to research workers in biochemistry, agricultural chemistry and the chemistry of foods is obvious, and indications are given of problems awaiting solution, since the present positions of many subjects are explained, together with indications of future problems requiring solution. About 1,400 references are included in the text. Its appeal to students of dairy science is also evident.

A new departure is a section on the chemistry of milk processing, which should be of special value to workers in milk technology, and in general food technology. The full treatment of variation in the composition of milk should appeal to teachers and instructors in agricultural education whose duty it is to convey the findings of research to milk producers and students. In the compiling of the book, full acknowledgement must be given to the volume of material collected from scientific literature. In this direction, I am grateful for the amenities provided by the Library of the National Institute for Research in Dairying and the services rendered by the Librarian, Miss D. Knight, B.A., and the Assistant Librarian, Miss D. Atkins. I

also wish to thank the editors of various scientific journals for permission to refer to data and to quote and reproduce tables and figures. (*Biochem. J.*, *J. Dairy Res.*, *J. Hyg.*, *J. Agric. Sci.*, *J. Dairy Sci.*, *J. Biol. Chem.*, *J. Agric. Res.*, *Le Lait*, *Milchw. Forsch.*, *Biochem. Z.*, *Z. Physiol. Chem.*, etc., and various departmental, college and university bulletins.) In particular, I thank the Controller of H.M. Stationery Office for permission to quote and reproduce diagrams from Tocher's " Variations in the Composition of Milk " (also with the courtesy of the author) and to refer to various publications of the Medical Research Council, the Ministry of Health and the Ministry of Agriculture. I also thank the Publications Department of the U.S. Department of Agriculture for permission to quote and reproduce tables and figures from their publications.

I wish also to acknowledge the assistance derived from various books on biochemistry, food and dairy science. Text-books by the following authors and publishers have assisted in this respect : Plimmer's " Chemical Constitution of the Proteins " ; Haldane's " Enzymes " ; Lane-Claypon's " Milk and Its Hygienic Relations " (Longmans, Green & Co., Ltd.) ; Richmond's " Dairy Chemistry " (Charles Griffin & Co., Ltd.) ; Clayton's " Theory of Emulsions and their Technical Treatment " and " Colloid Aspects of Food Chemistry and Technology " (J. & A. Churchill Ltd.) ; Hunziker's " Condensed Milk and Milk Powder " (Hunziker) ; Rogers' " Fundamentals of Dairy Science " ; Sutermeister's " Casein and its Industrial Applications " ; Palmer's " Carotinoids and Related Pigments " (Reinhold Publishing Corp., New York ; Chapman & Hall, London).

Finally, thanks are due to Dr. E. H. Tripp, the Editor of this Series, for help in correcting the MS. and proofs and for his generous advice and help throughout, and to the Director of this Institute, Professor H. D. Kay, O.B.E., Ph.D., D.Sc., for his interest.

W. L. DAVIES.

READING,
October, 1935.

AUTHOR'S PREFACE TO SECOND EDITION

THE reception given to the First Edition has been gratifying, and a second edition has been called for rather quickly. A second edition, moreover, is demanded by the rapid advances made in dairy chemistry in the meantime.

In this edition no change has been made in the method of presenting the subject. Most of the chapters have been elaborated and newer knowledge has been worked into the text, but the continuity of treatment has been preserved. Incorrect or misleading statements in the First Edition have been corrected and obscurities cleared up. I wish to thank the large number of reviewers for their kindly criticisms, most of which have been very helpful.

More information has been added, particularly to the sections on the composition of milk, fat and fat oxidation, lactose, casein, mineral constituents, enzymes, physical chemistry, and the nutritive value of milk. This information is adequately supported by references for further study, if required.

I wish again to acknowledge my indebtedness to the various scientific journals mentioned in the Preface to the First Edition for my sources of information of newer material, and to Dr. E. H. Tripp, Editor of this series, and Messrs. Chapman & Hall Ltd., Publishers, for their efficient services in the production of the work.

W. L. DAVIES.

READING.

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PART I

THE COMPOSITION OF MILK

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THE CHEMISTRY OF MILK

CHAPTER .I

INTRODUCTORY AND GENERAL CONSIDERATIONS

1. Biological Considerations

THE importance of the nutrition of the newly-born offspring cannot be overestimated, and the composition and properties of such nutritive fluids intended by nature to be the last means of contact between mother and offspring must need be both comprehensive and complex. As soon as conditions of separate existence obtain, the newly-born animal is called upon to assimilate food-material for growth and energy purposes, and in order to do so brings into play and exercises its respiratory, muscular and digestive apparatus, with the result that in a short time fullest advantage is taken of the food-material at its disposal.

Under conditions of separate existence the food-supply must completely satisfy the requirements of the developing offspring, and as such must differ markedly in composition from that of the fluids on which the embryo was sustained during its uterine development. The slow utilisation of nutrients from the amniotic fluid and the maternal blood-stream is sharply replaced at birth by a different type of nutritional scheme involving principally the development and exercising of prehensile, digestive and excretory apparatus, coupled with developments of a respiratory system and, in varying degrees, that of muscular systems associated with movement.

In Mammalia, the food-material necessary for the offspring is secreted in special glands—the mammary apparatus—and the " milk " is suckled by the young immediately after birth, usually spontaneously. The liquid thus secreted is of a whitish or creamy colour, varying in consistency from watery to creamy. Its main characteristic is that it is a physiological fluid comparatively rich in compounds of high calorific value containing, in the main, at least three compounds which are characteristic of milk alone, namely, milk-fat, lactose, and casein. The inorganic elements of nutrition are present in small but well-balanced amounts. This fluid also possesses physical properties, some of which have a

direct bearing on the limited capabilities of the digestive tract of the offspring. Thus the main protein is precipitable at the low hydrogen-ion concentration (pH 6·2) by the rennin in the stomach of the offspring, bringing down completely the fat, which is in the form of finely-divided globules in the soft curd. The digestion of the curd can then proceed slowly, the fat globules being slowly liberated for their subsequent saponification and absorption in the intestines. This precaution obviates the troubles accruing from the entry of large amounts of fat into the intestines. Unlike other physiological proteins (albumin and globulin), casein, when precipitated by rennin in the stomach, or under slightly more acid conditions at its isoelectric point (pH 4·6), commences the process of giving volume to the stomach and of starting muscular action and movement in that organ. With this is also associated the development of gastric secretion, while the buffering effect of the curd is a safety factor in maintaining the all-important low acidity for the proper functioning of the young stomach.

Immediately after birth a special type of fluid—*colostrum*—is secreted. Colostrum contains a high content of solid material, differing from the normal milk secreted later in its content of globulin. Various functions have been ascribed to this fluid, amongst the most important being the purging or cleansing of the alimentary tract coupled with a disinfecting action, and the seeding of the intestines with the proper flora to deal with the milk later. Various immunological factors connected with some species of animals are conveyed from mother to offspring by means of the colostrum. Possibly also the high proline content of globulin is instrumental in the formation of hæmoglobin for the rapidly increasing blood volume of the newly-born animal.

The flow of colostrum is replaced slowly by a fluid which finally assumes the composition of normal milk. That this change is not sudden is significant in that the process of making the alimentary tract accustomed to the strange milk compounds, and to digestion and ingestion, is gradual. The period during which milk is secreted subsequently—the lactation period—naturally depends on the progress made by the young animal both in growth and in fending for itself in obtaining natural food, and by the preparation of the mother for the requirements both *in utero* and subsequently of another embryo or embryos. The cessation of lactation, or the " drying-off," is marked by a diminution in yield and in the content of solid matter in the milk. This consists mainly in a lowering of the lactose content and an increase in common salt (or chloride) content ; the milk is thus rendered less appetising to the suckling, which naturally

turns to other material for sustenance and so aids the mother to
" dry off " by discontinuing the encouragement of secretion in
the mammary glands.

Some significance must also be attached to the unique composi-
tion of milk-fat which, as will be described later, is made up of
the glyceryl esters of a variety of fatty acids from butyric acid (C_4)
to stearic acid (C_{18}). Such a range does not occur in any other
fat, and there is no doubt that the inclusion of the lower fatty
acids enables the modified catabolic capacity of the newly-born
animal to break down and utilise the fat immediately for its own
special requirements. The presence of a considerable amount
of an unsaturated acid—oleic acid—can be interpreted similarly.
The fat carries with it the fat-soluble vitamins A and D, essential
for growth and ossification respectively.

Further, it has to be appreciated that, during suckling, the
offspring takes in milk which (a) has not been subjected to a
higher temperature than that of the mother nor to a lower
temperature than that of the udder, (b) possesses its natural
content of gases, notably carbon dioxide, and very little of the
gases of the atmosphere, (c) has not been exposed to sunlight,
to processing or to metallic contamination from surfaces of
plant and utensils, and (d) has not been infected with micro-
organisms other than those from udder sources or from the
outside of the teat and the mouth of the suckling. Milk drawn
into a vessel and subsequently consumed may therefore be
taken to differ slightly from the milk directly taken from the teat
during suckling.

2. Environmental and Special Conditions

Milks from different species of mammals will differ in com-
position, not only for reasons due to the length of time since
parturition, but also from causes directly associated with its source.
These causes may be enumerated as follows :—

(a) Differences in degrees of development *in utero* of the
embryos of species.

(b) Variations in the ratio of size and weight of the offspring to
those of the mother.

(c) The varying immediate post-natal energy requirements of
the offspring.

The newly-born young of the domestic animals—cow, sheep,
goat and horse—are generally capable of comparatively speedy
locomotion within an hour of birth, whilst puppies, kittens and
piglings are helpless. The offspring of rabbits, rats and other
vermin, at birth, are incapable of much individual movement

and are incomplete in body development (absence of hair and unfinished development of body and limb). It is clear that this latter group requires a greater concentration of muscle-building material (protein) in the milk than the former group, which, on the other hand, requires more efficient energy-producing constituents such as fat and carbohydrate.

Table I gives the composition of the milk of various mammals, the species being approximately arranged in descending order of degree of development at birth.

The most apparent differences in the composition of milk from different species lie in the weak solution of nutrients secreted by the " equine group," the slightly higher protein content in that of ruminants, and the high protein and low sugar contents of the milks of Carnivora and Rodentia. Animals confined to special habitats, such as the reindeer and marine mammalia, secrete milk of high calorific value and all types show a very high fat-content. The hippopotamus, a tropical aquatic animal, on the other hand, secretes milk of comparatively low fat-content.

The offspring which is highly developed in bone and prehensile habits at birth receives a milk low in ash and protein but high in sugar. Human milk does not fall into any of the above groups and generally is characterised by its variable composition. It resembles ass's milk most closely and that of the equine milks generally.   .

Certain variations in the composition of milk ash also may be correlated with the stage of embryonic development at birth and with the subsequent rate of development of the suckling.

3. Sources of Milk of Economic Value

The use of milk of other animals as food for man dates back to early history, when domestication of animals for the service of man commenced. It is quite probable that animals kept by nomadic tribes served a dual purpose of providing a certain quantity of milk and use as beasts of burden. Flocks of small ruminants, such as sheep and goats, were also used for the supply of milk. With the general cessation of nomadic existence, the value of the larger ruminants came into prominence chiefly from the requirements of tillage and of meat supply. Later on, the value of domesticated animals further increased, due to the inventions which were responsible for the depletion in the number of wild animals, the chief of which might be mentioned—the discovery of gunpowder. Governmental interest, land settlement and general progress in crop-growing were additional factors leading to more intensive agricultural pursuits and population, which

TABLE I. *Composition of Milk from Various Species of Mammals (Percentages)*

Species	Water	Fat	Sugar	Casein	Other Protein	Ash
Mare (a) . .	90·68	1·17	5·77	1·27	0·75	0·36
Mare (b) . .	89·80	1·17	6·89	1·84		0·30
Mare (c) . .	—	1·14	5·87	1·30	0·75	—
Ass (a) . .	89·88	1·50	6·09	0·73	1·31	0·49
Ass (b) . .	—	1·37	6·19	0·79	1·06	—
Mule (a) . .	91·50	1·59	4·80	1·64		0·38
Mule (b) . .	—	1·92	5·69	2·63		—
Zebra . .	—	4·80	5·34	3·03		—
Camel (a) . .	86·57	3·07	5·59	4·00		0·77
Camel (b) . .	—	2·87	5·39	3·49	0·38	—
Llama . .	86·55	3·15	5·60	3·00	0·90	0·80
Cow .	87·32	3·75	4·75	3·00	0·40	0·75
Goat (a) . .	82·34	7·57	4·96	3·62	0·60	0·84
Goat (b) . .	—	4·33	3·61	2·91	0·76	—
Buffalo (a) . .	86·04	4·63	4·22	3·49	0·86	0·76
Buffalo (b) . .	—	7·51	4·77	4·26	0·46	—
Reindeer (a) . .	68·20	17·10	2·08	8·40	2·00	1·50
Reindeer (b) . .	—	17·09	2·82	8·38	1·51	—
Sheep (a) . .	79·46	8 63	4·28	5·23	1·45	0·97
Sheep (b) . .	—	9·29	5·04	4·08	0·80	—
Elephant . .	67·85	19·57	8·84	3·09		0·65
Pig (a) . .	84·04	4·55	3·13	7·23		1·05
Pig (b) . .	—	9·54	3·30	3·76	1·45	—
Pig (c) . .	—	7·09	3·44	3·26	1·55	—
Pig (d) . .	—	6·32	3·19	3·71	1·65	—
Pig (e) . .	—	6·77	—	6·22		0·97
Cat (a) . .	81·63	3·33	4·91	3·12	5·96	0·58
Cat (b) . .	—	4·49	4·79	3·79	3·30	—
Cat (c) . .	—	4·80	4·80	3·79	3·11	—
Cat (d) . .	—	4·98	4·71	3·69	3·29	—
Dog (a) . .	75·44	9·57	3·09	6·10	5·05	0·73
Dog (b) . .	—	11·62	3·24	4·80	2·64	—
Dog (c) . .	—	12·19	3·23	4·84	2·43	—
Rabbit . .	—	16·71	1·98	8·17	2·21	—
Guinea-pig (a) . .	—	7·31	2·31	4·60	0·49	—
Guinea-pig (b) . .	—	6·96	2·02	4·79	0·61	—
Rat . .	68·3	14·8	2·8	9·2	2·6	1·5
Porpoise . .	41·11	48·50	1·33	11·19		0·57
Whale .	48·67	43·67	—	7·11	—	0·46
Caaing Whale .	—	43·76	-	—	—	—
Hippopotamus .	—	4·51		—	—	—
Human (a) . .	88·50	3·30	6·80	0·90	0·40	0·20
Human (b) . .	—	3·74	6·37	0·80	1·21	—
Human (c) . .	87·41	3·76	6·29	0·91	1·23	0·31

were naturally followed by an amelioration in type and yield of the domestic animals used as sources of food for the community. The outcome of such a development is the present-day highly efficient milk-producing animal—the dairy cow—which yields a valuable source of food as milk, of remarkable constancy of composition and stable physical properties.

The goat has also been domesticated and bred for milk yield, being particularly adapted for mountainous areas. Other animals, all ruminants, whose milk is used for human consumption, are the sheep, buffalo, llama and the reindeer. The milk of the horse, ass and camel has an economic value in some parts of the world. Apart from the use of these milks for potable purposes in the raw form, quite an appreciable portion of their economic significance arises from their use in the manufacture of special products of more or less local importance only. As examples, " kumiss " or fermented mare's milk is an important beverage in Tartary, whilst " keffir " is another type of soured milk in some demand in Southern Europe. The innumerable types of small cheese manufactured in the Alps from goat's milk are considered as delicacies of world-wide repute.

4. The Scientific Study of Milk Composition and Behaviour

By far the most widely produced, the most generally known and studied milk is that of the cow, and the term " milk " is usually taken to refer to cow's milk unless otherwise stated. Milk and products manufactured from it represent an important and considerable portion of the food supply of nations and a great part of the agricultural prosperity of certain parts of the world is linked up with the manufacture of milk products. The studies concerning milk and its products have dealt mainly with the composition, technology, nutritive value, analysis, bacteriology and bio-chemistry of the products, and the amount of work done on each section has been voluminous.

It is interesting to realise that successful attempts to preserve milk as its by-products, cheese, butter or fermented products, have been made for a period extending over a score of centuries, and that the principles of what was termed the " *art* " of manufacture a century ago still obtain in the present " *scientific* " manufacture of the products. On the other hand, scientific research into the methods of manufacture and control commenced barely sixty years ago and, compared with the age of the dairy industry, that of dairy science is comparatively young. Research in dairy science may roughly be divided into two branches, namely, (*a*) the studies of fundamental problems, such as milk

secretion, physical equilibria, rennet action and cheese-ripening, composition, nutritive value and storage qualities, and (*b*) subsidiary studies of faults and taints and hygienic quality. The two branches are interrelated, and a good grounding in fundamental knowledge is necessary for the elucidation of problems concerned with taints and faults.

5. Fundamental Requirements in Milk Composition and Properties

When dealing with milk as a commercial article of food or as a raw material for the manufacture of by-products, it must be understood that in order to safeguard its wholesomeness and its marketable value, it must be subjected to a series of processes, some of which are of a drastic nature. Although milk was not intended to undergo such treatment, it can withstand much processing without economic loss by adopting suitable precautions.

Milk is a special emulsion of the oil-in-water type, and with the oil of lower density than the aqueous phase, *surface creaming* can occur. For marketing purposes it is essential to preserve the property of the appearance of a " cream line," and the distinction or magnification of the depth of cream layer visible in the bottle has called for much study. The stability of the emulsion under various treatments rests on the nature and properties of the fat globule/water interface, and it can be said at the outset that the conditions at that interface govern the colloid chemistry of milk and most dairy products. For example, the state of the fat in the globule and the possibilities of the regeneration of the adsorptive conditions at the interface after heat-treatment are influential factors governing the physical behaviour of milk.

The stability of milk to the *action of heat* is another important requirement, and the conditions guarding against total or even partial coagulation by heating up to 100° C. and above have been well established.

The ideal to be aimed at as regards *composition* is that the producing animal secretes compounds of maximum calorific value in the milk instead of replacing large organic molecules (such as lactose and protein) by inorganic ions and non-protein nitrogenous compounds. The three main sources of energy and nutritive value in milk are fat, protein and lactose, and the composition is ideal when the amounts of these compounds are at or above an expected average level. Variation in milk composition amounts, in brief, to the occurrence of milk falling short in the level of production of one or more of these compounds. Protein must account for most of the nitrogen and casein for most of the protein of milk.

The *constancy of the osmotic pressure of milk* has been well established from the related property, the depression of the freezing-point of the water of milk, by the concentration of the water-soluble materials which are present. Evidence may be obtained from the freezing-point for the detection of added water, but a genuine milk low in non-fatty solids will show a normal value for the freezing-point depression. In this connection the particular constituents responsible for lowness of solids-not-fat can be detected.

On the manufacturing side, constancy of buffer value, a constant rate of lactic-acid production by the addition of " starters," and a general *reproducibility of behaviour* under corresponding conditions (*e.g.*, in cheese-making) in consecutive batches are expected requirements of milk. An abnormality of composition or behaviour at once upsets the normal routine and time-table in the preparation of a product. Linked up with this is the economic value of uniformity in the quality of a product from the marketing point of view.

Finally, *a uniform, taintless, hygienically clean article of food* which can with confidence be expected to behave normally in the hands of others less acquainted with its properties, for a reasonable length of time, is required.

CHAPTER II

THE COMPOSITION OF MILK

6. Classification of the Constituents

THE composition of the milk from various mammals has already been given in Table I (p. 7). Considerable variation in composition may be observed in the multiple data given for some species.

The range of constituents found in cow's milk is given in Chart I, with indications as to the amounts usually found present and to doubts of the presence of some. The range of the constituents at once brings into prominence the complexity and the comprehensiveness of milk composition.

The constituents may be classified in the following ways :—

(A) (1) *Fat* and (2) *Solids-not-fat*. Since milk is a heterogeneous system of a fat phase dispersed in an aqueous phase, and since the fat-content of milk is of paramount importance, it seems a simple and logical method to subdivide the *total solids* or dry matter of milk into (1) the dry matter of the fat phase, and (2) the dry matter of the aqueous phase. Such treatment is always used when dealing with the compositional quality of a large number of samples, while legal considerations of milk quality, except for addition of foreign material such as preservatives, etc., only go as far as this mode of subdivision. Reliable analytical methods have also been devised and standardised so that the composition of milk in regard to contents of fat and solids-not-fat can be quickly determined. The presumptive legal standard for milk in Great Britain is a minimum of 3 per cent. of fat and 8·5 per cent. of solids-not-fat. Samples falling below these minima in composition are presumed to have had a portion of the fat abstracted or to contain added water, respectively, unless the contrary is proved.

(B) (1) *Compounds characteristic of milk* and (2) *subsidiary compounds* generally found in biological fluids.

Class (1) is comprised of the fat, casein and lactose of milk. The trace of citric acid should perhaps be included in this class also. Included in Class (2) are : water, albumin, globulin, non-protein nitrogenous compounds, the mineral salts, enzymes and various cells. Carotinoid pigments and fat-soluble vitamins would naturally fall into the same class as the fats.

CHART I

CONSTITUENTS OF MILK

*, present in traces ; (?), doubtful ; (HL), heat-labile ; (O), unstable in air ; Capitals, known to be essential to life in young animals ; Figs., percentages ; (F), present in the fat phase ; (C), present in the colloidal state ; (S), present in true solution.

PROTEINS

CASEIN (2·7), ALBUMIN (0·5) and globulin (0·15) containing amino-acids as follows (percentages of proteins) :

	Casein	Albumin
Glycine	0–0·4	0·4
Alanine	1·5–1·8	2·4
Valine	7·2–7·9	1·0 3·3
Leucine	9·3–10·5	14 –19
Phenylalanine	3·2 3·9	1·2 2·4
TYROSINE	4·5–6·5	0·9 1·9
Serine	0·4–0·5	1·8
CYSTINE	0·25	1·7–4·0
PROLINE	7·6 8·7	3·8–4·0
Hydroxyproline	0·2	?
Glutamic acid	20 –21·8	10·1 12·9
Hydroxyglutamic acid	10·5	10·0
Aspartic acid	1·4 4·1	1·0 9·3
TRYPTOPHANE	1·5–2·2	2·7
Arginine	3·8–5·2	3·0–3·5
HISTIDINE	2·5–3·4	1·5 2·6
LYSINE	6·0–7·6	8·4–9·9
Methionine	0·4	?
Dodecanoamino acid	0·75	?
Ammonia	1·6	1·3
Phosphorus	0·85	—

Proteose* (?).　Lacto-mucin* (?).　Alcohol-soluble proteins* (?).

FATS AND RELATED COMPOUNDS

Butter-fat (F) consisting of the mixed glyceryl esters of the following atty acids (variable in amount) :

Butyric	.	.	.	.	3·0	(percentage of butter-fat).
Caproic	.	.	.	.	1·6	,,	,,
Capryllic	.	.	.	.	1·3	,,	,,

Capric.	.	.	.	3·0	(percentage of butter-fat).
Lauric.	.	.	.	3–6	,,	,,
Myristic	.	.	.	6–16	,,	,,
Palmitic	.	.	.	25	,,	,,
Stearic	.	.	.	4–8	,,	,,
Arachidic	.	.	.	1	,,	,,
OLEIC (O)		.	.	30–39	,,	,,
LINOLEIC (O)	.	.	.	3	,,	,,

also dihydroxystearic and linolenic (?).

Lecithin (0·04) and cephalin* (F) (O), Carotene and carotinoids* (xanthophylls) (F) (O).   Cholesterol* (0·015) (F) and ergosterol* (F).

SUGARS

Lactose (glucose-galactoside) (S) (4·9) ; Glucose * (?) (S).

NON-PROTEIN NITROGENOUS SUBSTANCES

Lactochrome* (0·05) (S).
Creatine * and creatinine* (S).
Urea* (S).
Thiocyanic acid* (?).
Orotic acid* (?).
Hypoxanthine, xanthine and uric acid*.
Choline* (?), trimethylamine *, trimethylamine oxide*.
Methyl guanidine* (?).
Ammonia* (0·0003) (as ammonium salts).

ENZYMES				PHOSPHORUS COMPOUNDS IN MILK
Diastase (HL) .	.	.	.	(Free phosphate).
Peroxidase (HL)	.	.	.	Phosphate loosely bound to casein.
Reductase (HL)	.	.	.	(Casein phosphorus).
Lipase (HL) .	.	.	.	(Lecithin and cephalin).
Protease (galactase) (HL) .	.	.	Diamino monophosphatide.
Catalase (HL)	.	.	.	Three acid-soluble organic phosphorus compounds (Phosphoric esters).
Lactase (?) (HL)	.	.	.	
Phosphatase (HL)	.	.	.	
Oleinase (?) (HL)	.	.	.	

VITAMINS

Fat-soluble	Water-soluble
A (growth-promoting, anti-xerophthalmic).	B_1 (anti-beriberi).
D (anti-rachitic).	B_2 (anti-pellagra).
E (reproductive).	B_3 (?).
	B_4 (?).
	C (anti-scorbutic) (O) (HL).

MINERAL OR INORGANIC CONSTITUENTS

POTASSIUM (S).	PHOSPHATES (C, S).
SODIUM (S).	CHLORIDES (S).
CALCIUM (C, S).	Sulphates* (S).
MAGNESIUM (C, S).	Citrates (S).
Zinc*.	Carbonates.
Aluminium*.	IODINE*.
COPPER*.	Silica*.
IRON*.	Fluorine*
MANGANESE*.	

Unidentified substances.
Various cells.
Water.
Dissolved gases, including carbon dioxide, oxygen, nitrogen.

(C) *Major and Minor Constituents.* The major constituents account for the bulk of the dry matter (*e.g.*, the constituents given in Table I) and are easily determined by the usual analytical methods. The minor constituents consist of all other compounds found in traces and requiring special methods of analysis for their determination ; their functions, if any, in milk need to be determined by special examination.

7. Average Composition of Cow's Milk

Owing to the academic, technical and legal aspects of the composition and variation in the composition of milk, the literature on this subject is voluminous. In the study of this literature considerable difficulty is experienced in separating (*a*) the analytical results for the samples of milk from herds and those from individual cows, and (*b*) the results for samples from herds or cows under conditions of normal management analysed for cow-testing purposes only, and those from cows or herds suspected of giving low-quality milk, which have come under observation for advisory purposes. Another slight difficulty in comparing results from different workers lies in the possibility of variation in methods of analysis, particularly in the methods of determining solids-not-fat, protein distribution, lactose and ash.

Table II gives the average composition of milk with regard to contents of fat and solids-not-fat according to various authorities.

The value, 8·89 per cent. of solids-not-fat, is closely agreed to by all investigators, but a considerable variation in the average fat-content is met with (3·25–3·95 per cent.). This is to be expected, since content of fat shows more variation due to differing conditions (to be discussed later) than that of solids-not-fat.

TABLE II. *Average Proximate Composition of Milk*

Authority	Number of Samples	Fat %	Solids-not-fat %	Country
Tocher [1]	676	3·95	8·78	Scotland
Crowther [2]	4,220	3·70	8·78	England
Cranfield [3]	732	3·71	8·75	,,
Golding et al.[4]	312	3·88	8·91	,,
Golding et al.[5]	3,115	3·89	8·89	,,
Golding et al.[47]	106	3·82	8·86	S. England
Golding et al.[47]	1,498	3·82	8·81	,,
Van Slyke [6]	5,552	3·90	9·00	U.S.A.
Fleischmann [7]	—	3·40	8·85	Germany
,,	—	3·25	8·75	N. Germany
Farrington and Woll [8]	- -	3·70	8·90	U.S.A.
Eccles [9]	—	3·90	9·00	,,
Babcock [10]	—	3·69	9·14	,,
Oliver [11]	—	3·25	9·15	—
Willoughby [11]	—	3·80	9·20	—
Droop Richmond [12]	330,000	3·78	8·74	England
Bailey [13]	127	3·87	8·65	U.S.A.
Abderhalden [14]	—	3·70	9·10	Germany
Cornalba [48]	—	3·75	8·80	N. Italy
Rogina [49]	379	3·86	—	Balkans

Average (all data) . . . 3·73 8·89
(SD, 0·22) (SD, 0·16)

Average, excluding references 7 and 11 3·78 8·89
(SD, 0·14) (SD, 0·16)

(SD = standard deviation).

The average solids-not-fat content of the fat-free serum is 9·24 per cent. (SD, 0·17).

Criticisms may be levelled at the figures quoted in Table II with respect to the number of samples of morning and evening milk included in calculating the averages. As will be seen later, there is a greater yield of morning milk of lower fat-percentage generally. The bulking of morning and evening milk would tend therefore to give a slightly lower fat-percentage. The content of solids-not-fat would not be appreciably altered. Again, it is unknown to what extent the inclusion of results for samples of low quality influences some of the averages. It may be sufficient to

TABLE III. *Variations in the Proximate Composition of Milk*

Authority	Fat			Solids-not-fat		
	Maximum %	Minimum %	Average %	Maximum %	Minimum %	Average %
Richmond [12] .	6·39	1·03	3·78	10·60	4·90	8·74
Crowther [2] .	5·30	2·00	3·70	9·50	8·40	8·78
Cranfield [3] .	6·00	2·20	3·71	9·60	7·90	8·75
Tocher [1] .	7·50	1·66	3·95	10·66	7·00	8·80
Golding *et al.*[4] .	5·17	2·60	3·88	9·28	8·40	8·91

is experienced in the samples of milk from cows which are constantly giving low-quality milk ; the composition of milk from cows regularly giving milk of good quality is more uniform.

9. Incidence of Composition Falling below Standard

In the Sale of Milk Regulations, 1901, and for the purpose of the Foods and Drugs (Adulteration) Act, 1928, a sample of milk
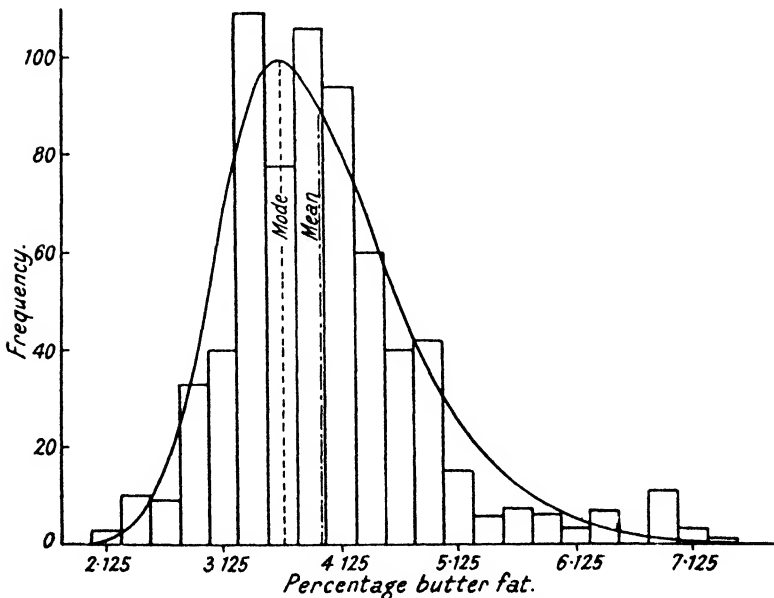
FIG. 2.—Percentage butter-fat distribution. Tocher. 676 samples.

containing less than 3 per cent. of fat or less than 8·5 per cent. of solids-not-fat is presumed not to be genuine unless the contrary

is proved. These limits are substantially lower than the average percentages (*v*. Table II) for genuine milk, but it is generally
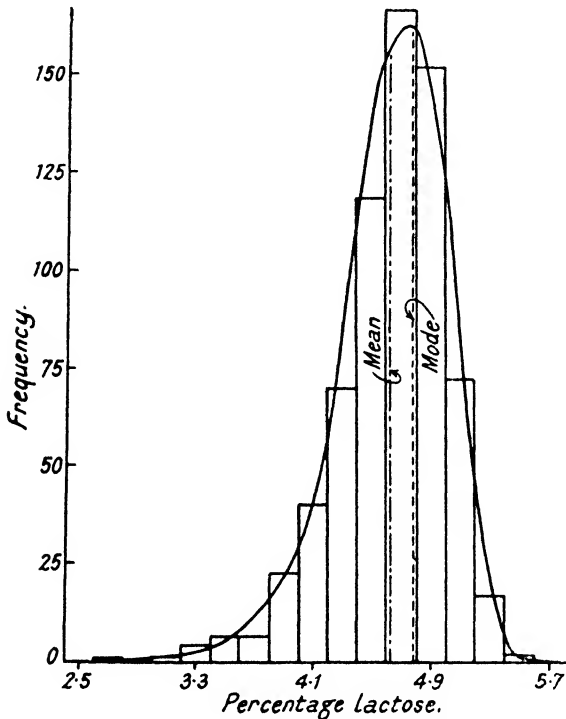
FIG. 3.—Percentage lactose distribution. Tocher. 676 samples.

recognised that a proportion of genuine milks can fall below one or both of these limits of composition. Low-quality samples need and are receiving further investigation, but at present do not

TABLE IV. *Percentages of Samples of Milk falling below the Legal Limits of Composition*

Authority	Tocher [1] %	Cranfield [5] %	Bailey [15] %	Monier-Williams [17] %	Min. of Agri. Bull. [18]	
					A %	B %
Samples below—						
8·5 per cent. S.N.F. . .	24·7	11·6	10·2	9·6	2·6	7·8
3·0 per cent. Fat .	8·3	8·1	35·7	7·7	4·9	3·2
No. of samples .	676	730	127	104	6,800	2,900

permit an exact estimation of this proportion ; there is, however, sufficient available evidence of the occurrence of low-quality samples and of the circumstances associated with such deficiencies. Table IV gives authoritative findings on the question.

TABLE V. *Detailed Composition of Milk. Per cent. of Whole Milk*

Authority	Water	Fat	Casein	Albumin*	Total Protein	Lactose	Ash	Total Solids
Van Slyke [6]	87·1	3·9	2·5	0·7	3·2	5·1	0·7	12·9
Babcock [10] .	87·3	3·6	3·0	0·6	3·8	4·5	0·7	12·7
Willoughby[11]	87·0	3·8	3·3	0·4	3·7	4·8	0·7	13·0
Oliver [11] .	87·6	3·25	3·40	0·45	3·85	4·55	0·75	12·4
Richmond [12]	87·1	3·9	3·0	0·4	3·4	4·75	0·75	12·9
Fleischmann[7]	87·75	3·40	2·80	0·70	3·50	4·60	0·75	12·25
Rogers [20] .	87·27	3·66	2·95	0·52	3·47	4·91	0·69	12·73
Tocher [1] .	87·25	3·95	2·42	0·74	3·16	4·64	0·70	12·75
Cranfield [3] .	87·54	3·71	—	—	3·25	(4 74)†	0 76	12·46
Farrington and Woll [8]	87·4	3·7	—	—	3·2	5·0	0·7	12·6
Abderhalden [14]	—	3·70	2 90	0 50	3·40	4·95	—	—
Cornevin [21]	87·75	3·30	3 00	—	—	4·80	0·75	12 75
Golding et al.[4] .	87·21	3 88	2·60	—	—	4·74	0·77	12·79
Davies [16] .	—	—	2·48	0 57	3·05			
Maximum [22]	90·0	7·8	—	—	4·5	6·0	0·9	10·0
Minimum .	82·0	2·3	1·5	0·5	2·0	3·5	0·6	18·0
Range—								
Richmond[12]	83·87– 91 55	1·03– 6 39	—	—	2·37– 4·26	4·41– 5·00	0·62– 0·78	8·45– 16·13
Cranfield [3] .	—	2 20– 6·00	—	—	2·70– 4·05	—	0·69– 0·88	—
Tocher [1] .	82·27 89·75	1·66– 7·50	1·65– 4·08	0·45– 1·40	1·98– 6·00	2·70– 5·50	0·47– 0·99	10·25– 17·73
Average (all data)	—	3·67	2·86	0·56	3·42	4·78	0·73	12·69

* Albumin *plus* globulin.   † Calculated by difference.

Table IV shows that as the number of samples examined increases the percentage of low-quality milk diminishes. There is no doubt that Bailey was dealing with special cases. Lesser,[19] during investigations on a herd of British Friesian cows, known to give milk deficient in solids-not-fat, found 80·1 per cent. of
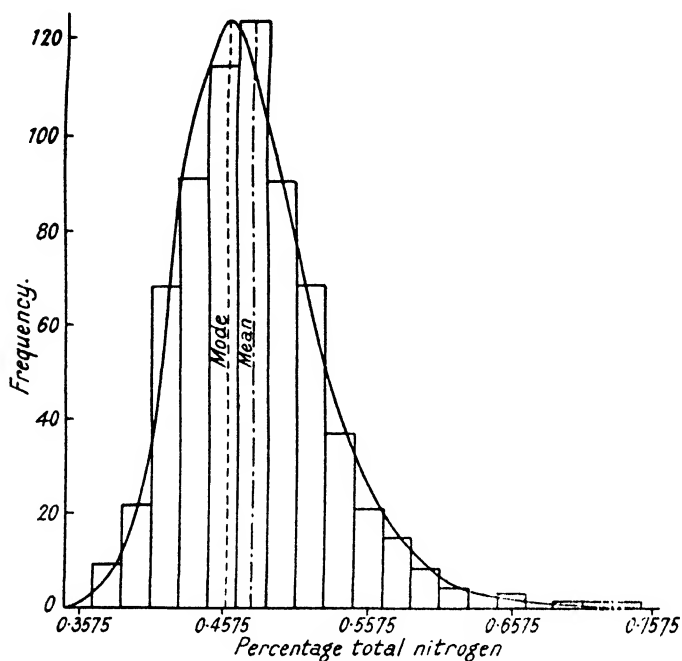
FIG. 4.—Percentage total nitrogen distribution.   Tocher.   676 samples.
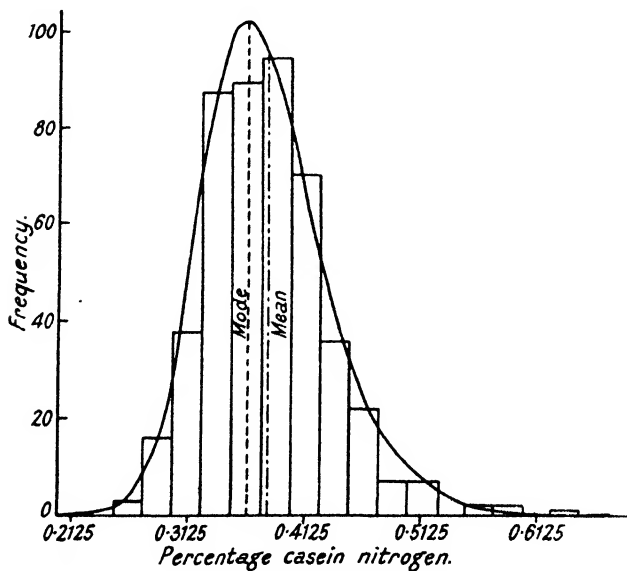
FIG. 5.—Percentage casein nitrogen distribution.   Tocher.   474 samples.
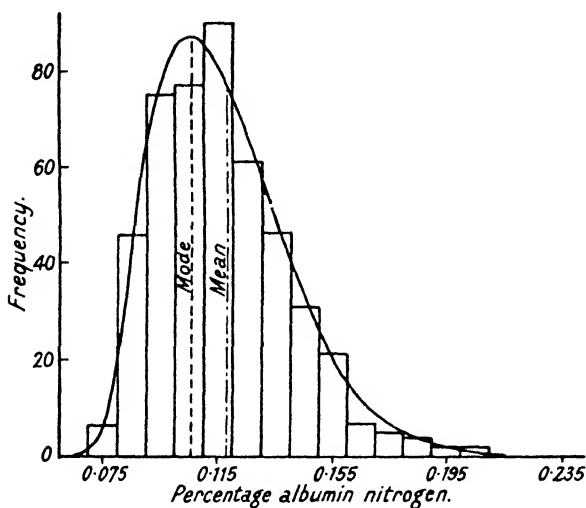
FIG. 6.—Percentage albumin (albumin *plus* globulin) nitrogen distribution. Tocher.   474 samples.
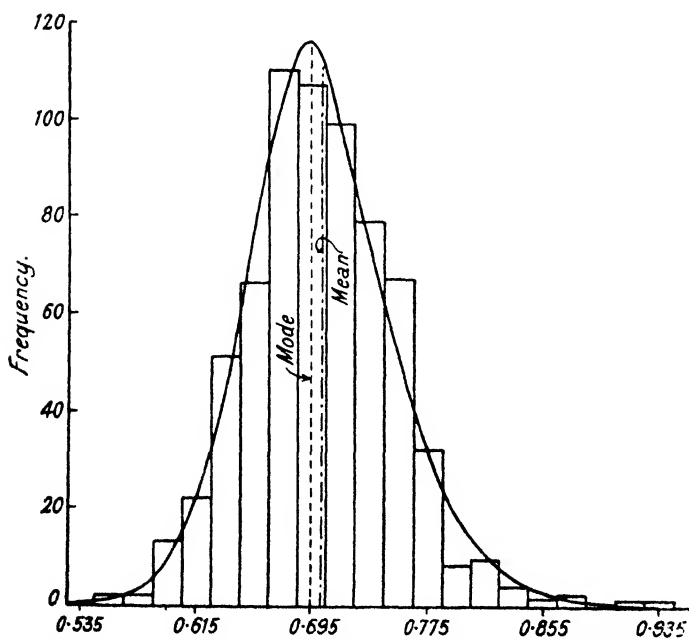
FIG. 7.—Percentage ash distribution.   Tocher.   676 samples.

1,226 samples (winter period, 1930), and 62·0 per cent. out of 718 (1931) to be below 8·5 per cent. Golding [4] observed that the fat-content fell below 3 per cent. in 13·7 per cent. of 1,074 morning samples, but that only 3·2 per cent. contained less than 8·5 per cent. solids-not-fat.

10. Detailed Composition of Milk (See Figs. 3–7).

Table V gives the average detailed composition and ranges of composition of milk observed by various investigators.

There is close agreement concerning the average lactose, total protein and ash contents, although it is unknown to what extent the total nitrogen content of milk has been used to calculate the total protein content ; 5 per cent. and above of the total nitrogen of milk is non-protein nitrogen.[16] Considerable differences are noted in the average casein content, and it is inadvisable to compare the results of different workers since different methods of analysis have been employed.

The detailed composition of milk from other mammals has been given in Table I (p. 7).

11. Circumstances Affecting the Composition of Milk

Cow's milk, as has been observed, is not a uniform article of commerce. The differences in composition mainly met with are chiefly due to different environment, inheritance and management. The factors concerned are dealt with *in seriatim* below :—

(A) *Breed of Cow*. Differences due to breed are of greatest

TABLE VI. *Influence of Breed on Milk Composition.
Percentages of Whole Milk*

Breed	Water	Fat	Protein	Lactose	Ash	Solids-not-fat	Ratio of S.N.F. to Fat
Jersey .	85·27	5·14	3·80	5·04	0·75	9·59	1·86
Guernsey .	85·45	4·98	3·84	4·98	0·75	9·57	1·92
Ayrshire .	87·10	3·85	3·34	5·02	0·69	9·05	2·35
Shorthorn	87·43	3·63	3·32	4·89	0·73	8·94	2·35
Friesian .	88·01	3·45	3·15	4·65	0·68	8·48	2·46
Shorthorn [23]	87·07	3·70	—	—	—	9·23	2·50
Aberdeen-Angus [46]	—	4·06	3·56	—	—	—	—

magnitude. Table VI illustrates the average composition of milk from several breeds of cows.

Generally, milk high in fat is also high in solids-not-fat, but,

with a lowering of fat-content, the ratio of fat to S.N.F. widens. The decrease in S.N.F. is accounted for by a decrease in all the organic constituents, lactose and ash showing the least percentage decrease. It must be understood that these figures (Table VI) represent the average of a large number of samples. As will be seen later for individual samples, variation in lactose and casein is mostly responsible for variation in S.N.F.

The breed characteristic shows up most prominently in the different fat-percentages of the milk. Table VII shows the variation in fat-percentage with breed (and the corresponding S.N.F. content in Drakeley's averages [24]).

Drakeley's figures (Table VII) are of comparative value only,

TABLE VII. *Average Fat Content of Milk from Large Numbers of each Breed*

	Tocher [1]	Drakeley [24]	
	Fat %	Fat %	S.N.F. %
Jersey . . .	5·43	5·18	9·30
Guernsey . . .	5·16	4·88	9·29
Kerry . . .	4·67	4·30	9·09
Welsh . . .	4·40	—	—
Ayrshire . . .	4·09	3·97	9·00
Shorthorns . .	3·91	3·78	9·04
British Friesians .	3·63	3·67	8·78
South Devon . .	—	4·02	9·25
Lincoln Red . .	—	3·76	9·00
Red Poll . . .	—	3·81	9·09

since they apply to selected cows under showyard conditions (6,566 samples).

The fact that cows of a particular breed give a low percentage of fat in their milk does not mean that the *total yield of fat* is low, since the *yield* of milk also varies with breed. Tocher [25] has shown that Friesian cows give a larger amount of fat per milking than Ayrshire and other cows, although the average percentage of fat for Friesian milk is 3·625 as against 4·085 for Ayrshire and 3·819 for other cows.

(B) *Individuality of Cows.* Individual cows kept under the same conditions of care, feeding and environment will show considerable variation in the composition of their milk, some yielding milk falling persistently below the presumptive standards. In the absence of any circumstances usually associated with the giving

of milk low in solids-not-fat (pathological conditions, end of lactation, etc.), it must be assumed that the production of such milk is a characteristic of the individual cows in question, due possibly to some factors not yet identified.

The variations are chiefly in percentages of fat, lactose and protein. Milk low in solids-not-fat is characterised by a low lactose content (high chloride content),[26] low total protein and a low percentage of total nitrogen as casein nitrogen.[16] Samples from cows regularly yielding milk of normal composition show little variation from day to day, whilst low-quality milk shows more variation in detailed composition with consecutive samples.

The following table (Table VIII) shows the variation in the fat-content of samples from individuals of various breeds.

TABLE VIII. *Variation in Fat-Content of Milk from Breed Individuals* [27]

Breed	Shorthorn	Friesian	Ayrshire
Fat per cent. Cow (a) .	4·01	4·00	4·00
„ „ „ (b) .	3·28	3·00	3·20
„ „ „ (c) .	3·27	2·90	3·40
„ „ „ (d) .	4·03	3·80	4·00

Although milk from individual cows may fall below the limits defined in the Sale of Milk Regulations, it is often asserted that the mixed milk of herds does not usually vary very much in composition from the average. This requires some qualification, as such an occurrence will naturally depend on (a) the size of the herd, and (b) the thoroughness of mixing of the herd's milk. Obviously, the bulking of the milk of as many cows as possible will lead to the composition being nearest the average.

(C) *Differences in Composition between one Milking and another and the Influence of Intervals between Milkings.* When cows are milked twice daily at equal intervals there is little difference in the fat-percentage and milk yield, although the morning's yield may be slightly greater and the percentage of fat therein slightly lower. When the night interval exceeds the day interval the fat-percentage is lowered in the morning and increased in the afternoon milking. Mackintosh [28] states that in the case of mixed milk from a herd, for each hour that the interval exceeds twelve hours, the fat is lowered 0·10—0·15 per cent., and for each hour the interval is under twelve hours the fat is raised 0·20

—0·25 per cent. The individuality of cows influences the results because some are more affected by the length of interval than others.   Economic and practical conditions often force the producer to milk at unequal intervals with the result that sometimes the mixed morning's milk falls below the presumptive standard of 3 per cent. fat.   With a night interval of fourteen hours, associated with a considerable proportion of newly-calved cows and grazing and other conditions conducive to high yields, the morning's milk from ordinary herds is often dangerously near and frequently under the 3 per cent. standard through no fault of the producer or of his cows.

Where milking is practised three times daily, at intervals of approximately eight hours, the yields at each milking are in close agreement but the percentages of fat are by no means uniform.

Table IX gives a summary of experiments carried out at Garforth [29] and Cambridge [30] (summarised by Crowther [2]) on the effect of intervals between successive milkings on the composition of milk.

TABLE IX. *Effect of Interval between Milkings on Composition*

Period of Experiment	Length of Night Interval	Fat %	S N F %	Length of Day Interval	Fat %	S N F. %
Garforth 14 days	15 hours	2·87	9·03	9 hours	4·26	9·00
28 ,,	12·5 ,,	3·18	8·95	11·5 ,,	3·80	8·99
21 ,,	15 ,,	2·94	8·83	9 ,,	4·40	8·79
14 days	12 hours	3·64	8·81	12 hours	3·45	8·92
14 ,,	16 ,,	2·33	8·97	8 ,,	4·47	8·92

Collins [31] has estimated the variations in fat likely to occur with different milking intervals (Table X).

Milking three times a day, Cruikshank, working with a herd of five cows, found similar results (Table XI).

These results are sufficient to confirm the accepted opinion that, when a long interval has elapsed since the previous milking, it is not unlikely that the fat-percentage will fall below 3·0.

Campbell,[32, 33] has studied the effect of night on milk production under winter conditions. When the fifteen-hour interval is

TABLE X.   *Estimated Variation in Fat-Percentage with given Intervals. Morning Milking at 6 a.m.*

Intervals—Night Interval First	12 and 12 %	13 and 11 %	14 and 10 %	14 5 and 9 5 %
Excess of fat in evening's milk over morning's milk . . .	− 0·18	+ 0·33	+ 0·70	+ 1·09
No. of tests . . .	22	192	18	391

TABLE XI.   *Three Times a Day Milking. Effect of Length of Interval*

After Interval	Fat %	Yield lb	Weight of Butter fat per Milking lb
Night : 12·5 hours .	2·59	119 5	3 10
Morning : 5·5 hours.	4·79	83·5	3 94
Afternoon : 5 hours .	4·88	63·0	3 08

between night and morning milkings, a larger proportion of milk is produced at the morning milking than is produced at night when there is a fifteen-hour interval between morning and evening

TABLE XII.   *Solids-not-fat in Morning and Evening's Milk. Night Interval—Fifteen Hours*

F.F.S. = fat-free serum.

Year	Evening Milk				Morning Milk			
	No of Samples	Fat %	SNF %	SNF in F.F.S %	No of Samples	Fat %	SNF %	SNF in F.F.S %
1928–29 .	337	4·69	8·84	9·28	352	3·28	8·99	9·30
1929–30 .	365	4·55	8·84	9·26	365	3·21	8·87	9·16
1930–31 .	365	4·46	8·84	9·25	365	3·22	8·98	9 28
1931–32 .	365	4·53	8·87	9·29	363	3·27	8·99	9·30
1932–33 .	118	4·39	8·77	9·17	120	3·38	8·78	9·09
Whole period .	1,550	4·54	8·84	9·26	1,565	3·25	8·94	9·24

milking. Night itself, or factors operating at night, tend to high production of milk of low fat-content. More weight of milk and fat are produced *per hour* during nine-, twelve- and fifteen-hour night intervals than during intervals of the same length during the day, the milk-increment being greater than the fat-increment. Milking at midday and midnight, more milk and fat are produced *per hour* from midnight to midday than in the other twelve-hour interval, and in this case the fat-increment is greater.

No great variations in the percentage of solids-not-fat appear to occur in connection with intervals of milking. Table XII gives the results for periods covering over four consecutive years at the National Institute for Research in Dairying.[5]

(D) *Variations in Composition during Milking.* In the actual process of milking the first-drawn milk is lowest in fat-content ; successive portions steadily increase in fat-content and the strippings are richest in fat. Van Slyke's [6] results, given in Table XIII, illustrate this variation.

TABLE XIII. *Variation in Fat-Content of Milk during Milking*
(*Percentages of Fat*)

Portion	Cow A	Cow B	Cow C
First	0·90	1·60	1·60
Second	2·60	3·20	3·25
Third	5·35	4·10	5·00
Strippings	9·80	8·10	8·30

The maximum amount of fat will be given by quickness combined with thoroughness of milking.[28] An increase up to 30 per cent. in weight of fat may be obtained, especially after a long interval. Daily milk-recording is a help towards uniform efficiency in milking.

During milking the percentage of solids-not-fat in the fat-free serum naturally remains constant.

· The value of the complete drawing of the strippings is at once apparent from Table XIII. Gilchrist [34] has pointed out in interpreting results of an experiment on similar lines to that reported in Table XIII that, if the milkers had failed to draw the strippings (average volume of half a pint), the average percentage of fat would have dropped from 2·94 to 2·46 at the 5 a.m. milking, from 4·30 to 3·66 at the 12.30 p.m. milking, and from 4·46 to 3·86 at the 6 p.m. milking. Both Gilchrist and Crowther have stated that the temporary employment of unskilled milkers will cause serious decreases in the percentage of fat.

(E) *Variations Due to Age.* (*a*) *Fat.* Speir [35] has deduced the following relations between the age of a cow and the percentage of fat in the milk (Table XIV). His data were collected from observations for six months on 903 Ayrshire cows at approximately equal periods of lactation.

From data provided by the Scottish Milk Records Association, Tocher [1] has calculated the regression of the average percentage of fat due to the increasing age of the cows. The regression equation arrived at was : Fat $= 3\cdot9842 - 0\cdot0312x - 0\cdot0094x^2$, where the class unit was one year, and $x =$ age of cow.

TABLE XIV. *Effect of Age of Cow on Percentage of Fat in Milk. Speir* [35]

Age of Cow Years	Number of Cows	Average Yield in Six Months (Gals.)	Fat % (average)
2	30	362	3·83
3	147	377	3·87
4	164	403	3 76
5	137	421	3·66
6	110	438	3·63
7	88	465	3·63
8	80	468	3·69
9	50	461	3·63
10	36	457	3·64
11	28	464	3·60
12	16	493	3·48
13	10	428	3 42

(*b*) *Other Constituents.* The regression of the other constituents with age is linear (Table XV).[1]

TABLE XV. *Regression Equations for some Constituents of Milk.* ($x =$ Age in Years)

Constituent → y	Regression Equation
Ash	$y = 0\cdot6994 - 0\cdot0011x$
Lactose	$y = 4\cdot6075 - 0\cdot0327x$
Solids-not-fat	$y = 8\cdot7824 - 0\cdot0316x$
Casein nitrogen	$y = 0\cdot3780 - 0\cdot0034x$
Albumin nitrogen	$y = 0\cdot1187 + 0\cdot0027x$
Total nitrogen	$y = 0\cdot5076 - 0\cdot0012x$

It will be observed that a decrease occurs in all constituents with age except in albumin nitrogen.

(F) *Variation Due to Period of Lactation.* Table XVI, compiled by Crowther,[2] from the records of four sets of experiments in which the cows were housed for the greater portion of the day, shows the influence of the period of lactation on the composition of milk.

The percentages of fat and solids-not-fat tend to fall for the first three months of lactation and to increase thereafter.

TABLE XVI. *Influence of Period of Lactation on Composition of Milk*

Period of Lactation Months	No. of Cows	Average % of Fat	No. of Cows	Average % of S.N.F.
1	8	3 78	6	8·95
2	12	3·40	7	8·72
3	10	3·35	5	8·74
4	6	3 38	4	8·84
5	2	3·56	2	8·81
6	4	3 86	3	9·03
7	1	4 05	1	9·00
8	5	4·05	5	9·17
9	4	4 17	4	9·10
10	2	4·27	2	9·29
11	3	4·70	3	9·49

Tocher [1] finds that the minimum amount of fat occurs at the fourteen to sixteen week period. The average after calving is 3·97 per cent., falling to 3·85 per cent. at seventeen weeks, after which there is an increase, that at thirty-eight weeks being 4·18 per cent. The minimum of solids-not-fat occurs at seventeen to twenty-one weeks, being 8·88 per cent. at two weeks, 8·69 per cent. at twenty weeks and 9 per cent. at forty-one weeks.

The variation in milk composition during the lactation period is influenced by (*a*) breed of cow, (*b*) the condition of the cow before calving, and (*c*) the management and feeding after calving. Drakeley [24] has observed an initial decrease in fat-percentage and then an increase till near the end of the lactation period ; the time required for the minimum to be reached varies with the different breeds from thirty-five to 105 days after calving. The fat-range varies from 4·48 to 5·61 per cent. for Guernsey cows to 3·7 to 4·10 per cent. for Red Polls. He has found similar variations but

of lesser magnitude (0·3 per cent.) for the solids-not-fat content. The second, third and fourth months of lactation are therefore mostly associated with low milk composition.

Mackintosh [28] states that a cow in good condition before calving, with consistent good management and feeding of balanced rations, will maintain a higher fat-percentage than under conditions of careless management.

(G) *Climate and Weather Conditions.* Mackintosh [28] sum-
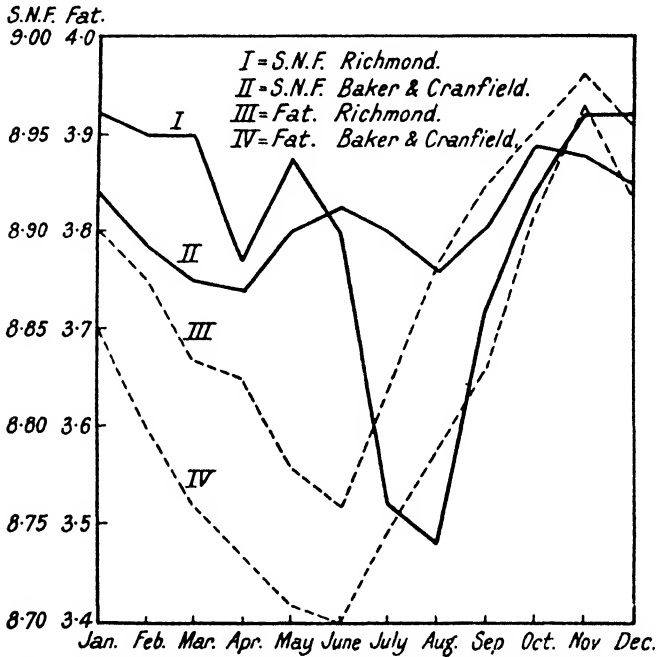
Fig. 8.—Monthly variations in fat and solids-not-fat percentages.

marises as follows the effect of climate and weather conditions on *fat-percentage* under the headings (a) season of the year, (b) temperature, and (c) sudden weather changes :

(a) *Season of the year.* Fat-percentage follows a general curve. It is lowest in summer and highest in winter. The influence of other factors, such as the proportion of autumn calvers, may cause the difference to be slight (Fig. 8).

(b) *Temperature.* Low temperature (colder weather) tends to increase and warm weather to lower fat-percentage. Experimentally controlled conditions, which, however, seldom occur on farms, have been found to increase fat by 0·15–0·20 per cent. for each 10° F. fall in temperature.

(c) *Sudden changes in weather* should be taken into considera-
tion in conjunction with other conditions, such as temperature,
comfort, water and food supply, and exercise.

Moderate *exercise* increases fat-percentage over that associated
with rest periods. Four comparisons between rest and exercise
showed increases of 0·27, 0·37, 0·12 and 0·47 per cent. of fat.
During summer the effect of differences in night and day tem-
perature and rest and exercise cancel each other.

Tocher [1] has observed that the percentage of fat is significantly
less for the period January to March than the average for the
remaining months, whilst fat-percentage is significantly greater
for the period July to September. For the period July to Sept-
ember the average percentage of solids-not-fat is less than the
average for the other nine months, *i.e.*, it is associated with a
higher average percentage of fat.

Houston and Hale,[36] in agreement with Ragsdale and Brody,[37]
Hayes,[38] and Weaver and Mathews,[39] have found that the per-
centage of solids-not-fat is depressed during the summer period,
but that fat-percentage is not significantly influenced by the
season of the year. The morning percentage of butter-fat is
more subject to slight variation by seasonal influences than the
evening percentage.

Drought conditions in summer may cause an appreciable lower-
ing of milk composition, due possibly to the combined effects of
(a) insufficient water supply, (b) irritation by insects, and (c) the
physiological necessity of secreting a liquid lower in calorific value
to suit the naturally smaller needs of a potential suckling during
hot weather.

(H) *Kind and Quality of Food.* The results of a large number
of experiments show that, where cows are suitably fed, the butter-
fat content of milk cannot be altered appreciably by food, and
that any effect on the content of solids-not-fat is still more diffi-
cult to trace. The question remains, however, whether under-
feeding affects the *composition* of milk. Workers at the Cornell
University Experimental Farm collected the milk and butter-fat
records of a neighbouring badly-nourished herd for a year,
managed the herd themselves for two years under liberal condi-
tions, and then returned the herd to the farmer in the fourth year.
The result showed that whilst the yield increased 42 per cent.
during the second and third years over that of the first and fourth
years, the difference in percentage of fat was only 6 per cent.
Starvation conditions therefore depressed the *yield* appreciably,
but influenced the quality only to a slight extent.

Attempts to raise the solids-not-fat in milk from cows per-

sistently yielding low-quality milk, by feeding balanced rations supplemented with ample vitamin-containing foods, tonics and well-balanced mineral matter, have been fruitless (Lesser [19] and unpublished work). Roadhouse and Henderson [41] find that the feeding of large quantities of molasses or of common salt has no effect on the composition of milk. A change to spring pasture after indoor feeding has, however, been found to increase the S.N.F. content, especially in the case of cows giving low-quality milk.

The effect of changes of feeding is often over-emphasised, probably due to ignorance of the effects of associated factors, and it is easy to attribute results to the feed. Mackintosh [28] summarises the situation as follows :

(*a*) Continuous underfeeding produces less milk with slightly less fat than cows would yield under adequate feeding.

(*b*) Continuous overfeeding improves condition and maintains yield, but does not cause high fat-percentages. An increase of protein in the diet may temporarily increase the fat-percentage. Reducing a high ration to a standard amount will also cause a temporary increase in fat.

(*c*) *A change to pasture* means an increase in digestible protein, exercise and a more laxative diet. Autumn calvers may show an increase in fat-percentage in May, whilst spring calvers may show a decrease in fat-percentage.

(*d*) *The feeding of special oils and fats*, such as soya bean, linseed, coconut, cottonseed and palm-nut oil, will sometimes cause temporary increases. Such increases may be due to abnormal feeding. Some fats decrease the percentage of fat. Golding *et al.*[42] found that 6–8 oz. of cod-liver oil given daily depressed the fat-percentage, and that the depression continued for six weeks.

The preparation of the cow in good condition before calving, and adequate feeding after calving, cause most cows to give more and richer milk for a few months than if the cows are calved in a lean or low condition.

(I) *The Effect of Disease.* Milk is usually altered in composition by digestive ailments and by diseases responsible for a fall in yield (Bergema [43]). The lactose content is usually appreciably decreased with a consequent increase in chloride and ash content. Fat generally is more likely to be increased than diminished. Albumin is likely to be increased, casein to be lowered, and total protein to remain constant. In general, the expected level of amounts of special milk constituents in the solids-not-fat portion (casein and lactose) is lowered.

Koestler and Elser [44] have observed a diminution in yield through the effects of *foot and mouth disease*, but the influence on

milk quality depends on whether the udder is inflamed or not. If not inflamed, there is a moderate change in composition consisting of increased fat, protein and ash content and a decreased lactose content. The fat may rise to 10–15 per cent. and the protein to 4–5 per cent., whilst the lactose decreases to 3–4 per cent., but rarely under 3 per cent. The freezing-points of these samples were normal, casein was higher, and albumin much higher than in normal milk, whilst the chloride content was uniformly increased.

In the case of *mastitis*, the casein and fat contents are markedly reduced, the lactose content is reduced considerably, and chlorides are increased enormously (100–200 per cent.). The Koestler number, $\dfrac{(100 \times \text{Cl per cent.})}{(\text{Lactose per cent.})}$, also rises to 3 and above.

Changes occurring in the composition of milk when a cow dries off follow the same tendency as those experienced by a cow with foot and mouth disease without udder infection.

Davies [16] has found that the milk of some cows consistently giving milk of low non-fatty-solid content is similar in composition to that from cows suffering from mastitis, although there are no indications of the udder being inflamed. Such milk is much less uniform in composition when consecutive weekly samples are analysed, the difference being mainly reflected in the lactose and chloride content. Normal milk, on the other hand, is remarkably uniform in composition when judged on the analyses of consecutive weekly samples.

It can thus be stated that the major part of the effect of disease on the composition of milk is to decrease the lactose content and increase the chloride content, leaving unchanged the depression of freezing-point. The lowering of the solids-not-fat below the presumptive standard by disease can easily be distinguished from watered milk by carrying out determinations of chloride and freezing-point.

(J) *Hormonal Control of Lactation.* The effect of administering thyroxine to lactating cows on the composition of milk has been studied by Graham [50] and Folley.[51] Graham found that thyroxine causes a marked increase in the production of milk-fat. Folley found that daily subcutaneous injections of thyroxine (0·2 mg. per kg.) into cows in declining lactation caused a marked increase in milk secretion and in production of milk-fat and non-fatty solids ; the treatment also increased the percentage of these constituents in the milk.

(K) *Abnormal Conditions.* Exceptional conditions, such as are likely to excite or alarm cows, probably have a marked effect on

the fat-content of milk. Cows, for instance, which normally give high fat-percentages will, under showyard conditions, give milk of low fat-content. In 1922, out of 109 cows entered for the milking trials of the Royal Agricultural Society of England's Show, 7 were disqualified for having below 3 per cent. of fat in their milk. The corresponding figures for 1923, 1924 and 1925 were 92 (13), 83 (16) and 123 (30) respectively. Table XVII shows

TABLE XVII. *Influence of Showyard Conditions on Fat-Percentage of Milk yielded from Various Exhibit Breeds*

Breed	No. of Animals	No. of Animals yielding Milk below 3% Fat	Percentage
Guernsey	116	1	0·9
Jersey	193	2	1·04
Ayrshire	90	6	6·7
Kerry	84	9	10 7
Non-pedig. Shorthorn	99	11	11·1
Red Polls	152	24	15 8
Pedigree Shorthorns	230	41	17·8
Lincoln Red	86	26	30 2
British Friesian	165	57	34 5

similar results compiled from the figures from the Milking Trials, 1920–25, of the British Dairy Farmers' Association Annual Show.

Sudden changes of temperature [45] and the employment of inexpert milkers will also cause a lowering of the fat-content of milk. There is also evidence that even a change of expert milkers affects the composition.

During periods of sexual excitement, milk may show abnormal fluctuations of quantity and composition. The fat-content is decidedly above the average for the two or three days immediately preceding the period of active sexual excitement, but the milk obtained in the first milking after the commencement of the awkward manifestations is generally very low in quantity and poor in fat, while at the next milking the quantity of milk and the percentage of fat are usually high.

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CHAPTER III

THE COMPOSITION OF MILK (*continued*)
SPECIAL VARIATIONS

12. Day-to-day Variations

THE study of the possibility of day-to-day variations in milk composition is of importance in connection with the practice of taking " appeal-to-cow " samples at farms in order to determine whether the original sample was adulterated or naturally poor in quality. There is sufficient evidence to show that daily variations in fat and solids-not-fat are of considerable magnitude.

Buckley (quoted in [1]) has found the following variations (Table XVIII) in the fat-content of the milk of one cow in the Moundsmere herd, whilst Tocher [2] quotes also the following variations in the solids-not-fat content of the milk of one Ayrshire cow.

TABLE XVIII. *Daily Variation in Fat (Buckley) and Solids-not-fat (Tocher) in Milk from Individual Cows*

Day		1	2	3	4	5	6	7	8
Fat per cent.	a.m.	2·5	3·5	3·0	1·9	2·3	3·0	2·5	2·8
	p.m.	3·1	6·6	5·4	4·0	6·2	4·1	4·6	6·3
S.N.F. per cent.	Day's Sample	8·2	8·3	8·2	8·4	8·6	8·3	8·7	8·4

Tocher (*loc. cit.*) also cites the case of the herd milk of twenty-four cows (examined for thirty-nine days) in which the mean values of solids-not-fat varied as much as 10 per cent. in one day. This variation, however, is far from being general. The tendency of milk to vary from day to day may be associated with the individuality of a particular cow or cows, but this will tend to vanish in the milk from a large herd, since it is not likely that simultaneous variation will occur in the same direction. But it is always possible that, *on a given day*, the variations of a large proportion of the herd may be in the same direction, a possibility which becomes more marked as the number in the herd decreases. Some ordinary market milk may be taken from a herd of not

more than eight cows and day-to-day variations may even be wider than those cited by Tocher.

In studying the performance of individual cows, it may generally be stated that a good producer of butter-fat is also a good producer of solids-not-fat. With herd milk, on the other hand, day-to-day analyses of milk from the same herd reveal that when fat is relatively high the solids-not-fat are relatively low, and that there is a tendency for a herd to give a constant amount of *total solids*.

13. Variation in Composition Obtained by Fractionating the Regular Intervals between Milking

The following observations on the composition and quantity of milk have been made on cows milked at two-hourly intervals after the morning milking (Mackintosh and Davies, unpublished data) :—

(*a*) The unaccustomed treatment of the cows at first may be partly responsible for the high chloride and low lactose contents of the first and second two-hour samples ; after a considerable period (two to four days) under this treatment the composition of the samples still tends in this direction. This points to the fat-free serum not being secreted as a liquid of constant composition during the fractions of the interval between milkings.

(*b*) The fat-contents of the first, second and third two-hourly samples after milking out in each case were, without exception, very high.

(*c*) The *flush* of milk was found to be produced after the second two-hour sampling, that is, approximately four hours after milking out in the morning.

(*d*) The solids-not-fat in the fat-free serum varied during the treatment, but tended to a maximum at the fourth two-hourly milking, that is, at the normal evening milking time.

(*e*) Reversion to the usual times of milking showed the composition of the milk at evening milking to be similar in composition to that after taking the consecutive two-hourly samples throughout the day.

(*f*) There was very little difference in the composition of the consecutive two-hourly samples, whether the udder was stripped at each milking or when samples (100 ml.) only were taken.

Table XIX gives the results for some of the days of a two-hourly milking experiment, together with the analyses of samples at normal milking times for three cows. (The non-fatty solids are calculated also on the basis of the fat-free serum to avoid the effect of the variable fat-content in studying their variation.)

No account of the investigations of the composition of milk abstracted at short intervals during the night or over a whole period, or consecutive periods, of twenty-four hours has been met with in the literature.

TABLE XIX. *Composition of Milk of Two-hour Sampling during the Day*

Time of Milking	Cow A			Cow B			Cow C		
	Fat %	S.N.F. %	F.F.S.* %	Fat %	S.N.F. %	F.F.S.* %	Fat %	S N.F. %	F.F.S.* %
Day 1 7.30 a.m.	2·60	9·36	9·61	3·85	9·31	9·68	2·65	9·45	9·71
10 a.m.	7·60	8·89	9·62	7·90	8·92	9·69	7·75	9·14	9·91
12 noon	8·05	8·53	9·28	7·05	8·83	9·50	5·95	8·81	9·37
2 p.m.	4·00	8·97	9·34	5·95	9·01	9·58	4·30	9·18	9·59
4.30 p.m.	2·45	9·38	9·62	4·35	9·32	9·73	3·45	9·68	10·02
Day 2 7.30 a.m.	2·60	9·26	9·51	2·60	9·19	9·43	2·45	9·36	9·60
10 a.m.	6·90	9·07	9·74	6·30	8·93	9·53	5·70	9·26	9·82
12 noon	5·90	8·82	9·37	6·35	8·71	9·30	4·10	9·26	9·66
2 p.m.	6·35	8·91	9·57	8·10	8·71	9·48	3·60	9·26	9·61
4.30 p.m.	4·00	9·26	9·63	5·85	9·04	9·60	3·55	9·85	10·21
Day 3 7.30 a.m.	2·85	9·26	9·53	2·05	9·20	9·39	2·65	9·22	9·47
10 a.m.	6·45	9·08	9·71	5·90	8·85	9·40	6·50	9·04	9·67
12 noon	3·50	9·12	9·45	5·00	8·97	9·44	5·00	9·17	9·65
2 p.m.	5·85	8·86	9·41	5·70	8·91	9·44	7·20	8·91	9·60
4.30 p.m.	4·90	9·25	9·73	3·95	9·43	9·82	4·05	9·78	10·20
Day 6 7.30 a.m.	3·00	9·24	9·53	2·65	9·37	9·63	4·35	9·31	9·73
10 a.m.	5·90	8·95	9·51	7·55	8·75	9·46	7·25	8 77	9·46
12 noon	5·25	9·24	9·79	6·70	9·38	10·06	4·00	9·82	10·23
2 p.m.	5·75	9·24	9·80	6·35	9·56	10·21	3·90	9·77	10·17
4.30 p.m.	4·80	9·33	9·82	6·20	9·38	10·00	3·30	9·75	10·08
Day 8 7.30 a.m.	3·60	8·93	9·26	3·45	9·23	9·56	4·95	9·03	9·50
4.30 p.m.	5·05	9·03	9·51	5·25	9·42	9·95	6·75	9·57	10·27

* F.F.S. = Solids in fat-free serum.

14. The Composition of Colostrum and of Milk during the Colostral Period

As stated in Section 1, the first secretion which can be drawn from the udder immediately after parturition is a thick

viscid fluid differing greatly in composition from that of the milk obtained a week after calving. This secretion—*colostrum* or *beestings*—is characterised by a higher proportion of albumin and globulin (the general, soluble proteins of the same nature as were provided for the fœtus *in utero* by the maternal blood-stream), a higher percentage of ash and chloride and a lower lactose content. The change from colostrum to milk which takes place during the first few days after calving must of necessity be a gradual one. Engel and Schlag [3] have studied the composition of colostrum and its gradual change in composition over a period of seven days (Table XX).

TABLE XX. *Composition of Colostrum and its Change of Composition during the Seven Days after Calving*

Time after Calving Hrs	Spec. Grav.	Chloride (NaCl) %	Acidity N/9 (Ml. Alk. per 10 ml.) °C.	Freezing-point °C.	Total Protein %	Casein %	Albumin and Globulin %	Lactose %	Fat %	Ash %	Water %	Coagulation on boiling
Immediately	1·067	0·153	4·14	−0·605	17·57	5·08	11·34	2·19	5·10	1·01	73·01	+
6	1·044	0·163	3·24	−0·555	10·00	3·51	6·30	2·71	6·85	0·91	79·54	+
12	1·037	0·156	2·52	−0·500	6·05	3·00	2·90	3·71	3·80	0·69	85·47	+
24	1·034	0·156	2·43	0·575	4·52	2·70	1·48	3·98	3·40	0·80	87·23	+
30	1·032	0·152	2·21	−0·570	4·01	2·56	1·20	4·27	1·99	0·53	86·37	+
36	1·032	0·100	2·25	−0·570	3·95	2·77	1·03	3·97	3·55	0·81	87·78	+
48	1·032	0·149	2·16	−0·550	3·71	2·63	0·99	3·97	2·80	0·83	88·56	+
72	1·033	0·137	2·25	−0·575	3·56	2·70	0·97	4·37	3·10	0·84	88·14	−
96	1·034	0·135	2·07	−0·555	3·76	2·65	0·82	4·72	2·80	0·83	88·15	−
120	1·033	0·131	1·91	−0·575	3·86	2·68	0·87	4·76	3·75	0·85	87·33	−−
168	1·032	0·113	2·02	0·570	3·31	2·42	0·60	4·96	3·45	0·84	87·87	−−−

From Table XX it can be observed that chloride, total nitrogen, casein, albumin plus globulin, ash, dry matter and specific gravity fall regularly during the progressive change into milk. The titratable acidity also falls, possibly owing to a lower concentration of protein and mineral salts of considerable buffer value. The lactose content is variable whilst the fat is irregular. The freezing-point remains practically constant.

The variation in protein composition can best be demonstrated by the findings of Crowther and Raistrick [4] (Table XXI).

The most striking difference between colostrum and milk is the high globulin content. This protein causes the milk to coagulate on boiling up to the third day after calving, but there is no doubt that a lower heat stability during the latter part of this period also influences the appearance of the coagulum on boiling. Colostrum is coagulated by acetic acid and mercuric chloride; rennet coagulation occurs at a slower rate than with milk.

TABLE XXI. *Distribution of Nitrogen in Colostrum. Percentage of Whole Milk. Crowther and Raistrick*

Milking	Total Nitrogen	Casein Nitrogen	Albumin Nitrogen	Globulin Nitrogen	Non-protein Nitrogen
1	2·40	0·75	0·14	1·32	0·19
2	2·01	0·68	0·17	1·02	0·14
3	1·44	0·59	0·14	0·59	0·12
4	0·97	0·51	0·11	0·31	0·04
5	0·76	0·46	0·07	0·20	0·03
6	0·75	0·46	0·06	0·20	0·03
7	0·69	0·42	0·06	0·18	0·03
8	0·65	0·46	0·05	0·12	0·02

Fleischmann [5] gives the following composition for the ash of colostrum (Table XXII):

TABLE XXII. *Composition of the Ash of Colostrum. Percentages of Ash*

Constituent .	K_2O	Na_2O	CaO	MgO	Fe_2O_3	P_2O_5	Cl	SO_3
Percentage .	7·23	5·72	34·85	2·06	0·52	41·43	11·25	0·16

The iron content of colostrum is approximately seventeen times that of normal milk. This and the high proline content of the globulin are significant from the aspect of the production of hæmoglobin in the rapidly-growing newly-born animal.

All mammals secrete colostrum and its significance will be dealt with in a later chapter (see Section 185).

15. Other Secretions

A secretion can usually be drawn from the udder for some time before parturition, whilst many cases of pre-lactation occur or can be initiated by udder manipulation. In these cases the early milk drawn is of the same nature as colostrum.[6] The secretion drawn from the udder of pregnant heifers has been examined by Woodman and Hammond,[7] and it is interesting to compare them with the secretions obtained from the udders of non-pregnant cows which have been dry from two to three months (Table XXIII).

Globulin was found present in appreciable amounts in every secretion analysed. It is known that this protein is derived unchanged from the blood-stream and that it occurs only in traces in normal milk. It seems that when the synthetic pro-

TABLE XXIII. *Composition of Secretions from Udders of Pregnant Heifers and of Dry Non-pregnant Cows (Woodman and Hammond).*

Heifer No.	1	2	3	4	Cow " Dry "	
					2 mths.	3 mths
Weeks Pregnant	15	22	28	—		
Amt. of Secretion (ml.)	45	100	350	80	—	—
Sp. gr.	1·026	1·110	1·030	1·060	1·026	1·015
Total solids %	10·41	40·80	15·63	24·68	10·95	6·16
Ash %	0·72	0·80	0·77	0·50	0·87	0·73
Fat %	0·12	1·50	4·76	2·66	0·28	0·18
Total protein %	8·07	37·90	7·61	20·31	9·30	4·54
Casein %	2·36	—	4·02	2·58	2·58	1·57
Globulin %	3·44	34·50	2·53	14·75	3·00	1·11
Albumin %	1·61	1·00	0·49	1·57	2·30	1·08
Non-protein %	0·66	1·90	0·57	1·41	1·42	0·78
Lactose %	1·19	Trace	2·43	0·70	0·48	0·64

cesses of the mammary glands are operating at their maximum capacity, there is either an inhibition of the transference of globulin from the blood-stream, or that the great volume of milk secreted during lactation dilutes the globulin to such an extent that only small quantities appear in the milk. The appearance of globulin in considerable amount in a mammary secretion is undoubtedly associated with an abnormal functioning of the mammary glands such as occurs before and immediately after parturition, when " drying off " is advanced or when the udder is inflamed.

16. The Problem of Low Solids-not-fat. " *Isotonic Diluent* "

The possibility of the content of solids-not-fat falling below the presumptive standard of 8·5 per cent. has already been discussed in Sections 9, 11, 12. The results of Lesser's [8] investigations provide a striking example of persistently low solids-not-fat occurring during winter conditions. Lesser investigated the proximate composition of the samples only, but Davies [9] has made more detailed analyses of some representative samples. The result of these investigations may be summarised as follows :

(*a*) The constituent mostly responsible for the deficiency in solids-not-fat is *lactose*. It has invariably been found that samples low in quality are always high in chloride content, and that samples high in solids-not-fat are low in chloride content. By

using the approximate relationship between the lactose and the chloride content of milk adopted by Mathieu and Ferré [10] (Davies [11]) (lactose per cent. $= 7 \cdot 0 - 19 \cdot 6 \times Cl$ per cent.), a rough value of the lactose content can be gauged from an easily determinable chloride content. The chloride content of milk may thus be regarded as a valuable " single value " property of milk, from which a picture of the nature of the S.N.F. of milk can be obtained. An appreciable variation in the chloride content of consecutive weekly samples from cows giving milk with low solids-not-fat has been observed ; the percentage of S.N.F. varies directly with the lactose content. No such variation has been observed in high-quality samples, which are extremely regular in chloride content. It is obvious that the maintenance of the osmotic pressure of the milk is effected by the substitution of ionised chlorides for lactose, and that the lowness in S.N.F. is reflected in the fact that from osmotic considerations 1 molecule of common salt (2 ions) is isotonic with 2 molecules of lactose, which weigh $11 \cdot 7$ times that of a molecule of the salt.

(b) *The protein distribution* in milk low in S.N.F. differs somewhat from that of good quality or normal milk, and is also variable in consecutive weekly samples from the same cows. The main differences, apart from a low total nitrogen content generally, are (i) a low casein content, (ii) a high albumin and globulin content, the distribution of these two proteins being also variable, and (iii) a high non-protein nitrogen content. That is, the character of the nitrogen distribution tends to revert to that of the abnormal secretions mentioned in the last Section (15). The yields in most cases are normal and even high, so that the above-mentioned question of globulin from the blood-stream being diluted by a flow of milk does not appear to be probable.

In this connection, on reviewing the literature concerning the percentage of the total nitrogen of milk accounted for as casein nitrogen, it has been observed on a large number of samples of *normal* milk (as inferred from its regularity of composition) that casein nitrogen accounts roughly for 76 per cent.[9] of the total.

Analysis of the results for 148 samples of morning milk from a typical herd of Shorthorn cows [12] has shown that casein nitrogen is 76·5 (SD, 2·16) per cent. of the total nitrogen. The results of sixty-one other samples examined by Davies (unpublished work) gives the figure 76·7 (SD, 1·09) per cent. Tocher [13] also finds that 76·1 per cent. of the total nitrogen is casein nitrogen (mean of 474 samples). The uniformity of such results, obtained by different investigators, warrants the use of this figure (76 per cent.) as a rough standard of composition for normal milk.

In samples low in S.N.F., the percentage of casein nitrogen falls below this figure.   The results of the analyses of a number of samples of milk have been examined further with a view to determining what portion of each sample analysed could be regarded as " *normal milk* " containing 76 per cent. of its nitrogen as casein nitrogen.   This was done from the casein nitrogen figures.   The remaining fraction was regarded as the " *diluting fraction*," and since all milk samples studied up to the present have been found to have the same freezing-point as

TABLE XXIV.   *Composition of the " Isotonic Diluent " in some Samples of Milk of Low S.N.F.   Davies.*

Cow	A			B	C	D	X	E
Dilution %	28·3	24·4	28·3	18·0	13·0	21·2	15·3	8·9
% Total N .	0·414	0·421	0·430	0·437	0·499	0·421	0·443	0·444
Protein N .	0·271	0·287	0·286	0·238	0·398	0·321	0·264	0·363
Albumin N	0·081	0·209	0·247	0·238	0·108	0·021	0·045	0·363
Globulin N	0·191	0·076	0·039		0·290	0·300	0·219	
Non-protein N . .	0·143	0·135	0·148	0·199	0·101	0·100	0·179	0·081
In whole milk sample % S.N.F. .	6·08	6·51	6·56	6·88	7·77	7·82	7·71	8·08
Cl . .	0·218	0·204	0·197	0·176	0·154	0·130	0·108	0·134

normal milk (Golding [14]), this fraction has been termed the " *isotonic diluent*."

Other generalisations have been arrived at with regard to the amounts of other nitrogenous products in milk.   Thus *protein nitrogen* has been found to account for 94 per cent. of the total nitrogen, whilst the percentages of *albumin* and *globulin* nitrogen have on the average accounted for 12 and 6 per cent. respectively.[9]   With this information in hand, the amount of the nitrogen in the " normal milk " fraction has been calculated for each group in the distribution, and, by subtraction from the analytical results for the whole sample, the nitrogen allotted to the

diluent has been obtained by difference.   These results have then to be converted into percentages of the diluent, and by this means the composition of the " isotonic diluent," irrespective of origin, has been compared for as many analysed samples as are available.

This has been carried out by Davies [15] for thirty-eight samples with surprising results as to the total nitrogen content and nitrogen distribution found for the diluent.   Table XXIV shows some of the results obtained.

The average total nitrogen content of the diluent (thirty-eight samples) was 0·458 (SD = 0·036) per cent.

By plotting the chloride content of each sample against the
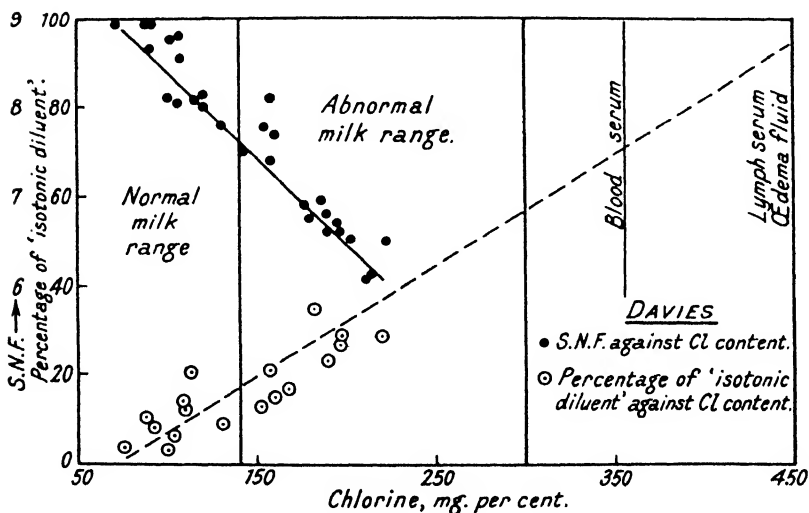
FIG. 9.—Trend of percentage of solids-not-fat and of isotonic diluent with the chloride content of milk.

dilution as calculated from the figures for casein nitrogen, the extrapolated value for 100 per cent. dilution was 0·360, which approximates closely the chlorine content of a hypothetical lactose-free milk calculated according to the formula of Mathieu and Ferré[10] (0·355 per cent.).   The total nitrogen and chlorine contents of blood serum are 0·440–0·550 and 0·355 per cent. respectively, whilst the chloride content of lymph serum is higher (0·365 per cent., 0·450 in lymph).[16]   (See Fig. 9.)

The diluent seems to be related in composition to either blood or lymph serum, and probably more to the latter, owing to its lower content of albumin and globulin.   One may therefore infer that the secretion of milk low in solids-not-fat of this nature is a

reversion in part to the type of secretion during the aforementioned periods of " drying off," or immediately before and after parturition.

It is noteworthy that the same changes are experienced in the composition of milk during cases of udder infection such as *mastitis*. Under such conditions (as mentioned in Section 11(I)), together with a drop in yield, a milk high in chloride and low in lactose is secreted, while the casein content is lowered and the albumin and globulin, especially the latter, are increased. In some cases, a thick liquid somewhat similar in composition to colostrum may be drawn from the udder.[17] The explanation of this change in composition is that the inflamed membranes secrete comparatively large quantities of a nitrogen-containing exudate rich in chlorides. The efficiency of the mammary gland is considerably lowered in respect of the elaboration of the special milk constituents, casein and lactose, and the solids-not-fat suffer by the high chloride content, since the secretion must of necessity be of the same osmotic pressure as normal milk.

After an attack of mastitis, the membranes of the udder might recover from the purulent condition but still remain in a quasipathological or catarrhal condition, when considerable quantities of a liquid rich in chlorides and unelaborated nitrogen compounds would be secreted as a " *transudate*." This would result in a continuation of giving milk low in S.N.F. due to a chronic slightly diseased fraction of the effective secretory area inside the udder. While it may be said that all cases of low S.N.F. are not due to the after-effects of mastitis, it must be pointed out that the particular trend taken by milk composition under conditions of secreting low S.N.F., where past history does not reveal the occurrence of such udder-infection, is similar to that where infection has occurred. There are no physiological or histological data available at the present time to support the theory that some of the secretory areas are not functioning properly in elaborating the essential milk constituents to their expected level.

The problem of low solids-not-fat content in milk seems to be largely a physiological one. Lesser [8] found no improvement in the low level of S.N.F. in the herd which he examined by feeding plenty of green matter, and cod-liver oil and malt extract as supplements to generous balanced rations, so that the fault was not due to faulty nutrition. On the other hand, a distinct improvement was experienced when the cows were put out on spring grass, when a number of factors may be taken to operate together in improving the performance of the cows. One of the chief of these factors is undoubtedly the digestible protein content of

young grass, while other nutritive factors, free range, congenial temperature and a general toning up of the system influence milk secretion in the same direction. A reversion to nearly the same level was experienced early in the following autumn and persisted through the winter (Lesser, unpublished work).

17. Legal Considerations of the Quality of Milk

The possibility of milk falling below the legal standards of 3 per cent. fat and 8·5 per cent. S.N.F. has been discussed in Sections 9, 11 and 12, but it still remains that such samples are taken to be *adulterated* unless the contrary is proved. That is, such samples are regarded as either having a portion of the fat abstracted or as containing added water.

The sale of milk is regulated in this country by a series of Acts passed between 1875 and 1928 and various Regulations made by Government Departments under these Acts. The last Act was the Foods and Drugs (Adulteration) Act, 1928. It is interesting to note that no definition of milk has been attempted by legislative authorities ; the Geneva Congress has defined it as " the integral product of entire and uninterrupted milking of the female milk cow in good health and well nourished and not overworked. It ought to be collected in the proper manner and contain no colostrum." *

A table giving the milk standards in many parts of the world is given in the Minutes of Evidence of the 1901 Interdepartmental Committee (Cmd. 484, p. 434).

The essence of English law for the sale of milk is that the genuine article as it comes from the cow be offered for sale, so that, in principle, no standard of composition is implied. But the composition varies so much that it is impossible to decide with certainty whether a given sample is genuine or not, and analysts generally are disposed to classify as abnormal or adulterated any sample which differs in composition from most of the samples examined. The conception of the analyst is that milk of low quality should not be offered for sale, such milk as an article of food being equivalent to adulterated milk. The findings of an analyst may form the grounds for a *criminal* prosecution, amount-

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* According to the Federal Standard of the United States, " milk is the fresh, clean lacteal secretion obtained by the complete milking of one or more healthy cows, properly fed and kept, excluding that obtained within 15 days before and 10 days after calving, and containing not less than eight and one half (8·5) per cent. of solids-not-fat, and not less than three and one quarter (3·25) per cent. of milk fat." The standards prescribed in the United States vary according to the State ; thus it is 2·4 per cent. of fat in Wyoming and 4 per cent. in Vermont but the great majority have adopted a 3 per cent. standard.[18]

ing to a case of *fraud*. Again, the fact that milk must be sold fresh, before the vendor usually has an opportunity to analyse his material, amounts to a condition of hardship, since he is constantly open to being proceeded against for a criminal offence through circumstances out of his control. Although the legal requirements of milk composition appear quite simple, the matter has raised acute discussion and argument in many court cases.

On the other hand, there is no doubt that milk offers an opportunity for adulteration by unscrupulous vendors in the direction of adding water or separated milk, or skimming off of some of the cream, and that legislation is necessary " to protect the many from the unlawful practices of the few." It would appear that two important directions in which the law should be revised are : (*a*) the selling of milk below standard should be regarded, not as a *criminal*, but a *civil* offence, in which case the supplying of milk below specification could be regarded as a breach of contract ; and (*b*) methods of analysis which are known to yield unimpeachable evidence of the difference between watered milk and genuine milk low in solids-not-fat should be recognised. In the latter case the recognition of the freezing-point method for detecting added water is essential. Such tests are recognised in the Dominions, on the continent of Europe, and in the U.S.A.

18. Factors in the Handling of Milk which may Cause Low Quality

An analysis by Messrs. Bibby of milk-prosecution cases has revealed that one-third of the total number is due to inadequate bulking of the milk of the herd. Thus there is a tendency to milk the heavy milkers first, so that the first churns under the cooler may be of poorer quality. This difficulty may be surmounted by using an " equaliser " under the cooler, and by managing the milking so that the produce of all types of cows is mixed as uniformly as possible. When milk is bottled on the farm the variations in composition are still more apparent, and can only be overcome by bulking to a considerable volume before bottling. The value of recording the yields of individual cows to help in overcoming these practical difficulties is at once apparent.

There is usually a considerable difference between the fat-content of morning and evening milk, sometimes amounting to I per cent. (see Table XII, p. 26). Whenever possible, the morning and evening milk should be bulked, since this will overcome the occasional occurrence of the fat being below standard in morning milk. Although this may not usually be practicable, owing to the exigencies of trade and transport requirements, it is

certain that the more that herd milk is bulked the smaller is the chance of it falling below standard.

The rising of cream is another factor to be contended with, and, after a period of settling, all milk should be agitated either by plunging or other methods of stirring until a satisfactory homogeneous fluid is obtained. This procedure is important for milk tapped off from the bottoms of churns for retail. During the processing of milk the volumes of milk and the necessary agitation suffice to give a product usually well above the standards of composition, although an appreciable percentage (7–8 per cent.) of the milk in churns may be below the standards (see Table IV, p. 18). (In Messrs. Bibby's analysis of cases one-third of the prosecutions were due to the vendor neglecting to mix the milk before selling.)

19. The Usual Methods of Calculating the Amount of Adulteration

The usual formulæ based on the " law of mixtures " are used for calculating the amount of added water or abstracted fat, the basis of 3 per cent. fat and 8·5 per cent. S.N.F. being adopted as the standards. Thus :

(1) Minimum per cent. fat abstracted $= \left(\dfrac{3 - \text{Fat per cent.}}{3} \right)$ $\times$ 100

(2) Minimum per cent. added water $= \left(\dfrac{8 \cdot 5 - \text{S.N.F. per cent.}}{8 \cdot 5} \right)$ $\times$ 100

The use of such formulæ is not justifiable in the case of genuine milk below the standard in composition, but is applicable to adulterated samples which contain at least 3 per cent. fat and 8·5 per cent. S.N.F. before adulteration.

20. Attempts at Correlating the Amounts of certain Milk Constituents

(*a*) *Casein and Fat.* Van Slyke [19] states that " to find the percentage of casein in milk when the percentage of fat is known, subtract 3 from the percentage of fat in milk, multiply by 0·4 and add this result to 2·1." This rule is clearly applicable only to milk containing 3 per cent. of fat and over. The rule has been found to be approximate for 80 per cent. of the samples studied (which were bulk samples for cheese-making). Casein *plus* albumin can be calculated by adding 2·9 instead of 2·1. The rule has been found not to hold for the milk of many individual cows or for the milk of cows in advanced lactation.

(b) *Vieth's Ratio.* Vieth gives the average proportion of lactose, protein and ash as 13 : 9 : 2. Richmond's [20] findings agree almost exactly with this ratio, his ratio being 12·7 : 9·1 : 2. The ratio holds best for bulked herd milk, but individual samples may show great divergencies.

(c) *Ash and Protein.* The formula, ash $= 0.36 + 0.11 \times$ protein, holds fairly accurately for bulked milk. Sherman [20] finds that the formula, ash $= 0.38 + 0.10 \times$ protein, agrees most closely with the results. That the latter is more accurate is borne out by Tocher's figures [2] (ash $= 0.38 + 0.10 \times 3.19 = 0.699$ (found 0·691)).

(d) *Chlorides and Lactose.* These two constituents account for roughly 75 per cent. of the osmotic pressure of milk, and many attempts have been made to link up their concentrations in milk on a complementary basis, *i.e.*, the variations in chloride and lactose content follow a linear law.

Kopatschek [21] states that the lactose-plus-chloride content multiplied by 44 is a constant, and that samples of milk giving a constant below this value may be suspected of being adulterated.

Bouin [22] states that the lactose is five times the weight of the ash, with remarkable regularity throughout the lactation but not in the colostral period. Lactose $= 6.6$ times ash is more applicable to normal milk.

Koestler [23] has suggested that the value $100 \wedge \%\text{Cl} \div \%$ lactose (*the Koestler number*) is of value in diagnosing milk from cows with diseased udders or otherwise giving abnormal milk. The value for normal milk is usually less than 2, but milk samples giving values above 3 may be regarded as abnormal. This latter value applies to milk containing 0·14 per cent. Cl and above.

Mathieu and Ferré [10] have suggested that the lactose (grams per litre) plus the lactose equivalent of the chlorides (as NaCl) (NaCl $\times$ 11·9) in grams per litre, does not fall below 70. They have termed this figure the *constant moléculaire simplifiée* (C.M.S.). When values below 70 are given, the samples are suspected of containing added water. Owing to variation in fat-content the values have later been calculated on the fat-free serum basis, the minimum then being 75.[24] This relation between lactose and chloride has been much discussed on the Continent for the past twenty years as a means of detecting added water. When it is considered that the addition of 15 per cent. water lowers the C.M.S. from 75 to 64, the method merits some commendation. As in other directions, the greatest difficulty is to fix the minimum value for the C.M.S.

It has already been pointed out that the relationship C.M.S.

$70 =$ Lactose (g.p.l.) $+ 11\cdot9 \times$ NaCl (g.p.l.), or, in its simplified form, per cent. Lactose $+ 19\cdot6$ Cl (per cent.) $= 7$, is useful for calculating the lactose content from the easily-determinable chloride content. On analysis, the above expression reveals that a hypothetical chloride-free milk would have 7 per cent. lactose and a lactose-free milk $0\cdot355$ per cent. chloride : the lactose content drops by 1 per cent. from 7 for each $0\cdot051$ per cent. chloride content. It is interesting to note that the chloride content of lactose-free milk (hypothetical) approximates that of blood serum.

Sundberg [25] arrives at the equation : lactose $= 7\cdot07 - 18$ Cl (per cent.), in which case $0\cdot392$ per cent. would be the chloride content of a lactose-free milk and $7\cdot07$ the lactose content of a hypothetical chlorine-free milk. Davies (unpublished work), calculating from the lactose and chloride contents of a large number of samples of variable composition, finds that the formula : Lactose (per cent.) $= 6\cdot26 - 13\cdot5$ Cl (per cent.), is representative of the lactose-chloride relationship for the amounts generally found present in milk.

Post [26] has suggested that the chlorides and lactose in milk account for nearly 80 per cent. of the osmotic pressure, and on the assumption that the freezing-point of milk is $- 0\cdot540°$ C., has suggested that the *cryolac number* ($1,000 \times$ depression of freezing-point due to lactose, chlorides and acidity) is useful for detecting added water. The cryolac number for genuine milk has been found to vary mostly between 410 and 430, so that the difficulty of setting the standard for comparison, especially necessary for the detection of from 5 to 10 per cent. of added water, has been experienced here also.

21. The Composition of Separated and Skim Milk

Skim milk is that obtained by settling milk so that creaming by gravity occurs. The separation of the fat from the lower layers is not complete, and there is no separation of solid impurities, accidental foreign matter or cells.

Separated milk is the product (along with cream and separator slime) of passing warm milk through a cream separator. This process consists of subjecting the milk to a centrifugal force which multiplies the gravity effect to such an extent that the cream separates very quickly, and quick separation is possible with a constant flow of milk. Solid and mucoid particles are centrifuged out of the milk at the same time and form a slime on the outer wall of the " bowl." A similar slime is obtained in the bowl

of the " clarifier," which is sometimes used as a step in the pasteurising process of milk.

The difference in composition between skim and separated milk lies mainly in the fat-content. Skim milk may contain above 0·5 per cent. of fat, whilst well-separated milk contains only about 0·05 per cent., and should not be above 0·3 per cent. The amount of fat in the separated milk depends on the control of the separator and on physical factors connected with the fat globules, such as size-distribution and rate of creaming. The average content of solids-not-fat is naturally appreciably higher than in the whole milk ; for example, if the original milk contained 3·5 per cent. of fat, the S.N.F. which was previously present in 100 parts is now present in 96·5 parts.

Separated milk is regarded commercially as a cheap article, since the removal of cream has depreciated its value both for potable and manufacturing purposes. Some cheese, mostly intended for the manufacture of processed cheese, is made from it. The manufacture of recognised brands of skim or separated milk cheese, such as Dorset Cheese, is not carried on extensively at the present time.

During the separating of milk a viscous *slime* of a dirty white to a distinctly brown colour forms on the walls of the bowl. This slime is composed of separated milk, dust and soil gathered during milking and transport, vegetable matter such as hay and other fodder material (dung and hay-dust also), hair from the cow and epithelial and gland cells from the udder. Milk from diseased cows would give mucus, blood and pus cells, and bacteria (*e.g.*, *M. tuberculosum*) in the slime. The differences in the amounts of certain materials, *e.g.*, enzymes, in separator slime and milk are often used as evidence for the accidental occurrence of some of them in milk.

Table XXV gives the composition of some samples of separator slime.

TABLE XXV. *Composition of Separator Slime (Percentages)*

	(20) 1	(5) 2	(20) 3	General
Water . . .	66·24	67·3	72·3	70
Fat . . .	0·50	1·1	3·1	1
Casein (or Proteins) .	22·0	25·9	18·1	20—25
Lactose . . .	0·5	} 2·1	4·0	2—8
Other organic matter .	7·75			
Ash . . .	3·01	3·6	2·5	3

22. The Composition of Milk Serum

" Milk serum " is the name of the clear liquid obtained from milk either by freeing it from casein and fat with acetic or lactic acid—the latter causing spontaneous coagulation during lactic fermentation—or by reagents which precipitate all the protein together with the fat. Such liquids are utilised for the measurement of some physical properties of milk, such as osmotic pressure, refractivity, optical rotation and refractive index. The last property has been used intensively for detecting the presence of added water. In this case precipitating agents are used which do not interfere with the subsequent determination of the physical property. This is usually effected by using such a strength of solution of precipitant as exhibits the same refractivity as the serum of normal milk. The two precipitants most generally used are copper sulphate and calcium chloride solutions.

Osmotic pressure is usually determined, without freeing milk from colloid material, by determining the freezing-point. Van der Laar [27] observed that the depression of the freezing-point in the blood and in the milk of the cow was the same irrespective of the composition of the blood or the milk or of a diseased condition of the udder. Many investigators [28] have studied the freezing-point of milk, and the range −0·529° to −0·569° C. has been found, with the majority of the results falling between −0·540 and −0·550° C. This property appears to be one of the most constant physical properties of milk, and it has already been pointed out (Section 17) that it is the most valuable test for the detection of added water.

There is no *a priori* reason why milk and blood should have the same osmotic pressure. The fact that these two fluids must be in dynamic equilibrium on different sides of a complex system of membranes does not necessarily mean the setting up of an equal concentration of ions and molecules on each side. Such an isotonic condition does not obtain with other secretory or excretory fluids in the animal body, *e.g.*, gastric juice, cerebrospinal fluid and urine. On the other hand, amniotic fluid is isotonic with milk, whereas allantoic fluid has a higher osmotic pressure. This isotonicity, it seems, is partly explicable on phylogenetic grounds and on the natural requirement of feeding the newly-born animal *per os* of a fluid isotonic with (*a*) the nutrient fluid it was bathed in *in utero*, and (*b*) maternal blood. It is also obvious that the mechanism of milk secretion has naturally been made as simple as possible, and in a way involving the least expenditure of energy if viewed on a thermodynamic

basis, for the mother.   On phylogenetic grounds also it would be interesting to compare the osmotic pressures of *sweat* and milk.

Milk from different quarters of a cow's udder might differ considerably in lactose content and in electrical conductivity, owing to variable chloride content.   Jackson and Rothera [29] have found that the variation in chlorine and lactose is always reciprocal ; this is well illustrated by the types of milk given during the gradual recovery of one or more affected quarters of the udder.

Staub [30] has shown that the freezing-point depression is due to three main classes of compounds : lactose, chlorides and residual substances ; the last group, which consists of citrates and phosphates in true solution, contributes uniformly $0.14°$.   The remaining $0.41°$ is accounted for by chlorides and lactose (compare Post's *cryolac number*, equivalent to $0.425°$).   In colostrum, or in milk from diseased udders, the residuals account for 0.16–$0.20°$, which may be accounted for by a higher non-protein nitrogen content, with the result that the reciprocity of the contents of chlorine and lactose, and hence the " cryolac number," will be slightly affected in such cases.

Table XXVI gives a comparison of the composition of milk serum and blood serum.

TABLE XXVI.   *Composition of Milk Serum and Blood Serum* (*Parts per Thousand*)

	Milk Serum [31]*	Blood Serum [32]
Na_2O	0.77	4.314
K_2O	1.49	0.258
CaO	0.651	0.115
MgO	0.133	0.043
Cl.	0.81	3.686
P_2O_5 (inorg.) . . .	1.42	0.073
Citric acid . . .	2.37	—
Lactose	57.5	—
Dextrose . . .	—	1.035

* Calculated from elemental composition for purposes of comparison.

The results for milk serum were obtained on the liquid obtained for filtration through a porcelain filter, which is the only method of obtaining a serum that preserves its composition as in milk. In both sera it will be observed that the basic elements are in excess, and that in the whole liquids they are undoubtedly combined with

the proteins, since the isoelectric points of all the proteins found in blood and milk are on the acid side of neutrality. In blood serum 25–30 per cent. of the calcium is non-diffusible,[33] but all the inorganic phosphorus is diffusible. Citrates account for some of the basic elements of milk. The sodium, potassium, chlorine, and citrates of milk are totally diffusible, being in true solution in the serum, whilst inorganic phosphorus and calcium and magnesium are partly in solution and partly in chemical or physical combination with the suspended material.[31] The acidity of fresh milk must be largely due to *soluble* acid phosphates, since the titration values of sera are roughly the same as for the milk from which they are separated.

All inorganic phosphorus and the basic elements are dissolved in acetic acid sera and also in trichloracetic acid sera. All the elements are in solution at pH 4·6, if the milk is first brought well to the acid side of that point with acetic acid, and then brought back to the isoelectric point of casein and albumin with sodium acetate. The slow development of lactic acid by fermentation causes all cations to be in true solution at $pH < 4·6$.

23. Composition of some Freak Milks

(a) *Milk in Fœtal Breasts.* Halban [34] describes a puerperal involution in the mammary glands of the newly-born human embryo. It is well known that both sexes secrete milk when newly-born—often termed *sorcerer's milk*. The secretion appears to be due to a hormone not associated with the fœtus but due to placental tissue. Thus Freux [35] and Haterius [36] obtained slight lactational effects in guinea pigs and rabbits by feeding placental powders, and Philipp [37] obtained an increase in secretion by

TABLE XXVII. *Composition of Milk in Fœtal Breasts (Human)*

	Schlossberger	Gessner		
	%	1 %	2 %	3 %
Water	96·75	95·705	96·30	89·40
Total solids	3·25	4·295	3·70	10·60
Fat	0·82	1·456	0·82	1·40
Casein	} 2·83	0·557	—	} 2·80
Albumin		0·490	—	
Lactose		0·956	—	} 6·40
Ash	0·05	0·826	0·05	

implanting placental tissue into women after child-birth, but only if placenta from a well-advanced pregnancy were used. Billard [38] considered it an imperfect milk loaded with leucocytes, as it frequently ends in abscesses. Schlossberger [39] gives an imperfect analysis of a sample, whilst Gessner [40] gives a complete analysis of one together with other less perfect analyses (Table XXVII).

Table XXVIII gives the analyses of milk obtained from a maiden bitch (I),[41] a he-goat (II),[42] and three analyses of human milk (III—V),[43] secreted after the infant had died.

TABLE XXVIII. *Composition of Various Freak Milks*

	I	II	III	IV	V
Sp. Gr. . .	1·069	—	1·039	1·025	1·023
	%	%	%	%	%
Total solids .	29·00	—	21·50	18·30	18·63
Fat . .	2·20	26·50	5·00	6·15	7·80
Sugar . .	0·32	2·60	2·19	1·27	3·50
Protein . .	23·20	9·60	12·96	9·00	5·65
Ash and extractives .	3·28	0·78	1·35	1·88	1·68

The ash in every case contained 70 per cent. NaCl and 25 per cent. $Ca_3(PO_4)_2$. Casein was entirely absent from III, IV and V.

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PART II

THE CONSTITUENTS OF MILK

CHAPTER IV

MILK-FAT OR BUTTER-FAT

24. Introductory

FAT, biologically, is a stored product which, owing to its high percentage of carbon, has the highest calorific value of all food nutrients. Associated with its insolubility in aqueous media is its comparatively low chemical activity ; this enables it to withstand breakdown during the autolysis of the other classes of compounds closely associated with it in animal and vegetable matter. Thus, the fat in cheese suffers little degradation during the ripening process. Under conditions of carbohydrate fermentation, factors likely to cause fat-splitting, such as lipase action, are almost totally inhibited, and it is only by prolonged exposure of a large amount of surface, such as in emulsions, that hydrolysis of fat through the influence of hydrogen-ion concentration can be detected in appreciable amount. Fat-splitting occurs best at neutrality or at alkaline pH and, when once converted into fatty acid or soaps, fat is catabolised rapidly. The fat-splitting enzymes, the *lipases*, are often found associated with stored fat in its natural condition and are specific in their action. Thus lipases from various sources will show different rates of liberation of free fatty acids and a different distribution of such acids, although it is recognised that it is the unsaturated acids, *e.g.*, oleic acid, which are liberated in greatest amount.

25. The Composition of Butter-fat

(*a*) All fats and oils are mixtures of triglycerides of acids of the aliphatic series. Some oils contain hydroxy acids and variation occurs in the amounts and distribution of unsaturated acids. The fatty acids contain an even number of carbon atoms ranging from butyric (C_4) to carnaubic (C_{24}) acid. That only acids containing an even number of C atoms are present supplies evidence of the mode of their breakdown in the animal body, which occurs in steps of two, or multiples of two, C atoms.[1]

The alcoholic portion of the fatty esters is the trihydric alcohol, glycerol, $CH_2OH.CHOH.CH_2OH$. The substitution of one, two or three OH groups by one, two or three fatty acid radicals (of one acid) yields *simple* mono-, di- or tri-glycerides and the esters are

usually named by adding -*in* to the root name of the acid, *e.g.*, *monobutyrin* or *tripalmitin*. If two or three different acid radicals replace the OH groups, *mixed* glycerides are obtained, *e.g.*, *dibutyro-palmitin*. Natural fat very rarely contains simple glycerides. The number of combinations of acids to form mixed esters is large when the effects of the unsaturated acids have also to be considered. Thus, there are possible the following broad classes of glycerides : tri-saturated, di-saturated—mono-unsaturated, mono-saturated—di-unsaturated and tri-unsaturated. Although the possibilities of structural isomerism are large, the glycerides are in the racemic form and the only optically active glycerides known are those prepared from optically active acids.[2] Optically active mono-, di- and tri-glycerides have been prepared.[3]

The following fatty acids are found in major amounts in milk fat :—

Butyric, $C_3H_7.COOH.$	Myristic, $C_{13}H_{27}.COOH.$
Caproic, $C_5H_{11}.COOH.$	Palmitic, $C_{15}H_{31}.COOH.$
Capryllic, $C_7H_{15}.COOH.$	Stearic, $C_{17}H_{35}.COOH.$
Capric, $C_9H_{19}.COOH.$	Oleic, $C_{17}H_{33}.COOH.$
Lauric, $C_{11}H_{23}.COOH.$	

Traces of linoleic, linolenic and of other unsaturated acids containing from 10 to 22 C atoms, and of saturated acids containing up to 22 C atoms, have also been found in butter-fat. All the numbers of the above list are saturated acids, except oleic acid, $CH_3(CH_2)_7.CH = CH(CH_2)_7.COOH.$

Hilditch and Jones [1] have produced strong evidence in support of the presence of linoleic acid, $CH_3(CH_2)_5.CH = CH(CH_2)_2CH = CH(CH_2)_5.COOH$ in milk fat, since they consistently found the presence of an acid more unsaturated than oleic acid, as borne out by the iodine values of the C_{18} unsaturated esters obtained in the fractionation of the liquid esters.

Bosworth and Brown,[98] however, have failed to verify the presence of linoleic acid in butter-fat. By subjecting the methyl esters of the acids from a typical sample of creamery butter to fractional distillation (37 fractions), and examining each fraction for unsaturated acids by its iodine value, they were able to prove the presence of decenoic ($C_{10}H_{18}O_2$) and tetradecenoic ($C_{14}H_{26}O_2$) acids, but the presence of hexadecenoic and eicosenoic acids was doubtful ; they also suspected the presence of a C_{20}, C_{22} or a C_{24} acid containing two double bonds. They detected highly unsaturated acids, probably of the arachidonic type, and isolated from the four highest fractions a mixture of saturated acids of high

molecular weight, mainly lignoceric acid with small amounts of behenic and cerotic acids. They were unable by their exhaustive fractionating methods to detect linoleic and linolenic acids, but Bosworth and Sisson [99] isolated arachidonic acid as its octabromide. This work conflicts with the findings of Eckstein,[100] who found 0·17–0·25 per cent. of linoleic and 0·07–0·17 per cent. of linolenic acids in Michigan butter-fat.

Attempts at the isolation of individual glycerides in an unchanged state have met with little success ; factors such as mutual solubility and the high concentrations necessary for working in organic solvents render quantitative fractional crystallisation impossible. Attempts by the author (unpublished work) to crystallise out fractions from ethereal solution by the addition of acetone produced various fractions of identical iodine and saponification values, but the fractions contained no carotene. Amberger [6, 7] claims to have isolated small amounts of tristearin, triolein, palmito-distearin and stearo-dipalmitin by methods of fractional crystallisation, while tributyrin and tricaproin have been found absent.

(b) METHODS OF DETERMINING THE DISTRIBUTION OF FATTY ACIDS. (1) The first step in the resolution of a fat into its components is that of *hydrolysis* (or *saponification*), in which glycerol and fatty acids (or soaps) are formed. This may be effected by acids or alkalis, steam under pressure, or by enzymes. Acids and alkalis act as catalysts to the well-known ester-hydrolysis reaction, but commercially the caustic alkalis are used for the manufacture of soap :

$$C_3H_5 \cdot (OR)_3 + 3NaOH(H_2O) = C_3H_5(OH)_3 + 3NaOR(H_2O),$$
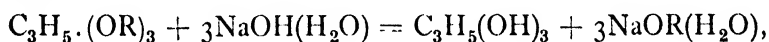
where R is any acyl group.

In laboratory work, alcoholic solutions of alkalis are used owing to the greater solubility of fat in alcohol and the shorter time necessary for quantitative hydrolysis. The *unsaponifiable residue* may be isolated by successive ethereal extraction of the diluted alcoholic liquid, whilst the fatty acids may then be separated by acidifying the caustic solution with mineral acid.

Where the nature of the unsaponifiable residue must be preserved as much as possible (for isolating carotene or obtaining concentrates of vitamins A and D from butter-fat), a cold saponification can be carried out. This is done by dissolving the butter-fat in alcohol (96–98 per cent.), adding solid caustic potash or soda, and shaking. The heat of the solution of solid alkali will be found to have saponified the fat in from four to six minutes and the temperature may have risen to 60° C. The

separation of the unsaponifiable matter and fatty acids is effected as for hot saponification.

(2) *Water-soluble and Volatile Fatty Acids.* Butter-fat is characterised by the presence of appreciable amounts of steam-volatile, water-soluble fatty acids, which serve to distinguish it from other fats and oils. The high Reichert-Meissl and Polenske values for butter-fat are due to this. Table XXIX gives the physical constants of the fatty acids of butter-fat.

TABLE XXIX. *Physical Constants of Fatty Acids of Butter-fat*
Holm and Greenbank in Rogers [124]

Acid Saturated	Formula	Molecular weight	Melting point °C.	Boiling point °C. 760 mm.	Boiling point °C. Reduced pressures	Solubility in 100 ml. Water	Solubility in 100 ml. Alcohol	Solubility in 100 ml. Ether	Volatility in Steam
Butyric .	C_3H_7COOH	88	−7·9	162		Sol.	Sol.	Sol.	Volatile.
Caproic .	$C_5H_{11}COOH$	116	−6·5	205		0·882 (15°)	,,	,,	,,
Caprylic .	$C_7H_{15}COOH$	144	16·5	237·5		0·25 (100°) 0·079 (15°)	,,	,,	,,
Capric .	$C_9H_{19}COOH$	172	31·3	269		V.sl. sol.	,,	,,	,,
Lauric .	$C_{11}H_{23}COOH$	200	48		102 (vac.) 225 (100 mm.)	Insol., V.sl sol. (100°)	,,	,,	Appreciably volatile.
Myristic .	$C_{13}H_{27}COOH$	228	58		250·5 (100 mm.)	Insol.	,,	,,	V. sl. volatile.
Palmitic .	$C_{15}H_{31}COOH$	256	64		138·5 (vac.) 268 (100 mm.)	Insol.	0·9 (20)	,,	Non-volatile.
Stearic .	$C_{17}H_{35}COOH$	284	69 3		291 (100 mm.)	0·034 (25°)	4·0	17 82(25)	,,
Unsaturated									
Oleic .	$C_{17}H_{33}COOH$	282	14		223 (10 mm.) 286 (100 mm.)	Insol.	14 02(25°) in 60% alc.	Sol.	Volatile in superheated steam (250° C.)
Linoleic .	$C_{17}H_{31}COOH$	280	Below −18	—	—	Insol.	Sol.	Sol.	

The fatty acids up to lauric acid are thus comparatively separable by steam-distillation, but some capryllic and most of the capric and lauric acids will be insoluble in the distillate. The further separation of these two groups raises some difficulty and in fat-analysis they are not usually subdivided. (The number of ml. of decinormal alkali necessary to neutralise the volatile fatty acids distilled from 5 grams of fat, subject to certain standardised technique and apparatus, is the *Reichert-Meissl number*, whilst the number of ml. of the same alkali required to neutralise the steam-volatile insoluble acids is the *Polenske number*.)

26. Separation and Determination of Fatty Acids

Various analytical methods have been used to determine the fatty acid distribution in fats. The most successful have been

those taking advantage of the difference in the solubility of their salts in organic solvents and by the oxidation of the unsaturated acids before the distillation of the methyl esters. The unsaturated acids have been separated either by freezing out the lithium salts of saturated acids from acetone solution at ice-salt temperature or freezing out the acids themselves from the same solvent by cooling with ether-solid carbon dioxide mixtures.

As remarked on the glycerides, the presence of a number of acids exerts a considerable effect on the properties of each acid and this complicates the attempts at separation. The presence of oleic acid makes the fractional crystallisation of the lower acids difficult, and so is used only when an estimate of the higher saturated fatty acids is needed.

In the salt-solubility methods, the lead salt-ether method has been successful. Lead oleate is soluble in warm ether whereas lead palmitate and stearate are not. In this case also there is the objection that the solubility of the lead salts of the saturated acids is increased by the presence of lead salts of the unsaturated acids. The salts of barium, potassium, lithium and silver have also been used in attempts at separation in this manner (v. Lewkowitsch,[5] Paal and Amberger [8]).

Fractionation of the acids by distillation *in vacuo* has failed, whilst fractionation in steam is not complete for the lower fatty acids. This is undoubtedly due to the solubility effects of the non-volatile acids which, by dissolving the volatile acids, depress their vapour pressure, and hence their partial pressure in the vapour phase, so that quantitative distillation is very much prolonged. Oleic acid is also slightly volatile in steam. In common with all methods of fractional distillation, no clear-cut separation is obtained.

The most satisfactory results have been obtained by the fractional distillation of the methyl and ethyl esters, but oleic esters appear in every fraction. This can be overcome by calculating the oleic acid in each fraction from its iodine value. Holland and Buckley [9] have combined crystallisation and distillation methods. Hilditch and his collaborators [10, 11] carefully oxidise the fat in acetone solution with potassium permanganate. By this treatment, all the unsaturated esters are broken down into mixtures of lower fatty *acids* and semi-acidic glyceride derivatives of azelaic acid. Thus a natural mixture of fully saturated and mixed saturated-unsaturated glycerides can be quantitatively resolved into a corresponding mixture of neutral and acidic products. This gives a simple method of ascertaining the proportion of fully saturated glycerides in a fat and a means of separating

them in pure condition. Further information of a quantitative nature is afforded by the fractional distillation of the methyl esters of the two fractions.

Hilditch and Jones [4] criticise the methyl-ester distillation method of Crowther and Hynd [12] in that the presence of methyl oleate in every fraction indicated that the fractional distillation was not effective. Holland and co-workers [9, 13] claim too high an accuracy for their distillation method, which Armstrong, Allan and Moore [14] state can only attain an accuracy of a unit per cent., even with extreme care in the preparation and isolation of fractions of individual esters.

Hilditch has applied his method with much success to the analysis of butter-fat from various sources. Table XXX gives

TABLE XXX. *Distribution of Fatty Acids in Butter-fat.*
Percentages

Acid	Crowther & Hynd [12]	Holland et al [9, 13]	Browne [11]	Frog & Schmidt Neilson [16]	Hilditch [4, 10]		
					A	B	C
Butyric	4·6	2·2–4·2	5·45	3·40	3·4	3·1	3·2
Caproic	1·7	1·3–2·4	2·09	3·30	1·8	1·9	1·7
Capryllic	1·3	0·5–1·0	0·49	1·90	0·9	0·8	0·8
Capric	1·3	1·2–2·0	0·32	3·00	1·9	2·0	2·3
Lauric	5·4	4·5–7·7	2·57	3·70	3·1	3·9	4·3
Myristic	17·7	15·6–22·6	9·89	12·90	9·7	10·6	10·8
Palmitic	16·0	5·8–22·9	38·61	20·80	27·6	28·1	28·4
Stearic	3·7	7·8–20·4	1·83	6·20	12·2	8·5	9·4
Oleic	48·3	25·3–20·4	32·50	27·00	34·3	36·4	33·1
Linoleic	—	—	—	—	4·4	3·7	5·4
Dihydroxy-stearic	—	—	1·00	—	—	—	—
Unidentified	—	—	—	9·80	—	—	—
Residue	—	—	—	8·00	—	—	—
Arachidic (?)	—	—	—	—	—	—	—

the analyses of butter-fat as found by various investigators. Table XXXI gives the results of analysis by Hilditch and Sleight-holme [17] of five samples of butter-fat collected at different seasons of the year and from cows fed on liberal quantities of soya and coconut cake. The molar percentages of the fatty acids are also given for these butter-fats as well as the rough average amounts of each fatty acid found present.

Hilditch summarises his findings as follows :

(*a*) The proportion of fully saturated glycerides is about 30 per cent.

TABLE XXXI. *Fatty-acid Distribution in Butter-fat at various Seasons of the Year and from Cows fed on Oil-cakes*

Butter	I		II		III		IV		V		Approx. composition %
	Autumn-fed		Coconut-cake		Soya-cake		Early summer pasture		Pasture-fed December		
Acid	%	Molar %	%	Molar %	%	Molar %	%	Molar %	%	Molar %	%
Butyric	3·1	8·4	3·4	9·0	3·6	9·6	3·3	8·0	3·5	9·2	3
Caproic	1·7	3·5	2·0	3·9	1·5	3·0	1·3	2·7	1·7	3·4	2
Capryllic	1·6	2·7	1·1	1·7	1·7	2·8	1·2	2·0	1·3	2·2	1
Capric	2·1	2·9	3·2	4·3	3·8	5·1	2·2	3·0	3·1	4·2	2
Lauric	3·4	4·1	7·3	8·3	6·5	7·5	4·0	4·7	4·1	4·7	4
Myristic	6·9	7·2	17·1	17·2	10·6	10·7	10·4	10·9	11·1	11·5	11
Palmitic	29·0	27·1	27·0	24·1	26·3	23·7	26·1	24·3	27·3	25·0	28
Stearic	7·6	6·4	4·8	3·9	8·3	6·7	6·5	5·4	11·5	9·5	9
Arachidic	0·9	0·7	—	—	1·2	0·9	—	—	0·6	0·5	—
Oleic	40·1	33·9	31·7	25·7	32·9	27·0	40·9	34·6	31·3	26·1	33–34
Linoleic	3·6	3·1	2·4	1·9	3·6	3·0	4·1	3·5	4·5	3·7	4–5

Glyceride distribution :   Per cent.
    Mixed fully saturated .   .   .   .   .   . each   30
    Mixed mono-oleo-disaturated .   .   .   .   . ,,   36
    Mixed di-oleo-mono-saturated .   .   .   .   . ,,   34

(*b*) The saturated acids are distributed more or less evenly throughout both the fully-saturated and the mixed saturated-unsaturated glycerides. (Arup,[18] working on the Reichert-Meissl, Kirschner, iodine and other values of fractions of Irish butter-fat, separated by chilling at various temperatures, arrived at a similar conclusion.)

(*c*) The saturated-unsaturated portion of the fat (*circa* 70 per cent.) is made up of mixed glycerides, the molecular proportions of the acids being 104 mols. of saturated to 100 mols. of unsaturated. The amount of mono-oleo-disaturated glycerides in the original fat is at least 36 per cent., and there cannot be more than 18 per cent. of triolein (or 36 per cent. of dioleo-mono-saturated glycerides). It is quite probable that very little triolein is present.

(*d*) The results do not necessarily hold in detail for butter produced from widely varying sources.

(*e*) The percentage of stearic acid shows considerable variation.

(*f*) The Kirschner value gives amounts of butyric acid about 20 per cent. too high; this is due to the presence of caproic acid.

(*g*) There is a slight tendency for the lower fatty acids to associate with the unsaturated acids more than with the higher saturated acids.

(*h*) There is consistent evidence of the presence of a small percentage of an acid less saturated than oleic acid.

(*i*) Acids from butyric to myristic are present in fairly constant amounts in all butter-fats, whilst the amounts of palmitic, oleic and linoleic acids are not widely dissimilar from those found in the body- or storage-fats of the same class of animal. Palmitic acid is the most constant in proportion of any of the component acids.

(*k*) Changes from indoor to outdoor conditions in the life of the cow and *vice versâ*, the general character of the diet, and seasonal changes in temperature cause considerable changes in the composition of the fat, whilst the effect of added fat to the diet is of a relatively minor order. The greatest change is in the variation of the amount of unsaturated acids present.

27. The Influence of Season and Food on the Composition of Butter-fat

The season of the year and the food fed to the cow have been found to influence the composition of butter-fat. The composition is influenced mostly as regards (*a*) the amount of volatile fatty acids (*e.g.*, as measured by the Reichert-Meissl value), and (*b*) the amount of unsaturated acids (*e.g.*, as measured by the iodine value). Generally, the amounts of these two classes of acids in butter-fat are lowered by cold temperature conditions coupled with a lack of green food (fresh grass, kale, etc.), and feeding, even generously, on silage or dry forage. Further, the keeping of cows on a lower plane of nutrition than would be demanded by their body-weight and potential yield of milk causes the composition of butter-fat to take the same course.

Hilditch and Sleightholme,[17, 36] while examining butter-fats produced at different times through the winter period, found that the iodine value dropped from a high value (41·3) in the early autumn, when there was still abundant grass and kale, to lower values (31·6, 34·8) in March, but rose again to 41·6 in May after the cows had gone out on to spring grass. The value 34·8 also was given by butter-fat from animals on a ration liberally supplemented with soya-bean cake (high unsaturated-acid content).

In this connection, the above workers have calculated the " association ratio," or the number of molecules of saturated acid per molecule of unsaturated acid in the non-fully saturated glycerides, and have tabulated the limiting values for the molar percentages of the four types of mixed glycerides in butter-fat (Table XXXII).

The increase in oleic acid content of butter will be reflected in

TABLE XXXII. *Limiting Values for Molar Percentages of the Four Types of Mixed Glycerides in Butter-fats. Glycerides (mols. per cent.)*

Butter	Iodine value	Association ratio	Fully saturated	Mono-unsaturated di-saturated	Di-unsaturated mono-saturated	Tri-unsaturated
II	31·6	1·11	41·3	33·9–46·3	24·8–0	0–12·4
V	34·5	1·07	39·6	33·3–46·8	27·1–0	0–13·6
III	34·8	1·07	38·2	34·0–47·9	27·8–0	0–13·9
CI	36·0	1·07	33·7	36·5–51·4	29·8–0	0–14·9
A	38·0	1·04	33·8	35·0–50·6	31·2–0	0–15·6
B	39·4	1·03	31·5	35·8–52·1	32·7–0	0–16·4
I	41·3	0·92	29·1	31·0–51·0	39·9–0	0–19·9
V	41·6	0·94	27·2	33·0–52·9	39·8–0	0–19·9

a disproportionate increase in *softness* of the butter-fat, since not only is the ratio of unsaturated to saturated acids increased in the saturated-unsaturated mixed glycerides, but the quantity of the latter is increased considerably. The fully saturated glycerides, which are the highest-melting components of butter-fat, are reduced in amount with increase in oleic acid. The practical significance of the iodine value in studying the " hardness " or " softness " of butter-fat is at once apparent.

In the winter of 1929–30, samples of butter-fat collected at various times showed a similar, but not so marked, trend in iodine values (Table XXXIII).

TABLE XXXIII.[17] *Variation in Composition of Butter-fat in the Winter Period*

Date	Conditions	Iodine value	R.M. value	Polenske value	Kirschner value
20/11/29	Indoor feeding .	42·2	27·0	1·8	21·3
19/12/29	,,	38·3	29·3	1·7	22·6
5/ 2/30	,,	38·9	27·3	1·6	21·4
26/ 2/30	,,	36·5	29·7	2·0	23·8
17/ 3/30	,,	37·3	30·6	1·8	24·8
27/ 4/30	Spring pasture .	43·3	28·4	1·9	23·5
3/ 6/30	,,	44·2	24·6	1·8	20·6

Channon, Drummond and Golding,[37] in their work on the feeding of cod-liver oil to cows, determined the iodine values of the butter in the winter season of 1922–23, and found the same general trend of observations as above.

Holland and Buckley,[38] working at a similar period of the year, found constant but extremely low iodine values for the butter-fats. The cows were in early lactation, and the ration was dried hay and " grain mixture," which partly explains the low iodine values. (A substantial amount of " roots " was included in the experiment recorded in Table XXXIII.)

Hilditch and Sleightholme [17] found that when liberal amounts of coconut cake (coconut oil contains 50 per cent. lauric acid and 17 per cent. myristic acid) were fed to cows, the butter-fat showed a rise in lauric acid from 4 to 5 per cent. and in myristic acid from 7 to 17 per cent. Capryllic and capric acids are present in fair quantity in coconut oil, but the butter-fat was normal in these acids. It seems that the lower acids, capryllic to lauric, are transformed to other products by the animal. Where soya-bean cake was fed liberally there was a slight rise in the oleic acid content of the butter over that from the feeding of coconut cake, but the linoleic acid content was of the same order, although the ratio linoleic : oleic acid in soya-bean oil is 2 : 1. Linoleic acid from the cake is used up by the cow, and the excess is not passed on to the milk.

Cranfield [39] considers that the amount of unsaturated acids are higher after feeding groundnut cake (arachis oil has 55–65 oleic and 20–25 per cent. linoleic acid) than cottonseed cake (25 oleic and 45–50 per cent. linoleic acid).

Iyer [40] has observed that rice polish used with a ration of linseed cake increases the volatile-acid content and diminishes the unsaturated acids of butter-fat (ghee). Moore [41] has found that silage made from lucerne hay, soya-bean hay, sorghum and maize silage produces butter of equal quality without any effect on texture. He has also found that changing from one food to another in the experiments is responsible for much variation in the constants of butter-fat.

Hansson and Olofsson [42] summarise their work on the influence of feeding-stuffs on the consistency of butter thus :

" Summer feeding with green fodder, especially young clover, tends to produce a soft butter of a high iodine value (40). This effect is reduced by drying or ensiling the plants. Winter rough-ages such as hay, straw and roots have the reverse effect. The effect of farm-grown concentrates depends on their fat-contents. Thus barley produces a rather harder butter than oats, and leguminous seeds a very hard butter. The effect of purchased concentrates varies with the total fat-content and the nature of the fatty acids in the fats. Thus fats of high iodine value cause soft fats and *vice versâ*. For good consistency of winter butter,

oil cakes should have 5–6 per cent. of fat of iodine value not over 100–110. The effect is evident on the butter within eight days of commencement of feeding, while individual response by cows may vary up to 5 units in iodine value."

Hansson,[43] in commenting on the change in consistency of winter butter during recent years, states that the increased use of fat-poor oilcake meal, and the substitution of coconut and palm kernel cakes for sunflower and rape cakes, has had an unfavourable influence on butter consistency.

Arup [44] has shown that some Irish butter produced in the winter months, when analysed by the usually accepted methods, gave results which, when viewed from the standpoint of specified standards, would raise doubts as to the purity of the products. Thus a presumptive minimum limit of 24 is tentatively accepted for the Reichert-Meissl value. Fifty samples out of 310 were found to have R.M. values under 24, whilst the Polenske and Kirschner values were low in a parallel manner. It appeared, therefore, that the content of the lower fatty acids, especially butyric and caproic, was abnormally low. A possible explanation of this occurrence is that rigorous weather and possibly a low plane of nutrition caused the animals to utilise some of the lower fatty acids for their own maintenance, with the result that the concentration of these fatty acids in the butter-fat was lowered.

Brown and Sutton [55] have found that feeding menhaden fish-oil to cows not only lowers the yield and the percentage and total butter-fat in the milk, but that the characteristic unsaturated acids of the oil enter into the butter-fat in small quantities and the analytical constants of the butter-fat change to those of a mixture of that fat and the added oil. Recovery to normal on a standard ration is found to occur slowly. They are also of the opinion that normal butter-fat contains small quantities of an acid more unsaturated than oleic acid, possibly arachidonic acid. Mrozek, Schlag et al.[56] have found that feeding cows on fish-meal causes the size of the fat globules to increase and gives a butter-fat higher in iodine value and lower in Polenske value.

Buschmann,[57] investigating the value of fats in the food of dairy cows, has found that quantities from 0·4 to 1 kg. per 1,000 kg. body weight has no effect, but that larger quantities decrease the fat-content of milk. Rapeseed oil has the lowest limit and cottonseed oil the highest limit in bringing about this effect. The physical properties and composition of the butter-fat alters as if 18 per cent. of the oil has entered directly into the butter-fat. Sutton, Brown and Johnson,[58] feeding 1 lb. of maize oil daily,

have found no decrease in the fat-percentage, but that the iodine value of the butter-fat rises by 30 per cent., whilst the amount of the soluble volatile fatty acids decreases to half its normal value. The fatty acids also have a higher mean molecular weight.

Arup [59] reports from data obtained on the iodine and other values of Irish butter that as the percentage of oleic acid decreases that of linoleic increases, and that generally the percentage of linoleic acid is fairly constant. Winter feeding was found to lower the iodine value and advance in lactation to increase it.

Composition of Butter-fat from other Mammals. Dhingra [101] has found the milk-fats of Indian goats and sheep to have a similar composition ; these fats differ from those of cow's or buffalo's milk by containing more fatty acids of low molecular weight. The content of fully saturated glycerides is a function of the mean unsaturation. The general composition of the component glycerides are the same as for those of cow's or buffalo's milk-fat. Butters made from the milk of sheep or goats show a low Reichert-Meissl value (23·4 23·8), but otherwise follow cow's butter closely in their range of constants.[102]

Dhingra has also found that the amount of volatile fatty acids in the milk-fat of the Indian camel is lower than in that of the cow, buffalo, sheep and goat.

The fat of whale milk has an iodine value of 139, due to 38 per cent. of the fat being made up of C_{20} - C_{22} acids containing from two to six double bonds. There are also present 32 per cent. of C_{18} acids and 22 per cent. of C_{16} acids.[103]

28. Compounds Associated with Butter-fat

Butter-fat, as rendered from butter, contains a variety of fat-soluble materials more or less in traces. The yellow colouring matter is *carotene*, whilst traces of the sterol common to all animal fats, namely, *cholesterol*, are also present. *Lecithin* and *kephalin*, representing the phospholipids, are present in small amounts. The fat of milk acts also as a carrier of the fat-soluble *vitamins* A, D and E, which, as proved by Golding *et al.*[19] for A and D, are wholly taken out of milk by separating the cream and churning into butter.

(*a*) CHOLESTEROL, $(C_{27}H_{45}OH)$. Cholesterol is a monhydric alcohol containing at least one double bond.[20]

The structural formula is given on p. 223.

Klostermann and Opitz,[21] by determining the cholesterol before and after saponification, have found that there is little, if any, present as cholesterol esters in butter-fat.

Table XXXIV gives the cholesterol-content of butter-fat as found by various investigators.

TABLE XXXIV. *Cholesterol-content of Butter-fat*

Investigator	Range of cholesterol-content. % of fat
Klostermann and Opitz [21] . .	0·071–0·075
Boemer [22]	0·3116–0·4066
Kirsten [23]	0·3–0·43

Nakanishi [60] reports the cholesterol-content of the milk of various mammals as follows (milligrams per cent.) :

Sow, 145 ; rabbit, 109 ; cat, 63 ; bitch, 55 ; sheep, 22·5 ; human, 16·4 ; goat, 14 ; cow, 12·7 ; mare, 11·5. (If the fat-content of cow's milk is 4 per cent., this would give a figure of 0·32 per cent. cholesterol in the fat, which agrees with the values in Table XXXIV.)

Grimmer and Schwarz [24] have found that 4·5 per cent. of the slime obtained on centrifuging milk is cholesterol.

An isomer of cholesterol—phytosterol—is found in vegetable fats exclusively, and, although its chemical properties resemble those of cholesterol, its physical properties and those of its derivatives differ appreciably. The acetate test for phytosterol is used to detect the addition of vegetable oils or fats to butter. Cholesteryl acetate melts at 112·8° C. and phytosteryl acetate at 129·2. Jaeger [25] has prepared a table of the melting-points of mixtures of the two acetates (see Table LXXVIII, p. 223).

(*b*) PHOSPHOLIPIDS. This class of compounds represents mixed glycerides where one of the hydroxyl groups of glycerol is joined on to a choline-phosphoric acid radical (true lecithins) or to an amino-ethyl phosphoric acid radical (kephalins), the other hydroxyl groups being joined to the usual fatty acid radicals (limited to a few, mostly the higher acids). Egg lecithin contains oleic, linoleic and arachidonic acids.

The lecithins are chemically active and undergo autoxidation easily. In milk, they are easily destroyed by heat (Bordas and de Raczkowski [26]). Their isolation from milk is difficult since they are soluble in all organic solvents other than acetone. The sparing solubility of butter-fat in acetone and the solvent effect of the fat itself prevent any separation of the trace of lecithin by this method.

Table XXXV gives the percentage of lecithin in milk as found by different investigators.

TABLE XXXV. *Lecithin-content of Cow's and Human Milk*

Investigator	Cow's milk %	Human milk %
Glikin [27]	0·0516–0·1173	0·1329
Burrow [28]	0·049 –0·058	0·057–0·060
Dornic and Daire [29] . .	0·0595	—
Koch and Woods [30] . .	0·072 –0·086	0·078
(Kephalin	0·027 –0·045	0·037)

Osborne and Wakeman [31] established the fact that mixed phospholipids occur in milk in small amounts. They found monamino- and diamino-phospholipids differing in phosphorus and oleic acid contents and in their solubilities in organic solvents. They found 0·0027 per cent. of these compounds in milk, mostly in the casein with none in the fat and inorganic matter.

A large portion of the lecithin remains in the buttermilk on churning cream. The amounts left in the butter vary with the conditions of manufacture and range from 0·01 to 0·17 per cent.[32] Supplee and Cusick [33] quote a range of from 0·043 to 0·072 per cent., according to the method of manufacture of the butter. (See also [124].)

Perlman [106] has found that on removing cream of 15 to 20 per cent. fat from milk, about 40 per cent. of the phospholipids appear in the skim milk, but that on increasing the fat-content of the cream (up to 58 per cent.) the phospholipid content of the skim milk decreases uniformly.

· Heat alone does not destroy milk phospholipids, but some milk bacteria secrete a lecithin-hydrolysing enzyme. Mohr et al.[107] confirm the stability of milk lecithins to heat. They comment on the lack of agreement between various workers concerning the lecithin content of milk fractions and, by using improved methods of separation (absolute alcohol and chloroform), have found the following percentages : skim milk 0·016, whole milk 0·037, cream 0·169, butter 0·206, whey 0·007. Bird et al.[108] find that 25 per cent. of the fat of buttermilk is phospholipid (corresponding to 0·152 per cent. of the buttermilk). In the acid methods (Gerber and Babcock) of fat determination, only traces of phospholipids appear in the fat layer, but in the Röse-Gottlieb method from 6 to 17 per cent. is extracted. They found skim milk to contain 0·13 per cent. and buttermilk 0·27 per cent. of phospholipid.

Demair and Bleyer [109] extracted phospholipids from dried milk

with methyl alcohol and found a monophosphatide containing palmitic, stearic and oleic acids, together with choline and colamine. Kurtz et al.[110] found that the lecithin and kephalin fractions of milk phosphatide contained none of the lower fatty acids and no palmitic acid ; the acids present were : myristic 5·2, stearic 16·1, oleic 70·6, arachidic 1·8, C_{24} acids 6·3 per cent.

Lecithin is responsible for the fishy odour and taste of butter and of milk powders of high moisture-content. The mechanism of the action is hydrolytic-oxidative. The lecithin in the butter is acted on by the small amount of peroxides formed during the initial oxidation of the fat, and since the taint is more prevalent in butter made from acid cream, and in the presence of traces of

TABLE XXXVI. *Colour of Butter-fat and Milk Serum of Various Breeds on* (a) *Carotene-rich and* (b) *Carotene-poor Rations*

Breed	Carotene-rich ration				Carotene-poor ration			
	Butter fat		Blood serum		Butter fat		Blood serum	
Units	Yellow	Red	Yellow	Red	Yellow	Red	Yellow	Red
Ayrshire .	24·0	1·3	54·0	1·8	1·3	0·4	3·3	0·5
Holstein .	22·0	1·2	41·0	1·0	8·5	1·4	6·0	0·7
Jersey .	64·0	2·0	45·0	1·1	11·0	1·7	10·0	0·9

Butter-fat : 1-inch layer against Lovibond-tintometer standards.
Blood-serum : Extract of 10 ml. in 12·5 ml. as 1-inch layer against Lovibond-tintometer standards.

metallic catalysts, oleic acid and a certain concentration of activated oxygen (or peroxides) are necessary for the breakdown of the choline portion to trimethylamine.[34]

(c) CAROTENE. This is a yellow pigment (belonging to the carotinoid group) which occurs, usually associated with other members of the group, e.g., xanthophylls, in green vegetable material. Xanthophyll accompanies carotene in small amount in milk-fat, but the isomer of carotene, lycopin, which gives the red colour to tomatoes, red pepper and fruits, does not occur in milk.

The amount of carotene in butter-fat depends on the amount of green food in the ration ; also the vitamin A content varies with the carotene content from such a source. (This will be discussed later in the section on The Nutritional Value of Milk.)

Of the mammals whose milk is used for human consumption, only the cow gives milk characterised by the pronounced colouring

of the fat.   Human milk may at times be tinted by carotinoids.[35]
Coloured milk-fat or adipose tissue occurs only in those species
where the blood serum is coloured by carotinoids, but there is no
definite physiological information available to explain why cow's
milk has this characteristic.

Various breeds exhibit a difference in amount of pigmentation
of milk-fat and blood-serum, even when fed on identical rations.
Table XXXVI gives comparative figures for various breeds on
carotene-rich and carotene-poor rations.   The Channel Island
breeds of cattle give butter-fat of the highest yellow colour, and
the Ayrshire breed gives the lowest colour in butter-fat.

Xanthophylls are also transferred to butter-fat in amounts
comparable to their occurrence in the food, but they are not
changed to carotene by the cow.

Xanthophylls are more easily destroyed in the digestive tract
of cows.

(d) THE FAT-SOLUBLE VITAMINS.   These are present wholly in
the fat phase of milk and will be dealt with in the section on The
Nutritional Value of Milk.

29. The Autoxidation of Butter-fat

(a) " OILINESS " IN MILK.   It may be thought that, since fat
is the disperse system in milk, and being insoluble and relatively
inert chemically, it would be the last constituent to undergo
chemical change and cause a taint to develop.   Experience has
shown that this is far from being the case, and that (a) fat can
quickly undergo chemical action in the condition in which it
exists in milk, and (b) it requires little chemical action to occur
before an objectionable taint develops.   The flavour which de-
velops is variously termed oxidised, cappy, mealy, oily, " card-
boardy," or " tallowy," according to the degree of taint develop-
ment in milk.   A similar occurrence due to solar radiations is
termed a " sunlight taint."   The taint developed to a moderate
degree is similar to the taste of castor oil.   (Castor oil, of acetyl
value 150, is made up of a considerable amount of hydroxy-acids,
and the development of a similar taste in milk-fat may be due to
the formation in small amount of hydroxy-acids.)

There is no doubt that the taint is due to the incipient oxidation
of butter-fat, and that the dissolved oxygen of the milk is an
essential factor.   Milk, fully saturated with the gases of the
atmosphere, is roughly $N/900$ in oxygen-content at room tem-
perature.   When milk is stored, the dissolved oxygen is used by
the micro-organisms for respiratory purposes, and the rate at
which the oxygen is removed is roughly proportional to the

number of organisms per unit volume. This is the basis of the " methylene blue test " for milk quality. The organisms use up the oxygen in molecular form and this rate of requirement for oxygen may be termed the " *biological oxygen demand*." The dissolved oxygen cannot be made to act chemically on the fat unless there is some *activator* present, and only under conditions whereby the dissolved oxygen is activated is it possible to have a " *chemical oxygen demand* " in the milk and the oxygen to be used up by the fat. This is easily demonstrable by holding a tube of milk coloured with methylene blue close to a 100-watt lamp. Small bleached areas will immediately appear that can easily be distinguished and blotted out by swirling the blued milk in the tube. This shows that where the radiations of the lamp have activated the dissolved oxygen of the milk the fat has exhausted the oxygen sufficiently for the reducing agents in the milk to reduce the methylene blue. That is, the oxygen, activated by the radiations of the lamp, has combined with the fat ; the concentration of the oxygen has thus been sufficiently reduced to set up a reduction potential compatible with that shown by the change of methylene blue to leuco-methylene blue. The same condition is reached throughout the body of the liquid when bacterial multiplication and oxygen demand has brought the liquid down to the same oxidation-reduction potential, only taking a longer period of time to do so. It is noteworthy that once the oxygen-activating conditions obtain, the absorption of oxygen to combine with the fat is rapid even at ordinary or cold-storage temperatures.

It is obvious that the onset of fat autoxidation in milk depends on a variety of factors. Whether the development of a fat taint will occur or not depends to a great extent on the competition for dissolved oxygen between the micro-organisms and the fat after the activation of the oxygen. It is clear that with a large proportion of organisms the B.O.D. is high, and that the concentration of oxygen to be activated, were an activator present, would be low, so that the amount of chemical action on the fat would be small and no taint would develop before the milk would be consumed. Thus, milk of low count under the proper conditions would develop the taint first, whereas conditions of storage, *e.g.*, low temperature, which would be likely to depress bacterial growth, would be a further factor in hastening taint development.

Under commercial conditions the activators met with are mostly traces of copper dissolved off detinned surfaces of processing plant during the handling of milk, whilst during the summer months the exposure of milk of good hygienic quality to direct sunlight or to skyshine will cause a taint to develop.

Milk varies in its natural copper-content according to geo-graphical sources of production,[45] a range of from 0·25 to 0·75 parts per million having been recorded. From observations on a large number of commercial samples of processed milk, and from experiments where milk was made to contain varying amounts of copper (as lactate), the minimum amount generally required to produce a definite oxidised flavour in twenty-four hours in good quality milk stored at from 32–40° F. was 1·5 p.p.m.[62] It has also been established that exposure to sunlight of milk of a lower copper-content, or " coppering " a milk already exposed to sun-light to a lower limit than 1·5 p.p.m., will bring on the taint. Thus the effect of all activators is cumulative, and the multiplicity of conditions favouring taint development or otherwise justifies only approximate observations being made. It has been found that doubling or even trebling the iron-content of milk does not cause an oxidised flavour to develop within the time that milk is usually kept before consumption. Other metals used in the fabrication of milk-processing plant, such as nickel, chromium, manganese, aluminium and tin, have no appreciable effect on the development of this taint, although the first three and iron reduce considerably the keeping quality of dried milk, causing the development of a " tallowy " or " cardboard " flavour. The effects of traces of heavy metals entering milk or cream during processing may be further observed in the keeping quality of some by-products, particularly butter, when stored for an appreciable period. In the deterioration of butter during storage the rate of the reaction causing spoilage is considerably slower than that occurring in the finely-divided fat of milk and cream, so that the course of autoxidation can be studied on butter-fat in greater detail.

Kende [111] agrees that oxidised flavours in milk are due to the catalytic action of traces of heavy metals on the oxidation of finely-divided milk-fat. The tendency to give oxidised flavours is variable owing to the food of the cow adding varying amounts of protective substances (e.g., ascorbic acid) to the milk. Milk heated to 80°–85° C. is protected owing to the formation of some reducing substances in the process. The effect of traces of copper varies with the amount, from 2 to 4 p.p.m. giving the maximum of off-flavour. Kende postulates the presence of an enzyme—oleinase—as a causative agent in off-flavour development. The mechanism of its reaction is obscure, since the enzyme is not destroyed by pasteurisation. Elaidin is thought to be the com-pound giving the tallowy taste.

Csiszar [112] postulates two causes of oxidised or emery flavour, namely that the oxidation of butter-fat will cause a flavour :

(a) which is distinguishable from an oily rancidity (b) due to fat hydrolysis (or possibly the secondary effects of hydrolysis).

The possibility of the development of an oxidised flavour in the absence of catalysts has been explored by Guthrie and Brueckner.[113] From individual cows they took samples of milk not exposed to metallic contamination. During a three-day storage period 21 per cent. of samples from 155 cows developed an oxidised flavour, and there was no correlation between breed, stage of lactation, or age of cow and the incidence of the taint. The taint was more intense in winter than in summer, but the connection with the food of the cow was obscure. Pasteurisation at $71°$ C. for 30 min. decreased the incidence of, or prevented the appearance of, the taint. Kende maintains that the substances which protect milk-fat from spontaneous oxidation are transferred to the milk from green foods, especially hay and grass, whereas concentrated foods are poor in these factors. These protective factors are similar to the vitamins in their quantity and specificity ; thus ascorbic acid, in amounts of from 0·5 to 1·0 per cent., is claimed to be protective.[114]

The reducing substances formed by milk bacteria together with their oxygen demand are also protective factors. The protective substances present in a dry preparation of *Reductobacterium frigidum*, which can be added to milk in the proportion of 1 : 25,000 to prevent the development of oxidised flavour, are claimed to be solely products of bacterial metabolism,[111] although Ritter and Christen [115] found that the preparation gave the Tillmann's reaction for ascorbic acid and contained 5 to 7 per cent. of hydroquinone.

Brown *et al.*[116] have found considerable variation among individual cows with respect to the tendency for oxidised flavour to develop in their milk. They found that dry feeding increased but fresh pasture grazing decreased the tendency to flavour development, whilst feeding one quart daily of either tomato or lemon juice also reduced the tendency. It was also found that daily feeding of 0·5 gm. of ascorbic acid greatly decreased the occurrence of oxidised flavour.

Investigating the flavour in pasteurised milk, Roland *et al.*[117] found that the flavour appears mostly in milk of high fat-content and low bacterial count, and therefore milk of good quality both in composition and hygienic quality was more prone to show oxidised flavour.

Sharp *et al.*[118] state that there is an enzyme in milk which brings about the oxidation of ascorbic acid. This enzyme is not destroyed by holder pasteurisation at $145°$ F., but is almost

completely destroyed at 170° F. Traces of copper add consider-
ably to the catalytic action of the enzyme. They found that the
amount of destruction of ascorbic acid was proportional to the
intensity to which the oxidised flavour developed. Ross [119]
claims that homogenisation of milk and cream at pressures of
1,500 lb. per sq. in. prevents oxidised flavour, even in the presence
of copper.

(b) AUTOXIDATION OF RENDERED FATS. The fundamental
study of fat-oxidation has been carried out on the pure (one-phase)
fats or oils and not on fat emulsions. Consequently the importance
of means to make oxygen diffuse quickly into the body of the
fatty mass and the interfacial absorptive effects in a multiphase
system, especially when free acid or traces of heavy metals are
present, has not been fully recognised. In butter, for instance,
the fat/water-droplet interface is undoubtedly the site of the
chemical action involved in the deterioration of the fat,
since, at this interface are located : (a) the " curd " which is
the vehicle of the oxygen activators—the traces of heavy metals [46] ;
(b) the water, which acts as a solvent for free acids and as a medium
for fat-splitting to occur, the oleic acid especially being liberated
in greatest equivalent and dissolved to an appreciable concentra-
tion in the aqueous phase ; (c) the lecithin, a hydrophyllic colloid
acting as liaison between the aqueous and fat phases and relatively
a fairly reactive lipoid material ; (d) the oxygen, which can diffuse
from outside through the aqueous films and is naturally essential
for autoxidation to proceed ; and (e) the concentrating effects of
the spherical surfaces on the above reactants. King [47] states that,
on the basis of Rahn's theory of the structure of butter, the
minute water droplets are composed of buttermilk, and that this
type of water in butter is responsible for a greater amount of
spoilage than the *wash* water ; also that, generally, any changes in
the butter-fat, whether due to chemical or biological causes,
commence at the fat/water interface.

With rendered fats it is obvious that the above conditions do
not obtain. Nevertheless much valuable information has been
obtained by studying the autoxidation of pure fats. By the study
of the rate of oxygen-absorption it has been observed that a con-
siderable length of time elapses before a fat shows any appreciable
" oxygen-uptake." This period is termed the " period of induc-
tion," and when it is over, the fat will absorb oxygen at an increas-
ingly rapid rate (logarithmic) in a manner characteristic of an
autocatalysed reaction. This period of rapid oxygen-absorption
is contemporaneous with the region of tallowiness, as characterised
either by a positive Schiff's test for aldehydes first appearing, then

a positive Kreis test, or by very large increases in the peroxide-content of the fat. The " period of induction " may be regarded as a rough but valuable comparative index of the time during which a fat would be expected to keep in a wholesome condition.[48, 49] Experimentally, this period of induction may be cut down to a few hours by studying the rate of oxygen-absorption at elevated temperatures, and in this way valuable evidence has been obtained as to the shortening of the normal induction period by various agencies which might be suspected to shorten the life of a fat in its " sweet " condition under ordinary commercial conditions.

Pro-oxygenic Factors. The two most important factors in this direction have been found to be a high " acid value " of the fat, and the presence of traces of heavy metals such as vanadium,[50] copper, iron, nickel, manganese, chromium and cobalt.[51] These factors have been termed " *pro-oxygenic* " and are effective throughout the body of the fat. A rise in temperature has been found to decrease the period of induction considerably, whilst solar, ultra-violet or artificial-light radiations are also pro-oxygenic to the extent to which they can penetrate into the body of the fat. It can be said that any physical factor capable of activating oxygen or of adding energy to the system is pro-oxygenic. These factors also cause the rate of subsequent rapid oxygen-absorption to be increased considerably.

Anti-oxygens. Natural fats contain traces of non-fatty compounds which behave as " *anti-oxygens*," that is, which lengthen the period of induction considerably. These, in time, lose their property and after guarding the fat proper from oxidation are themselves oxidised to a non-anti-oxygenic form, after which the fat is attacked and shows signs of autoxidation. Mattill [52] has observed that various naturally-occurring sterols are not anti-oxygenic, but that the natural anti-oxygens can be concentrated in the unsaponifiable fraction of fats. He also suggests that these compounds are linked up with the physiological action of the fat-soluble vitamins and that in the preparation of the easily autoxidisable substances from a natural source the presence of anti-oxygens prevents undesirable changes. Hilditch and Banks [53] have found that boiling with water removes the anti-oxygens present in olive and linseed oils, but that traces of quinol added to the oils causes them to recover their oxygen resistance. They have also found that the induction period was shorter in the case of the fatty acids but longer on subsequent esterification. The shortest periods of induction are given by the synthetic glycerides of the distilled fatty acids. Mild treatment with

sodium hydroxide or boiling with dilute hydrochloric acid almost completely eliminates the induction period, but treatment with small quantities of concentrated sulphuric acid increases it. They suggest that the amount of anti-oxygen present is variable and depends on the oil. Newton [54] cites carotene as a naturally-occurring anti-oxygen, whilst Davies [51] has observed that, where lipase is active in butter from unpasteurised cream, the onset of peroxide-formation, even if catalysed by traces of heavy metals, is delayed, and that the lipolitic activity is destroyed before any
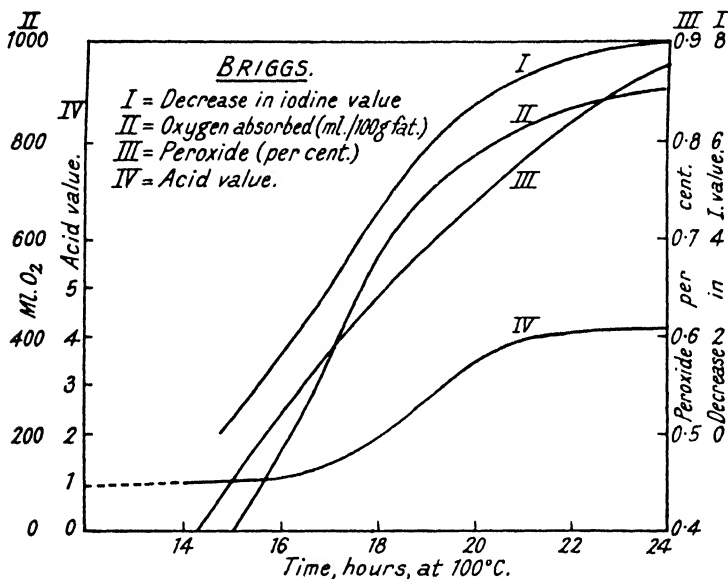
FIG. 10.—Changes in butter-fat when kept at 100° C. in an atmosphere of oxygen.

appreciable peroxide-formation occurs, thus pointing to the anti-oxygenic effects of lipase.

(c) RANCIDITY. Although the term rancidity is used loosely to describe any " off " flavour in fat, it is generally agreed among food chemists to apply the term only to the results of hydrolytic action, whilst the term " *tallowiness* " is applied to " off " flavours caused by oxidative changes, with the exception of the term " *fishiness* " for such detectable intermediate oxidative changes in butter and dried milk of high-moisture content. The term " *ketonic rancidity* " is used to describe the odour resembling deteriorated coconut oil which is caused by the action of " dry " moulds on butter-fat and coconut and other oil.

Owing to the relatively high percentage of the lower and

volatile fatty acids in butter-fat, slight hydrolysis produces a
strong smell characteristic of these acids, especially butyric acid.
Lipase activity very quickly causes " bitterness " in milk and a
strong taste and smell in butter where such activity has not been
destroyed in the manufacturing process, *e.g.*, in butter from
unpasteurised sweet cream.[51] Lipase in milk or cream can be
almost completely destroyed at pasteurisation temperature or its
effect can be submerged by controlled or natural ripening of
cream, since acidity is unfavourable to lipase activity. " Cold
dairy " conditions, or storage of cream at low temperatures,
especially during the winter months, is not favourable to the
development of acidity at a rapid enough rate to check lipase
activity, so that, on churning, the resulting butter shows a decided
rancid taint in a day or two. This is especially true for cream
separated from clean milk. Lipase liberates the lower and volatile
fatty acids, amongst others, and these are responsible for the
" off " odours and flavours. Butyric acid, found only in butter-
fat, is thus responsible for the " off " flavour more specifically
termed the " *butyric rancidity* " of butter. This type of rancidity
is characteristic of butter only. It must be realised that other
changes due to oxidation may occur simultaneously, since free
oleic acid is also liberated by lipase action.

Acidity of Butter. The rate of acid liberation, especially in a
polyphasic system like butter, is influenced by the acidity of the
cream, since the minute droplets of water in the butter are in
reality buttermilk, and the wash water is also in a slightly acid
condition.[47] In general, the acidity of butter (ml. normal alkali
per 100 gms.) is a function of the acidity of the cream. The
process of ripening cream is carried out in order to produce a
butter of appealing aroma and flavour. During the develop-
ment of lactic acid (up to 0·6 per cent.), two by-products,
diacetyl, $CH_3.CO.CO.CH_3$, and *acetyl methyl carbinol*,
$CH_3.CHOH.CO.CH_3$ are formed in small amounts. These
compounds enter into butter during churning and, with some
oxidation of carbinol to diacetyl during early storage, confer the
necessary flavour and aroma to the product. Diacetyl can be pre-
pared synthetically and is used extensively to give margarine a
butter flavour, about one to three parts per ten million only being
required to give the desired result.

The drawback of acidity is that it creates a condition favouring
oxidative changes, and there is some evidence that a relatively
high concentration of diacetyl giving a high-flavoured butter is
most conducive to the development of tallowiness or other
oxidative faults during storage.[62]

Rogers and Gray [63] have investigated the effect of cream acidity on butter deterioration during storage, and have concluded that butter from sweet cream is superior in keeping quality to butter from ripened cream. In practice, therefore, where cream of all grades and conditions is collected to a central creamery, it is first graded and then, since it is usually of too high an acidity to withstand pasteurisation, it is neutralised to reduce its acidity usually well below that of sweet cream. Thus in New Zealand an acidity of 0·09–0·12 per cent. is aimed at, whilst in Australia an acidity of about 0·11–0·13 per cent. lactic acid is usually reached by neutralisation. In South Africa the cream is reduced to an acidity below 0·2 per cent. Bicarbonate of soda or cream of lime is usually used for neutralising. The cream is then pasteurised by the flash method, and the fat globules are allowed to recover the physical properties essential for churning by being kept cold overnight in special vats.

The drawback of cream neutralisation is the lack of efficiency of control, since it is possible to over-neutralise, in which case a poor-keeping butter, usually of a soapy or limy flavour, is produced. Neutralisation and pasteurisation do not completely eliminate the faults associated with the development of a high acidity in the original cream, since the hydrolytic and enzymic changes might have proceeded into routes not corrected by neutralisation alone. It may, therefore, be expected that butter from cream which was highly acid before neutralisation would keep less well than that from less acid cream or from culturally ripened cream, both of which in their turn would keep less well than butter made from pasteurised sweet cream

(d) TALLOWINESS. Tallowiness is a taint which develops in pure fats or fat-rich material and is regarded as a state of comparatively advanced stage of oxidation of the fat when compared with other fatty taints such as oiliness in milk or fishiness in butter. The taint, which was once prevalent in stored butter, is now rare, but can easily be developed experimentally, for instance, by the incorporation of 1 p.p.m. of copper as copper sulphate into butter during working. When tallowiness develops under such conditions the carotene is first of all bleached in localised areas but later complete bleaching occurs. On further storage, butter turns to various stages of a brown colour. Volatile products from a sample of tallowy butter diffuse into non-tallowy butter stored with it in a confined space and bring about tallowiness in the wholesome sample.

All fats contain unsaturated acids and it is the chemical reactivity of this group of acids which is responsible for the taint. It has

been established by Holm and Greenbank [64] [65] that it is the oxidation of oleic acid which produces tallowy flavours. It is quite possible that the greater reactivity of traces of more unsaturated acids is responsible for the initial stages of oxidation, which later lead to the oxidation of oleic acid, it being the unsaturated acid present in greatest molecular equivalent in those fats which develops tallowiness.

Oxidation of fats which contain acids which are more unsaturated than linoleic acid in considerable amount causes polymerisation into resinous substances. These are the so-called drying oils, *e.g.*, fish and linseed oils. Aldehydes and other volatile degradation products are also formed in small amounts during the drying process.

The mechanism of the development of tallowiness has been the subject of much investigation and speculation. For a summary of the present position the reader is advised to consult Holm and Greenbank's section on the subject in *Rogers's Fundamentals of Dairy Science* (2nd edition, Reinhold Publ. Corpn., New York, 1935, pp. 87–94). Most fats show an induction period to oxidation before deterioration sets in, and this period may be regarded roughly as that in which the fat remains sweet and wholesome. When this period is over the rate of oxidation increases logarithmically and rapid deterioration sets in.

The induction period is that in which the antioxygens present in most natural fats exert their protective action on the fat proper. These protectors or inhibitors are present in small traces only and their stoichiometrical effect in the oxidation process is small. Their function as protectors is gradually destroyed by oxidation until the fat proper commences to oxidise and possibly their own oxidation products catalyse to some extent the initial stages of oxidation of the fat.

In the fat itself, the seat of initial oxidation is the double bond of unsaturated acids, and probably the first of a chain of compounds formed is a fat peroxide. A form of potential of active oxygen of this nature is built up and further oxidation results in the splitting of the fatty acid residue to simpler compounds. The increase in peroxide content at the end of the induction period can be easily followed by determining the amounts of iodine liberated from potassium iodide by 1 gram of fat in chloroform-acetic acid solution.[66] Later on, these simpler molecules are further broken down to give aldehydes, acids, etc., which confer the tallowy taste on the fat. The oxidation is autocatalytic and the products of the reactions increase the rate of oxidation considerably.

(e) THE ROLE OF TRACES OF HEAVY METALS IN FAT-OXIDATION.

Traces of heavy metals or active metallic surfaces are in common use as catalysts in many technical processes, *e.g.*, platinum in the contact process for sulphuric acid, nickel in the hydrogenation of fats, and small quantities of manganese, nickel and cobalt salts in the clarification of oils by the process of " blowing " at elevated temperatures,[67] whilst the use of various pigments, such as red ochre and red lead, as driers is another example of catalytic oxidation by metallic compounds. Ingle [68] states that the oxygen-carrying capacity of the various metallic compounds depends on the number of stable oxides which the metal can form. The readiness with which one oxide form can pass into another and the stability of the peroxides are other factors. In the " blowing " of oils the catalytic activity of the metallic oxides occurs in the following decreasing order : Co, Mn, Ni, Ce, Pb, Cr, Fe, U, Bi, Ag.

But in the case of milk-fat the metals do not conform to this order of activity. Briggs [50] finds that the most active metal in inducing autoxidation is vanadium, which is closely followed by copper, whilst iron, chromium, nickel and manganese are less potent and very similar in action ; tin, lead and aluminium have no significant action. Davies [51, 69] has also observed that when traces of metals, in amounts from 2 to 50 parts per million, are incor-porated into butter, copper has by far the most active catalytic action, 2 parts per million being sufficient to cause incipient tallowiness within six days of storage at room-temperature. Of approximately equal activity are the metals : iron, nickel, cobalt, chromium and manganese, while tin and aluminium have no effect.

The contamination of milk products with traces of heavy metals rapidly induces oxidation with a subsequent shortening of the keeping quality of the product.[34, 50, 61, 70, 71, 72]

Copper salts added to milk in amount equal to 1·5 parts per million [61] or from 10–15 p.p.m. of dry solids either induce an oily taint or produce tallowiness in the powder. The use of copper utensils in the handling of milk or the manufacture of stored milk products, or the use of tinned copper surfaces where the tinning has worn off, is a potential source of danger as regards the keeping qualities of milk-fat.[73] The solution of this problem naturally rests with the use of equipment which will cause the least or no contamination of milk or its products with heavy metals. The use of metals with suitable physical properties, both from the heat-exchange and fabrication points of view, is essential in dairy-equipment in order to economise working costs (steam, water, etc.) and space. Copper is satisfactory for this purpose, since it

is easily worked and has high heat-conductivity ; but owing to its corrosiveness it can only be used for dairy-equipment when coated with a thick plating of tin, and the purer the last coatings of tin the better. It must be understood that when milk of any temperature comes in contact with a metallic surface a certain amount of the metal dissolves and this effect is cumulative as the milk circulates through the various parts of a pasteurising plant. Great care is necessary to keep the tinned surfaces in good order to prevent the entry of copper into the product and thus to guard against the incidence of fat taints.

(f) OTHER FACTORS INFLUENCING FAT OXIDATION. LIGHT AND ACIDITY. Increased temperature [75] and light [74] have marked catalytic effects. Rise in temperature has the approximate effect that for every rise of 10° C. the reaction velocity is doubled.

The effect of sunlight is strongly catalytic, and it is inadvisable to expose milk of good hygienic quality or any fat to direct sunlight or skyshine for any length of time. The fat in milk occasionally develops a " cardboardy " taste—sunlight taint—through the effects of exposure to the sun in shop windows or in uncovered prams in the streets.

The effect of sunlight on milk has been studied by Whitehead,[76] who found that when fat was oxidised by the activation of the dissolved oxygen in milk, a reduction potential which could be detected potentiometrically or by methylene blue was produced in the milk. The same effect was produced by adding sodium oleate, but not sodium palmitate, to fat-free milk, thus demonstrating the effect of the unsaturated acid, oleic acid. Fay and Aikins,[77] however, found a similar fall of potential with both sodium oleate and palmitate. They also studied the effect of sunlight on the reduction of methylene blue in milk, and found that the addition of fat decreased the reducing time, the reason being that fat heightens the zone of reduction. Martini [78] has observed that the milk of different mammals exhibits different powers of reducing methylene blue under the influence of sunlight, sheep's milk being most active, whilst that of the cow is less active and loses its power very easily. He states that the power to reduce does not reside in the fat but is due to a water-soluble thermostable substance, probably glutathione, of which sheep's milk contains three times as much as cow's milk. It is apparent that some reducing substance is responsible for the actual reduction of methylene blue in milk, but of greater importance is the fact that the sweeping up of the dissolved oxygen, so that the reduction potential (rH) compatible with the incipient bleaching of the blue is reached, is brought about by the fat.

The effect of light on carcase fat has been studied by Lea.[79]

Increase in the acid-value of the fat also increases its suscepti-bility to oxidation.[65]

MOISTURE AND OTHER CONDITIONS GOVERNING FAT-OXIDATION. The part played by the moisture of butter and the importance of studying butter-fat deterioration in the light of the physical structure of butter has already been discussed (Section 29 (b)).

The moisture of milk powder plays an important part in determining the storage qualities of milk powder. This is dealt with later (Section 181).

Recent work on the physical nature of milk powders throws some light on the variations in the character of the oxidations met with. Lampitt and Bushill [80] have observed that the fat of spray-dried whole milk powder is only partly extractable by organic solvents (carbon bisulphide), but that after allowing the powder to absorb water in a humid atmosphere until the " critical mois-ture content " (8–10 per cent.) is reached all the fat becomes extractable. It appears that the fat is surrounded by a glassy layer of anhydrous lactose which, on the addition of sufficient water to form the hydrate, crystallises and exposes the fat to the action of solvents. With powder prepared by other methods, e.g., by the roller process, this does not occur, and presumably the lactose is present as the hydrate and the fat is not protected. The very dry powders (spray-dried) therefore have a very small area of fat exposed to oxidising conditions, and there is no continuous water-film to keep the fat surrounded by a medium of relatively high acidity or to dissolve and activate atmospheric oxygen, for it is undoubtedly through a water-film that the oxidation of the fat (its free oleic acid, for instance) must occur. With higher mois-ture-contents, on the other hand, a water-film is present and the attack is concentrated initially on the lecithin to give the fishy flavour and subsequently on the fat proper to give tallowiness.

The question of the amount of active surface of fat exposed is also a factor determining the onset of taint development. Dorner and Widmer [81] have found that under certain conditions the homogenisation of raw milk and cream causes oiliness to appear within a few hours and that the taint increases as the size of the fat globules diminish. They state that only pasteurised milk can be homogenised with safety without becoming rancid. The mixing of homogenised raw milk or cream with pasteurised milk also causes oxidised flavour to develop.

Weich and Bauer [82] have described some samples of milk as having an " emery " flavour. This was undoubtedly an oily taint

since the milk contained 3·8 p.p.m. of copper, as against 0·37 p.p.m. in the raw milk.

Kende [83] suggests that an enzyme such as *oleinase* may cause the appearance of an oily taint in milk indirectly. He also states that certain anti-oxygens appear in the milk seasonally and resist the development of the taint. These anti-oxygens are derived from the food, green food being especially rich in them.

30. The Development of "Fishiness"

"Fishiness" is a taint mostly confined to butter and occasionally, as mentioned above, to milk powder of high moisture-content. A fishy flavour in milk through the feeding of sugar-beet tops and molassed by-products (molassed beet-pulp and molasses), arises from the metabolism of betaine in these feeding stuffs.[84] The main tertiary metabolite of betaine is *trimethylamine oxide*. Traces of this compound enter into milk during the period of secretion. Its oxygen is activated and can combine with the unsaturated acids of butter-fat at the double bonds, the resulting addition compound being the cause of the fishy flavour. Milk from cows which allow an abnormal amount of blood constituents to enter their mammary secretion is more susceptible to a fishy flavour.

Supplee,[85] Cusick,[86] and Sommer [87] are of the opinion that the taint in butter is due to the *hydrolysis* of lecithin to produce trimethylamine, $N(CH_3)_3$, the compound which they take to be responsible for the fishy flavour. If it be assumed that "fishiness" is due to the liberation of the volatile base, it must be realised that its liberation due to hydrolysis is far from describing the mechanism of the reaction. The trimethylamine residue in lecithin forms part of choline, and the base cannot be liberated by simple hydrolysis from a C—N combination any easier than ammonia can be liberated from aminoethyl alcohol or from glycine by a similar reaction. Trimethylamine can only be liberated by a hydrolytic-oxidative action. The same authors state that traces of metallic salts accelerate the production of "fishiness" through catalysis of the hydrolytic action. Traces of heavy metals are more likely to prevent lipolytic action, and in the amounts they are present in butter can have no appreciable effect on the hydrogen-ion concentration. Lintzel and Fomin [88] have found that it is necessary to employ hot alkaline permanganate to break up choline into trimethylamine, and by regulated oxidation have obtained quantitative yields from choline. Betaine, the carboxyl derivative of choline, cannot be broken down by this method.

Again, Davies [34, 69] has been able, by the action of hydrogen

peroxide in the presence of ferrous salt (Fenton's reagent), to break the —C—N— combination and obtain considerable amounts of trimethylamine from both choline in aqueous solution and lecithin in alcoholic solution or brine emulsion. The mechanism of trimethylamine-liberation in butter is thus explained by the action of organic peroxides on the choline residue of lecithin causing a mild Fenton type of oxidation which is strongly catalysed by traces of copper, iron and other heavy metal salts. The fact that acidity (butter from cream ripened with starter) also encourages the development of " fishiness " is further evidence of the oxidative nature òf the taint development. Further, as was pointed out in Section 29 (*b*), the site of the reaction is at the fat/water interface in the butter, where the conditions of solution of oleic acid, lecithin, the concentration of curd and its metallic contaminants, lactic acid from the cream and oxygen diffusing in from the atmosphere, are optimal. Rogers states that the taint is a defect mainly of salted butters. The salt in aqueous solution is a solvent for traces of lecithin from the fat and aids the development of " fishiness " by bringing more of one of the important reactants into the sphere of reaction. Overworking or renovating butter brings more lecithin into solution in the brine and encourages the developing of " fishiness." In a way, the lecithin, being present only in traces as a chemically reactive lipoid, may be regarded as an anti-oxygen, bearing the brunt of the oxidation until it is oxidised to non-anti-oxygenic compounds.

There are also certain moulds, notably those with marked lipolytic properties (*e.g.*, *Oidium* and *Oospora* spp.), which tend to produce fishiness. This taint is common in farm butter made from self-ripened cream. These moulds have the property of liberating considerable quantities of oleic acid, which during its incipient oxidation catalyses the oxidation of lecithin, and " fishiness " is at once evident. Callaghan's [89] idea of " fishiness " being due to *Oidium* was therefore sound in principle. Such butter usually has a strong butter aroma and flavour, and the relatively high content of diacetyl seems to enhance the fishy flavour.

There is another possible mechanism for the production of " fishiness," but this particular " fishiness " is characteristic more of fish oils than of trimethylamine. During the intense oxidation occurring when drying oils are exposed to the atmosphere, it has been found [69] that many organic nitrogen compounds (casein, lecithin, choline, etc.), when mixed with a drying oil (linseed oil), in the course of a few weeks have caused the oil to develop a taste

and smell of cod-liver oil.   A considerable amount of volatile base can be distilled from the resinous oil, most of which is of an alkylated nitrogenous nature.   It appears that the aldehydes formed from the autoxidation of the oil have alkylated a fraction of the nitrogen and also liberated it from the —C—N— combination.   This explains the presence of the higher amines (*e.g.*, iso-amylamine) in low-grade liver oils.

Vakil [90] has observed that Indian cotton-seed oil retains a fishy odour which is difficult to get rid of, since oxidation increases the odour and " blowing " is observed to cause the workers to become violently sick.   Maxwell [91] has found choline and betaine in cotton-seed cake, but this has no bearing on the flavour of the oil.

The fishy flavour given occasionally to milk by cows that have been fed on beet by-products (beet molasses and dried molassed pulp), is due to a reaction between milk-fat and trimethylamine oxide, which is the main tertiary nitrogenous metabolite of the betaine of sugar beet.[104]   The oxide is chemically active and is capable of dissociating molecularly into (*a*) $N(CH_3)_3$ and O and (*b*) $NH(CH_3)_2$ and HCHO.   The active oxygen from reaction (*a*) can act on the fat to produce the initial stages of fat oxidation, and the trace of fishy flavour is probably due to this reaction, together with the association of some methylated nitrogen entering into organic combination with the fat.[105]   The fact that the taint is sporadic may possibly be due to ascorbic acid being occasionally low in milks where the taint has developed.

31. Ketonic Rancidity

This form of rancidity is due to the action of the " dry " moulds (*Penicillium, Monilia, Aspergillus, Cladosporium* spp.) on the lower fatty acids of fats, and their effect therefore is most marked on butter-fat and coconut oil.   These organisms do not possess strong lipase activity, and when their mycelium is smothered with fat or fatty acids their respiratory capacities are modified.   Their tendency to fructify on the surface of butter is evidence of the unfavourableness of the medium.   They nevertheless derive some energy from the lower fatty acids by breaking them down to methyl ketones containing one carbon atom less :

$$R.CH_2.CH_2.COOH \rightarrow R.CHOH.CH_2.COOH \rightarrow$$
$$R.CO.CH_3 + CO_2.$$
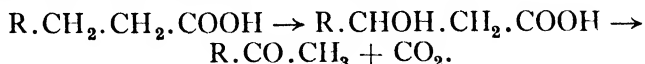
The methyl ketones are volatile odoriferous liquids and these, together with volatile acids, probably cause the odour and taste characteristic of ketonic rancidity.   Starkle [92] has observed the

formation of ketones (from methyl-amyl to methyl-nonyl) by the degradation of triglycerides with moulds, whilst Stokoe [93] has been able to fractionate these ketones from the products of the advanced action of moulds on coconut oil. Mundinger [94] has observed the same course of deterioration, and his paper may profitably be consulted as a reference to all types of fat breakdown.

A coconut flavour of common occurrence in sterilised milk has been traced to the occurrence of a small amount of fat breakdown to give methyl ketones.[95] The causative organism, as yet unidentified but showing certain similarities to *B. novus (Plectridium novum*, Huss), occurs as a resistant spore form in bottles of tainted commercial sterilised milk. Sporulation in laboratory cultures can be induced by heating at $100°$ C. for thirty minutes, which appears to destroy a growth-product inhibitory to the germination of the spores.

It is interesting to realise that while the dry moulds produce little acid and are mainly ketogenic in action, the *oily* moulds, which find butter a more favourable medium, produce a series of changes, namely, acid liberation, " fishiness," and finally " tallowiness." The main cause of this difference rests in the fact that the dry moulds cannot obtain sufficient glycerol from the glycerides to carry the oxidation beyond the ketone stage owing to the absence of lipase. The fat consequently " smokes," instead of being metabolised to its usual end-products.

Taufel and Thaler [96] have reported a delicate test for traces of methyl ketones in fats. The fat is steam distilled and the distillate extracted with pure salicylic aldehyde (freshly purified from its bisulphite compound). On the addition of concentrated sulphuric acid, traces of methyl ketones will turn the aldehyde to a deep rose or blue. The colour is extractable with butyl alcohol. Diacetyl will not give a colour by this method.

32. The Analytical Constants of Butter-fat

Fats are characterised analytically by determining various physical and chemical constants. Advantage is taken of peculiarities in composition to obtain a volume of evidence which can be utilised for identifying an unknown fat, or for detecting the adulteration of mixed fats.

(i) PHYSICAL CONSTANTS. These comprise specific gravity, refractivity, melting-point, and the solidifying-point of the fatty acids (Titer test).

(ii) CHEMICAL CONSTANTS. These consist of :

(a) The *saponification value*, which is the number of milligrams of potassium hydroxide required to saponify 1 gram of fat.

(b) The *Reichert-Meissl number*, which is the number of milligrams of decinormal alkali required to neutralise the volatile fatty acids distilled (under specially prescribed conditions in a standard apparatus) from 5 grams of fat.

(c) The *Polenske number*, which is the number of milligrams of decinormal alkali required to neutralise the insoluble volatile fatty acids from 5 grams of fat.

(d) The *Iodine value*, which is the percentage of iodine with which the fat can combine ; this is a measure of the unsaturation of the fat.

(e) The *Hehner value*, which is the percentage of water-insoluble fatty acids and unsaponifiable matter in a fat.

(f) The *Maumene number*, which is the maximum rise in temperature in degrees Centigrade when 50 grams of oil and 10 millilitres of sulphuric acid, both at an initial temperature of 20° C., are mixed in a standard apparatus.

(iii) VARIABLES OF FATS AND OILS comprise the following values :

(a) The *Acid value*, which is the number of milligrams of potassium hydroxide required to neutralise the free acid in 1 gram of the fat.

(b) The *Ester value*, which is the number of milligrams of potassium hydroxide required to saponify the neutral esters in 1 gram of fat.

(c) The *Unsaponifiable matter*, which is the percentage of the fat extracted by ether after complete saponification and solution in water.

(d) The *Acetyl value*, which is the number of milligrams of potassium hydroxide required to saponify 1 gram of the acetylated oil, and is a measure of the hydroxy-acids present.

(iv) OTHER VALUES, some of recent introduction, which are not so well known or commonly applied to the analysis of fats are the following :

(a) *Avé-Lallement value*, which is the value $b - (200 + c)$, where b is the equivalent as barium oxide of the insoluble barium salts from 1 gram of fat, and c is the equivalent as barium oxide of the soluble barium soaps (calculated from $a - b$, where a is the milligram equivalent of the saponification number (Sap. no. $\times$ 1·367)).

(*b*) The *Kirchner number*, which is the number of milli-grams of decinormal alkali required to neutralise the butyric acid (separated from the other volatile acids by reason of the solubility in water of its silver salt) distilled from 5 grams of fat, as in the Reichert-Meissl method.

(*c*) The *Thiocyanogen value*, which is the percentage of thiocyanogen taken up by the unsaturated acids of a fat when titrated in glacial acetic acid solution. Thiocyanogen is added at one double bond only, and by determining the iodine and thiocyanogen values it is possible to obtain from the differ-ence of the two values the amounts of the respective un-saturated constituents present (*vide* [97]).

Table XXXVII gives the range of butter-fat constants usually met with.

TABLE XXXVII. *Range of Butter-fat Constants*

Specific gravity (fat), 0·936–0·940 (15°C.)	Hehner number, 86·5–89·8.
„ „ (fatty acids), 0·907–0·914 (37·8° C.)	Saponification no., 219·7–232·6.
Refractive index (fat), 1·459–1·462 (15° C.)	Neutralisation values of fatty
„ „ (fatty acids), 1·437–1·439 (60 C.)	acids, 210–220.
Solidifying-point, °C. 19·1–24·5	Iodine value {fat, 25·7–37·9. {fatty acids, 28–31.
Titer test (fatty acids), °C. 35·8–38·0	
Melting-point (fat), °C. 28·4–33·3	Reichert-Meissl, 20·63–33·15.
„ „ (fatty acids), °C. 38–43	Mean molecular weight of fatty acids, 263.
Acid value, 0·4–35	Unsaponifiable matter, 0·31–0·4.
Heat of bromination, 6·6–9·5	Kirschner number, 19–26.
Avé-Lallement value, − 1·4 to − 2·0	Polenske number, 1·4–3·0.

An approximate relationship between the Polenske (P) and Kirschner values (K) is given by the formula $P = (K - 14) \times 0.26$.

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CHAPTER V

MILK SUGAR OR LACTOSE

33. General

THE carbohydrate which is present in cow's milk in the average amount of 4·8 per cent. is termed lactose ($C_{12}H_{22}O_{11}$).

Lactose was discovered and first isolated from cow's milk by Bartoletti in 1615. This sugar, otherwise termed sugar of milk, milk sugar, or lactobiose, is probably present in the milk of all mammals. Richmond and Papel [1] isolated a sugar from the milk of the Egyptian buffalo which was distinct from lactose. They termed it " *tewfikose*." The sugar of mare's milk can easily undergo alcoholic fermentation (to give *Yoghourt*), a property not possessed by bovine lactose. Richmond and Carter [2] claim that the sugar of human milk is not identical with bovine lactose.

The lactose content of the milks of various mammals varies from about 2 to 8·5 per cent. (see Table 1). Human milk is much richer in sugar than cow's milk, and lactose should be added to the latter therefore in the adaptation of that milk for infant feeding. No carbohydrate other than lactose occurs ordinarily in milk. It is improbable that lactose occurs elsewhere, although its isolation from two fruits has been reported. Sudborough [3] mentions its occurrence "occasionally in the vegetable kingdom," but it is doubtful if lactose occurs outside the animal kingdom.

The sweetness of milk is entirely due to lactose, which is much less sweet than ordinary cane sugar, sucrose.

34. Chemical Constitution

Lactose is a disaccharide which yields two aldoses, *d*-glucose and *d*-galactose on hydrolysis. Oxidation with bromine water or alkaline iodine yields the monobasic acid, lactobionic acid. Therefore one of the —CHO groups of the constituent hexoses is free and the other is implicated in the biose linkage. By oxidising lactose to lactobionic acid and exhaustively methylating the barium salt, methyl octamethyl lactobionate is formed. On hydrolysis with dilute acid tetramethyl galactose and tetramethyl γ-gluconolactone are formed.[5] The aldehyde group of galactose is therefore implicated in the linkage of the two hexoses while that of glucose is free and undergoes oxidation to form the lactobionic acid.

In a series of papers [4] published on the constitution of lactose, Haworth and his associates report their findings on the constitution of lactose. The reason for modification of their original formula was the revision of the structural formula for glucose. The elucidation of the structure of lactose required the determination of the constitution of the hexoses and the position of the biose linkage. Galactose had previously been found to possess an amylene-oxide structure. Derivatives of glucose were also found to have an amylene-oxide structure, not the butylene-oxide structure as previously thought. The possibility of an isomeric change of butylene- to amylene-oxide structure during the hydrolysis of lactose derivatives was ruled out as lactose did not itself display the behaviour of a labile or γ-sugar.

The constitution of the cleavage products of the methyl octamethyl lactobionate mentioned above established the position of the biose linkage. The two cleavage products were $2:3:4:6$ tetramethyl galactose and $2:3:5:6$ tetramethyl γ-glucono-lactone which gave a phenylhydrazide of the corresponding tetramethyl gluconic acid. Formula I was therefore established for methyl octamethyl lactobionic acid and Formula II for lactose.

(1) COOMe — CH—— ——CHOH — CH——
(2) CHOMe | CHOMe | CHOH | CHOH
    O   O
(3) CHOMe | CHOMe O   O CHOH | CHOH O
(4) CH ——— CHOMe | CH——— CHOH
(5) CHOMe CH——— CH C———
(6) CH$_2$OMe CH$_2$OMe CH$_2$OH CH$_2$OH

I.   II.
Methyl octamethyl   Glucose   Galactose
lactobionic acid.   residue.   residue.
                                Lactose.
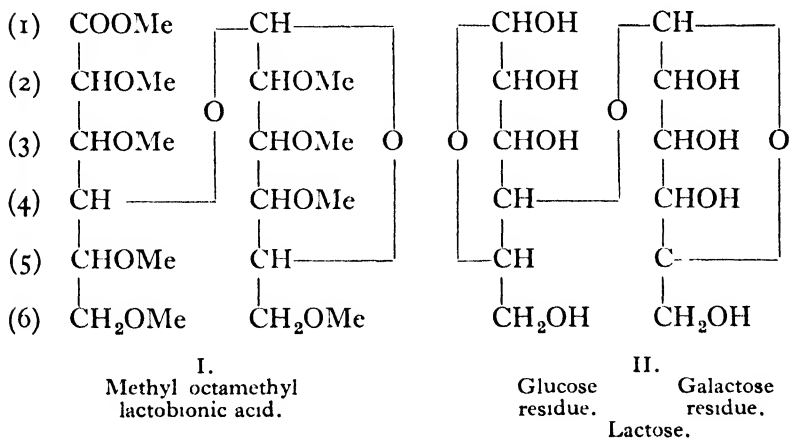
35. Physical Properties of Lactose

There are three physical modifications of lactose : α-lactose monohydrate, α- and β-lactose (anhydrides). The α-mono-hydrate is the sugar of commerce. The β-form has also entered into commerce owing to its superior dissolving properties. The α-*monohydrate*, which is the stable form and into which the other

two forms change in the presence of water below 94° C., is pre-
pared by crystallisation from aqueous solution at temperatures
below 94° C. It melts at 202° C. and has $[\alpha]$ + 89·4°. Freshly-
prepared aqueous solutions show mutarotation and, after twenty-
four hours, $[\alpha]$ decreases to + 55·5°. Heating the solutions or
the addition of alkali, particularly ammonium hydroxide, increases
the rate of change in rotation. This is of importance in the
polarimetric determination of lactose in milk products from which
a portion of the lactose has crystallised out, e.g., in sweetened
condensed milk.

On crystallising lactose solutions at temperatures above 94° C.,
crystals of β-lactose, of $[\alpha]$ + 35·0° and melting point 252° C.
separate out. This is the stable form at temperatures above
94° C. as proved by the method of preparation and by the fact that
the other forms change to it at the same range of temperature in
the presence of water. The crystals can be obtained by crystal-
lisation from boiling water. The crystals are purified by washing
successively with hot glycerol, alcohol and ether. On dehydrating
the α-monohydrate *in vacuo* at temperatures above 65° C.,
α-*lactose* (m.p. 228° C.) is formed. It may be preserved
indefinitely if stored out of contact with water. This form is
α-glucose-β-galactoside. β-lactose is β-glucose-β-galactoside [6] ;
the difference between these two forms therefore rests in the
form of the glucose residue present, whether α- or β-glucose.

Any of these forms, or their mixtures, in aqueous solution form
a mixture of the α- and β-forms, the composition of which consists
of 1·65 parts of β to 1 of α-lactose. This mixture has been pre-
pared in the solid form by evaporating a concentrated solution at
85° C. or by adding a mixture of alcohol and ether to a concen-
trated solution which has stood for at least twenty-four hours.
The dry product is a mechanical mixture of α-monohydrate and the
β-anhydride ; this is proved by the fact that its initial heat of
solution is intermediate between those of its constituents.

Hudson [8] summarises the change of one lactose form into
another by stating that the reaction α-lactose + $H_2O \rightleftharpoons$ α-hydrate
attains its equilibrium rapidly but that the equilibrium of the
reaction α-hydrate $\rightleftharpoons$ β-lactose + H_2O is only slowly established.

A substance, termed *isolactose*, which gave an osazone, m.p.
190°-193° C., has been prepared by Fischer and Armstrong [9] by
the action of kephir lactase on a concentrated solution of equal
parts of glucose and galactose.

For the preparation of β-lactose, Supplee and Flanigan [33] suggest
the rapid drying of a film of lactose solution on a hot surface at
such temperatures above 100° C. and for such a time that only

about 2 per cent. of moisture remains ; the heat remaining in the film must be sufficient to dry the product and maintain the crystallisation of β-lactose and not of the α-anhydride, *i.e.*, the temperature during crystallisation must not fall below 94° C.

The mutarotation of lactose, generally attributed to catalysis by H· and OH′, is accelerated by molecules and by ions other than H· and OH′. The catalytic effect of the anions of weak acids is much greater than that of the cations of weak bases. The catalytic effect of lactate ion, for instance, is small below a concentration of 0·1N, but increases rapidly at higher concentrations. The effect of other salts found in dairy products is small.

In determining the effect of solvent on rotation, it has been found that there is more of the high than of the low rotatory form of lactose in glycerol solutions. Therefore the specific rotation of lactose is increased in glycerol solutions ; in acetone and alcohol solutions it is decreased. It may be assumed that water is not an important factor in determining the ratio of the sugars at equilibrium. In the presence of salts the specific rotation is altered ; changes in the concentration of lactose or of the salt cause a change in the equilibrium rotation, thus pointing to the formation of molecular compounds in salt solution.[32]

Hockett and Hudson [28] have reported a new crystalline modification of lactose. When powdered α-lactose hydrate is shaken with methyl alcohol containing 1 to 5 per cent. hydrochloric acid, the crystals change to a needle type. This was found to be a mixture of 5 parts of α-lactose and 3 of β-lactose, having an initial $[\alpha]_D$ at 20° C. of 67·9° and a final value of 55·2°.

The hydrate is insoluble in alcohol-water mixtures containing 95 per cent. or more of ethyl alcohol, in methyl alcohol and ether. Pyridine dissolves about 2 per cent. its weight, whilst warm dilute or concentrated acetic acid dissolves an appreciable amount which crystallises out in the same form on cooling. Cold water dissolves about 16 per cent. and hot water 40 per cent., from which it crystallises out on cooling, although supersaturated solutions may easily be formed. The hardness of the crystals, low solubility and low sweetness impart to the taste a sensation similar to that of fine sand.

Table XXXVIII gives the various physical constants for the three forms of lactose.

36. Chemical Properties

The hydrate, $C_{12}H_{22}O_{11}.H_2O$, has the following elementary composition (per cent.), C, 40·00 ; H, 6·11 ; O, 48·89 ; water of crystallisation, 5·00. Commercial samples may contain traces of

TABLE XXXVIII. *Physical Constants of the Three Modifications of Lactose (see also [36])*

Physical constant	α-hydrate	α-anhydride	β anhydride
Density	1·5453	—	1·59
Cubical expansion (per °C.) .	9·11 × 10⁻³	—	—
Specific heat	0·299	—	0·2895
Heat of combustion (cal/1 gm.) .	3663–3953	—	3737–4162
Heat of formation (cal., mol.) .	535·6	—	610·8
Initial heat of solution (cal./1 gm.)	— 12	7·3	— 2·3
Final ,, ,, ,, ,, ,,	— 11·4	7·9	— 2·7
Temp. coeff. of heat of solution (cal. gm.)	0·1	—	—
Heat of transition to β-anhydride .	+ 1·0	+ 1·0	—
,, ,, equilibrated mixture to β-anhydride	—	+ 1·3	—
[α]ᴅ	+ 89·4°	—	+ 35·0°

extra moisture, fat, nitrogenous compounds, and mineral matter (chlorides, sulphates and phosphates). Traces of glucose and ethyl alcohol may also be present.

Lactose reduces Fehling's but not Barfoed's solution. It also reduces ammoniacal solutions of silver oxide to silver. A variety of oxidation products may be obtained by different oxidising agents used in varying concentrations (Table XXXIX).

TABLE XXXIX. *Oxidation of Lactose with Various Oxidising Agents (see also [36])*

Oxidising agent	Product
Bromine (neutral solution) .	Lactobionic acid.
Alkaline bromine or iodine .	,, ,,
Hydrogen peroxide (on Ca salt of lactobionic acid) . .	Galactoarabinose.
Autoxidation (air-dilute acid) .	Levulinic and formic acids.[10]
Concentrated nitric acid . .	Oxalic and carbonic acids.
Nitric acid, 25–30 per cent. .	Mucic and saccharic acids (1·6 dicarboxylic acid from galactose and glucose resp.).
Dilute KMnO₄, acid solution .	Traces of racemic and tartaric acids.
Heat, alkali	Formic acid, pyrocatechin, succinic acid.

When milk is heated a certain amount of acid is formed from the lactose,[10] and the autoxidation of lactose to give small amounts

of formic and lævulinic acids is probable. Kometiani [25] ascribes the increase in acidity partly to the formation of formic and lactic acids from lactose by heating.

Hydrogenation (in the presence of nickel) gives the three sugar alcohols sorbitol, dulcitol, and lactositol. Reduction in aqueous solution converts the aldehyde group to that of a primary alcohol. Thus sodium amalgam yields dulcitol and sorbitol.

Lavolini [27] has suggested a method for differentiating between lactose and glucose in solution. Both sugars reduce Schweitzer s reagent, but glucose gives a reddish-brown flaky deposit of cuprous oxide ; lactose, however, causes the solution to become turbid only, because the reduction product remains in the hydrosol form.

On heating to 110° C. no change occurs in the hydrate but all the water of crystallisation is lost between 110° C. and 130° C. [11] Slight browning occurs at this range showing slight decomposition. A small amount of carbon dioxide is evolved. [12] Above 130° C. further decomposition occurs with formation of the hexoses. At 150–160° C. a yellowing not accompanied by any loss of weight occurs, whereas it turns brown at 170° C., emitting a characteristic odour of burnt sugar and losing 30 per cent. of its weight. The mass at this temperature consists mostly of anhydrous lactose, with some lactocaramel and a substance insoluble in water. By taking advantage of the solubility of anhydrous lactose in alcohol, the sugar can be extracted first from the mass and the lactocaramel extracted subsequently with water. Its empirical formula has been found to be $C_{12}H_{22}O_{10}$ and an apparently identical substance has been prepared by dehydrating lactose at 185° C. for ten to twelve hours at 4–5 mm. pressure. [13] This compound has been named *lactosan*, since it is probably 5 galactosyl-glucosan, and it further polymerises at 105° C. in the presence of anhydrous zinc chloride. Lactocaramel melts at 203·5° C.

Webb [34] has studied the factors associated with the browning of lactose in heat-treated products. The phosphate radical has a specific effect in causing the browning. The brown colour is more marked with increase in concentration of OH ions, amino acids, ammonium salts, and oxygen. Traces of heavy metals retard and sodium sulphite prevents colour formation ; traces of formaldehyde cause a deeper brown to develop whilst large amounts prevent colour development. At the pH of milk, both the reactions that are considered necessary for browning to develop can occur, namely, humin formation from the action of amino acids on sugar, and the formation of lactocaramel. No effective means have been found to prevent the slight browning

of evaporated milk, but the shortening of the storage period and the lowering of the storage temperature will tend to make the fault less evident.

(c) IDENTIFICATION OF LACTOSE. Oxidation with 25 to 30 per cent. nitric acid yields the sparingly soluble *mucic acid*, which distinguishes lactose from all other sugars except galactose.

The formation of the phenylosazone may be applied as a confirmatory test (heating with 3 parts phenylhydrazine hydrochloride, 4 parts sodium acetate in 60 of water). Fine yellow needles melting at 200° C. separate out on cooling. These may be distinguished from similar derivatives of other sugars with melting-points near 200° C. (galactose and glucose) by the characteristic crystalline form of the lactosazone when viewed under the microscope or the *absence* of rotation when examined polarimetrically (the osazone in a 40 per cent. solution of pyridine in alcohol).

37. The Solubility of Lactose and its Crystallisation from Solution

The crystallisation of lactose from concentrated whey, and spontaneously in sweetened condensed milk and in ice cream is of importance to the dairy manufacturer. Leighton and Peter [17] have carried out pioneer experiments on the physico-chemical side of the problems of sandiness in condensed milk and ice cream.

Williams and Peter [26] report the occurrence of a diamond-shaped crystal of α-lactose hydrate, similar to crystals isolated from condensed milk, in ice cream. They attribute the formation of such crystals to the high viscosity of the mix.

With the possible exception of a small amount of caramelisation of lactose by exposure to high temperature for a considerable length of time, the chemical properties of lactose are unchanged in the manufacture of condensed products. In the case of evaporated milk, long periods of holding and forewarming and the use of prolonged sterilisation temperature cause considerable browning of the milk, whilst the re-processing of sweetened condensed milk and re-sterilisation of the evaporated product enhance the dark colour.

In sweetened condensed milk a considerable proportion of the lactose is present in the crystal form, and if these crystals exceed a certain size the product is gritty to the taste and liable to form a sediment of crystals. To avoid large crystals being formed the conditions must favour the rapid crystallisation of the greatest possible number of small crystals instead of favouring the unstable state of supersaturation which would subsequently give rise to large and growing crystal aggregates. In this direction a description of the solubility properties of lactose is essential.

When the hydrated α-form is dissolved in water a total of 62 per cent. changes slowly into the β-form. The latter is more soluble in water, but, since the reaction α——→β is slow, if powdered lactose hydrate is mixed with water, its saturated solution can be made at once. This defines the *initial solubility* of lactose. Since the β-form is more soluble, as the change to the β-form proceeds, more of the powder (α-hydrate) will dissolve ; this will proceed until the solution is saturated with the equilibrium mixture of α- and β-forms. This defines the *final solubility* of lactose. The initial is the true solubility and the
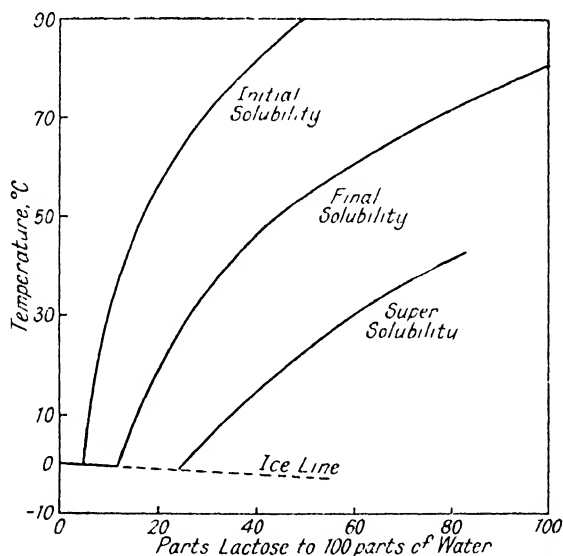
Fig. 11.—Solubility curves of lactose in water at various temperatures.

final solubility is the total amounts of hydrate and the β-form which can exist in solution at a given temperature.

The equilibrium between the two forms is attained very slowly at room temperatures and the rate is greatly accelerated by H and OH ions. The percentages of lactose which can be transformed in one hour are : 25° C., 51 per cent. ; 15° C., 17·5 per cent. ; 0° C., 3·4 per cent. At 75° C. the equilibrium is reached instantaneously. Leighton and Peter [17] have tabulated the values [14] for the initial and final solubilities of α-lactose mono-hydrate (Table XL). The solubility curves are given in Fig. 11.

Lactose solutions also show conditions of *super-solubility*. When cooling saturated solutions generally, the tendency to crystallise increases with lowered temperature at first and then decreases

TABLE XL.   *Initial and Final Solubility of Lactose (see Figure 11).
Leighton and Peter from Hudson's data*

Temperature		Initial Solubility		Final Solubility	
°C.	°F.	Lactose per cent.	Parts lactose to 100 parts of water	Lactose per cent.	Parts lactose to 100 parts of water
0	32	4·8	5·0	10·6	11·9
15	59	6·8	7·3	14·5	16·9
25	77	8·2	8·9	17·8	21·6
39	102	11·0	12·4	24·0	31·5
49	120	15·0	17·6	29·8	42·4
64	147	21·0	26·6	39·7	65·8
74	165	26·0	35·1	46·3	86·2
89	192	37·0	58·7	58·2	139·2

until the material passes into the amorphous phase and it is possible with moderate super-cooling to keep solutions without crystal nuclei forming. Ostwald [15] has proposed the term " metastable " for the condition where nuclei do not readily appear and " labile " for the condition induced by a greater degree of super-cooling where crystallisation will, if initiated, spread rapidly through the solution. Miers [16] has shown that a definite boundary line exists between these two states and, in the metastable state, crystallisation can be induced only by liberal seeding with lactose crystals or by those of an isomorphous substance. The inoculation point or the point of true saturation is a definite limiting temperature.

As regards the velocity of crystallisation, the rate first increases with super-cooling and then sharply decreases due to increase in viscosity and temperature effects. It is obvious that the growth of a crystal depends on a supply of material by diffusion and the dissipation of the heat of crystallisation.

On cooling lactose solutions to saturation point, if crystallisation occurs, α-monohydrate will separate. Some of the β-form will then be changed to the α-hydrate and this will separate at the rate that this reaction proceeds, and this rate is slow at low temperatures. The rate of crystallisation is greater than the transformation rate at the temperatures used in the manufacture of some milk products ; the transformation rate is therefore an important factor in manufacture and its acceleration, say, in the crystallisation of lactose from whey, may very well be considered.

Prevention of " Sandiness."   To prevent " sandiness " in

sweetened condensed milk the lactose crystals must be as small as possible. Thus with crystals up to 10 microns (0·01 mm.) in length, a smooth velvety product is given, whereas a pasty product is obtained when a length of 10–15 microns is reached, above which grittiness may be observed.

The favouring of mass crystal formation during the cooling of condensed milk is effected by providing a " forced crystallisation period," which must of necessity represent a condition of high supersaturation and minimum viscosity. An abundance of small nuclei can be initiated by seeding with powdered lactose, or a small quantity of a previous batch of cooled condensed milk, immediately before the forced crystallisation period is commenced. The proper temperature of forced crystallisation depends on the lactose concentration in the condensed milk.

Herrington [35] has lately studied extensively the crystallisation properties of lactose. When aqueous solutions of lactose, saturated for the temperature-range 46°–96° C., either alone or in 0·7 per cent. agar, were allowed to cool at various degrees of super-cooling without agitation, less supercooling was found necessary for the more concentrated solutions, but there was no sharp line of demarcation between the metastable and labile states. As the temperature or crystallisation was lowered, the rates of crystal formation and of crystal growth passed through maxima (at about 30° C.). Lactose, precipitated from solution by ethyl alcohol, was of varying composition.

The crystalline habit of α-lactose monohydrate varied greatly under different conditions of crystallisation. The principal factor governing the crystalline habit was the rate of crystal growth, or " precipitation pressure," which could be measured by the ratio of the actual lactose concentration to the solubility. The influence of sucrose on the crystalline habit was due to its pre-cipitating effect on lactose. Sufficiently rapid crystallisation of both α-hydrate and β-anhydride caused the formation of needles, those of the former being straight and those of the latter curved.

The α-hydrate loses water of crystallisation slowly at 80° C., small crystals parting with it more rapidly than large. Crystal size does not appear to affect the rate at which the β-anhydride takes up water.

38. Hydrolysis of Lactose

A number of weak acids (*e.g.*, citric) which easily hydrolyse sucrose have no effect on lactose under similar conditions. Complete hydrolysis of lactose can be effected by strong mineral acids.

Thus, 1·0 N acids will effect complete hydrolysis of lactose solutions held at 90° C. for ninety minutes.

Certain species of yeasts (*Torula*) are capable of fermenting milk sugar to form carbon dioxide and alcohol. *Torula cremoris* and *Torula sphærica* [18] ferment lactose rapidly at body temperatures and cause the foaming of certain creams in summer. Hunziker [19] states that on incubation at 95° F. the milk sugar in cream is completely destroyed by these organisms in seventy-two hours. The fermentation of lactose by the combined action of yeast and lactic-acid bacteria forms the basis of the manufacture of *kephir* and *koumiss*. Only yeasts which contain *lactase* can ferment lactose, since the hydrolysis of the molecule to hexoses must precede alcoholic fermentation. The lactase of the intestinal tract of animals and the lactase found in the crude emulsion of almonds can hydrolyse milk sugar ; the former converts lactose to 2 molecules of glucose, the galactose being changed to glucose during the hydrolysis.

39. Lactic Acid Fermentation

Milk sugar is easily converted into lactic acid by the lactic acid bacteria. One molecule of lactose theoretically yields 4 molecules of lactic acid, but in the lactic acid fermentation of milk the amount of acid produced is much less than is thus indicated owing to the formation of other products, *e.g.*, butyric acid, carbon dioxide, and acetyl methyl carbinol. A portion of the lactic acid combines with the calcium of casein to form calcium lactate, thus setting free the casein and coagulating it when its isoelectric point is reached (pH 4·6).

An acidity of 0·20 per cent. lactic acid gives milk a sour smell, and a sour taste at 0·25–0·30 per cent. The milk curdles at an acidity of 0·5–0·7 per cent. The maximum titratable acidity which can develop in milk is approximately 1 per cent. ; with whey, on the other hand, which has a lower buffering capacity than milk, only approximately 0·7 per cent. acidity is reached. Other forms of bacteria, *e.g.*, *Bacillus acidophilus* and *B. bulgaricus*, are capable of producing from 2 to 3 per cent. of lactic acid in milk.

It is generally agreed that the step before the actual fermentation process is the splitting of lactose to hexoses by the endo-enzyme—*lactase*—of the bacteria,[20] after which, under conditions of either moderate or low oxygen tension, lactic-acid fermentation supplies the energy to the organisms. Suzuki, Hastings and Hart [21] state that from 90–98 per cent. of the lactose fermented goes to form lactic acid and the rest to alcohol, aldehydes, and esters.

40. Commercial Lactose

The source of commercial lactose is the whey obtained in cheese manufacture which, according to Konig and to Berry,[22] contains on the average about 4·79 per cent. of lactose (with a total solids-content of 6·9 per cent.). The chief economic difficulties connected with the preparation of lactose from whey lie in the rapid loss of lactose by fermentation, and in obtaining enough raw material for handling at a central depot, as the cost of transporting a liquid containing 93 per cent. of water is, as a rule, prohibitive.

Up to 1880, lactose was manufactured only in Switzerland, where small cheese factories obtained, by a crude method of evaporation, a " sugar sand " which was refined at a central depot. More modern methods employ equipment similar in principle to that used in cane- and beet-sugar practice. The use of the vacuum pan for evaporation assists in obtaining a white product, since lactose solutions are susceptible to heat.

The first step consists in separating the trace of fat in the whey by centrifuging. Whey butter is made from this fraction. The centrifuged liquid is evaporated in a vacuum pan or multiple-effect evaporator at 60° to 70° C. until a product containing 55–60 per cent. of total solids is obtained. This product, containing 38–42 per cent. of lactose, is cooled slowly so that crystals as large as possible separate out, and crystallisation is allowed to proceed for one to five days. The crystals are separated by centrifuging in hydro-extractors lined with wire gauze of 40 mesh, on the surface of which the crystals form a cake containing from 85–90 per cent. of crude lactose, representing a yield of 3·5–4 per cent. of the original whey. The crude lactose is then dried (92–93 per cent. sugar), ground, dissolved in water at 95° C., and filtered free from albumin in a press, decolorised with bone-charcoal, concentrated and crystallised. The crystals are separated by centrifuging, dried and ground. Two crops are taken in the refining process.[23]

The mother liquors are used for the manufacture of animal foods either by using bulky foods, like dried brewers' grains, as fillers, or as whey paste. The latter may contain from 48 to 60 per cent. of dry matter, composed of lactose, protein, ash, and lactic acid.

41. Quality and Uses of Lactose

The regulations laid down in the " British Pharmacopœia " state that lactose must be white, free from acid and poisonous metals, dissolve completely to a clear solution in water and not contain more than 0·25 per cent. of ash. Very little free moisture

is present in powdered lactose ; when the moisture is determined by the oven method (100° C.) all the water of crystallisation is slowly driven off ; this also applies in the determination of moisture by the " volatile solvent " method.

The specifications in the U.S.A. require lactose to be a fine, dry, odourless powder of not less than 99·7 per cent. purity as determined by the polarimetric method ; not containing more than 0·020 per cent. nitrogen or more than 0·020 per cent. of fat, and yielding not more than 0·050 per cent. of ash. It must be neutral to litmus and free from heavy metals, whilst a 10 per cent. solution must be colourless and free from solid impurities.[24]

For bacteriological use, lactose must be free from ethyl alcohol (iodoform test), contain not more than 0·15 per cent. free moisture and no chlorides or sulphates. Glucose should be absent and a 10 per cent. solution sterilised for thirty minutes at 120° C. should not show a pH value greater than 4, and should remain acid on boiling.

Large quantities of lactose are consumed in milk and some of its products, and considerable quantities are used in making infant foods and for the modifying of cow's milk for infant feeding. The fact that it can pass the ileo-cæcal valve unchanged and encourage the growth of certain organisms only in the large intestine gives it a certain therapeutic value, but its laxative and diuretic effects, probably due to its dehydrating action, are the most prominent.

Lactose is also used in the manufacture of confectionery and for coating medicines in tablet form.

42. Evidence of the Presence of other Sugars in Milk and Milk Products

Glucose may be present in very small amounts in normal milk, but there is no evidence up to the present of an increased amount in milk of abnormal composition containing infiltrated blood constituents (globulin and sodium chloride). Whitnah,[29] by determining the rotatory powers of milk sera before and after fermentation with washed yeast, found indications of from 0 to 0·35 per cent. of glucose in normal milk. The amount was apparently not dependent on the amounts of the other constituents present. Jones [35] has published evidence of the presence of a reducing sugar in milk other than lactose (which might be glucose or a sugar fermentable by yeast) ; the amount is greatest (0·11 per cent.) in milk given at the end of lactation. An average value for normal milk is 0·06 per cent.

Polonovski and Lespagnol report the discovery of two new

sugars [31] in human milk as well as a gluco-protein.[30] The gluco-protein yields a reducing substance on hydrolysis. They found that the rotatory power of the whey of human milk was less than that corresponding to the reducing power of the lactose. They concluded that some constituents less dextrorotatory than lactose accounted for the difference.

The sugar was called *gynolactose*. Its reducing power was less than two-thirds that of lactose and half that of glucose. The Selivanoff test was negative. It yielded a small amount of osazone and was partly hydrolysed by warm dilute acids. Another sugar, *allolactose*, was also found in human milk. The properties of these two sugars were found to be :

Gynolactose ; melting point, $205°$ C. ; $[\alpha]_D - 27°$. Glucose and galactose were formed on hydrolysis.

Allolactose ; melting-point, $165°$ C.; $[\alpha]_D + 20°$. A hologluco-side giving glucose and galactose on hydrolysis.

Malted Milk contains maltose and small amounts of glucose as well as lactose.

Sweetened condensed milk contains sucrose, small amounts of glucose, levulose (from hydrolysis of the sucrose) and levan, as well as lactose.

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CHAPTER VI

THE PROTEINS OF MILK. CASEIN

43. General

THE class of organic compounds called the proteins are the most complex with which the chemist has to deal and their physiological and nutritive significance is at once apparent when it is considered that a large percentage of the body substance of animals has been elaborated from them and that they are the most important nitrogen-containing nutrients from which the supply of enzymes and other mobilised nitrogen compounds necessary for the proper efficiency of the animal organism is derived. Thus, proteins account for from 17 to 21 per cent. of the fat-free bodies of farm animals. They are of prime importance biologically since they form the basis of the cytoplasm and nucleus of every living cell.

Proteins are built up of a variety of " building stones " termed amino acids. These compounds exhibit a wide variation of structure and are classified into the following groups :

(i.) *Aliphatic monoamino - monocarboxylic acids :* glycine, d-alanine, l-serine, d-valine, l-leucine and d-isoleucine.

(ii.) *Aliphatic monoamino - dicarboyxlic acids :* l-aspartic, d-glutamic and d-hydroxyglutamic acids.

(iii.) *Aliphatic diamino-monocarboxylic acids :* d-arginine and d-lysine.

(iv.) *Sulphur-containing amino acids :* cystine and methionine.

(v.) *Aromatic amino acids :* l-phenylalanine and l-tyrosine.

(vi.) *Heterocyclic amino acids :* l-tryptophane, l-histidine, l-proline and l-hydroxyproline.

These amino acids, together with ammonia (making 20 units in all), are condensed together by various methods (in greatest part by the condensation of —COOH and —NH$_2$ groups to form —CO—NH—) and in various combinations to build up the protein structure. Hydrolysis of proteins either with acids, alkalis or proteolytic enzymes breaks up the protein structure into simpler units, the breakdown to amino acids being complete when chemical reagents are used. This forms the basis of protein analysis since from the hydrolysates most of the amino acids present in the original protein can be separated, or the

distribution of the various forms of nitrogen (amide, basic, monoamino, etc.) can be determined.

The structure of proteins has also been studied from the synthetic standpoint by the preparation of condensation products of various amino acids to form polypeptides ; a product containing 18 molecules of amino acids showing many of the properties of naturally occurring proteins has been prepared. The complexity of the problem of determining the structure of proteins is at once obvious when the number of combinations possible from a varying number of molecules of each of the 19 amino acids, the number of types of linkages possible (carbonyl-imino, deketopiperazine, etc.), and the varying effects of diamino and dicarboxylic groupings are considered.

Some proteins do not contain all the amino acids listed above and are termed "incomplete" proteins. The animal body depends for the building up of tissue and enzymic protein mostly on the amino acids of the proteins derived from foods, and the absence of one or more amino acids from a food protein is significant in that respect. *Gelatin*, for instance, contains no tyrosine, whilst *zein* contains no tryptophane, and since both these amino acids are essential physiologically, disturbances have been met with in feeding these proteins as a sole source of nitrogen for experimental animals. The amino-acid composition and the completeness of proteins are thus important matters in the biological considerations of protein composition.

Proteins have been classified according to their physical properties and composition. One broad classification is that of (*a*) simple and (*b*) conjugated proteins, those in the former group being made up of amino acids only, whereas in the latter a prosthetic grouping such as phosphoric acid (phosphoprotein), carbohydrate (mucoprotein), lecithin (lecithoprotein), a chromogenic grouping (hæmoglobin, hæmocyanin), or nucleic acid (nucleoprotein), is present. The prosthetic group usually confers on the protein certain special properties either in the direction of causing a considerable increase in molecular weight, or of being capable of performing special physiological functions.

44. The Proteins of Milk

In the domain of protein chemistry there is no protein more important nor one on which so much work has been done as the special protein of milk—*casein.* Casein occurs as its calcium salt in milk. (Milk when fresh usually exhibits a pH of 6·6 or thereabouts, and since the isoelectric point of casein is at a pH of 4·6, the casein must be combined to a certain extent with a basic

radical). In average samples of milk it is present to the extent of from 2·2 to 3·5 per cent., with an average value of 2·86 per cent. (see Table V). Casein is a phosphoprotein, that is, a phosphoric acid radical enters into its chemical structure, the linkage being stable irrespective of radical exchanges brought about by variations in pH of the medium in which the protein is maintained. The phosphoric acid is not bound ionically, but treatment with 1 per cent. caustic soda for twenty-four hours or two to three days with trypsin will bring all the casein phosphorus into solution.[1] The ready availability of the protein and its favourable physical properties, especially that of its insolubility at the isoelectric point, enable it to be isolated in pure form in large quantity. This has led to its being more extensively investigated than any other protein and to its exploitation in many directions as an article of commerce.

Other proteins of milk belong to the *general* class of proteins, that is, their composition and properties do not justify their being termed specific proteins. The albumin of milk—*lactalbumin*—although not identical with the albumin of blood serum conforms to the properties of the various water-soluble proteins found in nature. It is present in milk in amounts varying from 0·4 to 0·7 per cent., the average being 0·56 per cent.

Lactoglobulin is also present in milk in amounts roughly half those of lactalbumin, although some investigators cite lower values (*e.g.*, Bell [2] states that lactoglobulin is present to the extent of 0·05 per cent.).

Davies [3] gives the following protein distribution in normal milk (Table XLI).

TABLE XLI. *Protein Distribution in Normal Milk (Davies)*

Sample	Shorthorn	Ayrshire	Friesian
Per cent. of total N :			
Protein nitrogen . .	94·1	94·7	94·8
Casein ,, . .	76·1	78·4	76·6
Albumin ,, . .	12·6	10·2	14·1
Globulin ,, . .	5·4	6·2	4·2
Non-protein nitrogen .	5·9	5·3	5·2
Per cent. of Milk (N × 6·38) :			
Casein	2·41	3·06	2·45
Albumin . . .	0·38	0·39	0·45
Globulin . . .	0·17	0·24	0·14
(Total nitrogen . .	0·497	0·609	0·501)

Abnormality in milk composition is reflected in (*a*) a lower casein-content, with a lower percentage of total nitrogen accounted for as casein nitrogen, (*b*) a higher albumin-content, (*c*) a much higher globulin-content, (*d*) a fluctuating albumin/globulin ratio, and (*e*) a higher non-protein nitrogen-content. It seems worth while to stress the importance of casein-content and of casein-nitrogen accounting for a high percentage of total protein-nitrogen (80 per cent.) and of total nitrogen (76 per cent. and above) as an index of milk quality and of the absence of abnormality of composition. (This has already been discussed at length in Section 15 (*b*).)

Other Nitrogenous Compounds. After removal of casein, albumin and globulin from normal milk, about 0·02–0·03 per cent. of nitrogen is still left in solution. A small fraction of this may be accounted for by a little protein-splitting, *e.g.*, deamidisation, when the protein is separated from milk by acid or heat treatment (trichloracetic acid at 70° C. for thirty minutes), or when lipins are partially degraded by the same treatment, as suggested by Kay [4] for blood. Kieferle and Glöetzl [5] state that the residual nitrogen is identical with that of blood and urine, and is composed of hexone bases, proteose, amino acids, urea, creatine, creatinine, uric acid and ammonia. There is no doubt that some of the minor nitrogenous constituents of the blood diffuse into the milk, and that this infiltration may be appreciable when other blood constituents, such as sodium chloride, enter during the secretion of various kinds of abnormal milk (mastitic and tubercular conditions, colostrum, and drying off). This may partly account also for the high non-protein nitrogen-content of milk low in solids-not-fat. Heating of milk to different temperatures and souring increase the amount of residual nitrogen appreciably. Pasteurisation has been found to increase it by 10·4, and boiling by 18·6 per cent.[5] From 30–40 per cent. of the residual nitrogen is precipitated by phosphotungstic acid in acid solution.

Traces of other Proteins. No satisfactory proof has been adduced of the actual existence of some other proteins reported to occur in milk. Lactomucin, a mucoprotein, has been reported by Abderhalden [6] and by Storch [7] to occur in butter. The presence of alcohol-soluble proteins has been reported by several workers, but they are co-precipitated with casein, or derived from casein during the ripening of cheese (*v.* Allen [8]).

45. Casein

(a) NOMENCLATURE. The term casein is used for the main protein of natural milk, although the British term " caseinogen "

for this form is still insisted on by some authorities. In Britain, the term " casein " is confined to the product obtained by the action of rennin on caseinogen to which Americans apply the term " paracasein." The present tendency is to fall in with the casein-paracasein form of nomenclature.

(*b*) ELEMENTARY COMPOSITION. Casein contains the elements carbon, hydrogen, oxygen, nitrogen, phosphorus and sulphur. Table XLII gives the elementary composition of the casein of cow's milk according to various investigators. Very good agreement is observed between the figures, especially in the nitrogen-content. The average value of 15·68 per cent. has been universally accepted, and the factor used in calculating the weight of casein from the nitrogen-content is 6·38. The difficulty of freeing casein from traces of butter-fat is perhaps the cause of the discrepancy in the values for carbon. The closely agreeing values for phosphorus and sulphur (both elements being very close in atomic weight) suggest the presence of equal numbers of the atoms of these elements in the casein molecule.

TABLE XLII.   *Elementary Composition of Casein (Percentages)*

	Hammarsten[9]	Van Slyke and Bosworth[10]	Langl[11]	Lehmann[12]	Chittenden and Painter[13]
Carbon .	52·96	53·50	52·69	54·00	53·30
Hydrogen .	7·05	7·13	6·76	7·04	7·07
Nitrogen .	15·85	15·80	15·65	15·60	15·91
Phosphorus .	0·85	0·71	0·88	0·85	—
Sulphur .	0·72	0·72	0·83	0·77	0·82
Oxygen .	22·77	22·14	23·19	21·19	21·74

Casein from the Milk of Different Mammals.   The compositions of the caseins prepared from the milk of different mammals are so nearly identical that it is impossible to deduce the source of casein by this method. The physical properties also point to a close relationship, if not actual identity, but Kohn[90] claims that goat's milk can be differentiated from cow's milk by comparing the rate of solution of the precipitated curd in sulphuric acid. Curd from goat's milk is precipitated by acid in a fine state of division and dissolves quickly in excess of acid.   (The test is not applicable for milk when sour or when preserved with formaldehyde.)

Table XLIII gives the elementary analyses of casein from the milk of different mammals.

Immunological reactions of proteins are generally accepted as the most delicate means of identifying the presence or absence of

TABLE XLIII.   *Elementary Analyses of Casein from Different Sources and of Paracasein*

	Human (Wroblewski[17])	Mare (Tangl[11])	Goat (Tangl[11])	Ass (Tangl[11])	Ewe (Tangl[11])	Rabbit (Burow[18])	Paracasein
Carbon . .	52·24	52·36	52·90	52·57	52·92	52·87	53·50
Hydrogen .	7·32	7·09	6·86	7·01	7·05	7·05	7·26
Nitrogen .	14·97	16·44	15·48	16·28	15·71	15·60	15·80
Phosphorus .	0·68	0·88	0·76	1·06	0·81	0·81	0·71–0·83
Sulphur . .	1·12	0·53	0·70	0·59	0·71	0·73	0·72–0·87
Oxygen . .	23·67	22·70	23·30	22·49	22·80	22·94	21·87

specific proteins.   Such tests on casein from different sources have failed to reveal any essential differences between them. Wells [91] states that " an immune serum for any one casein will give reactions with casein from any other species, even of remote relationship," and " casein from the milk of an animal from any given species shows a closer biological relationship to the casein of another species than it does either to the whey proteins or to the serum proteins of its own species."

Dudley and Woodman,[92] in their investigations on the optical activity of the amino acids isolated from the racemised casein of cow's and sheep's milk, concluded that, whereas the amino-acid contents of the two caseins appeared to be identical, there was evidence that the arrangement or order of these acids in the protein-molecule was different.   They suggested that the difference between the caseins arises in the intra-molecular arrangements of the various amino acids.

Abderhalden and Schittenhelm [33] found that the compositions of the casein from cow's, goat's, and human milk were so similar that variations in the proteins in each of these milks could not be detected by chemical analysis.

(c) MOLECULAR WEIGHT OF CASEIN.   From the elementary analysis of casein given in Table XLII, and the inferences that at least 1 atom each of sulphur and phosphorus and at least 1 molecular equivalent of certain amino acids must be present, the minimum molecular weight of casein is calculated to be roughly 4,300.   Cohn, Hendry and Prentiss,[13] applying the principle of the least common multiple, have concluded that the molecular weight is at least 12,800, or roughly three times the above.   They state that probably the molecular weight is fifteen times this value, namely, 192,000.   This corresponds to the formula

$$C_{9490}H_{13425}N_{2160}O_{2640}S_{45}P_{45}.$$
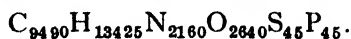
Recently Carpenter,[14] by ultra-centrifugal methods, has shown that the molecular weight is between 75,000 and 100,000. An analysis has shown : S, 0·785 ; P, 0·856 ; cystine, 0·488 ; tryptophane, 1·237 ; tyrosine, 5·550 ; histidine, 1·776 per cent. Consistent with these figures and the above range, the molecular weight would be 98,000. Svedberg, Carpenter and Carpenter,[15] dissolving casein prepared by Hammarsten's method in a phosphate buffer (pH 6·8), and examining by Svedberg's centrifugal sedimentation velocity method, have found that the material behaves as a mixture of protein molecules of different weights. A protein dissolved out of the product by hot acidified alcohol (33 per cent. yield) appears to be a chemical individual of molecular weight 375,000 $\pm$ 11,000. On the other hand, the same investigators [16] have found that casein prepared by the method of Van Slyke and Baker, also made up of a mixture of protein substances, is mainly composed of a fraction having a molecular weight of 75,000–100,000. If the casein were heated to 40° C. during the time of dissolving in phosphate buffer at pH 6·8, it has been found to have a molecular weight of 188,000, and no protein of molecular weight 75,000–100,000 can be detected.

(*d*) THE AMINO-ACID COMPONENTS OF CASEIN.   The determination of the amounts of the various amino acids contained in casein has been much investigated and has proved a fertile source of the discovery of other amino acids ; and it has also led to similar investigations on other proteins.

The first amino acid discovered was leucine (Proust,[19] 1818). Liebig [20] isolated a crystalline compound after hydrolysing casein with alkali, which Bopp [21] (1849) and Hinterberger [22] (1849) isolated from other proteins and called tyrosine. Serine, glutamic and aspartic acids were discovered later. This completes the list of amino acids which had been isolated from proteins up to 1892. The manner of isolation up to this time had followed the conventional organic methods, such as oxidation, reduction, destructive distillation, halogenation, etc. ; it was probably thought that the protein molecule was simpler in constitution than we now know it to be, and that the amino acids isolated were secondary decomposition products. At any rate, none of the studies threw any light on the real nature of protein composition until Emil Fischer [46] applied the method of hydrolysis and separation of the constituent amino acids.

Some improvements on Fischer methods have been introduced from time to time, particularly by Dakin [47] and Foreman.[24] Much information on the amounts of the various amino acids in casein and other proteins has been afforded ·by· these analyses, and

Table XLIV gives the results of such findings for milk, egg and serum proteins. (No results are available for lactoglobulin, but since the physical [48] and serological [49] properties of this protein have proved it to be identical with serum globulin, the results for the latter only are included in Table XLIV.)

On examination of the composition of the proteins, all of which are phylogenetically related, certain general similarities are evident, e.g., low glycine or even its absence, the general absence of

TABLE XLIV. *Amino-acid Content of Milk Proteins and Egg Proteins*

	Casein			Lactalbumin[35,37]	Ovalbumin	Ovovitellin	Serum globulin[43]	Serum albumin[45]
	Cow's milk[24]	Goat's milk[33]	Human milk[34]					
Glycine . .	0[1] 0[8]	0·5	0	0 0·37	0[38,39,40]	1·1[4] 0[38,39]	3·5	0
Alanine . .	0·9[1] 1·5[6] 1·9	1·5	1·2	2·5 2·41	2·22	Trace 0·75	2·2	2·7
Valine . .	1·0[1] 7·2[5] 7·9	—	1·3	0·9 3·30	2·50	2·4 1 87	†	—
Leucine . .	10·5[1] 9·4[6] 9·7	7·4	8·8	19·4 14·03	10·71	11·0 9·87	18 7	20·0
Isoleucine .	— —	—	—	— —	—	— —	—	—
Phenylalanine	3·2[1] 3·2[1] 3·9	2 8	2·8	2·4 1·25	5·07	2·8 2·54	3 8	3·1
Tyrosine .	4·5[1] 4·5[1] —	5·0	4·7[33]	0·9 1·95	-	1·0 3·37	2·5	2·1
Serine . .	0·5[8] 0·5[5] 0 4	—	—	— 1·76	1·77	- —		0·6
Cystine . .	0·1[4] 7·5[5] —	—	-	1·73[36] —	—	- -	0 7 0·9[21]	2·3 2·5[18]
Proline . .	3·1[1] 6·7[6] 7·6	4·6	2·9	4·0 3·76	3·56	3 3 4 18	2·8	1·0
Oxyproline .	0·3[2] 0·3[2] —	—	—	— —				
Aspartic acid .	1·2[1] 1·4[6] 0·6	1·1	1 0	1·0 9 30	2·20	0 5 2 13	2 5	3·1
Glutamic acid	10·7[1] 15·6[5] 15·9	12·0 11 3	11·0	10 1 12·89	9·10	12 2 12 95	8 5[43]	7·7[44]
Tryptophane .	1·5[1] 1·5[5]	+	1[36]	·†	·†	+	+
Arginine .	4·8[3] 3·8[5] 3·85[9]	—	-	3·2[16]	4·91	2·3 7·46	—	—
Lysine . .	5·8[2] 6·0[5] 6·25[9]	—	-	9·2	3·76	- 4·81	- -	—
Histidine .	2·6[8] 2·5[6] 1·83[9]	—	—	2·1	1·71	— 1·90	- -	—
Ammonia .	1·6[2] 1·6[6]	-	-	1·3	1·34	- 1·25		
$C_5H_{11}NO_2S$.	0·4[7] —	—	—	10 00*				
$C_{13}H_{24}N_2O_5$.	0·8[8]	—	—					

(1) Abderhalden.[25]
(2) Fischer.[26]
(3) Hart.[27]
(4) Mörner.[28]
(5) Osborne and Guest.[29]
(6) Hopkins and Cole[30]
(7) Mueller[31]
(8) Fischer and Abderhalden.[32]
(9) Vickery and White.[109]

* Hydroxyglutamic acid.

isoleucine and oxyproline, the low cystine-content, the high contents of leucine, proline and glutamic acid, and, amongst the diamino acids, a high lysine-content. As far as the figures go, there is no interpretable difference afforded by the analysis of casein from different mammals.

(e) THE SIMILARITY BETWEEN CASEIN AND VITELLIN. A striking similarity in composition is shown between casein and ovovitellin. Indeed, Lehmann,[50] Schwarzenbach [51] and others regarded vitellin as a mixture of albumin and casein, not on the grounds of both being phosphoproteins as we regard them at present, but because rennin would completely coagulate vitellin

from solution.   This idea was short lived, for Milroy [52] found vitellin to give the biuret but not Millon's test.   Levene and Alsberg [53] observed the high proline-content of both proteins conditioned by the need of that amino acid for hæmoglobin synthesis in the embryo.   Abderhalden and Hunter,[41] and Hugounenq,[54] observed and confirmed the similarity in the amino acid make-up of the two proteins, especially in leucine- and glutamic acid-contents, and in this way showed the similarity in physiological requirements of the young chick and mammal.   Plimmer and Scott [1] found vitellin to be digested more slowly by trypsin than casein, thirty-six days being required for half its phosphorus to be liberated into true solution, whereas most of the casein phosphorus was liberated in two to three days.   Bayliss and Plimmer [55] established the fact that both were phosphoproteins.   Plimmer and Scott [1] found later that the behaviour of both proteins in 1 per cent. caustic soda solution was identical.   The phosphorus groupings are stable towards dilute acids, but the phosphorus of nucleo-proteins is split off, thus affording a test for differentiating phospho- and nucleo-proteins.

(*f*) THE SULPHUR OF CASEIN.   The cystine-content of casein is low and accounts only for a small fraction of the sulphur-content of the protein.   A considerable amount of cystine is undoubtedly decomposed during the hydrolysis with strong acids.   Also this amino acid is destroyed, especially by strong alkali, in the casein purification processes.   The cystine-content of casein precipitated five times from sodium hydroxide solution is only one-tenth of that of the original casein.[110]   About 20 per cent. of the cystine of casein is liberated by 20 per cent. hydrochloric acid in 30 minutes, and the whole in six hours.   Pepsin does not liberate cystine from casein.   Mueller [31] partially accounts for the sulphur as being present also in an unidentified sulphur-containing amino acid, together with that present in the disulphide linkage in cystine.   Pirie,[56] taking advantage of the insolubility of its mercury salt in the presence of large amounts of other amino acids, has been successful in isolating *methionine* from casein hydrolysates.   This amino acid was also found extractable from strong acid solutions by butyl alcohol.   The amount yielded accounted for approximately 1 per cent. of the protein.   Other workers [108] have found only 0·41 per cent.

(*g*) THE NITROGEN DISTRIBUTION IN CASEIN.   The Van Slyke method [57] for determining the distribution of nitrogen in protein-hydrolysates into seven groups affords quickly-obtained evidence as to the character and amounts of the groups of amino acids

TABLE XLV. *Nitrogen Distribution in Casein. Percentages of Nitrogen*

	Van Slyke [58]	Crowther and Raistrick [48]	Dunn and Lewis [59]	Hoffmann and Gortner [60]	Davies [61]
Amide nitrogen . .	10·27	10·25	10·49	10·20	10·02
Humin nitrogen . .	1·28	1·20	2·13	1·51	—
Cystine nitrogen . .	0·20	1·24	0·48	1·05 ⎫	
Arginine nitrogen .	7·41	9·22	7·42	9·20 ⎬ 35·25	
Histidine nitrogen .	6·21	6·82	6·01	6·26 ⎭	
Lysine nitrogen . .	10·31	9·62	9·09	8·49 ⎫	
Amino N of filtrate .	55·81	54·76	58·78	54·12 ⎬ 64·73	
Non-amino N of filtrate	7·13	7·09	5·93	8·76 ⎭	
Recovery . . .	98·61	100·19	100·33	99·59·	

present. Table XLV shows the results obtained by this method by different investigators.

The Hausmann distribution into three groups is less instructive. Plimmer [62] found the following results for casein and the egg proteins (Table XLVI) :

TABLE XLVI. *Distribution of Nitrogen in Casein and Egg Proteins (Hausmann). Percentage of Protein (Plimmer)*

	Total N	Amide N	Humin N	Diamino N	Non-amino N
Casein . .	15·30	1·52	0·22	3·30	10·36
Ovovitellin .	15·29	0·84	0·25	3·84	10·26
Ovolivetin .	15·12	0·75	0·32	3·29	10·76
Ovalbumin .	15·51	1·34	0·29	3·30	10·58
Casein [63] .	15·62	1·61	0·21	3·49	10·31
Casein [64] .	15·87	2·10	—	1·84	11·93

(*h*) REMARKS ON THE ANALYSIS OF CASEIN. The total amino acids actually accounted for in the analysis of casein amounts to 97 per cent. This demonstrates that its amino-acid content is better known than that of any other protein of common occurrence with the exception of zein, the alcohol-soluble protein of maize (101 per cent.). An appreciable fraction of casein is still unaccounted for, since theoretical recovery should be between 110 and 120 per cent. (The amino acids are calculated as complete molecules and not as molecules minus the water of hydrolysis as they occur in combination.) The methods of analysis are

still unsatisfactory, but recent discoveries [47, 31] are still adding to our knowledge in this direction. Certain doubtful constituents have been isolated from casein hydrolysates alone, *e.g.*, the diaminotrihydroxydodecanic acid of Fischer and Abderhalden,[32] the hydroxy-tryptophane of Abderhalden and Kempi,[65] and the diamino acid of the formula $C_2H_6N_2O_2$ of Drechsel.[66] Abderhalden and Sickel [67] have concluded that the compound originally termed " hydroxy-tryptophane " may be phenyl hydroxypropionyl hydroindolylalanine.

The presence of compounds, which have not been isolated, in protein-hydrolysates other than amino acids, has been reported. Skraup and Krause [68] report the presence of 0·85 per cent. of methoxy groups and 1·13 per cent. of N-methyl groups. Geake and Nierenstein [69] report 0·55 and 0·33 per cent., and Herzig and Landsteiner,[70] 0·64 and 1·78 per cent. of these methyl groups, respectively. Although no methoxy or N-methyl compounds have been isolated from proteins, Dunn [71] found that carbon dioxide corresponding to 0·71 per cent. by weight of the casein was liberated during a period of twenty-four-hour hydrolysis, 0·31 per cent. being liberated in the first five hours. The sources of carbon dioxide are probably uramino acids, hydantoins and cyclic diacipiperazines.

In the analysis of nitrogen-distribution the amide nitrogen mostly comes from the free amide linkages, R—CO—NH$_2$, with traces from decomposed amino acids. The humin nitrogen, which is lowest in the purest samples of casein, comes from decomposed amino acids such as tryptophane [60] and part of the cystine. The Van Slyke method affords values only for the diamino acids and there is close agreement between the calculated values from the nitrogen-distribution and those determined by the Kossel and Kutscher method. In the Van Slyke method the value of cystine is too high and possibly some other sulphur-containing amino acid is precipitated by phosphotungstic acid ; methionine probably accounts for most of the sulphur of casein.

The form in which phosphorus is present in casein is still uncertain, although alkaline and tryptic liberation points to its probably being present as phosphoric acid. Casein is a relatively strong acid compared with other proteins and its titration curves (Fig. 18, p. 302) show a sharp inflexion between pH 6 and 7·5, in which range the secondary hydrogen of orthophosphoric acid is also neutralised. The titration curve of gliadin under similar conditions shows no such inflexion. About 2·5 per cent. of casein would be accounted for as phosphoric acid if the phosphorus is present as such.

46. The Properties of Casein

(*a*) PHYSICAL. Casein is a pure white, ashless, tasteless, odourless, non-crystalline solid. It is usually prepared as a fine, friable powder, but when dried quickly from a highly hydrated condition as thin films, it can be isolated as glassy transparent grains. It is very hygroscopic when dry, but is in equilibrium with the moisture of an atmosphere of average humidity, when it contains about 7–8 per cent. of moisture. A moisture-content above 8 per cent. lowers the keeping quality of commercial casein during storage and certain specifications require the moisture-content not to exceed 8 per cent. Heating at 70–80° C. for five hours causes such casein to lose about 6 per cent. of its weight, and there is still about 2 per cent. moisture left after drying *in vacuo* at 70° C. Heating above 90° C. causes loss of weight and decomposition. Pure casein is not coagulated by heat. The specific gravity is 1·259.

The heat of combustion of 1 gram of casein is 5,858 calories ; Lacquer and Sacquer,[93] however, report it to be 5,742 calories.

Solubility. Casein which has not been partly denatured by drying with alcohol and ether is soluble in water to the extent of 0·20–2·01 per cent. at 20–25° C., whilst at its isoelectric point it dissolves to the extent of 0·11 per cent. (25° C.). Treatment with ether and alcohol renders casein practically insoluble in water, and drying at temperatures from 80–100° C. also decreases the solubility. It is insoluble in alcohol, ether and in ordinary organic solvents. Hot 50 per cent. alcohol dissolves it with slight decomposition and, on cooling, the casein settles out as a ductile plastic mass. Pyridine will dissolve 0·09 per cent. at 20–25° C., whilst 50 per cent. pyridine in water will dissolve 0·56 per cent. at the same temperature. In phenol, casein gradually swells and dissolves, and it shows a similar behaviour in chloral hydrate and quinol solutions. Unchanged casein can be separated from these solvents by dilution.[74]

A slight contraction in volume occurs on dissolving casein in water, since the determined density of the solution is 0·625 per cent. greater than the theoretical value calculated from the mixture of dry substance and solvent.[75]

The Isoelectric Point of Casein. The isoelectric point may be defined as the hydrogen-ion concentration (usually referred to by the Sorensen index, *p*H) at which an ampholyte shows least or no combining capacity with acidic or basic ions, or when the ionic charges on the micelles neutralise one another (*i.e.*, $[H^{\cdot}] = [OH']$). Various methods have been used to determine this point, among

which are (*a*) the determination of the optimal mixture of acetic acid and sodium acetate for maximum precipitation, and (*b*) the method of electro-cataphoresis.[76] The isoelectric point of casein corresponds to a hydrogen-ion concentration of $2 \cdot 5 \times 10^{5}$ for (*a*) and $2 \cdot 4 \times 10^{-5}$ for (*b*), corresponding to a *p*H of $4 \cdot 4$ to $4 \cdot 6$. The optimum precipitation-point is at a hydrogen-ion concentration of $2 \cdot 4 \times 10^{5}$. Loeb [77] has shown that various physical properties of proteins, such as viscosity, total swelling, osmotic pressure and electrical conductivity are at a minimum at the isoelectric point.

(*b*) CHEMICAL PROPERTIES. *Colour Reactions.* Casein gives all the protein colour reactions, namely, the biuret, Millons, xanthoproteic, Hopkins and Cole (Adamkiewicz), and the Neubauer-Rhode. The absence of carbohydrate can be deduced from a negative Molisch's test, but galactose can be detected by the resorcinol reaction.

Combining Properties. When considered in relation to other proteins, phosphoproteins are relatively strong acids and casein behaves as such. Thus it is able to turn litmus red and slowly displace carbon dioxide from calcium carbonate. It is at least a tetrabasic acid, but the amino groups confer basic characteristics on it also. The acidic properties are, however, more pronounced, that is, the K_a of casein on the alkaline side of the isoelectric point is of greater magnitude than the K_b on the acid side of that point. The equivalent weight differs according to whether casein acts as an acid or a base. The following table (Table XLVII) shows the results obtained by different investigators on the combining powers of casein with acid and alkali and the corresponding equivalents :

TABLE XLVII. *Equivalent Weights of Casein*

Investigator	One gram of casein combines with ml $0 \cdot 1$N		Equivalent weight	
	Alkali	Acid	As acid	As base
Lacquer and Sacquer [73] .	8·81	—	1135·0	—
Lang [78]. . . .	8·30	7·0	1124·0	1428·5
Courant [79] . . .	9·50	—	1052·6	—
Spiro [80] . . .	8·57	—	1166·8	—
Spiro	8·70	—	1149·4	—
Matthapoulos [81] . .	8·83	—	1131·5	—
Pfyl and Turnau [82]. .	8·75	7·0	1131·5	1428·5
Van Slyke and Bosworth[10]	8·88	—	1125	—
Van Slyke and Bosworth.	9·00	—	1111	—

The agreement between different workers is fairly good and casein (1 gram) may be taken to combine with 9 ml. of 0·1N alkali and 7 ml. of 0·1N acid (either mineral acids or tartaric, oxalic or phosphoric acid).

The higher values of 5,000–6,000 for the equivalent weight calculated from the figures of Salkowski,[83] Hammarsten,[9] Lehmann and Hemple,[12] and Soldner [84] are probably due to working with acid salts or partly hydrolysed proteins. The combining powers of casein, both as acid and alkali, increase with temperature ; at higher temperatures casein combines with four times as much hydrochloric acid as at lower temperatures.

The maximum valency of casein is 8 according to the latest investigations. Theoretically it should be possible to prepare a series of eight different compounds of casein with a given base. Four of these have already been prepared.[85]

The Solubility of Casein in Acid, Basic and Salt Solutions. In Acids. Moist casein is totally soluble in dilute solutions of mineral acids and organic acids such as acetic, formic and lactic acids, but dry casein is less soluble. It appears necessary to prepare the surface of the solid to bring about a condition of hydration and swelling to accelerate the solution of casein in any solvent tested. The optimum concentration of acid necessary to effect rapid and maximum solution is 0·01N. Strong solutions of acids hydrolyse casein readily. Very little is known about casein acid salts and few have been isolated.

In Alkalis. Casein dissolves readily in dilute aqueous solutions of alkaline and alkaline-earth hydroxides and in dilute solutions of their soluble carbonates and bicarbonates. Dried and moist casein is soluble in strong solutions of alkali and alkaline-earth hydroxides, but the character of casein in solution is changed and hydrolysis into simpler compounds readily occurs by maintaining at a high temperature.

In Salt Solutions. Casein is slightly soluble in aqueous solutions of neutral salts, the amount dissolving varying with the hydrolytic dissociation of the salt in solution. The following figures represent the percentage solubility of casein at 25° C. in 0·1N solutions of the salts named : KBr, 0·18 ; NH_4NO_3, 0·45 ; $(COONa)_2$, 0·41 ; NH_4CNS, 0·93 ; C_2H_5COONa, 1·28 ; C_4H_9COONa, >2 ; CH_3COOK and KCN, over 2.[86]

It is also readily soluble in 1 per cent. solutions of sodium, potassium and ammonium fluorides and in 10 per cent. borax solution. It is quite soluble in alkali oxalates and sulphides, in 5 per cent. ammonium chloride and sulphate, and in dilute solutions of sodium and potassium cyanides.

In concentrated solutions of the acetates, propionates, and butyrates, casein swells but does not appreciably dissolve ; in strong solutions of nitrates and chlorides it remains pulverulent. The fact that it dissolves in these solutions after the lapse of a few days may be due to the formation of soluble degradation products.

The optimum concentrations of various salts to give maximum solubility of casein are given in Table XLVIII.

TABLE XLVIII.   *Optimum Salt Concentrations to give Maximum Solubility of Casein (Robertson)* [86]

Salt	NaCl	KCl	CaCl$_2$	BaCl$_2$	SrCl$_2$	MgCl$_2$	K$_2$SO$_4$	MgSO$_4$	N$_a$F	Na$_2$—HPO$_4$
Mol. concentration .	0·5	0·5	0·5	0·1	0·5	0·5	0·005	0·05	0·5	0·05
Per cent. casein soluble .	0·76	0·47	0·55	0·36	0·38	0·65	0·33	0·44	1·51	0·38

The State of Casein in Milk.   Brigando,[111] in surveying the knowledge of the state of casein in milk, states that the protein exists as a colloidal complex with $CaHPO_4$; acid and rennet casein are obtained by action on this complex ; acid casein is obtained by the change of $CaHPO_4$ to $Ca(H_2PO_4)_2$ and a progressive decalcification of calcium caseinate by added acids or souring. Pure casein should be salt-free, but it never is. If acid casein is prepared by precipitation at low temperature and is well washed, first with water and then with acid water (pH 4·7), a product low in ash is obtained, but excess of acid in the precipitation and vigorous stirring do not lower the ash content. Theoretically, rennet casein is an association of two salts—calcium paracaseinate, containing entrained $CaHPO_4$. Rennetting high-acid milk lowers the ash-content of rennet casein. The neutralisation of acid milks before rennetting gives rennet casein of normal ash content (about 7·5 per cent.). Rennet casein has a titratable acidity corresponding to 2·1 gm. of lactic acid/100 gm. of dry matter ; the corresponding value for acid casein is 8·1 gm./ 100 gm. dry matter.

The Precipitation of Casein from Milk or its Solutions.   Casein may be precipitated from solution or from milk in a variety of forms, *e.g.*, (*a*) in its free condition by adjusting the solution to the isoelectric point of casein, pH 4·6, either by the addition of dilute acid to caseinates or dilute alkali to casein acid salts ; (*b*) as a salt, either by saturation of its solutions with a neutral salt, *e.g.*, sodium or magnesium sulphate, or as its insoluble salts with a heavy metal, *e.g.*, copper caseinate ; (*c*) as paracasein, by the

action of the clotting enzyme, rennin (pH *ca.* 6·1) ; or (*d*) in combination with a large number of reagents referred to generally as " alkaloidal reagents."

(*a*) PRECIPITATION AT THE ISOELECTRIC POINT. As explained above, the isoelectric points of ampholytes such as proteins are of paramount importance. In solution, a protein dissociates and furnishes H˙ and OH′, one predominating over the other, usually the H ions being in excess. When casein is present in the form of calcium caseinate (in milk) at a pH of 6·6, gradual addition of dilute acid will liberate some calcium from casein combination until the isoelectric point is reached, at which stage the casein is not combined with calcium nor with the acid radicals of the added acid. In practice it is advisable to adjust the pH by using weak acids, such as acetic or lactic, and also, first of all, to bring the acidity slightly to the acid side of the isoelectric point, and finally to bring back the solution to the point of maximum precipitation by the addition of an alkali salt of the added acid. This means that the precipitation occurs from a weak acid-salt buffer mixture at pH 4·6 (*e.g.*, acetic acid-sodium acetate buffer). A more rapid liberation of combined calcium is effected in this manner and a casein containing less ash is obtained. In the preparation, for instance, of lactic casein by the " natural sour " method, the product is remarkably free from ash constituents, and this is effected by the gradual formation of lactic acid in the souring process, which gives sufficient time for the combined calcium to be freed as the increase in acidity proceeds. The preparation of lactic casein by the addition either of calculated amounts of lactic acid or a sour whey does not give a product so free of ash as the natural-sour process.

Dilute mineral acids, such as hydrochloric, sulphuric, nitric and phosphoric, and dilute organic acids, such as formic, propionic, tartaric and citric acids, have been used as precipitants for casein. The large-scale preparation of casein during the world war (for the manufacture of water-resistant glues for aircraft manufacture) was based on research [87] into the precipitation of casein from skim milk by mineral acids. Hydrochloric acid was used in the manufacture of " grain-curd casein," as it was termed, since sulphuric acid was found to leave a precipitate of calcium sulphate. The principle involved was to add sufficient mineral acid to cause maximum precipitation at the isoelectric point of casein free from foreign material other than that occluded or adsorbed. The addition of the correct amount of acid, rate of addition of acid, and adjustment of temperature gave a product in the form of uniformly sized grains, whence the term " grain-curd casein." The rela-

tively quick acidification caused from 2 to 3 per cent. of ash still to appear in the dry product.

Determination of Casein in Milk.[88]   For the determination of casein in milk the acetate buffer method is satisfactory. Ten grams of milk diluted to 50 ml. (40° C.) are treated with 1·5 ml. of 10 per cent. acetic acid and mixed by gentle swirling. After twenty minutes, 4·5 ml. of 0·25 N sodium acetate are added and again mixed. After standing for one hour the casein is filtered off and washed either with distilled water, or preferably with a dilute buffer solution containing the above quantities of acetic acid and sodium acetate per 50 ml. The filter and curd are digested in a Kjeldahl flask and the casein nitrogen determined by the usual distillation method (Moir).

On either side of the isoelectric point the precipitated casein becomes increasingly soft and colloidal until it goes into solution.

(*b*) PRECIPITATION BY SALTS. (i) *Neutral Salts.* The granulation or flocculation of colloidal material by adding concentrated solutions of neutral salts or of any liquid, *e.g.*, ethyl alcohol, likely to influence the degree of hydration or swelling of a colloid, is well known. The addition of electrolytes, in concentration greater than the ionic concentration within the colloidal particles, will reduce the osmotic pressure and the degree of swelling of the particles,[89] whilst with concentrated solutions the degree of hydration will be minimised to such an extent that precipitation will occur. Concentrated solutions of common salt or of magnesium or sodium sulphate will thus precipitate casein from solution whether it is present as kation, such as casein chloride, or as anion in calcium caseinate. This form of precipitation is different from that experienced at the isoelectric point, the precipitate being soft and rapidly dissolved by dilution. Lactoglobulin is co-precipitated with casein by a saturated solution of magnesium or sodium sulphate, preferably the former, in analytical work.

The deproteinisation of milk by copper sulphate or calcium chloride is applied in order to get a serum for refractometric measurements. In this instance solutions of salt are used which possess the same refractive index as normal milk serum. According to Ballowitz,[98] calcium chloride precipitates casein at *p*H 5·95–6·05, and on addition of calcium chloride to milk and boiling, the *p*H adjusts itself to this value. There is also a considerable amount of calcium adsorption which is at a maximum at the point of optimum precipitation.

(ii) *Precipitation by Heavy Metal Salts.* The combination of heavy metals with proteins has been studied by Vandevelde,[90] Osborne and Leavenworth,[100] and by Smythe and Schmidt.[101]

It was observed that proteins could bind metallic ions, removing them almost completely from the ionic state when low concentrations of heavy metal salts were present. A distribution law greatly in favour of metal in complex non-ionic combination as against the ionic form was found to hold. Davies [102] found, by diffusibility and potentiometric measurements, that only 4 per cent. of iron and 5·6 per cent. of added copper were diffusible from fresh milk, but that, at the isoelectric point of casein, 28·2 per cent. of iron and 43·2 per cent. of copper were diffusible; in fresh milk the fraction of copper in ionic form was $1·3 \times 10^{-6}$, but in milk of 0·50 per cent. acidity (pH 5·4) the fraction ionised was $159·0 \times 10^{-6}$, or over 100 times as much.

Lieben and Lowe,[103] in their investigations on the action of salts of heavy metals on proteins, found that silver and copper salts quantitatively displaced potassium from combination with casein, and that potassium at least was not combined with the NH_2-groups of casein. Under such alkaline conditions, of course, the possibility of adsorption of colloidal heavy metal hydroxides must not be overlooked. Ettisch and Schultz,[105] determining the affinity of casein for copper by potentiometric measurements while titrating with copper, found invariably that the two different chemical groupings were involved in the binding of the metal ; this affinity for copper increased with rising pH. Between pH 9·7 and 12·6 no less than eighteen steps could be recognised. A pH of 11·2 corresponded to the point of maximum affinity of one of the chemical groupings involved.

The use of copper sulphate solutions for the preparation of clear serum for refractometric work on milk involves the treatment of a known volume of milk with a quarter of its volume of 7·25 per cent. copper sulphate solution. Beckel [106] advocates the preparation of a serum by mixing the milk with half its volume of 17·5 per cent. copper sulphate solution, whilst Rothenfusser [107] has experimented with lead sera.

The copper-content of the precipitates has been much investigated (see Vandevelde [99] for bibliography) ; it has been found to vary from 1·5 to 5 per cent. for milk proteins, and the amount of copper precipitated with the protein is a fraction of the amount of added copper; the percentage of the total copper which is precipitated is greatest for low concentrations of the metal.

Acid mercuric nitrate is used to prepare milk serum for the polarimetric determination of lactose. Mercuric acetate in the presence of sodium carbonate and alcohol (Neuberg's reagent) will precipitate all protein and its degradation products from aqueous solution.

(c) PRECIPITATION OF CASEIN AS PARACASEIN BY RENNIN. Rennin, the enzyme of rennet, acts on the calcium caseinate of milk, causing the separation of a jelly-like curd (cheese-curd, junket) and a clear greenish liquid, whey. The reaction is very delicate since rennet can clot 400,000 times its weight of casein in milk ; the investigations of Fenger [93] show that rennet is capable of coagulating more than 2 million times its weight of fresh milk in ten minutes at 40° C. Shaking rennet inactivates it,[94] a phenomenon investigated by Rideal and Wolf,[95] who found that rennin destruction occurs at the air/liquid interface, this being accompanied by a rise in surface tension and pH of the solution.

The optimum temperature-range for rennet action is 36-45° C., acting best at 41° C., when it gives a firm curd. On each side of this range, soft curd and incomplete coagulation occur (18 per cent. only at temperatures from 15-20° C., and 50 per cent. at 50° C.). The more dilute the rennet, the longer is the time necessary for the formation of a firm clot.[96]

The precipitate formed by the action of rennin on casein solutions is called " paracasein." It possesses an elementary composition identical with that of casein, and its organic chemistry in general is also identical. It is difficult to imagine the occurrence of any deep-seated changes in the casein molecule during the short time that a minute quantity of rennin takes to coagulate milk. Nicholas [104] claims that casein and paracasein can be differentiated by serum precipitation methods. (The mechanism of rennin coagulation will be discussed later.)

(d) PRECIPITATION WITH THE " ALKALOIDAL REAGENTS." Tannic, picric, gallic, phosphotungstic, phosphomolybdic and trichloracetic acids, potassium mercuric iodide, potassium bismuth iodide, zinc chloride, potassium ferrocyanide, colloidal cupric hydroxide, and colloidal iron (hydroxide) can precipitate casein completely from solution. Some of these reagents precipitate protein only, whilst others can precipitate fractions of protein hydrolysates, e.g., phosphotungstates of the diamino acids. The compound formed with this precipitant and phosphomolybdic acid are analogous in composition to those formed with ammonia, and only the basic amino acids are precipitable. The nature of the compounds of casein with the other precipitants is unknown. Casein precipitated with trichloracetic acid and washed with water indefinitely will not revert into solution, but 30 per cent. of the precipitate is soluble in acetone,[97] as against the total solubility of serum globulin so treated.

Attempts to isolate casein from combination with alkaloidal

precipitants result in partial destruction of the original protein molecule.

It is inadvisable to use alkaloidal precipitants (other than trichloracetic acid) for precipitating the proteins of milk quantitatively. The precipitates are either too bulky and difficult to wash free from occluded or adsorbed nitrogenous compounds, or the line between precipitation of protein and that of non-protein nitrogenous products is not sufficiently clear cut. The best manipulation is obtained by the use of a precipitant which gives a fibrous curd, easily filtered and washed, such as is given by trichloracetic acid.

Whether precipitation by alkaloidal reagents involves the formation of a true protein salt is unknown ; possibly reactions variously described as sympathetic coagulation of two colloids (iron sol), weighting of the protein molecule, neutralisation of the charge on the colloid particle, causing segregation and precipitation, etc., come into play.

Precipitation by other Proteins. The acid nature of casein causes it to combine with bases of high molecular weight to form insoluble compounds, *e.g.*, with the protamines. Mixture with a heat-coagulable colloid such as egg-albumin will cause total precipitation of all protein from solution on heating (sympathetic coagulation). The surface-tension effect at an air/liquid interface can initiate such precipitation at lower temperatures. Gaseous bubbles in an egg-custard during cooking cause pear-shaped nuclei of coagulation, and the skin formed on the surface of heated milk is due to coagulated casein with entrained fat.

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CHAPTER VII

CASEIN (*Continued*)

47. The Homogeneity of Casein

THE fractionation of casein into fractions of different molecular weight has already been described in the previous chapter (p. 115). It appears generally that fractions are obtainable from casein which possess molecular weights that are simple multiples or submultiples of one another, and that the method of preparation of casein has some effect. Some form of partial polymerisation of simpler protein molecules seems to take place. Much of the evidence for this lack of homogeneity has been collected by Svedberg and his pupils ; Svedberg [1] is of the opinion that some proteins in their native condition in the cells of living organisms exist as proteins of low molecular weight, and that they are later converted into real proteins in the precipitating and purifying processes, especially by such salts as magnesium, sodium and ammonium sulphates. It is possible that this polymerisation of the native casein molecule can occur during its isolation and that the differences in the conditions of the methods of preparing casein carry the polymerisation to different degrees.

Elaborate investigations on the chemical and physico-chemical properties of casein have been conducted by Kondo,[2] and Linde-strom-Lang.[3] Both have found that the solubility of casein in dilute hydrochloric acid is not a constant value but is dependent on the amount of undissolved casein present. This was regarded as evidence that casein is not a homogeneous substance, but that it consists of a mixture of two or more proteins, similar in chemical composition but united in the precipitate as complexes of variable composition and solubility. Sorensen [4] regards the mode of combination of pseudo-globulin and euglobulin in the globulin fraction of blood-serum proteins, or the different fractions obtainable from the alcohol-soluble proteins of cereals to be of a similar type.

On subjecting commercial casein to fractional precipitation, Lindestrom-Lang and Kodama,[4] in their extreme fractions, obtained substances which differed appreciably in their solubility in dilute hydrochloric acid and in salt solutions. Later, with a carefully prepared sample of casein, the senior worker was able to separate the casein into fractions showing decidedly different

solubilities, and to show that the solubility was not a fixed value but depended on the original amount of casein taken for the solubility experiment.[3]   In the fractions thus isolated the phosphorus-nitrogen ratio ranged from a maximum of 0·0542 to a minimum of 0·0194.   Casein, precipitated from skim milk, has associated with it an alcohol-soluble protein [5] and no special precaution may have been taken to ensure the removal of this fraction.

Recent work by Cherbuliez and Schneider,[6] who have fractionated casein by the use of ammonium chloride solutions (3–5 per cent. solutions gave maximum solubility) into two components, have found that the insoluble portion (I) contains less, and the soluble portion (II) more sulphur and phosphorus than the original casein.   That there is no hydrolysis of the casein has been proved by the absence of a change in the formol titration, neutralisation and specific rotation values.   (II) is found easily soluble in 70 per cent. pyridine, (I) slowly soluble.   (II) is precipitated from ammonium chloride solutions at pII 3·6 and is more soluble in alkali than either (I) or the original casein.   The two fractions in 3 per cent. ammonium chloride require different degrees of saturation with ammonium sulphate for precipitation : 22 per cent. for (I), 32 per cent. for (II).   Rennet coagulation gives with (II) a softer curd than given either by (I) or the original casein.

Cherbuliez and Meyer [6] renamed these fractions $\alpha_1, \alpha_{11}, \beta$ and γ casein respectively, and perfected the method of separation by using acetone and hydrochloric acid alternately to precipitate from alkaline ammonium chloride solution.   Casein from six different sources and a freshly prepared sample gave widely different amounts of these fractions by identical methods of separation ; this was taken as evidence that the fractions were original components of the casein and not degradation products. The α_{11} fraction was responsible for the coagulation of the casein complex with rennin.

Carpenter and Hucker [12] have extracted casein with acid 70 per cent. alcohol and have fractionated the residue with potassium oxalate.   In this way, three proteins having molecular weights of 98,000, 188,000 and 375,000 have been separated from crude casein.   They have found that the alcohol-soluble protein of Osborne and Wakeman [5] is not the one having the molecular weight of 375,000.

Casein, in comparison with other proteins, shows a high degree of association, especially in the presence of salts, at its isoelectric point.   This is reflected in the very low solubility of casein at its isoelectric point.   It is possible that the ultra-centrifugal methods of approximately determining the molecular weight are influenced

by the amount of association of native casein molecules to different extents in the various methods of preparation employed.[8]

48. Racemised Casein

Solutions of proteins in dilute alkalis kept at ordinary temperatures (or at 37° C. preferably) for a number of days show a progressive decrease in optical rotation until a low and practically constant value is obtained. Dakin and Dudley [9] found that the optical rotation of a 10 per cent. solution of casein in 0·5 N sodium hydroxide fell from − 5° to - 3° in eighteen to twenty days at 37° C. The racemised casein was isolated by acidification and finally obtained as a light-brown friable powder containing C 53·55, H 7·03, and N 12·5 per cent., and in a yield only 20 per cent. of the original casein. Ammonia was evolved during the racemisation, and racemic proteoses were found in the mother-liquor from which the protein was isolated, this pointing to the occurrence of extensive hydrolysis accompanying the racemisation. This racemised casein could not be digested by pepsin, trypsin or erepsin when tested *in vitro*, nor was it digested when fed to dogs, since unchanged racemic protein was recovered from the fæces. A ten-day test revealed that it resisted the action of putrefactive bacteria.

Dakin [10] is of the opinion that the racemisation is due to a tautomeric change of the keto-enol type involving the —CO— group of the peptide linkage of the protein molecule and the H of the α carbon atom of the same amino acid :

$$—CO—NH—CH(R)—CO—NH— \rightarrow —CO—NH—C(R) = C(OH)—NH—$$
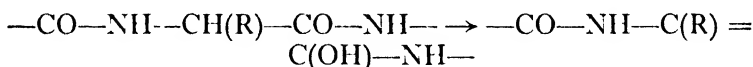
The α carbon atom is asymmetric and the formation of the double bond destroys the optical activity. The rupture of the double bond later would produce an equal number of *d-* and *l-* forms of the amino acid, so that the amino acids isolated would be optically inactive, being a racemic mixture. Dakin states that the non-racemisation of some amino acids in the protein molecule is due to the fact that the conditions necessary for racemisation of an amino group require the attachment of other groups to both amino and carboxyl radicals. Amino acids occupying terminal positions in the peptide chains of the protein molecule would retain their optical activity on hydrolysis of the protein.

Recently, Groh and Weltner [11] observed that the changes in the absorption spectra of proteins brought about by the enolisation of the peptide linkages were only discernible at high temperatures and at the end of racemisation. Two types of peptide

linkages were found, (a) those changing slowly to the enol form and then quickly to the inactive keto form, and (b) those yielding a stable enol form which should be detectable spectrographically.

Wright [13] bases his belief that the transformation of casein to paracasein is a colloidal phenomenon, and that it does not involve changes in the chemical structure of the casein molecule, on the fact that the two proteins show the same racemisation curve.

No material has yet been published on the racemisation curves of the various fractions of casein isolated as mentioned above. Halton [13] has found that the proteins obtained by the fractionation of wheat gliadin show different racemisation curves, and it remains to be seen whether the differences in internal composition of the casein complexes will be reflected either in the rate of racemisation or in the magnitude of the initial and final optical rotation.

49. Compounds of Casein with the Halogens

Numerous compounds resulting from the action of the halogens on casein have been reported. These may be in part simple substitution products or possibly such compounds accompanied by partial hydrolysis of the protein. Substitution in the aromatic nuclei apparently occurs, since the halogenated product usually gives negative Millon's (for tyrosine) and Hopkins and Cole reactions (for tryptophane).

Chlorine. Habermann and Ehrenfeld [14] chlorinated casein by the action of hydrogen chloride in the presence of potassium chlorate and obtained a brown product containing 13·28 14·04 per cent. of chlorine. Panzer,[15] in almost a similar manner but at a higher temperature, obtained a creamy product containing 8·32 per cent. of chlorine and 12·40 per cent. of nitrogen. The amino acids, leucine, glutamic and aspartic acids, arginine, histidine and lysine could be isolated from its hydrolysate. Part of the chlorine could be replaced by ethoxy-groups.[16] Salkowski [17] prepared a white product in a similar manner containing 6·76 per cent. of chlorine. This was soluble in hot water but was precipitable by sodium chloride. It was resistant to trypsin, pepsin, and the putrefactive bacteria, whilst indole-producing bacteria caused no indole formation, thus showing the destruction of tryptophane. Some unchlorinated phenylalanine still remained in the molecule, since a faint xanthoproteic reaction was given.

Hopkins and Pinkus [18] prepared a number of halogenated proteins having the following properties : xanthoproteic and biuret reactions positive ; Millon's, Hopkins and Cole and the lead acetate tests negative. The halogenated proteins are soluble

in hot alcohol, but difficultly soluble in cold alcohol, water, and organic solvents. They are strong acids, decomposing carbonates and forming stable salts with the heavy metals (Cu, Hg, Ag). They are soluble in dilute alkalis from which they are precipitated on neutralisation with acids.

Bromine. Hopkins and Pinkus [18] have prepared a brominated protein containing 11·2 per cent. of bromine.

Iodine. The action of iodine on casein has been studied by Liebrecht,[19] who prepared a series of compounds of different iodine content. *Periodocasein* (17·8 per cent. I), a yellow powder soluble in hot alcohol, was prepared by the action of powdered iodine on casein at 100° C., extracting the excess iodine with ether. Part of the iodine is extractable with thiosulphate, yielding *iodocasein* (5·7 per cent. I), which is a white powder, acid in reaction, insoluble in neutral organic solvents and containing both sulphur and phosphorus. The treatment of periodocasein with 10 per cent. sulphuric acid yields a third compound—*caseoiodin* (8·7 per cent. I) –soluble in hot 70 per cent. alcohol, from which it separates on cooling as white flocks which on drying yield a white powder.

Masui [20] iodised casein by treatment in alkaline solution (sodium hydroxide, carbonate and bicarbonate, and potassium bicarbonate) with iodine-potassium iodide solution at various temperatures. The iodised compound was precipitated with sulphuric acid and excess of thiosulphate. The maximum iodine content was obtained by solution in sodium carbonate. Iodised casein was digested *in vitro* by pepsin, trypsin, and tissue proteases, after which half the iodine could be precipitated by silver nitrate.

The halogens can be split off by autoclaving at 130° C. with water (5 6 atmospheres).

50. Desaminocasein

The treatment of casein with nitrous acid yields desaminocasein. Dunn and Lewis [21] treated casein in aqueous suspension with glacial acetic acid and sodium nitrite (eighteen hours at room temperature) and isolated the washed product in a yield of 90–97·5 per cent. of the original. The nitrogen-content had been lowered by from 0·22 to 0·68 per cent. The free amino nitrogen was greatly reduced due to the destruction of the ϵ-NH_2 group of lysine. Tyrosine was partially destroyed but in other respects the protein was only slightly altered. The nitrogen distribution corresponded closely to that of the original casein when allowance was made for the total absence of lysine. Steudel and Wohing [22]

have found that the action of nitrous acid in some way affects the cystine linkage in the protein molecule. The cystine-content of desaminocasein (by the Folin and Marenzi method) was found to be 1·681 per cent. as against 0·812 per cent. for pure casein. White,[69] however, found that the cystine content of deaminised casein does not differ from that of the original protein.

The lowering of the nitrogen content by treatment with nitrous acid does not account for all the nitrogen appearing as *amide* nitrogen on acid hydrolysis of casein (1·57 per cent.). Part of the amide nitrogen thus obtained comes from the destruction of amino acids. It appears that *amide* nitrogen is not appreciably affected by nitrous-acid treatment. Levites [23] could find no free $CONH_2$ groups in casein. Luck [24] has observed that only 67 per cent. of the ammonia of acid-hydrolysed casein is liberated by prolonged tryptic digestion of the protein, and that the tryptic-resistant fraction was an amidised glutamic acid-lysine peptide, and concludes that glutamine (or a glutamine-containing peptide) is an integral part of the casein molecule.

51. Other Derivations of Casein

(*a*) NITRO-CASEIN. The xanthoproteic reaction for proteins, which depends on the formation of a yellow colour (changing to orange in alkaline solution) on the addition of concentrated nitric acid, is due to the presence of aromatic nuclei in the protein molecule. According to Inouye [25] and Johnson [26] the compound responsible for the colour formation is tyrosine; the nitrated product is 3-nitro-4-hydroxyphenyl α-amino propionic acid. Von Furth [27] nitrated casein at room-temperature, using urea to counteract the effect of the nitrous acid formed, and obtained a yellow product soluble in alkalis to a deep-orange solution and reprecipitated by acids. The Millon's and lead acetate tests were not positive. In the absence of urea, more drastic hydrolysis or decomposition occurred, and a compound, xanthoprotein, very similar to nitro-casein, was formed. The ratio $S : NO_2 : N$ was 1 : 1·5 : 44. The nitro-caseins were slowly digested by trypsin yielding nitrated proteoses and peptones. Casein in aceto-formic solution with HCl and HNO_3 forms a nitrated compound having colloidal properties.[56]

(*b*) FORMALDEHYDE CASEIN. Formaldehyde forms more or less stable compounds with proteins, altering the properties appreciably. The main chemical property is that the protein loses its amphoteric nature and in suspension in water titrates as a weak polybasic organic acid to phenolphthalein. The basicity of the NH_2 groups is destroyed so that the acid dissociation constant of the protein is

increased considerably. This phenomenon forms the basis of the Sorensen [30] method of titrating proteins or their cleavage products. Since cleavage of the protein molecule involves the hydrolysis of the peptide linkage to —COOH and NH_2—, by submerging the basicity of the —NH_2, the carboxyl group can be estimated quantitatively by titration, and the method can be utilised as a comparative measure of the amount of cleavage which has occurred. Ammonium salts (formaldehyde forms hexamethylene tetramine, an un-ionised compound, with ammonia) and amino acids also react with formaldehyde, but owing to the influence of diamino acids quantitative titration is not attained, but by a further increase of the K_a of the acids by titrating in 75–90 per cent. alcoholic solution, quantitative results are possible.[28, 29] The casein thus treated is not acted on by rennin, trypsin, pepsin, or bacteria.

As regards change in physical properties, solubility in water and the capacity for hydration have been lost and the compound is insoluble in acids.   .

Blum [31] and Benedicenti [32] have prepared formaldehyde derivates of casein. The latter has found that each gram of casein combines with 0·0057 gram of formaldehyde (sixteen days at room-temperature). Steam distillation of the formaldehyde casein in suspension in water causes all the formaldehyde to distil over and the casein recovers its swelling properties. The biuret test, however, is now red and not violet, showing that some hydrolysis of the peptide linkages, probably during the steam distillation, has occurred.

Benedicenti suggests that the addition of formaldehyde to a protein occurs as a type of aldehyde-ammonia reaction : $RNH_2 + HCHO = RNH—CH_2(OH)$, and the ease of removal of the formaldehyde by steam distillation suggests that this reaction involving the acid amide groups may be the principal linkage of formaldehyde and proteins.

Blum,[31] on the other hand, suggests that the reaction involves the formation of methylene-imino compounds :

$$\begin{array}{c} —NH_2 \\ \\ —NH_2 \end{array} + HCHO = \begin{array}{c} —NH \\ \diagup \diagdown \\ —NH \end{array} CH_2 + H_2O$$
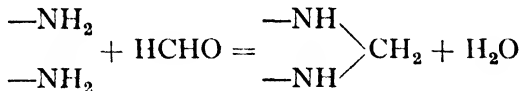
Some products from casein resembling bone and ivory are made by plasticising the material to the required shape and hardening in a bath of formaldehyde.

(c) METHYLATED CASEIN. The methylation of casein by the successive additions of methyl iodide to solutions of casein in alkaline absolute alcohol (Skraup and Krause [33]) and boiling under

reflux until alkalinity has disappeared gives a substance soluble in water but precipitated by ammonium sulphate. The product is a faint yellow powder, slightly acid to litmus, giving the biuret and the glyoxylic acid reactions but no reaction with Millon's reagent, and may be looked upon as a simple methylated casein. It contains C 52·50, H 7·37, N 14·22, S 0·61, P 0·87, and I 5·35 per cent. The methoxy group is present in amounts varying in different preparations from 1·52 to 2·07, and the N–methyl group gives values from 2·79–3·73 per cent.

Geake and Nierenstein [34] prepared methylated casein by the action of diazomethane in ethereal solution on casein in ethereal suspension, about four months being taken over the whole process. It was found that the methyl groups were split off by alkaline treatment, and it was found difficult to dissolve the product in dilute acid, several days heating on a water-bath being required, whilst solution in alkali required twenty hours. Analysis gave $-OCH_3$, 2·08 and $N(CH_3)$ 2·28 per cent. respectively, whereas for the original casein these groupings were found present in the amounts 0·49–0·55 and 0·87–0·88 per cent. respectively. Thus methylation increases the methyl groups in casein by 3·48 per cent. The hydroxyl group of tyrosine is not methylated since the Millon's test is still given.

The methylated casein of Herzig and Landsteiner [35] showed a higher degree of methylation. Edlbacher [36] methylated with alkali and methyl sulphate in aqueous solution. He was interested only in the " N methyl number " or the number of methyl groups per 100 nitrogen atoms which are bound to nitrogen by exhaustive methylation. This he found to be 19·7 for methylated casein.

Masui [37] found that the nitrogen-distribution of methylene casein was very similar to the original protein except for slightly less humin nitrogen.

(*d*) THE ACTION OF CARBON BISULPHIDE ON CASEIN. On boiling an alkaline solution of casein with carbon bisulphide, casein xanthate is formed which has been isolated by Uhl [38] as a clear golden yellow solution forming a copper salt, an amorphous brown powder containing 17·9 per cent. of copper. The reaction possibly takes the form :

$$\cdot\, R.NH_2 + CS_2 = R-NH-\underset{\underset{S}{\|}}{C}-SH$$
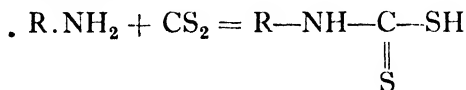
52. Various Degradation Products of Casein

By the action of various reagents casein can be broken down or decomposed into simpler products varying in complexity from

substances of large molecular weight to relatively simple compounds. The products and their complexity depend on the reagent used, the concentration of the reagent, and the duration and temperature of the reaction. The methods by which the cleavage of the casein molecule can be effected are numerous, but the few important ones which will be dealt with here divide themselves into two classes, (a) destructive decomposition and (b) hydrolysis.

(a) DESTRUCTIVE DECOMPOSITION may be effected by either destructive distillation, fusion with alkalis or by combined oxidation and hydrolysis. The products obtained by the first two methods are volatile substances, e.g., acids, aldehydes, etc., and gases such as ammonia, carbon dioxide, hydrogen sulphide and water vapour. This method of analysis is not instructive with regard to the true nature of the protein molecule and will not be considered further.

Hydrolysis with oxidation, on the other hand, yields some constructive information. When casein is treated with strong oxidising agents a variety of reactions occurs :

(i.) Sulphuric acid and manganese dioxide : acetaldehyde, propionic, butyric, benzoic acids, acetone, fatty acid members up to caproic acid, ammonia.[38]

(ii.) Chromic acid : as for (i.), nitrites and valeric acid.[39]

(iii.) Concentrated nitric acid : oxalic acid, hydroxyglutamic, leucinic and xanthomelanic acids. The formation of up to 30 per cent. of oxalic acid shows that the main reaction simulates that of nitric acid on sugars.[27]

(iv.) Potassium permanganate : formic, acetic acids, guanidine, oxaluric and oximino acids.[39]

(v.) Bromine : bromoform, bromacetic acid, bromanil, carbon dioxide, leucine, oxalic and aspartic acids.[40]

(vi.) Hypobromite : nitrogen, amino acids, valeric, oxalic and succinic acids.[41]

(vii.) Ozone : amino acids, aldehydes.[42]

Oxyprotosulphonic acid, formed by the oxidation of casein with potassium permanganate (1·5 per cent. solution), was isolated by Bodzynski and Zoja.[43] Later work by Buraczewski and Krause [44] and Schuberthowna [45] proved it to be a mixture of compounds, the latter worker resolving it into 7 fractions. It is evident that oxidation with potassium permanganate causes hydrolysis and oxidation of the protein-molecule simultaneously and the above name applies to a heterogeneous mixture of compounds.

(b) HYDROLYSIS. This type of degradation has afforded most evidence regarding the structure of proteins. Hydrolysis takes

place in stages, large fragments being broken off from the casein molecule. These substances are products of high molecular weight which resemble the parent substance in many respects and give some of the colour reactions for proteins. (α-casein, β-casein, isocasein, caseid, acid and alkali albuminates.) On hydrolysing further, these fragments are broken down into smaller units until finally the " *bausteine* " of the protein molecule, the amino acids and ammonia, only are present. The process of hydrolysis may be expressed thus : Casein → metaproteins → proteose → peptones → peptides → amino acids.

(i.) *Hydrolysis with Water and Heat.* Casein in contact with cold water for a period of several days goes partly into solution ; the soluble portion can be precipitated by alcohol and by lead acetate after the addition of ammonia.

Casein in suspension in water and heated for a short time loses some of its phosphorus [46] and its base-binding power is reduced. The phosphorus-content of the residue is o·49 per cent. Hydrogen sulphide is also split off.

Casein heated at 150° C. with water under pressure for three days yields proteoses and some amino acids.

Dry casein, heated above 100° C., is decomposed into two main protein fractions ; phosphoric acid is split off but ammonia is evolved only at high temperatures. The two fractions formed are termed (*a*) isocasein and (*b*) caseid.[47] Isocasein is similar in properties to casein except that rennin will not precipitate it from its solutions. Caseid, on the other hand, is different from casein in that it swells up in dilute alkali but does not dissolve, and contains only o·59 per cent. of phosphorus. When the calcium or barium compound of caseid is dissolved in nitric acid and the solution diluted, a precipitate is formed which now dissolves completely in sodium hydroxide.

When casein is dissolved in dilute potassium or sodium hydroxide solution (carbonate-free) and heated to 118–135° C., a precipitate is formed and the acidity of the solution increases. The precipitate resembles high-temperature casein and is termed β-casein.[48]

(ii.) *Hydrolysis with Acids and Alkalis.* All acids hydrolyse casein to a greater or less extent, and hydrolysis with hydrochloric acid of constant boiling-point or 20 per cent. sulphuric acid ensures complete breakdown to the constituent amino acids. In analytical work, owing to the ease of removal of all sulphate with baryta, the sulphuric acid hydrolysis is preferred, but in other cases either acid may be used. The time taken for complete hydrolysis may be shortened by carrying out the hydrolysis in an

autoclave at higher temperatures (three hours at 130° C., as against thirty to forty-eight hours at atmospheric pressure). Tryptophane and some cystine are decomposed in acid hydrolysis.

Hydrolysis with alkali follows the same course as that by acids, identical products being formed. Casein can be boiled for a considerable length of time with 0·1–0·01 N alkali without appreciable change, but longer contact with stronger alkali first liberates compounds not precipitated by rennin, then proteoses, and finally amino acids. Phosphorus is completely split off by 1 per cent. sodium hydroxide in twenty-four hours.[49] Under certain conditions alkali salts of protalbinic and lysalbinic acids are formed. These compounds have the power of holding metallic oxides in colloidal form.[50] Alkaline hydrolysis is rarely used in analytical work on proteins.

(iii.) *Hydrolysis with Enzymes.* A variety of proteinases can split casein into simpler compounds. The most important proteolytic enzymes are pepsin and trypsin. Pepsin [51] acts on casein when it is in the form of casein acid salt (pH 1·5–2·5), preferably as a salt of an acid having a high degree of dissociation, whilst trypsin [51] acts on casein only as a caseinate (pH 8–11). The optimum pH of these enzymes thus differ appreciably. A proteolytic enzyme, papain, not so well known as the above,[52] acts best on casein when not combined to an acid or basic radical or when combined with a small amount of base (pH 5). Rennin acts on casein best at a pH slightly to the acid side of the pH of fresh milk (6·1).[53] It may be stated generally that all proteinases, with the exception of pepsin, have a narrow range of specificity in the absence of activators. With some enzymes also their purification from the peptidases associated with them (*e.g.*, trypsin and kathepsin) narrows the range of their specificity.

(iv.) *Hydrolysis with Pepsin.* The hydrolytic action of pepsin can best be studied as a 0·2 to 1 per cent. solution in 0·4 per cent. hydrochloric acid acting on a 0·5 to 1 per cent. concentration of casein at 38–40°C. (with toluene). After the first twenty-four hours various degradation products, such as acid albuminates, proteoses and peptones, can be isolated. Pepsin cannot hydrolyse casein further than the " peptone " (or peptic digest) stage, since it cannot break down polypeptides, dipeptides or prolylpeptides.[54]

In the first stage of digestion, a precipitate of *pseudo-nucleins* separates out (together with undigested casein). On separating by filtration, the undigested casein can be separated by washing with 0·4 per cent. hydrochloric acid. The pseudo-nucleins are soluble in dilute alkali but are precipitated by strong acids. They are themselves strongly acidic and possess a high phosphorus content.

On neutralising the filtrate the *acid albuminates* separate out; these are insoluble in water, but soluble in dilute acids.

If an equal volume of saturated ammonium sulphate be added to the filtrate a precipitate of *primary proteose* is formed. After separating and saturating the liquid with ammonium sulphate completely, a precipitate of *secondary proteose* is formed. Both proteoses are indefinite mixtures with common properties, soluble in water, dilute acids and alkalis. The filtrate contains peptones, which may be precipitated with Siegfried's reagent (10 g. ferric ammonium sulphate, 200 g. ammonium sulphate, 250 g. water), and small amounts of peptides and amino acids.

(v.) *Hydrolysis with Trypsin* (or trypsinogenkinase). This is best carried out with a 0·5–1 per cent. solution of trypsin in 0·5 per cent. sodium carbonate on a 1 per cent. concentration of casein (with toluene) at 38–40°C. In early digestion, alkali albuminates, proteoses and peptones are principally formed, whilst peptones, polypeptides and amino acids are formed later. The scheme of separation of the fractions is essentially the same as that described for the peptic digest of casein. The alkali albuminate is insoluble in water and dilute acids, but soluble in dilute alkalis.

As mentioned above, whereas purified trypsin will not carry the digestion to the amino-acid stage, the presence of various peptidases in commercial trypsin widens the range of specificity, and hydrolysis to the ultimate stage is possible. In the alimentary tract the proteinase, *erepsin*, is rich in peptidases.

Holter, Lindestrom-Lang and Funder,[57] on treating the three fractions of casein obtained by treatment with alcohol and dilute hydrochloric acid, with pepsin, found a difference in the rate of digestion, especially with regard to the separation of the phosphorus-containing fraction. The P/N ratio of the phosphopeptones was the same in all cases, and the phosphorus linkage was similar in both parent and cleavage products. The peptic phosphopeptone was not the same as the tryptic phosphopeptone. The velocity of the cleavage of the substrate in this case was found to be dependent on its phosphorus content, the higher the phosphorus the higher the rate.

(vi.) *Hydrolysis with other Enzymes.* Rennin splits casein into two molecules of paracasein which on combination with calcium forms insoluble paracaseinate. Precipitation is dependent on the presence of free calcium ions and the nature of the precipitate is influenced by their concentration. Owing to the importance of rennin in cheese manufacture the action of this enzyme will be discussed later (see Sections 134–138).

Papain can split casein to the peptone stage with slow digestion of the peptones to the ultimate stage.

Arginase sets free half the original arginine of casein in three hours.[55]

53. Some Phosphorus Compounds in Casein

A method of distinguishing phosphoproteins from nucleo-proteins rests on the stability of the phosphorus-grouping in the former to the action of dilute acids. Dilute alkalis, on the other hand, split off the phosphorus (phosphoric acid) relatively easily.

The mode of combination of the phosphorus in casein has been much investigated, particularly with regard to the phosphorus-linkage in it and its degradation products. The presence of a peptic phosphopeptone isolated from the peptic digests of various casein fractions has already been mentioned.[57]

Rimington and Kay [58] reported that a product containing amino acids in association with phosphoric acid could be obtained from tryptic digests of casein. The phosphorus accounted for approximately 10 per cent. of the original phosphorus of the casein. Later work by the same authors,[59, 60] on the action of various proteolytic enzymes, acids and alkalis, and phosphatases on casein, has afforded information as to the state of combination of phosphorus in the protein molecule. The linkage involved appears to be of the ester type, phosphoric acid being united to some hydroxyl-containing constituents of the protein molecule, most probably hydroxy-amino acids. The study of artificially phosphorised proteins [60] has added support to this view.

Later Rimington [61] isolated a phosphorus-containing peptone, $C_{38}H_{62}O_{33}N_9P_{31}$, from tryptic digests of casein in a yield corresponding to 50 per cent. of the organic phosphorus. This " phosphopeptone " is a strongly acidic substance forming well-defined copper and barium salts containing nine equivalents of the metal. In titrations it acts as a nine-basic acid. The free acid has $[\alpha]_{5461}^{15} = -80 \cdot 5°$. One-ninth of the nitrogen is in the amino form whilst on hydrolysis all the nitrogen is converted into the amino form. It gives a positive biuret and ninhydrin reaction but none of the colour tests for the amino acids. Trypsin slowly attacks the peptide linkages and slowly liberates some of the phosphorus as phosphoric acid. The phosphorus and nitrogen contents are 7·05 and 10·13 per cent. respectively. On hydrolysis, hydroxyglutamic acid, hydroxyaminobutyric acid and serine can be isolated.

Posternak [62] (1923) had previously patented a process for preparing the phosphorus-nucleus of casein by means of a short tryptic digestion. Later he was able to prepare simpler phosphorus-containing polypeptides and came to the conclusion that the phosphorus-nucleus was composed of four serine-phosphoric acids. His phosphopeptone contained P, 5·86 and N, 11·90 per cent., a P/N ratio of 2 : 1.

Berggren [63] is of the opinion that the phosphorus of casein is more loosely bound than is usually thought, for she has shown that it is possible to prepare casein of a lower phosphorus-content by dialysis of milk than by the acid method. The lowest value for phosphorus content was 0·29 per cent. as against 0·80 per cent. found for acid-prepared material.

Grabar [70] and Levene and Hill,[71] have isolated the phosphorus-rich peptone, glutamyl serine phosphate (N/P − 4) from tryptic digests of casein. The latter workers precipitated the peptide with neutral lead acetate and isolated it as its brucine salt. The linkage of phosphoric acid to serine has been confirmed.

The properties of casein solutions and the action of rennin will be discussed later in the physico-chemical section (see Sections 134–138).

54. The Preparation of Pure Casein and Paracasein

Casein is usually prepared from fat-free milk by the addition of acids in slight excess over the amount required to give a pH of 4·6. Butter-fat is the most objectionable constituent of casein and must be removed from the milk, since it cannot be extracted completely from the dry product with organic solvents owing to the enveloping of the globules by dried casein. At the same time some denaturation of the casein by the solvents will occur, especially with alcohol, carbon bisulphide and petroleum ether. The milk can be passed through a centrifugal separator several times ; after a small amount of dilute alkali has been added it is centrifuged several times again. This removes practically all the fat and a casein almost free from fat results.

The curd from acid-treatment is washed with water, dissolved in dilute alkali and reprecipitated with acids several times until the product is free from ash. Casein is susceptible to treatment with alkali for long periods of time, and up to temperatures near 100° C., so that in the drying process low temperatures, and preferably a vacuum, should be utilised.

There are two general methods of preparing casein : (a) the method of Van Slyke and Baker,[64] and (b) Hammarsten's method.[65]

(*a*) VAN SLYKE AND BAKER METHOD. Fresh fat-free (see above) undiluted milk at 15° C. is used, this temperature being maintained during precipitation. A known volume of milk is poured into the precipitating vessel (3–5 l.), provided with a stirrer in the shape of a propeller, and a burette fitted with a tube with a finely drawn-out point set close to the propeller blades. Vigorous stirring of the milk (2,000–3,000 r.p.m.) is started and acid (either normal lactic or a mixture of normal hydrochloric to 1 to 2 parts of normal acetic acid) run in very slowly from the burette (45 ml. per litre of milk in thirty minutes). If acid is added too quickly local coagulation will take place. The acid is run in slowly until 60 ml. per litre have been added, after which the rate is decreased until the coagulation-point is nearly reached. This point can be detected by withdrawing a sample (5 ml.) and diluting it with an equal volume of water, when complete separation will occur, since the dilution of milk increases its acidity. When this point is reached the mixture is allowed to stand for three hours and the rate of stirring decreased to 500 r.p.m. Then the addition of acid is continued until complete precipitation takes place, this point being apparent by the formation of a layer of clear supernatant liquid above the casein. About 90 ml. of normal acid per litre is usually required. A fine granular precipitate can be obtained by adjusting the *p*H of the mixture to 4·6.

The curd is separated by centrifuging and then washed with successive amounts of distilled water until the casein fails to separate completely from the wash water (four to five washings). The solid is washed twice successively with 95 per cent. alcohol and three times with ether. The curd is dried by exposure to the air in thin films, when an impalpable powder is obtained. The ash-content ranges from 0·05 to 0·15 per cent. Traces of fat can be detected by dissolving in excess of lime water, when a milkiness appears.

(*b*) HAMMARSTEN'S METHOD. The fat-free milk is diluted with four volumes of distilled water and enough 10 per cent. acetic acid is added with constant stirring to make the excess of acid nearly 1 per cent. over that required to coagulate the casein. The clear liquid is syphoned off after settling, and an equal volume of water added, well stirred and allowed to settle. This is repeated a few times. The casein is then dissolved in the least possible amount of 0·1 N ammonium hydroxide, and the solution filtered through an asbestos pad. The casein is precipitated with acetic acid, then re-dissolved and re-precipitated twice. After washing with distilled water the casein is collected on a cheese-cloth filter, suspended in successive volumes of alcohol, and finally in ether,

after which it is exhaustively extracted with ether in a Soxhlet apparatus and dried in a vacuum at 60–70° C.

The acidity of coagulation may be adjusted to a pH of 4·6 to give a granular precipitate, and wash water at the same pH is an improvement. Lactic acid can be substituted for acetic acid.

Preparation of Paracasein. Fat-free milk is warmed to 38–40° C. in a suitable container and rennet (Hansen's rennin preparation) added at the rate of 0·12 ml. per gallon of milk. The temperature is maintained at 38–40° C. until precipitation is complete (indicated by the sinking of the curd to the bottom). The mixture is then heated nearly to boiling to destroy the rennin. After cooling and settling, the whey is siphoned off and an equal volume of distilled water added with vigorous stirring, this being repeated several times. The precipitate is finally removed by filtration and dried at low temperature.

Ashless Paracasein. Rennin causes calcium paracaseinate to be precipitated, and ash-free paracasein may be prepared from wet rennet curd prepared in the above manner as follows : The curd is suspended in water (5 l. for every 1 l. of milk used) and dilute 0·880 ammonium hydroxide (6 ml. to 1 litre of water) added with stirring until the paracasein is dissolved. After filtration through cotton wool it is precipitated with dilute acetic acid. This is repeated a few times. The paracasein is finally dissolved in an excess of ammonium hydroxide and 20 ml. of saturated ammonium oxalate (for each litre of milk originally used) added. After standing twelve hours the mixture is filtered through a double layer of paper and the paracasein precipitated with 1 per cent. hydrochloric acid. After washing free from chlorides, the precipitate is filtered, washed with alcohol and ether in succession, and finally dried in a vacuum over sulphuric acid in a desiccator.

55. The Properties of Paracasein

Paracasein is a white solid, insoluble in water, alcohol, and ether, but slightly soluble in neutral salts and completely soluble in warm 5 per cent. sodium chloride solution free from alkaline-earth salts. It is also soluble in alkali salts of weak acids and alkali hydroxides, and in alkaline-earth salts of weak acids and alkaline-earth hydroxides, and in hot 50 per cent. alcohol. Strong alcohol denatures paracasein and lessens its solubility in the above reagents.

It has acid properties similar to those of casein. On warming in the moist condition it can be drawn out into fine threads. This property has been utilised in the manufacture of cooked,

processed or loaf-cheese. Drying causes paracasein to lose its plasticity.

Paracasein has the following elementary composition : C 53·94, H 7·14, N 15·14, P 0·7–0·88, and S 1·01 per cent.

It forms salts with acids and alkalis in the same way as casein.

It forms *acid* paracaseinates with metals, those of NH_4, Na and K being water-soluble at low concentration, but those of Li, Mg, Ca, Sr, Ba, Mn, Fe, Co, Ni are insoluble One gram of paracasein combines with $2·25 \times 10^{-4}$ gram equivalents of the hydroxides of NH_4, Na and K and the amount of each basic element is double that in the corresponding acid caseinates. The alkaline-earth metals form two series of acid paracaseinates, the di-basic and the mono-basic.

Neutral and basic paracaseinates are also formed.

Salts with acids are formed as with casein. Van Slyke and Hart [66] state that paracasein lactates are formed during the ripening of cheese.

Heavy metals form insoluble salts with paracasein, but metals of Group II form compounds soluble in excess of the reagent.[67]

When the action of rennet on paracasein is prolonged it is broken down into different compounds, called paracasein A, B and C. These can be separated by neutralisation and precipitating A with calcium chloride, whilst B can be separated from the filtrate from A by precipitation with dilute acetic acid, and C by 60 per cent. saturation of the filtrate from B with ammonium sulphate. These are fragments of the protein-complex due possibly to the presence of small amounts of pepsin in the rennin used.

The numerous nitrogenous compounds found in cheese are cleavage products of paracasein by bacteria and enzymes. Intermediate decomposition-products have been called variously tyroalbumin, caseoglutin, tyrocasein, α-peptone, β-peptone, etc.

Strong acids and alkalis and the enzymes, pepsin and trypsin, hydrolyse paracasein in the same way as they hydrolyse casein.

It has already been stated (p. 126) that casein and paracasein can be considered identical so far as their organic chemistry is concerned. They show the same racemisation curves. One claim has been put forward for their serological differentiation.[68]

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CHAPTER VIII

LACTALBUMIN, LACTOGLOBULIN. NON-PROTEIN NITROGENOUS CONSTITUENTS OF MILK

56. General Considerations

THERE are two other proteins of importance in milk besides casein, namely, an albumin, or water-soluble protein—*lactalbumin*—and a globulin or a protein soluble in dilute neutral salt solution—*lactoglobulin*. In addition to differences in solubility and non-precipitation at their isoelectric points these proteins are not phosphorylated, contain a smaller percentage of nitrogen which is reflected in a lower amide-nitrogen content, and contain more sulphur than does casein. The albumin, for instance, contains 2·5 times as much sulphur as casein.

When rennet is used to precipitate casein, or when casein is precipitated by acids at its isoelectric point, these two proteins are quantitatively separated in the whey or filtrate respectively, and the latter method can be utilised for their combined isolation and determination.

Again, these two proteins can be quantitatively separated by utilising the property of globulin to precipitate from solutions saturated with neutral salts such as magnesium or sodium sulphate, preferably the former. In separating the proteins of milk for determining protein-distribution, the separation of the two proteins from the casein-free filtrate is not practicable owing to the large volume of liquid and washings ; but having determined the *casein* separately by precipitation at its isoelectric point, *casein and globulin* can be precipitated together by the addition of saturated magnesium sulphate to 10 ml. of milk, and adding some of the solid salt to saturate the water in this volume of milk. When kept at 30° C. for thirty minutes a granular precipitate easy to filter on a pleated filter is obtained. Washing is carried out by flooding with the saturated salt solution. The *total protein* is determined by precipitation from diluted milk (5 ml. to 50 ml.) in a solution, which contains a final concentration of 4 per cent. trichloracetic acid, at 70° C. for thirty minutes and allowing to stand and cool for thirty minutes before filtering. From the nitrogen-contents determined by the Kjeldahl method in the above frac-

tions (knowing also the *total nitrogen*) the nitrogen and protein distribution in a sample of milk can be calculated.[1]

In normal milk, *i.e.*, bulk milk, which on analysis of a large number of consecutive samples shows a uniform regularity in its nitrogen and protein distribution, the amount of albumin nitrogen approximately accounts for 12 per cent. and the globulin nitrogen for 6 per cent. of the *total nitrogen* (or 12·8 per cent. of the *protein nitrogen* is accounted for by albumin and 6·4 per cent. by globulin nitrogen). These average values will vary with samples from individual cows, being lower for samples high in solids-not-fat, but remaining constant for consecutive samples, whilst when solids-not-fat are low the values will be higher and more variable with consecutive samples (daily or weekly).[2] A peculiarity of milk low in solids-not-fat is the diurnal variation in the amount of non-casein protein matter, thus showing a fluctuating power of elaboration of the true milk constituent, casein, a property almost completely absent from milk high in solids-not-fat. It is important to observe that it is the globulin which varies the most.

The physiological significance of globulin in colostrum has already been discussed (see Sections 1 and 14), and the entry of globulin into milk under conditions of disease, etc., has been treated in Section 14.

57. Lactalbumin

Lactalbumin is not identical with blood serum albumin [3] but is similar to it in amino-acid composition (see Table XLIV). The high leucine-content is common to both but the glutamic-acid content of the serum protein is lower. The elaboration of albumin, therefore, calls for a distinct mammary synthesis and is not merely a transfer of albumin from the blood to the milk. The albumin of milk is identical with that of colostrum. Peskett [4] is of the opinion that there are traces of serum albumin in normal milk and that the amount increases when, during the secretion of abnormal milk, other blood constituents such as globulin and sodium chloride enter.

Lactalbumin differs from ovalbumin mostly in being more acidic, which is probably due to its higher content of dicarboxylic-amino acids, especially aspartic acid. The former protein also contains more leucine and lysine. A content of 10 per cent. hydroxy-glutamic acid [5] makes the dicarboxylic-amino acids account for over a quarter of the protein.

(*a*) PREPARATION OF LACTALBUMIN. Saturation of milk with magnesium sulphate precipitates casein and globulin ; the albumin in the filtrate can be precipitated by acidifying with

TABLE XLIX. *Elementary Composition of Lactalbumin and Lactoglobulin* [6] *(Percentages)*

	Lactalbumin	Lactoglobulin
Carbon	52·51	51·88
Hydrogen	7·10	6·96
Nitrogen	15·43	15·44
Phosphorus. . . .	Trace	0·24
Sulphur	1·92	0·86
Oxygen	23·04	24·64

acetic acid (0·25 per cent.) until the liquid is permanently turbid. After standing, the precipitate is filtered, redissolved in a little water and the precipitation with magnesium sulphate, filtration and acidification, repeated several times. The magnesium sulphate solution may be diluted to half-strength before adding the acetic acid. Finally the salt is removed by dialysis and the albumin precipitated with alcohol, washed with ether, and dried at a low temperature. Precipitation of lactalbumin from salt-free solutions with alcohol gives a product which dissolves easily in water, but when precipitated from a fairly concentrated salt solution a relatively insoluble albumin is obtained. Albumin is precipitated by saturation with ammonium sulphate, and the precipitate is readily soluble in water on diluting the solution.

Denatured albumin can be quickly prepared from the casein- and globulin-free filtrate by precipitation with trichloracetic acid, or by heat-coagulation of the diluted, acidified filtrate.

(*b*) PROPERTIES OF LACTALBUMIN. Albumin can be isolated in an optically active (lævorotatory) crystalline form which later denatures to another form which is not optically active.

Heat Coagulation. Denaturation. Albumins are heat-coagulable proteins and the behaviour of solutions of lactalbumin towards heat has been extensively studied owing to the importance of the subject in the heat-treatment of milk.

Chick and Martin [8] have observed two phases in protein coagulation : (i.) denaturation, which is the irreversible change preceding coagulation, and (ii.) flocculation, which is reversible and takes place only in the presence of electrolytes. Denaturation is the result of a chemical change in the protein-molecule, whilst flocculation is purely physical and is caused by the neutralisation of the charge on the protein-molecule. At the present time our knowledge of the nature of these two processes is obscure, and

the information on the nature of the alterations taking place in the heat-coagulable proteins when milk is heated has been mostly confined to the *amount of denaturation* at various temperatures and times of heating.

Generally, denaturation may be caused by heat, light, strong acids and alkalis, by surface-adsorption, pressure, by alcohol and acetone ; the term *denaturation* covers a number of reactions of proteins which are followed by loss of solubility in water and in dilute salt solutions at their respective isoelectric points. There-

TABLE L. *Changes of the Nitrogen Distribution in Milk when heated at Various Temperatures for Thirty Minutes. Kieferle and Glöetzl (N as mg. %).*

Total	Raw	145 4°	185°	212°	239° F
Total N . . .	540·4	537·8	537·3	540·5	537·2
Casein . . .	348·3	335·6	347·8	383·0	390·6
Albumin . . .	75·7	71·7	53·3	13·9	8·0
Proteose . . .	44·6	46·0	45·7	42·4	36·4
Peptone . . .	45·2	59·4	60·0	66·0	68·4
Total residual N .	123·6	138·0	148·6	152·0	160·0
Amino . . .	4·12	4·17	4·39	5·45	5·53
Creatine . . .	2·42	2·60	2·61	2·96	2·81
Creatinine . .	3 39	3·58	3·56	4·03	3·73
Ammonia . .	1·12	1·32	1·37	1·47	1·59
Urea . . .	13·80	14·38	15·83	15·93	17·41
Uric acid . .	2·78	2·44	2·42	2·82	2·63
Residual N . .	31·12	32·56	35·00	37·80	41·12
Titratable acidity * .	0·180	0·177	0·176	0·190	0·190
Lactose (%) . .	4·11	4·09	4·08	4·00	4·00

* As per cent. lactic acid (calculated from the Soxhlet-Henkel degrees published).

fore denatured proteins can be dissolved only in their ionised condition, that is, in solutions of dilute acids and alkalis. The proteins then can be precipitated from solution by the addition of ions of opposite sign. Solutions of denatured proteins are of the suspensoid type and not of the emulsoid type characteristic of normal proteins.

Rupp [9] has found that on maintaining albumin solutions at various temperatures for thirty minutes, no coagulation occurs at 62·8°, 5·71 per cent. is coagulated at 65·6°, 12·76 per cent. at 68·3°, and 30·87 per cent. at 71·1°C. The heat-coagulation is assisted by acidity, the optimum pH for the mixed soluble milk proteins being 4·5,[10] which is very close to the isoelectric point of

lactalbumin (4·55 [11]). Heating milk to 70°C. thus causes considerable protein coagulation, although complete coagulation is never reached, whilst a small amount would be coagulated at " holding " pasteurisation temperature (145° F.).

Kieferle and Glöetzl [12] have carried out detailed experiments on the changes in the nitrogenous constituents of milk by heating for thirty minutes at various temperatures (Table L). It can be seen that the nitrogenous compounds undergoing breakdown are the albumin (which in this case is albumin *plus* globulin) and the proteose, and since the increase in protein, precipitable as casein (denatured albumin at 212° F. and above), does not wholly account for denaturation, a certain amount of decomposition has occurred in addition to coagulation. The figures for

TABLE LI. *Nitrogen Distribution in Milk Proteins*

	Lactalbumin	Lactoglobulin
	%	%
Amide N	7·93	7·57
Humin N	1·82	2·16
Cystine N	2·18	1·90
Arginine N . . .	7·56	10·79
Histidine N . . .	4·44	3·96
Lysine N	12·54	8·58
Amino N of filtrate . .	59·84	62·97
Non-amino N of filtrate .	2·65	1·13

total residual nitrogen and peptone also bear this out. About 5 per cent. of the albumin nitrogen, therefore, becomes coagulated at holder-pasteurisation temperature. No increase in diffusible nitrogen has been observed by Mattick and Hallet [13] on heating milk for thirty minutes at temperatures ranging from 40–90° C. (104–209° F.).

It is evident that heating, even at 185° F., has not caused much coagulation of the albumin since there is no increase in the apparent casein-content. The casein would be determined by precipitation at a *p*H of 4·6, at which point any coagulation albumin would also be precipitated. Heating at temperatures of 212° F. and above causes large increases in what appears to be casein nitrogen, but is really coagulated albumin and globulin, about 50 per cent. of the albumin nitrogen appearing in this form at 212° and 66 per cent. at 239° F.

Chemical Properties. The elementary composition of lactal-

bumin is given in Table XLIX. The nitrogen-content is lower than in casein and the weight of protein must be calculated from the nitrogen-content by using the factor 6·48.

It gives all the protein reactions as well as a strong blackening on boiling with lead acetate. Its high sulphur-content is outstanding. Albumins in general differ from closely related globulins in this respect (*cf.* seed albumins and globulins).

The amino-acid content of albumin is given in Table XLIV. Table LI gives its nitrogen distribution (Van Slyke).

A pure aqueous solution of albumin shows a neutral reaction to litmus. It possesses a slight reducing power and forms an osazone.[14]

Osborne [15] gives the empirical formula of lactalbumin as of the order of $C_{644}H_{1064}N_{166}S_8O_{214}$, corresponding to the molecular weight 14,796. Sjögren and Svedberg,[16] by ultra-centrifugal methods, have found that lactalbumin is not homogenous and give values for molecular weight varying from 12,000 to 25,000. They have also found that the lactalbumin with the properties observed in the purified product does not exist in milk, but that it is formed during the process of preparation especially by the action of a high concentration of ammonium sulphate. The bulk of the material from which lactalbumin is formed in the separating processes has a low molecular weight not exceeding 1,000, and the high molecular weight of the final product is the result of gradual aggregation of the material of low molecular weight originally present in the milk. The molecular weight of lactalbumin, like that of casein, is markedly unstable. This property is characteristic of milk proteins and may be of considerable physiological importance in nutrition.

58. Lactoglobulin

Lactoglobulin is the other heat-coagulable protein of milk in which it is present to the extent of 0·2 per cent. Osborne and Wakeman,[17] however, were able to isolate only 0·52 grams per litre of milk.

This protein was first isolated from milk by Sebelin [18] and by Emmerling.[19] Milk is saturated with sodium chloride to remove casein, filtered, heated to 35° C., and after removing the small amount of precipitate it is saturated with magnesium sulphate. Purification is effected by dissolving in sodium chloride solution and reprecipitating with saturated magnesium sulphate.

A. H. Palmer [35] has concentrated casein-free whey by freezing, from which lactoglobulin was separated by precipitation with

sodium sulphate. On analysis he isolated a crystalline globulin which was insoluble in water within the pH range 4·5-5·5.

Properties. Lactoglobulin is soluble in dilute solutions of mineral salts and dilute acids but is precipitated from solution by half saturation with $(NH_4)_2SO_4$ or complete saturation with $MgSO_4$. On heating globulin solutions to 72° C. complete coagulation occurs.

Lactoglobulin is a pentavalent acid and its acidic are greater than its basic properties which explains in part its precipitation with very dilute acids and its solubility in weak alkalis.

The elementary analysis of globulin (Table XLIX) shows it to contain 0·24 per cent. of phosphorus stated by Osborne and Wakeman [17] to belong to a phosphatide, probably in a phosphatide-containing protein fraction. The amino-acid content of serum globulin is given in Table XLIV and the Van Slyke distribution of nitrogen in Table LI. Lactoglobulin approaches nearer to casein in composition than does lactalbumin, especially in its contents of sulphur and of dicarboxylic amino acids. Casein has more amide nitrogen than both the soluble proteins. The aggregation or condensation of 4 molecules of globulin, together with phosphorylation of the hydroxyl groups would give a product closely resembling casein in composition and molecular weight.

59. The Globulin of Colostrum

Crowther and Raistrick [23] have resolved colostral globulin into two fractions, *euglobulin*, which is insoluble in distilled water, and *pseudoglobulin*, which remains in solution. Taylor [24] has shown that the insoluble form is readily converted to the soluble form by hydrolysis, although Hammarsten [25] doubts the existence of these two proteins as derived from serum. These fractionation phenomena can partly be explained by the aggregation theory of Svedberg [16] or possibly by assuming that the recovery of soluble properties by part of the protein is accompanied by a lowering of the molecular weight. There is no doubt that lactoglobulin shares with casein and lactalbumin the property of instability of molecular weight.

Lactoglobulin is identical with blood-serum globulin.[23-26]

Howe [28] has found definite zones of sodium-sulphate concentrations which can precipitate euglobulin and pseudoglobulins I and II from blood plasma, thus confirming the work of Porges and Spiro.[29] Howe [30] has later applied his sodium sulphate method to colostrum and has found that the same fractions of globulin occur in that liquid.

The physiological significance of these globulin fractions is

evident when it is realised that the blood of a newly born calf contains no euglobulin or pseudoglobulin I, but that after feeding on colostrum these proteins appear in the blood. Their appearance in the blood is transient and their concentrations greatly decrease after the first day, even when the calf is receiving colostrum ; the animal does not recover the high concentration of these proteins characteristic of the adult animal until the age of twelve to fourteen months is reached. Pseudoglobulin II appears to be the basic normal globulin of blood and is present in a constant amount even if the calf is not fed on colostrum.

Colostrum contains agglutinins and various antibodies which are removed by the same concentrations of sodium sulphate as precipitate the globulins ; and this is true for blood serum also. The intimate association of the globulins with these immunological factors brings into prominence the significance not only of the globulins but of the permeability of the placenta of the mammal to both globulin and the immunological factors. Thus, in the case of animals with epithelio-chorial placentæ, colostrum plays a more important part than in animals with hæmo-chorial placentæ. The human placenta, for instance, is permeable to pseudoglobulin I and the blood serum of a new-born infant contains as much pseudoglobulins I and II as that of adults. The feeding of human colostrum makes up for the deficiency of euglobulin, but human colostrum is secreted about twelve hours *after* birth, and nursing is not spontaneous but has to be stimulated.

60. Evidence of Traces of other Proteins in Milk

Traces of other proteins appear in milk. A mucoprotein, *lactomucin*, has been isolated from butter.[31, 32] This protein was formerly thought to constitute the protein-layer surrounding the fat globules in milk. The adsorbed protein on the fat globules of cream has been found to have a high arginine and low histidine and cystine contents. The protein is not identical with either casein albumin or globulin, but resembles haptein.[36]

The various degradation products of casein which can be isolated from ripened cheese have already been discussed (Section 51). Some of these are alcohol-soluble (caseoglutins). Osborne and Wakeman [33] have isolated from casein a protein soluble in 50 to 70 per cent. alcohol and in very dilute acetic acid. This was probably one of the fractions of casein isolated in quantity by Lindestrom-Lang, Carpenter and others.

A bacteriostatic factor in milk, *lactenin*, is stated by Jones and Simms [34] to be associated with whey proteins, and to be in combination with calcium phosphate. They have found it to contain

carbon, hydrogen, oxygen and nitrogen. The material has been isolated in a dry form which is 200–500 times as active as dried skim milk. It is insoluble in water and keeps for at least three months at low temperature.

The occurrence of proteose (or albumose) to the extent of 0·28 per cent. (proteose nitrogen, 0·045 per cent.) has been reported by Kieferle and Glöetzl [12] (see Table L). The filtrate from the precipitation of milk proteins by trichloracetic acid gives a decided biuret reaction so that it may be concluded that small amounts of proteose and peptone are present in the residual fraction of milk after protein precipitation.

61. The Residual Nitrogen Fraction

The non-protein fraction of normal milk amounts to approximately 6 per cent. of the total nitrogen (0·030 per cent. approximately of the milk) and is, strikingly, the same amount as the globulin nitrogen. In samples from individual cows this proportion may vary widely, especially in the direction of accounting for more of the total nitrogen. When milk is low in solids-not-fat the non-protein nitrogen increases appreciably, and although there is an increase in the globulin content it cannot be correlated with the non-protein nitrogen. It may generally be stated that when milk is high in chloride and the expected level of lactose elaboration is not reached, the level of secretion of casein is also reduced and is accompanied by an increase in the relative amounts of the milk-soluble proteins and a higher non-protein nitrogen-content.[2] In normal milk about 40 per cent. of the nitrogen is precipitated by phosphotungstic acid in acid solution, but when the amount of non-protein nitrogen is higher only 25 to 30 per cent. is precipitated, which indicates that the fraction from normal milk more nearly approximates in composition to a protein hydrolysate than to that from abnormal milk.

Table L shows the result of the detailed analyses of milk heated for thirty minutes at different temperatures. It will be seen that most of the minor substances are those occurring in the non-protein nitrogen fraction of blood serum. They are, of course, present only in very small quantities ; urea, the constituent in greatest amount, is present to the extent of only 0·03 per cent. Amino, ammonia, uric acid, creatine and creatine nitrogen appear to be present in amounts of the same order of magnitude. These substances do not seem to have any significance in milk and their presence can be explained only by infiltration into the milk from the blood. Their osmotic effect is insignificant.

Mattick and Hallet [13] have found no increase in the amount of dialysable nitrogen on heating milk for thirty minutes at various temperatures. The diffusible nitrogen ranges from 0·017 to 0·027 per cent., or from 3·4 to 5·4 per cent. of the total nitrogen. Saito [35] has obtained a compound giving a strong ninhydrin reaction by the dialysis of milk. This compound accounts for 2·3 to 2·8 per cent. of the total nitrogen. Boiling for half an hour and lactic fermentation increase the free ammonia nitrogen and the amino nitrogen. The coagulation of milk, on the other hand, decreases the amount of dialysable nitrogen.

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CHAPTER IX

THE MINERAL CONSTITUENTS OF MILK

62. The Ash of Milk compared with that of Embryos

ALTHOUGH there is on the average only 0·75 per cent. of mineral constituents in milk their importance from the nutritive and technological standpoints is at once realised. It can be seen from Chart I that milk is complete as regards the elements needed for embryonic and post-natal development of the offspring.

In 1874 Bunge [1] asserted that the composition of the ash of the fully grown fœtus of a given species of mammal presented at least an analogy with the composition of the ash of the maternal milk. So close a parallelism was observed between the ash com-

TABLE LII. *Ash of Embryos and Milk of the same Species. Percentage of Ash*

	Dog		Cat	Rabbit
	Embryo at term	Milk	Embryo at term	Embryo at term
P_2O_5 . .	39·82	36·08	40·23	41·94
CaO . .	35·84	33·03	34·11	35·02
MgO . .	1·61	1·66	1·52	2·19
Cl . .	7·34	13·91	7·12	4·94
Fe_2O_3 . .	0·34	0·10	0·24	0·23
K_2O . .	8·49	12·98	10·11	10·84
Na_2O . .	8·21	5·37	8·28	5·96

Alkali Ratio of Milk and Embryos and of Vegetables

		Ratio
		K_2O Na_2O
Carnivorous . .	New-born body	0·8 : 1·0
	Milk . .	1·75 : 1·0
Herbivorous . .	New-born body	1·2 : 1·0
	Milk . .	5·6 : 1·0
Human milk . .	—	1·3–4·3 : 1·0
Vegetables . .	—	14·0–110·0 : 1·0

positions in the cat, dog and rabbit that he formulated the generalisation that " the epithelial cells of the mammary gland select from the mineral constituents of the plasma exactly those inorganic substances and in their right proportions which are necessary for the further development of the newly-born embryo." Table LII supports this generalisation.

The human species undoubtedly is an exception to Bunge's law (see Table LII) just as it is to other generalisations (such as Rubner's growth laws). Hugounenq [2] states that Bunge's law probably applies only to mammals with comparatively short pre-natal life, that depend on milk for the complete development of their complement of ash. In the dog, for instance, the suckling period amounts to 25 per cent. of the total development

TABLE LIII. *Ash of Human Embryos and of Human Milk*

	Full term Human Embryo		Human Milk	
	Hugounenq [3]	de Lange [3]	Bunge [1]	Soldner [4]
P_2O_5	35·28	37·61	21·30	14·5
CaO	40·48	38·89	14·79	18·9
MgO	1·51	1·37	2·87	2·0
Cl	4·26	6·36	19·73	16·3
SO_4	1·50	—	—	3·3
Fe_2O_3	0·39	1·69	0·18	0·05
Na_2O	6·20	6·54	35·15	31·1
K_2O	8·12	8·80	10·43	7·9
CO_2	1·89	—	—	—

period, but only to 5 per cent. in man, who stays relatively much longer in the uterus than any other mammal. Thus milk is much more important in most mammals than in man. The predominance of P over Ca in the ash of embryos shows the presence of some acid phosphates, as in milk.

Table LIV shows the variation in composition of the ash of milk from various mammals. The composition runs parallel to the stage of development of the embryo when born. The ash of rabbit's milk (2·5 per cent. of ash) is remarkably high in phosphate, lime and potash, whereas that of mare's milk and of human milk are strikingly low in chlorides and sodium. The iron-content of milk from all sources is very low (2 to 4 parts per million). Human milk is much poorer in all elements than milk from other species.

M 2

TABLE LIV. *Composition of the Ash of Mammalian Milks and of the Hen's Egg (Percentage of Milk)*[7]

	Man	Dog	Pig	Sheep	Goat	Cow	Horse	Guinea pig	Rabbit	Hen's egg
P_2O_5	0·059	0·508	0·308	0·293	0·284	0·191	0·131	0·288	0·997	0·403
CaO	0·049	0·455	0·249	0·245	0·197	0·167	0·124	0·242	0·891	0·117
MgO	0·007	0·020	0·016	0·015	0·015	0·023	0·013	0·024	0·055	0·012
Cl	0·047	0·166	0·076	0·130	0·102	0·137	0·031	0·100	0·136	0·096
SO_4	0·003	—	—	—	—	0·003	—	—	—	0·003
Fe_2O_3	0·001	0·002	0·004	0·004	0·004	0·002	0·002	0·001	0·002	0·004
Na_2O	0·025	0·078	0·078	0·086	0·062	0·097	0·014	0·070	0·198	0·245
K_2O	0·080	0·138	0·095	0·097	0·130	0·178	0·105	0·075	0·252	0·186

TABLE LV. *Composition of the Ash of Cow's Milk Percentages (and Gram Equivalents in 100 g.) of Ash*

Authority	Richmond[5]	Babcock[6]	Fleisch-mann[12]	Schrodt and Hansen[13]	Sche-pang[14]	Orla-Jensen[15] (average)	Storch[16]
P_2O_5	29·33 (1·24)	24·29 (1·03)	21·57	24·11	24·80	26·89	28·69
CaO	20·27 (0·72)	20·01 (0·71)	24·68	21·45	20·90	27·32	21·93
MgO	2·80 (0·14)	2·42 (0·12)	3·12	2·54	2·25	2·42	2·87
Cl	14·00 (0·39)	14·28 (0·40)	16·38	14·60	14·55	13·57	13·73
SO_3	Trace	3·84 (0·01)	—	4·11	2·55	2·96	—
Fe_2O_3	0·40	0·13	0·31	0·11	0·05	—	—
Na_2O	6·67 (0·22)	10·01 (0·32)	11·92	10·94	8·41	5·82	9·44
K_2O	28·71 (0·61)	25·02 (0·53)	25·71	25·42	30·33	23·63	25·31
CO_2	0·97 (0·04)	—	—	—	—	—	—

63. The Composition of the Ash of Cow's Milk

The ash of cow's milk is the white to grey material left after drying and incinerating milk. Table LV gives the average composition of the ash (the figures in brackets representing the number of gram equivalents of each constituent in 100 grams of ash). When the equivalents of the acidic and basic constituents are added up it will be noticed that the latter predominate and so milk ash is always alkaline in reaction. This condition does not obtain in milk itself since the incinerating process drives away certain acid radicals, such as citrate and carbonate, and the calcium attached to casein is set free.

The average ash-content of normal milk lies between 0·69 and 0·77 per cent. Table LVI gives the average value according to various investigators.

TABLE LVI.   *Average Percentage of Ash in Milk*

Richmond [5]	.	0·75	Deysher [9] .	. 0·70
Babcock [6] .	.	0·72	Tocher [10] .	. 0·70
Orla-Jensen [8]	.	0·72–0·77	Golding [11] .	. 0·774

(The incineration of milk requires considerable care to prevent loss of the easily-volatilised alkali chlorides, and the temperature employed should not rise above low red-heat (450–550° C.). Undoubtedly some of the figures from which the above averages were drawn are low values.)

More than half the ash is composed of calcium phosphate. Of all the radicals present, phosphoric acid is present in greatest equivalent, whilst of the metallic radicals calcium is in greatest equivalent, closely followed by potassium. Sodium is present in roughly half the equivalent of potassium. Chloride, a variable constituent, especially in milk samples from individual cows, is present from a third to a half of the equivalent of phosphoric acid. The greater weight per cent. of the potassium over that of lime is frequently overlooked. The ratio of potassium to sodium will vary according to the chloride-content. More sodium enters with an increase in chloride-content. The Na/Cl relationship in milk is linear and can be expressed by the equation :

$$Cl \ (mg. \ \%) = Na \ (mg. \ \%) \times 1·24 + 18·1.^{94}$$

The percentage of ash in an average sample of bulk milk has been connected with the amount of other constituents by various formulæ. Thus the proportion of lactose : protein : ash has been given as 13 : 9 : 2 (Vieth's ratio), whilst Richmond [5] connects the protein and ash contents by the formula, *Ash* = 0·36 + 0·11 *protein*. Better agreement is given by Sherman's modification of this generalisation, viz., *Ash* = 0·38 + 0·10 *protein*.

64. Salt Combinations in Milk

The drastic treatment which the mineral matter in milk undergoes during incineration naturally changes the nature and mode of combination of the various radicals in the original milk, and the ash by no means gives a true picture of the salt combinations of milk. Many attempts have been made to distribute the various radicals into salt combinations (Table LVII).

By removing the solid phase by ultra-filtration methods the phasic distribution of the constituents can be determined. In this way it has been shown that all the sodium, potassium and chlorine are in true solution, whilst calcium, magnesium and phosphate are partly in solution and partly in suspension. The constituents

are thus mostly in the ionic form, forming part of a polyionic system which is governed by the requirements of ionic equilibria.

TABLE LVII. *Salt Combinations in Milk*

Salt	Soldner[17]		Van Slyke and Bosworth[18]		Porcher and Chevallier[19]		
					Percentage of milk		
	% of milk	% of ash	% of milk	% of salt combinations	I	II	III
NaCl	0·096	10·62	—	—	0·109	0·095	0·100
KCl	0·083	9·16	—	—	0·092	0·085	0·080
CaCl₂	—	—	0·119	2·90	-		
KH₂PO₄	0·116	12·77	—	—	0·100	0·100	0·110
K₂HPO₄	0·084	9·22	0·230	5·61	0·110	0·110	0·100
Mg(H₂PO₄)₂	—	—	0·103	2·51	0·016	—	0·019
Mg₂(HPO₄)₂	0·034	3·71	—	—			
Ca₂(HPO₄)₂	0·067	7·42	0·175	4·27	0·106	- -	0·113
Ca₃(PO₄)₂	0·081	8·90	—	—			
Na citrate	—	—	0·222	5·41	—	—	—·
K citrate	0·050	5·47	0·052	1·26	0·067	0·054	0·075
Mg citrate	0·037	4·05	—	—	0·076	0·058	0·070
Ca citrate	0·213	23·55	—·	—·	0·178	0·203	0·180
CaO in protein combination	0·047	5·13	0·054	1·31	0·061	—	0·060

Our present knowledge of such systems is far from being sufficiently adequate to deduce salt combinations, and it is doubtful whether the cited salt combinations (Table LVII) have any constructive value in attempting to define the actual mineral salts present. The intervention of a colloidal solid phase further complicates the problem.

65. Partition of the Inorganic Constituents

(*a*) GENERAL CONSIDERATIONS. The work on the distribution of the inorganic constituents between the liquid and solid phases of milk has been confined mostly to the partition of calcium and phosphorus. Van Slyke and Bosworth,[18, 20] in investigating the composition of milk serum and the insoluble constituents, have obtained the following results for two samples of milk and serum (Table LVIII) :

The constituents of milk may be grouped into three classes : (i.) those in true solution, such as lactose, citric acid and chlorine, sodium, potassium ions, and non-protein nitrogen ; (ii.) those

TABLE LVIII. *Composition of Milk and of Milk Serum* (*Van Slyke and Bosworth*)

Constituents	Sample I			Sample II		
	Milk 100 ml	Serum 100 ml	Con-stituents in serum (%)	Milk 100 ml	Serum 100 ml	Con-stituents in serum (%)
Lactose	—	—	—	5·75	5·75	100
Casein	3·35	0·00	0·00	3·07	0·00	0·00
Albumin	0·525	0·369	70·29	0·506	0·188	37·15
Other N	—	—	—	0·049	0·049	100·00
Citric acid	—	—	—	0·237	0·237	100·00
P (organic and in-organic)	0·125	0·067	53·60	—	—	—
P (organic)	0·096	0·067	70·00	0·087	0·056	64·40
Ca	0·128	0·045	35·16	0·144	0·048	33·33
Mg	0·012	0·019	75·00	0·013	0·007	53·85
K	} 0·354	0·352	99·44	0·120	0·124	100·00
Na				0·055	0·057	100·00
Cl	0·081	0·082	100·00	0·076	0·076	100·00
Ash	—	—	—	0·725	0·400	55·17

partly in solution and partly in suspension, such as calcium, magnesium, inorganic phosphate, and albumin ; and (iii.) those entirely in colloidal suspension, *e.g.*, fat and casein.

(*b*) CONSIDERATIONS OF OSMOTIC PRESSURE. When the osmotic pressure of milk is considered it is obvious that the constituents of group (i) (*v.s.*) contribute most, whilst those in group (ii.) add a relatively small quota, and those in group (iii.) do not contribute at all to the osmotic pressure. Following their suggestions of salt combinations in milk (*v.* Table LVII) Porcher and Chevallier [21]

TABLE LIX. *The Partial Depressions of the Freezing-point caused by the Various Salt Combinations in Milk*

Salt	Amt %	Δ° (partial)		Salt	Amt %	Δ° (partial)	
NaCl	0·095	0·065 }	0·111	K_2SO_4	0·015	0·004 }	0·008
KCl	0·085	0·046 }		$NaHCO_3$	0·025	0·004 }	
KH_2PO_4	0·100	0·036 }	0·067	Lactose	5·000	0·293	
K_2HPO_4	0·110	0·031 }		Non-protein N	0·060	0·021	
Ca citrate	0·203	0·017 }		Colloidal com-plex		0·025	
K citrate	0·054	0·007 }	0·027	Total Δ		0·552	
Mg citrate	0·058	0·003 }					

have stated that the various salts contribute the following quotas to the freezing-point depression (0·552°) of normal milk (Table L).

(From Table LIX it is seen that the lactose and chlorides contribute 0·40° to the Δ. This is the basis of the " cryolac number " for milk (Post [22]), the minimum value for which is 410, corresponding to a quota of 0·410° provided by the lactose and chlorides. It may be deduced that this assumption necessitates the complementary regulation of isotonicity when either of these constituents of milk varies.)

(c) SUBSTANCES PARTLY IN SOLUTION AND PARTLY IN SUS-PENSION. Data on the distribution of phosphorus, calcium and

TABLE LX. *Percentages of Casein, Phosphorus. Calcium, and Magnesium in Milk (with Distributions) (Van Slyke and Bosworth)*

Col. 1	2	3	4	5	6	7	8	9	10	11	12	13	14
								Phosphorus					
Cow	Stage of Lactation	Total Protein	Casein	Calcium (total)	Calcium (% sol.)	Magnesium (total)	Magnesium (% sol.)	Total	Total insol.	Org. in casein	Inorg. (insol)	Ratio org. P : Insol. inorg. P : 1	Sol. P. % of Total P.
1	3 days	4·35	3·48	0·161	45·7	0·016	91·0	0·127	0 045	0 025	0·021	0·83	64·3
2	1 month	3·31	2·73	0·138	37·0	0·014	86·0	0·115	0 056	0·019	0 030	1 86	51·7
3	1 „	3·53	2·78	0·136	40 0	0·018	78 9	0·101	0·052	0 020	0·032	1·62	48·9
3	11 months	4·91	4·09	0·156	34·3	0·017	91·8	0·111	0·058	0·020	0·029	0 98	48·3
4	3 „	3·93	3·09	0·148	23·1	0 018	69·6	0·128	0·072	0 022	0 050	2·26	44·0
5	3 „	3·45	2·88	0·140	38·0	0·016	79·5	0·094	0 017	0 020	0·026	1·29	50·4
5	7 „	3·15	2·70	0·120	36·2	0·015	91·2	0·087	0·054	0·019	0·034	1·79	38·4
6	5 „	4·05	2·92	0·141	26 4	0·016	79·4	0·101	0 005	0 021	0 045	2 15	35 3
7	6 „	4·07	3·40	0·146	35·0	0·014	84·0	0·106	0·052	0·024	0 027	1 14	51·6
7	10 „	4·80	3·56	0·152	29 6	0·018	71 8	0·101	0·007	0·025	0·042	1 65	33 7
8	7 „	4·39	3·58	0·151	29·2	0·015	77·1	0·116	0 061	0·025	0 035	1·39	47·5
9	5 „	4·33	3·47	0·150	29 3	0·017	73·7	0·104	0·067	0·025	0·043	1·73	35·1
10	9 „	3·65	3·10	0·141	38·5	0·017	83·0	0·110	0·049	0 022	0 027	1 22	55·5
11	10 „	4·17	3·36	0·138	25·9	0·017	70·8	0·109	0·066	0·024	0·042	1·74	39·8
12	11 „	4·35	3·14	0·166	25·0	0·019	64·4	0·106	0·077	0 022	0 055	2·47	27·0
13	12 „	5·71	4·97	0·217	30·9	0·024	69·1	0·131	0·087	0·035	0·052	1·46	33·7

magnesium have been collected by Van Slyke and Bosworth [18, 20] in their investigations on the relations existing between casein and these constituents. They have analysed sixteen milk samples from thirteen individual cows at different stages in their lactations, and obtained values for casein, the different classes of phosphorus compounds and the soluble and insoluble calcium and magnesium (Table LX).

In this investigation Van Slyke and Bosworth have used 0·0071 as the phosphorus-content of casein (other workers differ as regards this figure, viz., from 0·85 to 0·88 per cent.) in calculating the organic phosphorus in casein ; the figures in column 11 are likely to be low and the ratio " organic P : insoluble in-

organic P " (column 13) are therefore slightly low. The ratio (column 13) shows wide variations (1 : 0·83 to 1 : 2·47), but the validity of such an observation will not be modified by small differences in the phosphorus-content of casein. The ratio should be fairly constant if the inorganic phosphates are in combination with casein, so the assumption that no such combination occurs is justifiable. Other investigators have observed that some of the calcium phosphate and casein are in chemical combination, but the important result of this investigation has disproved that assertion.

Table LX also shows that there is a considerable variation in the milk of individual cows both with respect to the total calcium and magnesium and the amounts in true solution. Total calcium has been found to vary from 0·126 to 0·217 and the soluble calcium from 0·034 to 0·073 per cent., whilst total magnesium varies from 0·014 to 0·024 and the soluble magnesium from 0·012 to 0·016 per cent. ; one cow was responsible for all the high and another for the low values, the former being at the beginning and the latter at the end of her lactation. Only a trace of magnesium is in the insoluble form, as compared with the greater part of the calcium.

With reference to the form in which the insoluble calcium and phosphorus are present in milk, analysis of the figures of Table LX, by calculating on an equivalent basis (acids and alkalis) has been found by Van Slyke and Bosworth to give a quantitative relationship between the bases (Ca and Mg) and the acids (phosphate and casein), according to the theoretical requirements of dibasic phosphate and calcium caseinate containing eight equivalents of calcium. The figures also show that the compounds may be present as acid caseinates and tricalcium phosphate.

Fractionation of milk by *ultra-centrifuging* at different speeds for different times and analysing the slime have given an uneven distribution of the inorganic phosphorus, but there is still the same balance between the bases (Ca and Mg) and the acids (casein and phosphoric acid). This can only be the case if the inorganic phosphorus is present solely as $CaHPO_4$ (neutral phosphate), for otherwise the balance between bases and acids would be altered by an alteration in the *relative* amount of inorganic phosphorus present, $Ca(H_2PO_4)_2$ giving an excess of acid and $Ca_3(PO_4)_2$ an excess of base. The insoluble inorganic phosphate of milk is thus proved to be the neutral phosphate, $CaHPO_4$.

The ratio. inorganic P : organic P, has been found to vary in

the above centrifugates, thus confirming that there is no combination of the calcium phosphate with the casein.

The serum obtained by ultra-filtration is slightly acid and shows the same titratable acidity as the original milk. Table LXI gives the results for eight samples.

TABLE LXI. *Titratable Acidity (ml. 0·1 N alk. per 100 ml.) of Milk and its Serum*

No	1	2	· 3	4	5	6	7	8
Milk . .	4·8	6·2	4·2	6·0	6·4	4·4	7·0	6·6
Serum .	5·0	6·2	4·2	5·8	6·4	4·4	6·8	6·4

(*d*) THE EFFECT OF SOURING ON THE MINERAL CONSTITUENTS PARTLY IN SUSPENSION. Van Slyke and Bosworth [20] have later studied the effect of lactic acid production by bacteria on the state of the soluble and insoluble constituents. Developed acidity has been found to render soluble those substances which in fresh milk are insoluble. Thus, all calcium, magnesium and inorganic phosphorus become completely soluble.

The state of the insoluble constituents at different stages in the souring process has also been studied. The insoluble calcium as $CaHPO_4$ is quickly brought into solution, *e.g.*, 8·7 per cent. in 4 hours, 27·4 in 8 hours and 100 per cent. in 13½ hours. The calcium as caseinate is acted on more slowly, none being dissolved in 5 hours but all in 25 hours. Magnesium is brought completely into solution in 11½ hours. The combination between calcium and both phosphoric acid and casein is a function of hydrogen-ion concentration. Thus at the isoelectric point of casein (*p*H 4·6) maximum (and total) solubility of calcium is reached. Whey from milk which has soured contains, therefore, a considerably higher amount of calcium and phosphorus than whey from the rennet coagulation of milk at a comparatively low degree of acidity.

Gyorgy [23] also has found that the amounts of Ca and P in solution increase with rise in acidity of milk, and that at the isoelectric point of casein no calcium remains in protein-combination. He has also determined the relation between diffusible and indiffusible inorganic phosphorus in milk, using ultra-filtration methods of separation ; he has found that from 40 to 55 per cent. is in the diffusible and 45 to 60 per cent. in the indiffusible form (Van Slyke and Bosworth have found a lower amount of diffusible phosphorus, viz., 30 to 45 per cent.).

Tryptic hydrolysis of milk at constant pH results in a liberation of considerable amounts of Ca and P into the dialysable form, and Gyorgy concludes from this that some dialysable Ca and P salts are adsorbed on the casein since, if calcium phosphate only were adsorbed, digestion of casein would merely result in the *precipitation* of the insoluble phosphate. His findings therefore conflict with those of Van Slyke and Bosworth, who have demonstrated the presence of $CaHPO_4$ in milk. Allen,[24] in discussing this conflicting evidence, attempts to explain the difference of opinion by stating that, (*a*) either ionic equilibrium has not been reached, or that (*b*) provided the laws governing the solubility products of the respective salts concerned are satisfied, it is possible for insoluble salts to remain in equilibrium with ions in solution. That no stable equilibrium exists is proved by precipitation of calcium phosphate and citrate when milk is heated. Mention must be made of the adsorptive effects on the surfaces of the colloidal material in milk which cause localised concentration of ions (micelle formation). When the adsorptive surface is minimised or its physical properties changed, such as by the two treatments cited above (digestion and heating), a change must occur in the concentration of adsorbed material and hence in the composition of the continuous phase.

Mattick [25] has determined the total and diffusible calcium and phosphorus, using parchment membranes and dialysing in 3 per cent. sodium chloride solution (25 ml. milk in 150 ml. of 3 per cent. saline, for eighteen hours). This arbitrary method showed 24·2 to 30·6 per cent. of the calcium and 28·7 to 38·4 per cent. of the phosphorus to be diffusible. The method has much to recommend it for comparative purposes but the values are undoubtedly lower than the absolute values.

Wardlaw [26] has found 35 to 55 per cent. of the total phosphorus and 30 to 40 per cent. of the total calcium in milk to be diffusible. He has observed in dialysing large quantities of milk against small volumes of water that in twenty-four hours an isotonic equilibrium (as determined by the freezing-point method) on each side of the membrane is established.

Lampitt and Bushill [96] have found that heating or acid dissociation causes a slight alteration in the amount of dialysable phosphorus. About 40 per cent. of the total P, 50 per cent. of the inorganic P and 20 per cent. of the organic P are dialysable.

Jordan, Hart and Patten [27] have subdivided the phosphorus of milk into (*a*) total, (*b*) soluble and (*c*) inorganic. A 0·2 per cent. hydrochloric acid solution has been used for extracting soluble phosphorus and precipitating the proteins. In the inorganic

phosphorus fraction, therefore, all the soluble and insoluble phosphates will appear, owing to more acid conditions than those corresponding to the isoelectric point of casein prevailing. According to their results, from 65 to 80 per cent. of the total phosphorus is in the inorganic form.

Lenstrup [28] divides the phosphorus thus :

```
                          Total P
                             |
        --------------------------------------------
        |                                           |
   Acid-soluble P                            Acid-insoluble P
        |                                           |
   ------------------                   -------------------------
   |                |                   |                       |
Inorganic P    Organic P            Lipoid P                Protein P
```
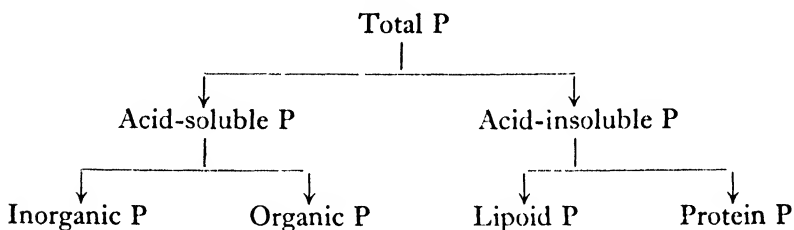
Acid-soluble P is separated from insoluble P by the use of picric acid as a precipitant. Inorganic phosphorus is then precipitated from the filtrate as magnesium ammonium phosphate and the organic phosphorus is in the residual filtrate.[28, 29] About 83 per cent. of the total phosphorus is found to be precipitated as magnesium ammonium phosphate, which can be considered as inorganic.

With respect to the seasonal variations in the different phosphorus groups, Lenstrup has found that the acid-soluble organic phosphorus is practically constant, and the inorganic phosphorus is distinctly higher from September to May. The casein phosphorus (acid-insoluble) is also constant, so that the only group subject to seasonal variation is the *acid-soluble inorganic phosphorus.*

The phosphorus partition in milk has been studied by Kay and Graham,[97] who have developed a simple method of separation. Their work has been concentrated on the fate of the acid-soluble P by the action of phosphatase. The amount of ester-P is from 0·15 to 0·25 of the total inorganic P, *i.e.*, from 7–21 mg. P/100 ml.

Hocheimer [98] has tabulated the P distribution in goat's milk for one lactation. The acid-soluble ester P averages 8 mg. per 100 ml., of which 3·3 mg. is hexose-phosphate P ; hexosepyrophosphate, adenosine and creatine phosphoric acid account for the remainder.

The acid-insoluble phosphorus is that of casein. During the precipitation, picric acid solution has no decomposing effect on this group, since the phosphorus-content of rennet-casein is identical with that from picric acid precipitation. Kay [30] states that the use of a strong acid, such as trichloracetic acid, for the

precipitation of protein acts on the lipoids so rapidly that the results of such determinations are valueless, and there is no doubt that the lipoid phosphorus of milk decomposes appreciably during the use of trichloracetic acid as precipitant.

66. The Range of Variations of the Mineral Constituents

The tendency to fluctuation in the composition of milk holds also for the composition of the mineral constituents, a large range of variation being experienced. Here also arises the difficulty of differentiating between normal variation and a desired variation, as for example from a feeding experiment. That variation is of technological importance may be deduced from the fact that the mineral constituents play an important part in determining the heat stability of milk, troubles like change in coagulation-temperature during the sterilisation of evaporated milk being encountered. Thus it is well known that success attends some batches of milk intended for condensing or drying better than others, and that salt variation is primarily responsible for these differences.

Table LXII gives the range of variations met with in the amounts of the various mineral constituents of milk.

Calcium. Cranfield, Griffiths and Ling [40] have determined the frequency distribution of CaO (673 samples from individual cows from fifteen herds). The figures show extreme variations of fiom 0·135 to 0·230 per cent., these values being given by single cows, but 316 samples were within the range 0·175 to 0·190 per cent., so that this range may be taken as an average. Crichton,[44] discussing 220 samples (twenty Ayrshire cows, three lactation periods), has found a range of from 0·120 to 0·212, with an average value of 0·166 per cent. Sommer gives the range 0·125 to 0·291 per cent.

Phosphorus. Cranfield, Griffiths and Ling (*loc. cit.*) have found a range of variation of from 0·175 to 0·310 per cent., but 80 per cent. of the samples fell between the range 0·210 to 0·255 per cent. Sommer gives the range 0·155 to 0·273 per cent., whilst Crichton has found it to be 0·166 to 0·291 per cent.

Magnesium. Only a few data are available for the amount of magnesium. Sommer gives the range of 0·005 to 0·039 per cent.

Sodium, Potassium and Chlorine. Results recorded for these constituents are few, except for chlorine. Average amounts of sodium have been found to lie between 0·043 and 0·c61 per cent. and of potassium between 0·164 to 0·193 per cent. Chlorine, on the other hand, has been found to range betwee 1 0·055 and 0·245, the higher values occurring in samples very low in solids-not-fat.[46] Porcher and Chevallier [21] have described a sample with a

TABLE LXII. *Variation in the Amount of the Mineral Constituents in Milk (Percentages of Whole Milk)*

Authority	CaO Min.	CaO Max.	CaO Mean	P₂O₅ Min.	P₂O₅ Max.	P₂O₅ Mean	MgO Min.	MgO Max.	MgO Mean	Na₂O Min.	Na₂O Max.	Na₂O Mean	K₂O Min.	K₂O Max.	K₂O Mean	Cl Min.	Cl Max.	Cl Mean
Forbes and Beigle [31]	0·115	0·150	—	0·156	0·201	—	0·0133	0·0168	—	0·0461	0·0807	0·0609	0·176	0·223	0·193	0·073	0·127	0·101
	0·104	0·153	0·128	0·146	0·201	0·173												
Forbes et al. [32]	0·113	0·179	0·144	0·147	0·206	0·179	0·0151	0·0212	0·0178									
Forbes et al. [33]	0·144	0·179	0·164	0·175	0·231	0·215	0·020	0·025	0·022									
Hess, Unger and Supplee [34]	0·138	0·165	—	0·158	0·190	—	0·005	0·009	—									
Hart, McCollum and Humphrey [35]	0·118	0·136	—				0·013	0·020	0·018	0·051	0·056	—	0·150	0·157	—	0·054	0·097	—
Katagama [36]	0·143	0·227	0·167	0·164	0·180	0·173												
	0·150	0·204	0·178	0·180	0·273	0·216												
Trunz [37]	0·168	0·222	0·191	0·153	0·296	0·225	0·0183	0·0253	—	0·036	0·050	0·043	0·148	0·174	0·164	0·074	0·100	0·085
	0·161	0·194	0·179	0·190	0·235	—	0·0195	0·0284	—	0·044	0·087	0·057	0·172	0·186	0·180	0·099	0·171	0·126
Meigs et al. [38]	0·122	0·160	0·140	0·204	0·230	—												
	0·174	0·203	0·185	0·179	0·224	0·199												
Sommer and Hart [39]	0·164	0·291	0·189	0·245	0·282	0·260												
	0·157	0·190	0·174	0·236	0·273	0·258												
	0·157	0·249	0·198	0·211	0·277	0·234												
	0·161	0·202	0·181	0·207	0·265	0·236												
Cranfield, Griffiths and Ling [40]	0·135	0·230	0·184	0·175	0·310	0·234												
Davies and Provan [41]	0·152	0·206	0·173	0·182	0·303	0·240												
Proks [42]	0·203	0·265	—															
Sommer [43]	0·125	0·291	—	0·155	0·273	—				0·070	0·090	—	0·175	0·190	—	0·100	0·120	—
Crichton [44]	0·120	0·212	0·166	0·166	0·291	0·229												
Sheehy [45]	—	—	—	0·162	0·236	0·205												
Golding et al. [11]	0·161	0·196	0·179	0·218	0·270	0·246												

chlorine-content of 0·242 per cent. If, as these authors suggest in their salt-combination table, potassium chloride is present to the extent of 0·085 per cent., the Na_2O-content wholly attached to chlorine would be of the order of 0·120 per cent., which would be considerably above the average values for normal milk. It is obvious that in samples of milk of high chlorine-content the content of sodium would be well above the average value indicated above (see " Salt Milk " below).

67. Variations in Soluble Calcium, Magnesium and Phosphorus

Table LXIII gives the range of variation and the mean values for soluble calcium, magnesium and phosphorus in milk. The

TABLE LXIII. *Variation in the Soluble Calcium, Magnesium and Phosphorus in Milk (Percentages of Total Constituents)*

	Calcium			No. of Determinations	Magnesium			No. of Determinations	Phosphorus			No. of Determinations
	Min.	Max.	Mean		Min.	Max.	Mean		Min.	Max.	Mean	
Van Slyke and Bosworth [20]	23·13	45·09	32·96	18	64·40	91·16	78·88	16	26·98	64·31	44·05	17
Gyorgy [22] .	—	—	15·62	1	—	—	—	—	39·8	53·1	44·95	4
									—	—	17·00	1
Mattick [11] .	24·18	30·64	26·84	11	—	—	—	—	28·70	38·38	32·94	11

number of investigations in this direction have been few, and workers have used different methods for the determinations, *e.g.*, dialysis and ultra-filtration. On the whole, even when deducing from the comparative work of any one investigator, the range of variation shown by all constituents is considerable, and so the proportion is a highly variable quantity.

68. Factors causing Variation in Composition of Mineral Matter

Factors which generally affect the composition of milk also affect the composition of the mineral matter. Thus breed and

TABLE LXIV. *CaO and P_2O_5 in Milk of Highly Bred and Primitive Races of Cows (Katagama)*

	CaO			P_2O_5		
	Min.	Max.	Mean	Min.	Max.	Mean
Highly bred . .	0·143	0·227	0·167	0·180	0·273	0·216
Primitive . .	0·150	0·204	0·178	0·153	0·296	0·225

individuality, period of lactation, yield, and different quarters of the udder are factors depending on the animal, whilst feeding, season of the year, time of milking and disease are external factors.

(a) BREED and INDIVIDUALITY. Soxhlet [47] has expressed the opinion that highly bred races of cows give milk low in calcium, which has, however, not been supported by the work of Katagama.[36] Table LXIV gives Katagama's figures.

Katagama gives the following average $CaO : P_2O_5$ ratios for milks from different Continental and other breeds (Table LXV).

It is obviously difficult to compute accurately the differences in

TABLE LXV. $CaO : P_2O_5$ *in Milk of Different Continental and other Breeds of Cattle* (*Averages*) (*Katagama*)

Breed	$CaO : P_2O_5$ 1 :	Breed	$CaO : P_2O_5$ 1 :
Red Silesian	1·42	Voigtlander	1·26
Dutch	1·42	African Cross	1·26
Scheinfeld	1·39	Simmenthaler	1·21
Corean	1·39	East Prussian Dutch	1·21
Schwyzer	1·34	Ceylon	1·18
Wilstermarsch	1·29	Redbrown East Friesian	1·15
Roumanian	1·28	Rumanian Cross	1·15
Pied East Friesian	1·28	Buffalo	1·02

mineral composition due to *individuality* alone since the other factors play their parts to varying extents. It has already been pointed out that the composition of milk varies to a greater extent among individual cows than do samples of bulked or herd milk, and the same principle holds as regards the composition of the mineral ingredients. Thus Forbes and Beigle [31] have shown that the milk of six cows of the same breed and in the same stage of lactation varied in (i.) ash, from 0·613 to 0·678, (ii.) calcium, from 0·074 to 0·098, (iii.) magnesium, from 0·008 to 0·013, (iv.) phosphorus, from 0·064 to 0·078, (v.) and chloride, from 0·073 to 0·127 per cent. The chloride-content of the milk of individual cows giving normal milk has been found to vary widely from cow to cow, but is fairly constant for one cow week by week in the absence of other factors that would upset the composition of the milk. The variation in chloride-content comes under a different class from that of Ca, Mg and P, owing to the fact that chlorides

are the salts mostly responsible for maintaining the isotonicity of milk. Such changes are not so marked in bulked milk.

(b) PERIOD OF LACTATION. Trunz [37] and Schrodt and Hansen [13] have given the composition of the ash of milk at different periods of lactation (Table LXVI).

TABLE LXVI. *Composition of Milk Ash at Different Periods of Lactation (Percentage of Whole Milk). Trunz*

Trunz	Cow I				Cow II			
	Colostrum	1/3	2/3	3/3 of period	Colostrum	1/3	2/3	3/3 of period
Ash . .	0·705	0·598	0·599	0·675	0·684	0·651	0·701	0·771
K_2O . .	0·174	0·168	0·165	0·148	0·176	0·186	0·186	0·172
Na_2O .	0·050	0·036	0·036	0·048	0·051	0·044	0·046	0·087
CaO . .	0·205	0·168	0·169	0·222	0·181	0·161	0·180	0·194
MgO .	0·025	0·019	0·017	0·025	0·028	0·020	0·020	0·025
Cl . .	0·089	0·076	0·074	0·100	0·101	0·099	0·131	0·171
P_2O_5 .	0·180	0·147	0·151	0·153	0·168	0·162	0·161	0·158
CaO/P_2O_5 .	1·14	1·14	1·12	1·45	1·08	1·00	1·12	1·23

Percentage of Ash. Schrodt and Hansen

	Colostrum, Day of Calving	Milk, 1 Day after Calving	Milk, 10 Days after Calving	Milk from Advanced Lactation
K_2O . .	17·40	18·17	24·12	20·61
Na_2O . .	10·10	11·94	8·72	16·15
CaO . .	22·99	26·69	22·69	20·97
MgO . .	6·88	3·07	2·92	2·75
Fe_2O_3 .	0·42	0·05	Trace	0·19
SO_3 .	2·82	3·94	4·10	3·74
P_2O_5 . .	34·30	23·87	30·73	22·18
Cl . .	6·85	16·01	8·30	17·63

From the above figures (Table LXVI) it may be observed that :

(i.) The ash is higher in colostrum and at the end of lactation than in the period of normal production ;

(ii.) Chloride increases markedly at the end of lactation ;

(iii.) Potassium is high a few days after calving and falls towards the end of lactation, whilst sodium follows the opposite rule and varies in the same way as the chloride ;

(iv.) Phosphoric acid is higher in colostrum than in normal milk ;

(v.) More calcium and magnesium are present in colostrum and towards the end of lactation ; and

(vi.) The ratio $CaO : P_2O_5$ increases as lactation advances.

In work by Forbes and Beigle [31] on the mineral metabolism of the milch cow, many data are given as to the variation in the mineral constituents of milk over a period of eleven weeks for six cows. Daily fluctuations in mineral composition were avoided by analysing composite samples from periods of twenty days. Calcium again showed an increase with advancing lactation, whilst magnesium was lower in the early period of lactation than later.

Meigs, Turner *et al.* [38] also give the data for the milk of two cows throughout their lactation periods. One cow showed very little fluctuation in the Ca and P contents of her milk, whilst the other showed the gradual rise in CaO as lactation advanced, with a constant P_2O_5-content.

Sommer and Hart,[39] in studying the composition of the mineral constituents of milk with respect to the differences in the temperature of heat-coagulation (manufacture of evaporated milk), have been led to conclude that CaO and MgO are higher at the beginning and the end of lactation than in the intermediate period, and that the inorganic phosphorus decreases and the ratio $CaO : P_2O_5$ increases towards the end of lactation (Table LXVII).

TABLE LXVII. *Variation in CaO- and P_2O_5-contents of Milk with Period of Lactation* (*Sommer and Hart*) (*Percentages of Whole Milk*)

Period	Cow A		Period	Cow B		Period	Cow C	
Days	CaO	P_2O_5	Days	CaO	P_2O_5	Days	CaO	P_2O_5
10–15	0·195	0·250	14–21	0·185	0·277	9–16	0·221	0·263
28–35	0·175	0·205	68–75	0·165	0·245	39–45	0·187	0·244
82–89	0·171	0 204	98–104	0·169	0 267	76–81	0·195	0·239
112–118	0·181	0·217	133–138	0·174	0·252	100–107	0·192	0·234
140–145	0·177	0·211	157–164	0·157	0·262	145–152	0·187	0·227
166–173	0·164	0·196	201–208	0·160	0·236	170–178	0·183	0·225
211–218	0·167	0·205	226–234	0·181	0·267	194–201	0·186	0·231
236–244	0·168	0·215	250–257	0·190	0·263	232–239	0·195	0·235
260–267	0·174	0·205	288–295	0·187	0·257	258–264	0·223	0·247
308–315	0·175	0·207	—	—	—	285–292	0·157	0·211
334–340	0·207	0·197	—	—	—	314–321	0·249	0·223
347–354	0·218	0·233						
374–281	0·291	0·273						

(*c*) INFLUENCE OF YIELD. Except for showing the inverse relationship between fat and yield (*e.g.*, Hammond and Hawk [48]) little work has been done on the variation of other constituents,

especially ash, with yield. Taylor and Husband [49] have found that the milk from an animal on a constant diet varies in its percentage content of constituents inversely as the volume, except that the lactose percentage varies directly with volume, this being apparent at the beginning and end of lactation. There is thus a higher chloride-content with low yield and a lower with high yield. Yield, nevertheless, is a production of four quarters of the udder, and it would be more accurate to correlate yield with composition on the product of one quarter only. The work of Mattick and Hallet [50] on the yield and composition of milk from the four quarters of the cow over a lactation period supports the principle formulated by Taylor and Husband so far as ash and casein-contents are concerned.

(d) Differences in Different Quarters of the Udder. Quite measurable differences occur in the mineral composition of milk from different quarters of the udder, although such differences are naturally not apparent in ordinary milk samples. Table LXVIII [42] shows figures for the maximum, minimum, and differences in calcium-content of the milk from four quarters of each of five cows.

TABLE LXVIII. *Calcium-content of Milk from Different Quarters of the Same Cow (Maxima and Minima) (Proks) (as CaO)*

Cow	Maximum		Minimum		Difference	
	% of Milk	% of Ash	% of Milk	% of Ash	% of Milk	% of Ash
A	0·187	25·57	0·185	24·84	0·002	0·73
B	0·189	24·88	0·176	22·06	0·013	2·82
C	0·173	24·46	0·161	23·10	0·012	1·36
D	0·159	24·70	0·153	22·87	0·006	1·83
E	0·153	21·82	0·145	20·72	0·008	1·10

Benton [51] concludes from her studies on the composition and physico-chemical properties of milk from each quarter that the four quarters are separate physiological units. Mattick and Hallet [50] have studied the properties of the milk from each quarter of one selected cow over a lactation-period. In each quarter, ash and casein were approximately correlative, and the yield inversely correlative to both. There were definite differences in the ash of the milk from each quarter, but that from the two front quarters agreed more closely in composition than that from the fore and hind quarters.

(e) INFLUENCE OF FEEDING. It is difficult to obtain evidence that feeding specifically affects the composition of the mineral constituents of milk, since the problem is complicated by natural daily fluctuations in the amounts of the constituents, lag in the response to a particular feeding-stuff, the effect of multiple factors and difficulties connected with the management of such experiments. Crichton [52] reviews the matter in detail and cites

TABLE LXIX. *Addition of $CaCO_3$ and $CaHPO_4$ to Ration. CaO- and P_2O_5-contents of Milk (Zaykowsky)*

Cow	Period	Extra Mineral Intake per Head per 10 Days		Milk Yield Kg	Ash %	CaO %	P_2O_5 %
		CaO g	P_2O_5 g				
A	1	—	—	113·4	0·711	0·148	0·189
	2	242·7	307·7	121·8	0·784	0·168	0·239
	3	—	—	107·6	0·751	0·149	0·208
	4	288·4	—	119·3	0·767	0·177	0·222
B	1	—	—	100·6	0·797	0·155	0·166
	2	288·4	—	106·0	0·805	0·177	0·184
	3	—	—	99·8	0·800	0·161	0·179
	4	242·7	307·7	103·5	0·882	0·173	0·215
C	1	—	—	107·6	0·747	0·145	0·165
	2	288·4	—	112·9	0·806	0·171	0·175
	3	—	—	110·5	0·768	0·165	0·170
	4	242·7	307·7	127·5	0·837	0·172	0·179
D	1	—	—	111·7	0·797	0·172	0·179
	2	242·7	307·7	120·3	0·876	0·190	0·199
	3	—	—	110·9	0·800	0·175	0·176
	4	288·4	—	118·3	0·920	0·180	0·188

various workers who have obtained negative results. Weiske [53] has found no effects in feeding experiments, and Von Wendt [54] has fed various salts with no results. Neumann [55] has fed 100 gm. of tricalcium phosphate per head per day and found a slight fall in milk calcium and phosphorus followed later by a small rise after three to four weeks. Hart, McCollum, and Humphrey,[56] from observations on one cow over a period of three and a half months, have found that wide variations in K, Mg, and P in the feeding do not influence their amounts in the milk, whilst Becker,

Eckles and Palmer [57] have observed that under conditions severe enough to cause osteomalacia, the Ca and P contents of the milk remains normal. But Zaykowsky,[58] experimenting with four cows, added $CaCO_3$ and $CaHPO_4$ to a basal ration (ten-day periods) and obtained increased amounts of CaO and P_2O_5 in the milk, wh'ch were, however, accompanied by a higher yield (Table LXIX).

Lauder and Fagan [59] fed calcium phosphate to cows and concluded that no interpretable increase in calcium and phosphorus occurred in the milk. They found a fluctuation in the P_2O_5-content of the milk of one cow, morning and evening, of from 0·23 to 0·29 (average 0·25) and a fluctuation of from 0·21 to 0·29 per cent. in the milk of the same cow over a period of two and half months, which demonstrates the difficulties of obtaining and interpreting a small increase due to feeding. Forbes and his co-workers,[60] feeding $CaCO_3$ and bone-meal, and Sommer and Hart,[61] feeding 100–200 gm. of $CaCO_3$ per head per day, found no measurable differences in the calcium-content of the milk or in its physical properties. The latter workers added cod-liver oil also, as a source of vitamin D to facilitate calcium absorption, but found no effect. They also investigated the effects of feeding grass and grass supplemented by bone-meal. No increase was observed on feeding bone-meal but there was a slight increase in phosphorus on grass.

No effect in mineral distribution and concentration was observed by Mattick and Wright [62] on feeding large quantities of $CaCl_2$, NH_4Cl, $NaHCO_3$ and Na_2HPO_4 to cows. They concluded that it was impossible by this means to alter milk composition, although the dehydrating influence of ammonium chloride suppressed milk yield, which was accompanied by a rise in the concentration of the milk constituents. Mattick [63] and Sheehy and Senior [64] give contradictory results of the effect of feeding cod-liver oil to cows. The former has found that the percentage of calcium is distinctly higher and that the rennet test takes a longer time. The experiment was not controlled and the rise in calcium comes within the range of normal fluctuations. The latter found the usual daily fluctuations in calcium and phosphorus but no measurable increase in either constituent through the feeding of cod-liver oil.

R. O. Davies and Provan [41] have investigated differences in the composition of the mineral constituents of milk from cows on winter rations and on pasture. Two groups of cows, one on a control and the other on a low-protein ration, were used, samples being taken just before, and again after a fortnight on grass. Out

TABLE LXX. *Calcium and Phosphorus Contents of Milk before and after Letting out to Grass* (*Davies and Provan*)

	CaO %		Total P_2O_5 %		Inorganic P_2O_5 %	
	Before	After	Before	After	Before	After
Expt. I.						
Group A.						
1 . .	0·161	0·162	0·215	0·214	0·138	0·138
2 . .	0·162	0·170	0·252	0·275	0·163	0·169
3 . .	0·172	0·177	0·267	0·285	0·177	0·186
Group B.						
1 . .	0·182	0·206	0·228	0·258	0·141	0·155
2 . .	0·199	0·201	0·287	0·303	0·190	0·200
3 . .	0·185	0·195	0·279	0·287	0·180	0·188
Expt. II.						
Group A.						
1 . .	0·175	0·182	0·251	0·258	0·168	0·167
2 . .	0·137	0·152	0·218	0·221	0·141	0·150
Group B.						
1 . .	0·180	0·192	0·220	0·241	0·147	0·166
2 . .	0·169	0·174	0·224	0·234	0·152	0·148
Expt. III.						
Group A.						
1 . .	0·167	0·164	0·182	0·192	0·123	0·129
2 . .	0·143	0·147	0·199	0·203	0·121	0·123
Group B.						
1 . .	0·170	0·182	0·217	0·231	0·134	0·142
2 . .	0·163	0·172	0·227	0·241	0·150	0·156

TABLE LXXI. *Composition of Milk as affected by Pasturage* (*Hess, Unger and Supplee*)

	Antiscorbutic-free Period %	Grazing Period %
Total solids . . .	11·62	11·8
Fat	3·37	3·44
Lactose	4·73	4·56
Ash	0·600	0·670
K_2O	0·150	0·157
Na_2O	0·051	0·056
CaO	0·138	0·165
MgO	0·009	0·005
P_2O_5	0·158	0·190
Cl	0·054	0·097

of fourteen animals, twelve gave an increase in lime and inorganic phosphate and thirteen showed an increase in total phosphate (Table LXX). The authors suggest that the increases are remarkable in that an increased yield of milk was given, and they give as factors conducive to such a result, (a) an improvement in the health of the animals through being left out at night, (b) the higher nutritive value of spring grass compared with winter rations, and (c) the specific effect of natural herbage on secretion. They also state that the magnitude of the change depends on the composition of the winter ration.

The beneficial effects of pasture have also been observed by Hess, Unger and Supplee.[34] The pasture period followed one in which the cows were fed for three weeks on an antiscorbutic-free diet. Considerable increases in calcium and phosphorus were observed (Table LXXI).

The higher chloride-content on grass is noteworthy. This is accompanied by higher values for soda and potash and a lower lactose-content. The poor quality of the milk as regards solids-not-fat is also worthy of note, and only a slight rise is experienced on going out to grass.

(f) SEASON OF THE YEAR. The results of Cranfield, Griffiths and Ling [40] indicate that the P_2O_5-content of milk is slightly influenced by the time of the year, showing a small rise in spring and falling again at the end of summer. Lime shows a steady fall in late spring and summer, reaching a minimum in August, after which a sharp rise occurs. The $CaO : P_2O_5$ ratio reaches its maximum in August. The stage of lactation, which has a considerable effect on the values, has not been taken into account. The results are contradictory to those of Davies and Provan,[65] who found a high rise in calcium on going out to grass.

Supplee [66] found that the ash of commercial milk used for the manufacture of dried milk (New York and Wisconsin) was at a maximum in late summer and lowest in April. Phosphorus is at a maximum in January and lowest in summer, while potassium varies in a reverse manner.

Experience on the Continent has shown that milk contains a greater amount of inorganic salts in winter than in summer, since phosphoric acids and sodium salts are at their maximum in winter. The calcium is also at its highest in winter and decreases gradually till the summer ; the Ca/P ratio, however, is higher in summer than in winter.

Milks of high titrable acidity (acid milk), soft-curd and heated milks differ in the proportion of soluble and insoluble calcium phosphate they contain ; these differences can be overcome by

the adjustment of Ca and P to the proper ratio. Acid milks are rich in Ca but low in P, whilst soft-curd milks are high in P but low in Ca.[99]

(g) THE INFLUENCE OF DISEASE. SALT MILKS. Diseases, especially those of the udder, cause profound changes in milk composition, and although knowledge of the changes in mineral composition in general is sparse, that on the chloride-content is fairly comprehensive, and many workers have investigated the mineral distribution in so-called " Salt Milks." Table LXXII gives the results of such analysis.

TABLE LXXII. *Analyses of " Salt Milk." Percentages of Ash*

	K_2O	Na_2O	CaO	MgO	P_2O_5	SO_3	Cl
Bogold and Stein.[67]							
Sound Milk .	20·59	13·02	21·55	2·72	26·42	3·66	15·58
Salt Milk 1 .	21·69	14·97	20·93	2·21	22·02	3·48	18·65
Salt Milk 2 .	10·96	33·17	11·70	2·16	15·63	6·73	25·23
Salt Milk 3 .	11·09	31·29	14·61	1·16	15·34	3·92	29·19
Hashimoto.[68]							
Salt Milk . .	8·94	36·54	7·44	1·74	17·38	1·34	33·63
Schrodt.[69]							
Drying Off .	8·52	45·85	8·04	1·82	9·70	5·68	24·35
Udder Catarrh .	10·56	24·92	16·17	2·70	24·56	1·56	24·52
Storch.[70]							
Tuberculous glands . .	5·08	42·37	7·52	—	8·76	—	44·64
Sound glands .	12·64	21·79	19·24	—	22·22	—	27·99

Conditions of disease generally lower the calcium, potassium and phosphorus contents and increase considerably the sodium and chloride contents. The same trend in composition is observed during the drying off of the udder, whilst the same change is observed for conditions of udder catarrh and tuberculous glands. Conditions of disease lower appreciably the solids-not-fat content of milk. Low solids-not-fat are almost invariably accompanied by high values for chloride. Koestler [71] has observed that abnormalities in secretion are accompanied by changes in composition which obey a general rule. Chloride, sodium and sulphate (as also albumin and globulin) increase, whilst calcium phosphate and potassium (also lactose) are diminished. The chloride-lactose (Koestler) number $\left(\dfrac{100 \times \text{Cl per cent.}}{\text{lactose per cent.}} \right)$ has been suggested as an index of abnormal milk. For normal cows it does not exceed 3·5, above which the milk can be considered abnormal (Davies [65]).

The chloride-content of milk compatible with a Koestler number of 3·5 is roughly 0·145 per cent., so that, according to Koestler, all milk having a chloride-content above this value may be considered abnormal. A wide range in chloride-content of milk may be met with. Most samples of milk of good quality rarely contain more than 0·100 per cent. chloride. The salty taste is perceptible at 0·120 per cent. and is easily detectable at 0·130 per cent. The lowered lactose-content probably enhances the salty taste at these figures. Cases have been met with of over 0·220 per cent. chloride.[46]

(*h*) INFLUENCE OF OTHER FACTORS. The calcium-content of the soil, although it may increase slightly the calcium-content of herbage, has been found by Wurster [72] to have no effect on the calcium-content of milk.

Proks [73] cites a case of salty milk given by a cow used also for land work. The milk was low in lactose and calcium, high in ash, phosphate, chloride and magnesium.

Gowen and Tobey [71] have observed that inanition causes a rise in ash and chlorides accompanied by a diminution of yield. They attribute this effect to a lowering of blood glucose since they could bring about the same result by lowering the blood glucose of well-fed cows with insulin or phlorizin.

Base Exchange Studies on Milk. Adjustment or the removal of calcium and phosphorus from milk by base exchange has been investigated by Lyman, Browne and Otting. Their object was to modify the curd tension of milk by decreasing the ionic calcium. The replacement of Ca by Na ions from a sodium zeolite decreases the titratable acidity, but this can be overcome by artificially increasing the acidity of the milk to 0·3 per cent. before treatment. When 22 per cent. of Ca and P are removed from the milk, the ability to clot with rennet is lost unless the milk is boiled, in which case better coagulation is obtained if mineral acids, instead of weak organic acids, have been initially used to increase the titratable acidity. The Na/K ratio of the milk is not disturbed if the zeolite is revived with a solution of chlorides of K and Na in the proper ratio ; reviving the zeolite with sodium hydroxide is necessary to remove phosphate. The ash of the treated milk is lowered even if mineral acid is used for acidifying, but is increased if lactic or citric acid be used.

69. Citric Acid and Citrates

Although citric acid is an organic compound, it will be treated in this chapter owing to its similarity in properties to the phosphate and its combination with calcium in milk.

Citric acid is a general but variable constituent in milk, and its study is accompanied by the disadvantage of the unreliable methods of its determination. Two main methods have been used for its determination, namely, (a) that depending on the isolation of its insoluble salts, and (b) that depending on its oxidation to acetone dicarboxylic acid or acetone. The calcium and barium salts are less soluble in hot than in cold aqueous media, and autoclaving at about 120° C. gives almost quantitative precipitation. In the heat-treatment of milk the precipitation of calcium and magnesium citrate and phosphate accompanies the phenomenon of heat-coagulation of milk.[75] Calcium citrate is

TABLE LXXIIA. *Citric Acid-Content of Milk (Percentages)*

	Method	Samples	Citric Acid		
			Max	Min	Average
Kunz	Pentabromacetone	5	0·198	0·155	0·177
Sommer and Hart	Denijes	16	0·248	0·191	0·231
Bosworth and Prucha[85]	Denijes	2	0·224	0·203	0·213
Hess[34]	Barium Salt	2	0·130	0·080	0·105
Supplee and Bellis[86]	Pentabromacetone	17	0·182	0·121	0·145
Sherwood & Hammer[79]	Denijes	335	0·330	0·070	0·180
Kieferle[87]	Pentabromacetone	104	0·400	0·200	0·270
Scheibe[88]	—	32	0·298	0·082	0·132
Steuart[89]	Both (Dried milk calculated to liquid milk)				0·150

insoluble in 50 per cent. alcohol, but the determination is complicated by the insolubility of other salts, *e.g.*, lactates.

Careful oxidation of citric acid in acid solution gives acetone dicarboxylic acid and acetone. Denijes[76] has found that on oxidation with permanganate in the presence of mercuric salts, a mercury-acetone-dicarboxylic-acid complex of constant composition is precipitated. The reaction is not quantitative owing to the instability of the dicarboxylic acid even at ordinary temperatures. This method has been perfected by Beau[77] and applied by Sommer and Hart,[78] Sherwood and Hammer[79] and Salant and Wise.[80]

If oxidation is carried out in the presence of bromine, pentabromacetone is formed,[81] which has the disadvantages of being a liquid of relatively high solubility and volatility. The quantitative conversion of citric acid to acetone is a matter of doubt in both these methods. Pratt[82] oxidises to acetone, which he estimated by distillation into Denijes' reagent, but Willoman[83] found

erratic results by this method. Lately Kometiani [84] has evolved an iodometric method for the determination of citric acid in milk. Lampitt and Rooke [95] have evolved an excellent method, based on the formation of pentabromacetone, for the determination of citric acid in milk products.

Table LXXIIA gives the citric acid-content of milk as found by different investigators.

The variation is from 0·07 to 0·40 per cent., with an average of 0·18 per cent. The cause of variation is due to differences in analytical methods, but the age of samples might also play a part since Bosworth and Prucha,[85] Van Slyke and Bosworth,[20] and Hammer [90] have found that various organisms present in milk ferment citric acid during the souring process. Scheibe and Hinkel,[91] who were the first to discover the presence of citric acid in milk, and Sommer and Hart [78] have confirmed its presence without doubt.

Variation of Citric-acid Content of Milk. Sherwood and Hammer [79] have found that the citric acid-content of milk is not affected by breed, period of lactation, or change to pasture grass. Considerable variations occur in successive milkings from individual cows, but it is evident that defects in the methods of determining this constituent do not justify too close an interpretation of the results.

Scheibe [86] finds that the citric acid of milk does not arise from that of the food, since feeding a high citrate-containing diet or starvation conditions do not vary the amount appearing in milk.

There is insufficient evidence to state conclusively that cows on pasture give milk with a higher citrate-content, although Hess, Unger and Supplee,[34] and Supplee and Bellis [86] have submitted data which tend to that conclusion. The latter workers also find great variations within a breed, and that variations due to the kind of food are inconclusive. The variation in the citrate-content of milk from the four quarters of the same cow has been shown to be less than that from one individual to another [87]; that is, milk samples secreted simultaneously agree fairly well in citrate-content.

Importance of Citric Acid in Milk. In the past the antiscorbutic factor in milk was considered to be associated with the citric acid-content, and the diminution in antiscorbutic value by heat-treatment of milk was held to be due to lowering of the citrate in solution.[92] The analogy was undoubtedly derived from the association of citric acid with high antiscorbutic potency of some fruit juices. It remains to be solved whether there is any connec-

tion between the presence of citric acid and of vitamin C (ascorbic acid) both in fruit juices and milk.

One of the functions of citric acid in milk is that of forming calcium and magnesium salts which are not highly ionised, the radical thus helping to maintain the salt-balance in milk. The technological importance of this fact enters into the manufacture of evaporated milk. During the sterilisation of the product (115° C. for several minutes) the evaporated product should coagulate into a tender jelly, which, on shaking, yields a smooth creamy liquid of sufficient viscosity to prevent fat-separation during storage. Milk varies in the readiness with which this coagulum is formed. Thus some samples require such a high temperature before forming the gel as to give a dark-coloured product, whilst others may give too thick a curd. Sommer and Hart [39] have found that the factor which controls the temperature of heat-coagulation is the balance of the calcium, magnesium, phosphate and citrate, and that the addition of small quantities of calcium prevents the coagulation of some samples at a certain temperature, whilst the addition of soluble phosphate and citrate prevents others. In general, it has been found that evaporated milk contains a slight excess of calcium, so that the correct balance has to be adjusted by the addition of sodium phosphate or bicarbonate (at the rate of from 4 to 10 oz. per 1,000 lb. of evaporated milk). Sommer and Binney [93] have found that the same factors influence the coagulation of milk in the alcohol test.

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CHAPTER X

THE ENZYMES OF MILK

70. The Enzymes Present in Milk

MILK is one of the natural secretions which contains a few of the enzymes of common occurrence. The work done on the enzymes of milk is in considerable confusion, and the literature contains much contradictory evidence; this is partly due to the lack of uniformity of methods and material and partly to the difficulty in differentiating between the enzymes native to milk and those of micro-organic origin. Colostrum is particularly rich in certain of these constituents, *e.g.*, amylase and catalase, and it is a matter of contention whether most of the enzymes present in normal milk have any function, or whether they are secreted only in traces during a normal flow in a similar manner to the globulin of milk. That they are present as rudimentary, non-functioning constituents seems to be supported by the fact that aseptically drawn milk shows little autolysis for a considerable period of time and does not show signs of rapid autolysis like blood or a yeast suspension. On the other hand, an enzyme-rich material, such as an avian egg with the embryo destroyed, very soon shows signs of autolysis. It is only in abnormal secretion, such as in colostrum, end-of-lactation milk, and milk from diseased udders, that the enzymes appear to be functional and are able to promote autolysis.

The synthetic nature of enzyme action also must not be neglected and it is quite possible that traces of the enzymes which help to build up the milk constituents in the mammary gland appear in the secretion. There is sufficient evidence of the presence in milk of enzymes capable of breaking down fat, casein and lactose.

It is important to note that the assay of enzymes in milk has mostly been carried out on substrates foreign to milk; this points to the fact that milk itself is a poor substrate although its pH comes approximately near to the optimum for many of the enzymes.

Table LXXIII gives the enzymes present in milk, together with some particulars of the substrates on which they are studied, and the range of pH in which they are active. The esterases are subdivided into true lipases, which act on neutral fat, mono-buty-

rinase, which is identified by its action on mono-butyrin, and phosphatases. An enzyme, salolase, which splits phenyl salicylate, has also been reported as occurring in milk.

TABLE LXXIII. *The Enzymes of Milk*

Enzyme	Substrate	pH Range	Thermal Inactivation Point, °C.
A. *Hydrolytic*			
(i.) *Esterases*			
Lipase [1, 6]	Butter, Milk ⎱		80°
Oleinase [2] .	Milk ⎰	5·5- 8·5 (8·0)	
Mono-butyrinase [3,4 5]	Mono-butyrin ⎰		
Lipase [7] . .	Milk ⎱		Below 62·5
Phosphatase [88, 89] .	Phosphoric esters	6–10 (9·0)	
Salolase . .			
(ii.) *Protease*			
Galactase [8, 1] .	Milk proteins	6·4–7·2	75–80°
(iii.) *Carbohydrate-hydrolysing*			
Lactase [9, 10] . .	Lactose	5–7·5 (6)	
Amylase [11, 12] .	Starch soln.	5·8–6·2 (5·9)	60 70°
B. *Dehydrogenases*			
Reductase or xanthine dehydrogenase, Schardinger enzyme	Xanthine,[11] methylene blue[15] and acetaldehyde ⎰	5·5–8·5	
C. *Enzymes acting on* H_2O_2			
(i.) Peroxidase [13] .	H_2O_2	4· 6	Above 70°
(ii.) Catalase [16] . .	H_2O_2 and peroxide	6 ·9 (7)	ca 65°

71. The Enzymes in Detail

(i.) LIPASE. (*a*) *Methods of Detection.* The requirements of a dependable method for the detection of lipase are that a natural fat be used as substrate, that the fat be well emulsified in aqueous suspension, and that a preservative which prevents micro-organic growth but does not inhibit lipolytic action, be used.

Rice and Markley [7] suggest the use of cream of 40–50 per cent. fat to which cane sugar has been added in sufficient amount to saturate the water (two of sugar to one of water). The cream is boiled to destroy its enzymes and dissolve the sugar. To the cool mixture the enzyme solution is added, and incubation at 38–40° C. is carried out for a considerable time, three to thirty days, according to the amount of enzyme present. Acidity is determined at the beginning and the end of the period by titration with 0·1 N alkali of 10 grams of the sample diluted to 50 ml. in presence of phenolphthalein. If the quantity of enzyme is considerable the smell of butyric acid is soon perceived. The titration-value is taken as the criterion of lipolytic activity. Control

experiments, using the same quantity of boiled enzyme-solution, should be carried out alongside the actual determinations.

· Other methods of a less refined nature than the above have consisted either of selecting a convenient ester or oil as a substrate and, after suspending in water, adding the enzyme-solution and titrating after a period of time. The esters of lower fatty acids have frequently been used owing to their higher solubility. Emulsifying agents such as bile salts or gum arabic have been used, but in some experiments only frequent shaking has been depended on. The titration of the fatty acids liberated in such cases may be in error owing to the solubility of the acids in the substrate, and titration in alcohol is recommended.

Methods, other than titration, of determining the liberated acids have consisted of : (a) using olive oil as substrate and determining the time necessary for sufficient acid to be produced to permit easy emulsification of the mixture in contact with sodium bicarbonate,[17] (b) determining colorimetrically by ferric chloride the salicylic acid liberated from its esters [18] ; (c) measuring surface-tension in cases where the products appreciably change the surface-tension of the solution [19] ; (d) determining the hydrogen-ion concentration [20] ; and (e) determining the electrical conductivity.[21] Marfan and Gillet [22] have used a 1 per cent. solution of monobutyrin as substrate and have measured lipolytic activity as the number of drops of 0·5 N sodium carbonate necessary to neutralise the butyric acid liberated in 10 ml. by 1 ml. of milk at 25° C. in twenty minutes. This method detected the activity not of a true lipase but of mono-butyrinase.

(b) *Evidence of the Presence of Lipase in Milk.* Rogers,[6] using fresh milk mixed with an equal volume of heated butter-fat, has found an increased acidity (0·9 ml. 0·1 N acid per 100 ml. of milk) over the control after nineteen days. Formaldehyde was used as antiseptic in the proportion of 1 to 1,200. Later, Rogers, Berg and Davis [23] prepared butter from fresh and heated (60°C.) cream. After forty-eight and ninety-two days at 23° C. the acidity of the butter from the unheated cream showed a small but distinct increase over that from the heated cream. Davies [24] has also observed significant increases in the acidity of butter made from unpasteurised sweet cream over that from similar cream which had been pasteurised. Rogers, Berg and Davis (*loc. cit.*) have also found that ethyl butyrate is hydrolysed appreciably by cow's milk, the action being weakened by heating at 66° C. and destroyed by heating at 80° C. This, of course, does not indicate the presence of a true lipase in cow's milk.

Rice and Markley,[7] using the sugar-cream method described

above, have proved definitely that there is a true lipase resembling pancreatic lipase in milk, and they are of the opinion that butyric rancidity of butter and acid rancidity of fats and oils are due to a true lipase. They have also found that rancidity in sweetened condensed milk results from the addition of a small amount of raw milk to the product, and that the condition is undoubtedly due to the lipase of the raw milk splitting the fat in the condensed milk.

Further evidence of the presence of a native lipase in milk has been offered by Csiszar,[90] Ramsey and Tracey,[91] and Hileman and Courtney.[92] The activity of the enzyme in frozen milk and its general fat-splitting properties, together with the fact that the rancid milk inhibits bacterial growth, have been advanced as evidence that the enzyme is not of bacterial origin.

The condensing of fresh milk in a vacuum pan usually gives a product which smells strongly of butyric acid within twenty-four hours, a condition which is not given by pre-pasteurised condensed milk.

Negative findings of lipase in cow's milk have been reported by Palmer,[25] Vandervelde,[26] and Thatcher and Dahlberg.[27]

The presence of lipase in human milk has been extensively investigated. Hippius [28] and Moro [29] have found a true lipase present in amounts well above that in cow's milk, whilst Resch [30] has found lipase present at all stages in lactation.

(c) *Butyrinases*. *Mono-butyrinase*. As has been stated above, the ability to split synthetic or simple esters such as alkyl butyrates, or mono-butyrin, is evidence of the presence only of the specific butyrinases and not of true lipases. Much of the early work on the enzymes of milk from various species has been carried out on such substrates, and the existence of such enzymes in milk has been effectively proved.

The experiments of Rogers, Berg and Davis [23] prove that ethyl butyrate is appreciably hydrolysed in milk, whilst Marfan,[5] Gillet [4] and Grimmer,[3] amongst others, have established the presence of a mono-butyrinase, the last-named isolating the enzyme from the milk glands of the cow.

The presence of these enzymes in human milk has also been proved.

(d) *Conditions under which Lipolytic Activity Occurs in Dairy Products*. In the handling of milk and dairy products under manufacturing conditions, lipolytic activity becomes evident in the following cases :

(i.) **In butter from unpasteurised sweet cream kept at ordinary temperatures.**

(ii.) In cream ripened under " cold dairy " conditions. This occurrence is prevalent in winter when the cream is stored below 10° C. The ripening organisms do not form the desired acidity necessary to check lipase-action and lipolysis becomes evident as butyric rancidity in the butter. The acidity in ripened cream prevents enzymic lipolysis even in unpasteurised cream. The method of control consists of storage at higher temperatures or the use of a " starter " to ripen pasteurised cream.

(iii.) During the concentration of unpasteurised milk (or cream), whether sweetened or unsweetened. The milk used commercially is always pasteurised to lower the bacterial count before condensation, but in the concentration of fresh milk of low count, say for nutrition experiments, considerable lipolysis occurs. The temperature of concentration is favourable for lipase-action, whilst the action of bacteria is checked by the increasing concentration of solutes in the medium.

The inhibition of lipolysis occurs in the presence of high acidity, or by the destruction of the enzyme by heat, or its inactivation by poisons, such as traces of heavy metal salts. The inactivation of lipases by traces of heavy metals is well known.[32, 24] In dairy products this inactivation is parallel to the power of the metal to catalyse autoxidation, the most potent activator, copper, almost completely inhibiting lipolysis, whilst the metals of the iron group, chromium and manganese, have a lower inhibiting effect ; tin and aluminium have no effect. The hypothesis of lipase being broken down first under the influence of traces of metals in butter and thus acting as a natural inhibitor to oxidation has been advanced.[24]

Heating for 20 minutes at 63° C. kills the enzyme, whilst concentrations of 0·2 per cent. of fluoride and 0·1 per cent. hydrogen peroxide are toxic. Homogenisation of milk activates the enzyme. There is an antagonism between lipase and the factors which cause fat oxidation in milk. The activity of the enzyme is seasonal, being most active in midwinter and least in June-July. The factors governing the secretion of lipase in milk are connected with the lactation cycle, the amount generally increasing with advanced lactation and abnormality of the udder.[92]

There are also some micro-organisms which have definite lipolytic effects on butter-fat. *Oidium* and *Oospora spp.* are capable of using glycerol as their source of carbon, and thus possess a high lipase-content. Their effect on butter is to liberate much oleic acid, thus making the butter appear oily. The autoxidation of

this product may then give rise to " fishiness " and, later, to " tallowiness."

(e) *Phosphatase*. There are indications of a phosphatase being present in milk.[88, 89] The range of acid-soluble ester phosphorus varies between 7 to 20·8 mg. per 100 ml. of milk ; by the agency of the enzyme it is converted into inorganic phosphorus. The enzyme has a *p*H range of from 6–10, with the optimum at
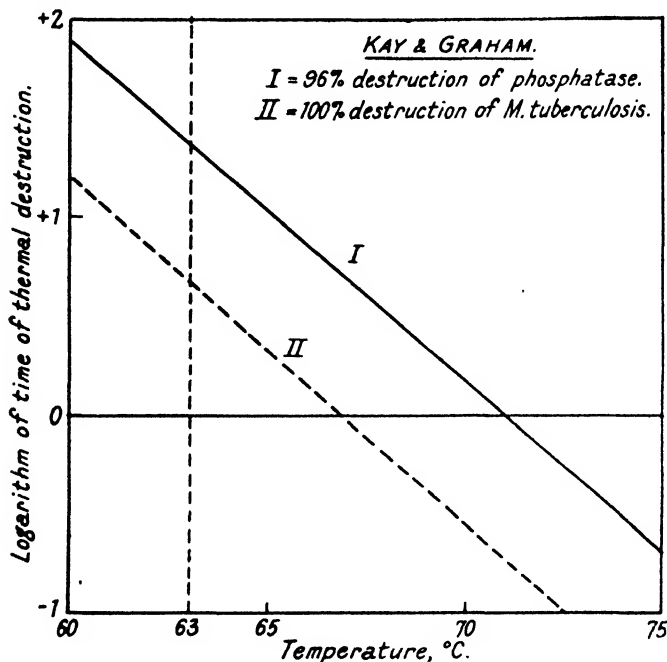
FIG. 12.—Thermal destruction of milk-phosphatase at temperatures near pasteurisation showing that *M. tuberculosis* is completely destroyed somewhat before destruction of 96 per cent. of the phosphatase (63° C., the minimum temperature of official pasteurisation).

*p*H 9, thus falling into line with the other phosphatases. The pasteurisation of milk by the holding method completely destroys the enzyme ; this has been developed as a test for holder pasteurised milk (see Fig. 12). (See Section 145 also.)

Salolase. The presence of an enzyme in milk capable of splitting phenyl salicylate to phenol and salicylic acid has been reported by Vandevelde,[10] but Grimmer [3] was not able to detect it in extracts from the glands of cows, although he was successful in obtaining it from extracts of the mammary glands of sheep, pigs, goats and mares. Its presence in human milk is agreed

upon by all workers. There is a little doubt whether the effect is due to enzyme action,[29] and the reaction of the medium plays an important part in favouring the reaction. Kullmann [45] could not detect its presence in sterile cow's milk, and concluded that the reaction, when found, was due to bacterial action.

Milk contains no salol and the function of the enzyme in milk is obscure.

72. Proteases

Babcock and Russell [8] named the normal proteolytic enzyme of milk *galactase*. This enzyme brings about the slow decomposition of milk-proteins into peptones and simpler degradation-products. Babcock [33] states that it plays an important part in cheese-ripening. Thatcher and Dahlberg,[1] using improved methods, have shown the presence of a protein-splitting enzyme in normal milk. They found that, in the separation of milk, the factors which increased the percentage of casein in the total nitrogen also increased the galactase-content. The ripening of cream did not increase the rate of proteolysis, so that more acid conditions did not favour the activity of the enzyme. Galactase is probably tryptic in nature, and its ability to break down proteins below the peptone-stage shows the presence of a probable peptidase in association with it. The enzyme is not of bacterial origin.

In butter the galactase-content depends on the curd-content, the distribution of the enzyme running parallel with the nitrogen-content of the milk fractions. Proteolysis is inhibited by 1 per cent. chloroform and 15 per cent. sodium chloride. Therefore galactase is inhibited in salt butter. Low temperature also inhibits proteolysis, so that the enzyme plays no part in the deteriorative changes of butter during storage.

Galactase is most active in the pH range 6·4–7·2 at temperatures of 37–42° C. In that pH range it is destroyed at 75–80° C.· but under more acid conditions at lower temperatures (72° C.).

Certain peptidases in milk are of bacterial origin.[40]

73. Carbohydrate-hydrolysing Enzymes

(1) LACTASE. Vandevelde [10] and Stoklasa [9] have proved the presence of a lactose-hydrolysing enzyme in milk. The products, glucose and galactose, must be formed before subsequent alcoholic fermentation can occur. The lactase activity is so low as to be hardly detectable in cow's milk, and it is doubtful whether the lactose of mare's milk (used for the manufacture of koumiss) is directly fermentable by a zymase, or whether a high lactase-content exists in such milk.

The action of lactase on lactose solutions was studied by Armstrong,[34] who found that the reaction is one of the second order, but that the mass constant shows a steadily falling value instead of a rising value as is found in other hydration processes. In the further interpretation of Armstrong's figures, Eyre and Davis [35] found that the course of the hydrolysis of lactose follows four successive parabolas (time against per cent. hydrolysis), as if four fractions of the enzyme were working, or that the course of hydration was governed by four impulses each of which showed its portion of the reaction curve.

(2) DIASTASE (Amylase). Although there is no starch in milk, amylase is present probably as a result of infiltration from the blood, which contains the enzyme in considerable quantity. The mammary gland is another source of this enzyme, since Grimmer,[3] in his experiments on gland tissue, obtained evidence of its presence even in species where little or no amylase could be detected in the milk.

Although Spolverini[31] was unable to detect the enzyme in cow's milk, Zaitschek [36] found evidence of amylolytic activity in all the samples of milk he examined. He used the method whereby the increase in reducing sugar was weighed (maltose). Koning [37] found that, although certain bacteria were capable of producing amylase, milk contained the native enzyme   He found that from 0·015–0·02 gm. of starch could be decomposed by 100 ml. of milk in thirty minutes, any increase in strength above this being regarded by him as abnormal or pathological. The first and middle milks were richer in amylase than the strippings. Heating milk for forty-five minutes at 68° C. destroyed the activity. Lane-Claypon,[38] working on aseptically drawn milk, found that 10 ml. of milk were able to hydrolyse from 1–2 mgm. of starch in three hours at 37° C.

Chrzascz and Goralowna [12] report a higher amylase-content in milk from diseased udders and in colostrum up to the fourth day. Giffhorn [39] has proposed the use of the amylase-content of milk along with the catalase-content for determining the quality of milk and for detecting milk from diseased udders. The amylase-content of old milk is low.

Amylase has an optimum temperature of 30° C. and an optimum pH of 5·8–6·2. One hundred ml. of normal milk will hydrolyse (dextrinise) 50–100 mg. of soluble starch in one hour at 30° C. Cream contains more amylase than skim milk. The quantity of amylase varies in the milk of individual cows. Only a small amount is found in whey, most of the enzyme being precipitated with the casein.

Amylase in milk is inactivated by heating at 60°-65° C. for one hour, and in colostrum at 65°-70° C.[40]   The destruction of amylase by heating above 60° C. has been used as the basis of a test for detecting insufficiently pasteurised milk.[41, 63]   The lead serum of the milk to be tested is mixed with starch solution and incubated for four hours at 37·5° C.   On adding a solution of 0·001 N iodine in potassium iodide the appearance of a strong *blue* colour indicates heating above 60° C., *red* or *orange*, heating below pasteurisation-temperature, insufficient holding, or the presence of appreciable amounts of raw or poorly pasteurised milk ; a *yellow* colour indicates raw milk or milk not heated above 50° C.

Kluge,[93] working on the lead serum of milk, has found that raw milk has a mean diastatic value of 1·312 (gm. of starch hydrolysed by 100 ml. of milk in 3 hours at 39° C., at pH 6·9).   The activity is reduced to 0·075 by momentarily heating the milk to 75° C. and completely destroyed by boiling.   The enzyme system is bound to albumin or globulin.

Amylase in Milk of Other Species.   The presence of amylase in human milk appears to be universally recognised, the amount being greater than that in cow's milk.[28, 29, 36]   Lagane [42] found the amylase-content of human milk to be greatly increased by the addition of hydrogen peroxide, and suggested that the increased activity was not due to a direct action of the amylase but to a side-action of the peroxidase also present in milk.   The starch was partly converted to maltose in such cases.   No such action was detectable in either cow's or goat's milk.

Schenk [43] found a considerable amount of amylase in human milk but none in the colostrum.   The milk of the cow, horse, dog, cat and the guinea-pig contained only traces, whereas neither the colostrum nor the milk of the goat contained amylase.   The markedly higher amount in the colostrum of many species of animals pointed to the enzyme originating in the colostrum. Schlack and Scharfnagel [44] found that human and occasionally mare's milk contained the most amylase.

74. Dehydrogenases

The " reductases " are enzymes capable of promoting the reduction of a given substance, and their action is easily detected by using substances that change colour as a result of the reduction. The enzyme capable of reducing methylene blue alone is a " *direct reductase* " (M.B.), but if the reduction is brought about by the agency of a reducing agent, *e.g.*, an aldehyde (formalin), the enzyme is known as an " *indirect reductase* " (F.M.B).   Other

names for the latter are aldehyde-catalase, aldehyde-reductase, formaldehydase, redukase, and the Schardinger ferment.[46]

That milk possesses a reducing power was first demonstrated by Vaudin,[51] using indigo, by Neisser and Wechsberg,[52] using methylene blue, and by Wynter Blyth,[53] using litmus. Since that time many papers dealing with various aspects of the question have been published, and much controversy has further complicated the work. It may be said that, even at the present time, the question of the nature of the enzymes is not agreed upon, e.g., the identity of the aldehyde- and the xanthine-dehydrogenase.

The Schardinger Enzyme. Schardinger [46] showed that when milk was fresh it did not reduce methylene blue except in the presence of formalin. (His solution—Schardinger reagent—contained 5 ml. of a saturated solution of M.B., 5 ml. of formalin, 190 ml. of water. He concluded that either (*a*) an aldehyde substance was necessary for the reduction of the methylene blue, this being as a rule formed gradually in milk by bacterial action and replaced in fresh milk by formaldehyde or other similar substance, or (*b*) that the reaction was due to the " living protoplasm " of the bacteria. Cathcart and Hahn,[54] along with Schardinger, favoured the latter view, for they also found that many bacteria had the power to reduce methylene blue, the property probably being attached to the cell protoplasm.

Later it was shown by many investigators that Schardinger's reaction was due to an enzyme, whilst the reduction of the M.B. alone was due to bacteria. Thus Smidt [55] showed that the three factors which could cause reduction of M.B. were lactose, enzymes, and bacteria. He found that fresh milk did not bleach M.B. alone, but did bleach F.M.B., and that the reaction was weakened by heating to 70° C. and destroyed by heating to 75° C. for twenty minutes. In the reduction of M.B. the rate was in direct relation to the number of bacteria present. Alkaline lactose at 45°–50° C. reduced M.B. in a few minutes, and boiling milk was also found to cause reduction.

Romer and Sames [56] showed that when milk had ceased to give the Schardinger reaction it could recover its power by the addition of 1 per cent. ferrous sulphate, but not if the ferrous sulphate solution had been previously boiled. Numerous observers had found that goat's milk did not contain an enzyme which promoted the reduction of F.M.B., but Wedemann [57] discovered that it could give the reaction if made alkaline to litmus or if ferrous sulphate were added.

Jensen [58] found that the Schardinger enzyme was concentrated in the strippings and that the time of reduction varied inversely

as the fat-content of the various samples drawn from the udder. The enzyme is not attached exclusively to the fat globules, since some action is obtained in skim milk. Sassenhagen [59] found that the F.M.B. reaction was not given by colostrum, but was given by the centrifuged cream of a later colostral period.

In these observations, which have been made on milk of low bacterial count, it is unknown to what extent the fat influences the rate of reduction of methylene blue. The fat globules present an enormous surface and when the system is activated, as by direct sunlight, the fat can easily account for quick bleaching of methylene blue. Aikins and Fay [60] have found that methylene blue accentuates the change in potential to the negative side when milk is exposed to sunlight, and that the fat hastens the time of reduction, since the zone of reduction is elevated. Variations of the dissolved oxygen content may also be the reason for different interpretations.

Xanthine Oxidase. Morgan, Stewart and Hopkins [61] demonstrated the presence in milk of an enzyme capable of oxidising the purine bases, xanthine and hypoxanthine, to uric acid and of using various oxidising agents to bring about the reaction. Dixon and Thurlow [62] obtained from milk a stable water-soluble casein preparation containing the enzyme in a form suitable for investigation. The enzyme was adsorbed only slightly by animal charcoal but completely by alumina and filter-paper, while still retaining its activity. The enzyme is concentrated on the surface of fat globules. Toyama [94] concentrated the enzyme by extracting the fat of a high-fat cream with ether and evaporating the residue *in vacuo.* The preparation had a low protein-content (5·1 per cent. N) and a high iron-content (33·5 p.p.m.). An albumin-like fraction of globulin is associated with the enzyme. Fat, in a finely divided state, as in milk, greatly increased the oxidation of the purine bases by the enzyme. They found that the range of the enzyme was from pH 5·5–9, and that the enzyme was destroyed at pH's below 4 and above 9. The reaction velocity was found proportional to the enzyme-concentration and independent of the methylene-blue concentration (Thunberg technique). Hypoxanthine reduced methylene blue twice as fast as xanthine, but on inhibition commencing (due to uric acid formed) the rates approached one another until inhibition was complete, after which the reaction velocity depended on the concentration of methylene blue. The enzyme also reduces nitrates to nitrites.

Differentiation of the Xanthine- and Aldehyde-oxidases. Wieland and Macrae [64] have found that the concentration of xanthine-

oxidase is low in fresh milk but increases on keeping or on agitation, probably by the union of fat-droplets on which the enzyme is adsorbed. They found that the xanthine-oxidase and the aldehyde-oxidase (Schardinger's enzyme) are separate enzymes, since the xanthine oxidase can be removed from whey by adsorption on calcium chloride leaving the aldehyde-oxidase in solution in a less active form.

Later, Wieland and Mitchell [65] found that if more xanthine than was necessary for the hydrogenation of methylene blue, and a large excess of aldehyde were used, all the dye is consumed in oxidising the purine. The addition of aldehyde increases the time for decolorisation and decreases the number of enzyme units. With less xanthine than the equivalent of methylene blue, all the base is changed to uric acid and decoloration is slower than in the presence of aldehyde. With p-benzoquinone the enzymic dehydrogenation of aldehyde occurs more rapidly without than with methylene blue, the reaction, however, being soon retarded owing to the destruction of the enzyme. The reaction on the xanthine (or hypoxanthine) is slower than with the aldehyde, but the rate is greater with a mixture of xanthine and aldehyde than with each constituent alone and more marked as the concentration of aldehyde diminishes. These results are taken to indicate that xanthine-oxidase and aldehyde-oxidase are different enzymes.

Inactivation of the Xanthine-oxidase. Para-benzoquinone has a gradual inactivating effect on xanthine-oxidase. Methylene blue increases the rate of oxygen uptake of a hypoxanthine-enzyme mixture, but p-benzoquinone has a strong retarding effect. Heavy metal salts (Cu, Ag, Hg, Pb, Fe, Zn, As_2O_3) retard the oxidation of xanthine in the presence of methylene blue, but Cr, Ni, Co, Mn, Cd, Tl have no effect. The retardation due to Hg, Ag and $HAuCl_4$ is inhibited by KCN if present in sufficient amount to form complex cyanides, but no effect is observed with Fe, Pb and Zn salts. Preliminary treatment of the enzyme with hydrogen sulphide causes a 50 per cent. loss of activity to xanthine plus methylene blue.[65] KCN alone has the same order of effect as it has on the aldehyde-dehydrogenase reaction or on dismutation.[64]

The enzyme is inactivated by alcohol, acetone, sunlight and ultra-violet light, but its activity is not inhibited by ether, chloroform, toluene and glycerol.

Reductases in Milk from Other Species. Human milk has been tested for a reducing enzyme by Gillet,[4] using the nitrate technique, but none was found. Other workers [37, 59, 66] have used Schardinger's reagent with the same negative result.

Goat's milk contains no enzyme capable of bleaching Schardinger's reagent.[57]

75. Enzymes Acting on Hydrogen Peroxide

These consist of (*a*) the enzyme capable of liberating active oxygen from hydrogen peroxide and organic peroxides, *peroxidase*, and (*b*) that which breaks down hydrogen peroxide into molecular oxygen and water, *catalase*. Both enzymes are found in milk.

These two classes of enzymes are functionally different, irrespective of their common substrate, and are indeed in many respects antagonistic. Peroxidase is directly concerned with the metabolic welfare of the organism containing it, the enzyme participating in the respiratory and other energy processes of the living cell. The enzyme is naturally required for the breaking-down of intermediate oxidation-compounds of long carbon chains, such as the fatty acids, and its action may be simulated by, or it may take part in, the catalytic oxidative effects of traces of heavy metals.

Catalase, on the other hand, appears to be a safety factor in the growth of organisms, preventing too high or even completely inhibiting a concentration of peroxide by transforming the potentially active oxygen of a peroxide to molecular oxygen. Hydrogen peroxide may thus be considered toxic to organisms having no catalase, but non-toxic to catalase-producing organisms.

The bearing of these facts on milk technology rests on the low, if any, catalase-content of the lactic-acid bacteria, as against the high catalase-activity of the proteolytes. The latter can thus resist the conditions of relatively high active oxygen concentration which exist in milk contaminated with traces of heavy metal salts, such as those of copper. The growth of the lactic acid bacteria is inhibited, with the result that quite a different distribution of organisms exists in the milk under such conditions. It is evident, therefore, that in the presence of copper there is no rapid sweeping up of dissolved oxygen, and local oxygen-activation can be utilised in the direction of chemical oxidation of the fat and thus cause the development of an oxidised or " tallowy " flavour.

(*a*) PEROXIDASE. Grimmer,[3] Koning,[11] Harden and Lane,[47] and Violle [48] have shown that peroxidase is an enzyme native to milk. Violle found that milk from diseased glands contains more peroxidase than normal milk. Blood contains much peroxidase ; its presence in milk is probably due to infiltration, and the amount increases with the increased infiltration of other blood constituents, such as globulin and salt, into the milk. Separator slime contains the greatest concentration of peroxidase of all milk fractions.

The guaiacum-tincture method was originally used for the detection of peroxidase but has largely been replaced by Storch's test,[49] in which *para*-phenylenediamine is used. In this test a grey-blue colour is produced in *raw* milk after the addition of a few drops of a 1 per cent. solution of the hydrochloride of the base and dilute hydrogen peroxide solution. Benzidine and ortol may also be used.

The mechanism of the peroxidase reaction has been much disputed. It is held that the reaction of the milk is important and that alkalinity is necessary to give the reaction. It has also been

SPITZER & TAYLOR.
I = 72·4°
II = 71·0°
III = 70·8°
IV = 69·9°
V = 69·6°
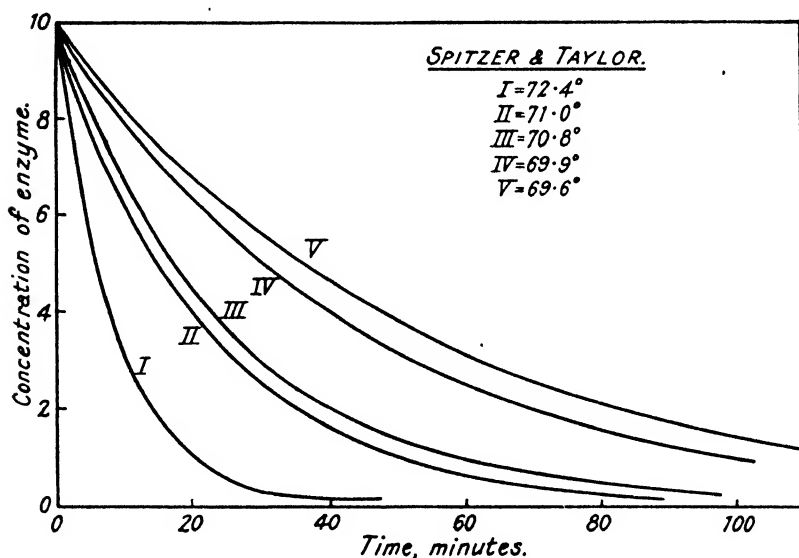
FIG. 13.—The rate of destruction of peroxidase on heating.

thought that iron is necessary to bring about the reaction. As has been stated above, traces of heavy metals can form a peroxide-peroxidase system which can easily bring about the reaction, and the presence of contaminating copper in milk can be detected by carrying out the peroxidase test on the cooled boiled milk, when a slow positive reaction will be given.

Zilva [13] states that heating milk to 70° C. has too slight an effect on peroxidase to be detectable by Storch's test, and therefore the test cannot be used to detect pasteurisation. But Koning has found that it is not active in milk heated to 72° C. for thirty minutes. Zilva has found that small amounts of alkali accelerate and that small amounts of acid retard the inactivation of the

enzyme. The range of temperature of inactivation, according to various authors (see Lane-Claypon,[38] p. 85), extends from 70° to 83° C., the lowest temperature being for a period of one hour's heating.

Peroxidase in Milk from Other Species. There is conflicting evidence as to the presence of peroxidase in human milk. Some observers have failed to detect the enzyme, others have obtained varying results, and it seems reasonable to suppose that when present it occurs only in very small quantities. The enzyme is present in colostrum, but when the glands are secreting normally it appears to be absent. Peroxidase has no effect on the progress of the infant.

Nursing rabbits on a diet deficient in vitamin B have been found to yield no peroxidase in their milk, but the addition of vitamin B to the ration immediately causes the peroxidase to return.[50]

Preparation and Properties of Milk Peroxidase. Elliot [67] describes a method for the preparation of milk peroxidase by fractional precipitation with ammonium sulphate. Too strong a solution of ammonium sulphate will cause the enzyme to be precipitated with the casein, whilst too weak a solution will cause contamination with catalase. The amount of salt to be added is determined beforehand on small quantities of milk to find the greatest amount of salt which can be added without diminishing the peroxidase activity of the filtrate. Further addition of solid ammonium sulphate to the filtrate completely precipitates the enzyme, which can be isolated in a dry form in the yield of 2·5 g. per litre of milk.

Milk contains peroxidase, according to the standard of Willstätter and Stoll,[68] to the extent of roughly 20–25 units per litre (or milk has a purpurogallin number of o·o20–o·o25).

The enzyme is active over a wide range of hydrogen-ion concentration. At pH 10, the enzyme is completely and irreversibly destroyed, whilst at pH below this, although the activity diminishes, neutralisation quickly enables the enzyme to regain its activity. On the acid side, a precipitate forms at pH 3·8–4·2, which dissolves at pH 3·2–3·6 with complete loss of activity, but neutralisation and standing cause partial recovery of activity. Milk peroxidase is more sensitive to conditions of acidity than plant peroxidases (*e.g.*, horse-radish peroxidase).

Some coloured hæmatin body is associated with the enzymic activity ; the nature of this pigment has not yet been studied.

Traces of H_2S inhibit peroxidase activity, as do other sulphydryl compounds. Solutions of cysteine, reduced glutathione, and denatured albumins interfere with the formation of blue colour

with H_2O_2 and benzidine or guaiacum, and slow down the action with p-phenylenediamine. The addition of boiled milk to fresh milk will therefore cause some interference with the colour test for peroxidase. The effect of sulphydryl groups is not due to inhibition but to a reducing action on the coloured products. Purpurogallin is not affected by them.

Nitrites are quantitatively oxidised by peroxidase and hydrogen peroxide. Tyrosine and tryptophane give coloured products; no oxidation of any of the following products by peroxidase-H_2O_2 is found to occur : formate, acetate, oleate, stearate, triolein, ethyl alcohol, glucose, glycerol, acetaldehyde, lactate, glycine, phenylalanine, histidine.

(b) CATALASE. This enzyme possesses the property of decomposing hydrogen peroxide into molecular oxygen and water (cf. the catalytic effect of nickel salts on the same reaction). Bach and Chodat [69] and Neumann-Wender [70] have shown that this catalytic activity is separate from the other enzyme actions.

Raudnitz [71] has demonstrated the presence of catalase in cow's milk, and a mass of confirmatory data has subsequently accumulated. Grimmer,[3] Harden and Lane [47] and others have shown it to be a secreted enzyme.

Chick has studied the process of Budde—*buddisation*—whereby milk is preserved with hydrogen peroxide. She has found that with large amounts of H_2O_2 the catalase activity is lost, and concurrently with this the milk becomes sterile, whilst milk rendered sterile by boiling and mixed with a small amount of raw milk recovers slowly its catalase activity.

Catalase is determined by measuring the volume of oxygen obtained by the splitting of added hydrogen peroxide under special conditions. Various forms of apparatus have been devised and used, but for investigations on milk Lobeck's apparatus [72] has been favoured. Zaykorskii and Alexseev [73] have suggested a titration method in which 5 ml. of 0·3 per cent. H_2O_2 are added to 2 ml. of milk diluted to 100 ml. and the excess peroxide is titrated after standing at room-temperature for thirty minutes. A control determination is made on 2 ml. of the same milk boiled. A catalase index representing the number of mgm. of oxygen liberated by 100 ml. of milk is a convenient method of expressing the results, that for normal milk being under 20, whilst values over 20 suggest abnormality of secretion.

Anderson and McWalter [95] have investigated the methods of determining catalase in milk, namely, the gasometric and the volumetric methods of estimating undecomposed peroxide. The methods are at best only empirical since the substrate gradually

destroys the enzyme. They describe a modified iodometric test for determining the amount of undecomposed peroxide, in which conditions have been chosen permitting the reaction to approach completion in a reasonable time with maximum destruction of substrate.

Distribution. The amount of catalase varies with the breed of cow [74] and with the fraction of the milking, the first portion being poor and the last portion rich ; milk containing a high percentage of fat does not necessarily contain more catalase. Reid [75] states that the catalase-content is high seven days before calving ; it increases in the colostrum and remains high for fourteen days, after which it decreases, and then at the drying-off period rises again to a high level.

Cream contains a higher amount than milk and separator slime contains still more. Bacteria and leucocytes increase greatly the catalase-content of milk, and the catalase-content has been suggested as the test for milk quality and the detection of milk from diseased udders.[58] The ease of its determination commends it for this purpose, but Reid is of the opinion that variation in catalase-activity does not justify the catalase test being used for the detection of udder abnormality, whilst the further complication of the problem by variation in the amount of catalase from bacterial sources is apparent. The titration method gives clear-cut differentiation between normal milk samples and those containing over 0·160 per cent. of chlorine, but the border-line cases are doubtful. In the detection of milk from inflammatory udders the leucocyte-content is the most reliable (95 per cent.), the catalase index is less reliable (80–85 per cent.), and the chloride figure still less reliable (75 per cent.).

Properties. Catalase is precipitated from milk with the casein, but, in the precipitation with half-saturation ammonium sulphate, tends to appear in small amounts in the filtrate and in greater amount with a lower degree of saturation.

Heating gradually inactivates catalase, temperature having a greater effect than the time of heating. During heating, acidity has a preserving effect. Complete inactivation is effected by heating at 90–92° C. for twenty to thirty minutes.[76]

Partial reactivation of catalase occurs in pasteurised milk when it stands at slightly elevated temperature under non-sterile conditions.

Other investigators give lower inactivation temperatures.

The optimum pH is 7 and slight inhibition is experienced under acid conditions. Shaking does not increase the catalase value although adsorption on the fat-globule surface is certain.[77]

Catalase has not been found uniformly present in human milk and increases have been associated with colostral characteristics and strippings from the gland. It has been suggested that the health of the mother affects the catalase-content, being least when in good health, although the amount of enzyme present does not affect the nutritional well-being of the infant.

76. Changes in Enzyme-content in Abnormal Conditions

Some of the enzymes normally present in milk as a result of infiltration from the blood are increased in amount under conditions connected with an abnormal state of the gland and possibly also of the cow.

Such conditions arise towards the end of lactation or when the milk approximates in character to blood serum, or in conditions of inflammation of the udder or mastitis. Koestler[78] has suggested that a high catalase-content in fresh milk denotes illness of the cow. Sassenhagen[59] has shown that a more powerful action on Schardinger's reagent is given by milk from an inflamed udder.

Vollrath[79] has found that in disease of the udder there is no rise in peroxidase-content but definite rises in catalase and reductases. Foot-and-mouth disease has the same effect.

Ullmann[80] also has found that the catalase-content is raised in cases of mastitis, but that the peroxidase- and amylase-contents are variable.

It is apparent that when the gland tissue is affected so as to allow increased exudation from the blood to the gland, even in a small degree, the catalase and possibly other blood enzymes tend to increase.

77. Enzymes Associated with the Micro-organisms of Milk

Lipase. It is doubtful whether the bacteria ordinarily contaminating milk have any fat-splitting properties. The fat of cheese also is very little affected by the bacterial population. On the other hand, moulds have a definite lipolytic action, which is shown either by ester-hydrolysis (*Oidium* spp.), or by oxidation of the lower fatty acids to the methyl ketone stage (*Penicillium*, *Aspergillus*, etc.). The formation of a mixture of methyl ketones in the development of a " coconut " taint in sterilised milk is a special instance of the latter form of fat breakdown, but is brought about by a bacterium.

Protease. The proteolytic bacteria contain a variety of enzymes : proteases, deaminases, decarboxylases, rennin, etc. Hudischka

and Komin [81] have found a peptidase able to split glycyl trypto-phane in cow's, goat's and human milk, but it is of bacterial origin. The rennin of bacteria is different from that of the young stomach in that it is highly thermostable and can also act on heated, or even autoclaved, milk. Thus Gorini [82] has found that the rennin of *B. prodigiosius* is destroyed only by heating at 100° C. for fifteen minutes.

It is beyond our province to describe bacterial proteolytic enzymes in detail, and it will suffice to point out that the stinking taint that occasionally appears in clean milk on long keeping is the result of proteolytic action.

Lactase. The most important enzyme in milk that acts on carbohydrate is *lactase*, which breaks down lactose to glucose and galactose, the hexoses being subsequently acted upon by zymase. This is the same series of reactions as occurs in the preparation of alcoholic fermented drinks.

The phosphatases and oxidases connected with the very important phenomenon of lactic-acid fermentation must also be mentioned.

Reductases. The increase in the " direct-reductase " content of milk through the growth of micro-organisms has already been referred to (Section 74). The reducing power is a property. associated with bacterial protoplasm, but Fred [83] has concluded that the reduction of methylene blue by bacteria must be both intra- and extra-cellular, since he obtains partial reduction in a solution of methylene blue in a collodion sac immersed in milk.

The Reductase Test. The *methylene blue* or *reductase test* has been extensively used as a test for the hygienic quality of milk, and is of some value in gauging roughly the progress of deteriora-tion due to bacterial multiplication. Fresh milk, free from dis-solved oxygen, will reduce methylene blue within two hours, but under ordinary circumstances free oxygen opposes the bleaching, and it requires the reinforcement of bacterial action to assist the natural reducing power of milk to cause decoloration. Barthel [84] states that two processes are involved : (*a*) exhaustion of the free oxygen in solution by the bacteria, and (*b*) the reducing action of bacteria in a suitable medium which overshadows the effect of the native-reducing enzymes of milk. Variable results have been obtained in attempting to correlate the rate of reduction of methylene blue with bacterial count (Table LXXIV [85]).

78. Lactic-acid Fermentation

As mentioned above, lactase hydrolyses lactose into glucose and galactose. Lactase is an endoenzyme of the true lactic acid

TABLE LXXIV. *Correlation of Bacterial Count with Time for the Reduction of Methylene Blue*

Quality	Orla-Jensen	Schweter	Fred	Barthel
Good. Count .	10,000	10,000– 1,100,000	1,000– 500,000	6,000
Reduction time (hrs.) .	7+	7–14	7–30	7–24
Fair. Count .	100,000– 3,000,000	50,000– 21,000,000	500,000– 2,000,000	60,000– 2,000,000
Reduction time (hrs.) .	2–7	2–7	2–7	2–7
Bad. Count .	3– 20,000,000	1,000,000– 142,000,000	15,000,000	2,000,000– 15,000,000
Reduction time (hrs.) .	0·5–2	0·25–2	0·25–2	1·5–2
Very Bad. Count .	20,000,000 +	70,000,000	10,000,000– 150,000,000	25,000,000– 250,000,000
Reduction time (hrs.) .	0·5	0·25	0·25 0 08	0 25–1·50

bacteria [85] and therefore only the lactose which is fermented is hydrolysed and the remaining lactose is unchanged. Other organisms such as *B. coli*, *B. acidi-lactici* and *B. bulgaricus* also contain lactase. It has been claimed that such organisms act directly on the lactose, and early workers seem to have followed this idea.

Glucose is more readily fermented than galactose. The first step is the formation of hexose phosphoric ester (zymophosphate, lactacidogen) from which the hexose is liberated in a reactive form, the changes to lactic acid and other compounds occurring in the following steps (Kluyver and Donker [86]) :

(*a*) Formation of a hypothetical 3-carbon compound

$$C_6H_{12}O_6 \rightarrow 2C_3H_6O_3.$$
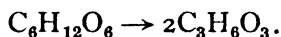
(*b*) Change of the intermediate compound to its stable isomer, lactic acid, or to acetaldehyde and formic acid

$$C_3H_6O_3 \rightarrow CH_3.CHOH.COOH$$
$$C_3H_6O_3 \rightarrow CH_3.CHO + H.COOH.$$
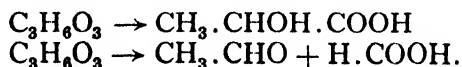
(*c*) Dehydrogenation ; acetaldehyde, acetic acid.

(*d*) Condensation reactions ; to give acetylmethyl carbinol, butyric acid and acetone.

(*e*) Protoplasm-regeneration reactions : hydrogen, glycerine.

The theories of Neuberg and his associates [87] mainly bring into play Cannizaro re-arrangements. The hexose liberated from the phosphoric ester is reactive and breaks into 3-carbon compounds, methyl glyoxal (or glyceric aldehyde), the former being stabilised to lactic acid :

$$CH_3CO . CHO + H_2O \rightarrow CH_3 . CHOH . COOH.$$
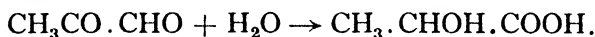
The kind and amount of by-products in lactic acid fermentation depend on the organism and the favourableness of the conditions of growth. The true " lactics " change 90–98 per cent. of the lactose into lactic acid, this being the chief source of energy either in the presence or absence of free oxygen. The products formed are a little succinic acid, acetic and propionic acids, and some carbon dioxide. Under unfavourable conditions the amount of acetic acid is increased and that of carbon dioxide is diminished.

The fermentation of lactose by coliform organisms gives a wider range of products, such as lactic and acetic acids, alcohol, hydrogen and carbon dioxide. The formation of a considerable amount of gas is useful in their detection.

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THE MINOR CONSTITUENTS OF MILK

79. The Pigments of Milk. General

THE white colour of milk is due to the scattering of reflected light by the ultra-microscopic particles (fat globules, calcium caseinate and phosphate). The colour can be simulated by colloidal dispersions of calcium caseinate or phosphate, or by an emulsion of butter-fat in which the particle size is of the same order as that of the fat globules in milk.

When the fat is concentrated in cream and the cream churned into butter the natural yellow pigment dissolved in the butter-fat appears. In milk this fat-soluble pigment gives a yellow tinge which is accentuated in cream. When the molten butter is filtered the colour is still more evident in the butter-fat. The pigment responsible for the golden yellow colour is a carotinoid—*carotene*—in association with which is a small amount of *xanthophyll*.

Another pigmenting substance in milk is *lactochrome*. It is water-soluble and appears when the colloidal matter is precipitated, as in rennet coagulation, the whey being of a yellowish colour with a green tinge. This pigment has no connection with carotene.

Milk, therefore, contains two classes of pigments, one soluble in the fat-phase, the other in the aqueous phase. Carotinoids only are found in the fat, and carotene, the predominating member of this group, is the same pigment as that which colours the fatty tissue and skin of cattle, especially those of the Channel Island breeds. Lactochrome is the only known water-soluble pigment in milk.

80. Carotene

This pigment is widely distributed in plants and is also found in many animals.[1] An isomer, *lycopin*, which causes the red colour of the skins of tomatoes, apples and red pepper does not occur in animals or in milk. Associated with the carotene are other members of the carotinoid group, the xanthophylls, which accompany carotene in small amounts in milk fat. Carotinoids

are synthesised by plants but not by animals. Animals, however, possess the power of storing considerable quantities of vitamin A, manufactured from carotene, in the liver.[7] Crystalline carotene has also been isolated from the suprarenals, testes and ovaries of cattle, but none from the thyroid and thymus glands.[22] The importance of carotene as the precursor of vitamin A has given rise to much investigation of the pigment.

The amount of carotene in the blood serum and the milk fat (Table XXXVI) varies directly with the amount of carotene in the food. Carotene-rich rations include grass, lucerne and all green foods and carrots, whilst cottonseed meal, straws, hay which has lost its greenness, naturally-fermented silage and timothy hay are carotene-poor feeding stuffs.

Of the species of animals whose milk is in common use as human food, the cow alone secretes milk which is characterised by a strong pigmentation of the fat. The butter-fat of the ewe, goat, buffalo and camel is non-pigmented, but the fat of human milk is occasionally coloured. Pigmented milk-fat occurs only in those species which have their blood serum coloured by carotinoids ; there does not appear to be a definite physiological explanation for this remarkable occurrence. It is also inexplicable why xanthophylls are not absorbed in quantities proportionate to the amounts in which they occur in the food ; they are not transformed into carotene by the animal but are broken down to a greater extent in the digestive tract of the animal,[2] which probably accounts in part for this anomaly.

81. Carotene.   Constitution and Properties

Carotene is an unsaturated hydrocarbon, having the empirical formula $C_{40}H_{56}$. Catalytic hydrogenation and other tests have shown that the molecule contains eleven double bonds with three less reactive than the others,[3, 4] the ultimate reduction-product being $C_{40}H_{78}$; the molecule is therefore dicyclic. The isolation of oxidation-products, including geronic acid, points to the presence of an ionone ring.[5] The formula :

$$\left[\begin{array}{c} \begin{array}{ccc} & CH_2-C(CH_3)_2 & \\ CH_2 & C-[CH:CH.C(CH_3):CH]_2\,CH: \\ & CH_2-C.CH_3 & \end{array} \end{array} \right]_2$$
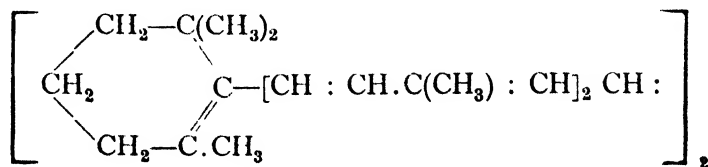
has finally been adopted for β-carotene, the precursor of vitamin A.
Naturally-occurring carotene has been fractionated into three

constituents all of which are represented by the formula $C_{40}H_{56}$. They are α-, β- and *iso*-carotene. The following table (LXXV) gives the physical properties of the three forms :

TABLE LXXV. *Physical Constants of Carotene Isomers* [6]

Carotene fraction	Melting-point	$[\alpha]_{Cd}^{20}$ in benzene	Absorption bands in CS_2 solution	$SbCl_3$ colour absorption bands
	°C.		mμ	mμ
α- . .	172–173	380	511, 478, 444	637, 583, 493
β- . .	180–181	<5	520, 485, 454	590
Iso- .	183–185		543, 503, 422	590

The ratio of α-carotene to β-carotene varies considerably in different plants, many carotenes being found to exist exclusively in the β-form. The carotene of the *corpora lutea* of cows is the β-form, and the interconversion of the α- and β-forms cannot occur *in vivo*. The third form, isocarotene, does not occur as a natural product, nor does it give rise to vitamin A in the animal body. It contains thirteen double bonds like the open-chain isomer, lycopene, that is, two more than natural carotene. The above formula is retained for β-carotene whilst two alternative ring structures each with an asymetric carbon atom are tentatively advanced for the optically active form, α-carotene :
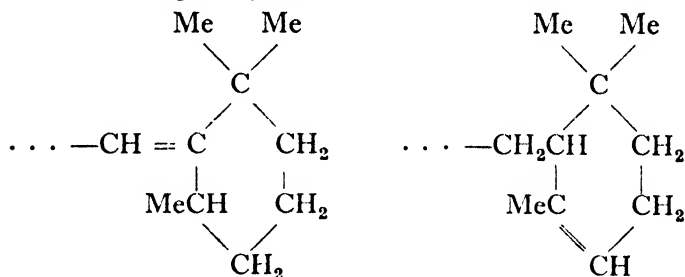
82. Carotene and Vitamin A

It is now generally agreed, although the purest carotene hitherto obtained possesses intense vitamin A-activity, that it is not identical with it. A colorimetric and spectroscopic method of differentiating carotene and the vitamin A of cod-liver oil has shown that the blue colours given in the antimony trichloride reaction are of a different shade, that of carotene being characterised by an absorption band at 590 mμ and that of vitamin A by a band at 608–612 mμ. In the ultra-violet, vitamin A shows a band at 320–330 mμ, which carotene does not give.[8] Pure caro-

tene also is strongly coloured and possesses an activity not greatly exceeding that of the best cod-liver oil concentrates, which are of a pale orange colour.

It may be concluded that carotene is transformed into vitamin A *in vivo*. Moore [7] and Capper [8] have provided evidence of this. Liver oils from rats on a vitamin A-deficient diet give no colour with the antimony trichloride reagent and no absorption band at 320–330 mμ, whilst liver oils from rats cured by doses of carotene show traces of yellow pigment and at least 99 per cent. of the chromogen is vitamin A, showing no intense pigmentation but an intense blue with antimony trichloride with a marked band at 610–630 mμ. The liver oils also show an absorption band at 328 mμ and intense biological activity. Since this band (328 mμ) is absent from pure carotene it has been concluded that the substance responsible for this band in the spectrum of liver oils of carotene-treated rats has been synthesised *in vivo* from the carotene.

Moore [7] has obtained direct evidence of the storage of vitamin A in the liver, where its concentration is about 100,000 times that in the body fats.[10] The vitamin A reserve of the average mammal per gram of liver-tissue is of the same order as that of the average fish, the superiority of the mammalian liver arising from its lower fat-content.[11] The diet of the mammal, however, can be arranged so as to ensure that the vitamin A content of the liver approaches a maximum, and a suitable animal can be " fattened up " on a diet rich in carotene or pre-formed vitamin A and so used as a factory for the concentration of the vitamin. The following range of vitamin A-concentration (in Lovibond blue units) illustrates this : butter-fat 0·02, cod-liver oil 1, liver oil of Jersey cow 100, cod-liver oil concentrates 400, liver oil of rat fed on red palm oil 600, concentrate of liver oil of rats fed on R.P.O. 2,000, concentrate from pig fed with R.P.O. 2,600. The last value (2,600 blue units) corresponds to 40,000 calculated on the cod-liver oil scale of Drummond and Hilditch. The biological activity of the best concentrates is not more than two or three times that of carotene. This slight superiority arises from the inefficient conversion of the very unstable carotene or the breaking down of the molecule during conversion.

Morton and Heilbron [12] have investigated the presence of carotene and vitamin A in butter-fat, both of which can be determined spectroscopically with fair accuracy, and their work supports Moore's views regarding the conversion of carotene into vitamin A *in vivo*. The vitamin A-activity of carotene extracted from plant sources has also been found to run parallel with the carotene-content.[13]

Fraps and Treichler [34] have found that pasture has a consider-
able effect in increasing the amount of vitamin A in butter. Thus
the butter-fat from cows on cottonseed meal, silage, and pasture
contains 33 units, that from cows on silage but no pasture 4 units,
whilst that from cows on cottonseed meal and hulls contained 2
units per gram.

83. Other Vitamins

(a) VITAMIN D. The fat-soluble anti-rachitic vitamin (D)
occurs in milk and the amount present is subject to seasonal
variation. It has been isolated in crystalline form, which has
every claim to be regarded as a vitamin in a state of purity, by
three sets of workers simultaneously. The products they
obtained are described in Table LXXVI.

TABLE LXXVI. *Properties of Crystalline Vitamin D as Isolated
by Various Teams of Workers*

Investigators	Melt.-pt. °C.	Rotation	Maximum U.V. Absorption	Analysis
Angus *et al.*[19]	123–125	$[\alpha]_{5461}$ + 260 in alcohol.	270 mμ	$C_{27}H_{42}O$
Reerink & Van Wijk[20]	115–117	$[\alpha]_{D}$ + 100 in ether.	—	$C_{27}H_{42}O$
Windaus [21]	122–123	$[\alpha]_{5461}$ + 169 in acetone.	265–270 mμ	$C_{27}H_{42}O$

Angus *et al.*[19] have named their product *calciferol*.

The small amounts of vitamin D which occur as such in milk are
accompanied by much larger amounts of the pro-vitamin, which
on irradiation can form the vitamin. There is no evidence as yet
whether the proportion of the pro-vitamin to the vitamin is con-
stant when seasonable variations of the vitamin are considered.

Supplee and his collaborators [23] have found that up to a certain
point the amount of vitamin D formed by irradiation bears a
direct relation to the energy applied, and that above this limit the
antirachitic potency is not increased. The source giving the
greatest amount of energy per unit time is best for the activation
of the pro-vitamin, and the quantum efficiency and formation of
vitamin D is greatest during the first few seconds of exposure.
Thus the maximum amount of the vitamin is found after the
application of $2 \cdot 5 \times 10^6$ ergs per second, which gives a vitamin-
concentration of 0·0025 mg. per litre, or twelve times the anti-

rachitic potency of non-irradiated milk. The short period of exposure (up to 15 seconds) does not greatly activate the dissolved oxygen or cause the " irradiation taint " usually following longer exposures. The irradiation also does not affect the composition or the digestibility of milk.[24] The naturally-occurring vitamin is present to about the same extent in human and cow's milk.[25]

The amount of vitamin D increases when green food is fed, and is not so easily destroyed by heat or oxidation as vitamin A. Kon and Booth [28] have found that the unsaponifiable fraction of butterfat has not the same vitamin D-content as the original butter, and that, whereas added vitamin D-concentrate, or the vitamin in cod-liver oil, can be completely recovered in the unsaponifiable fraction, a considerable loss occurs during a similar concentration of the vitamin of butter-fat.

84. Vitamin B

This accessory factor consists of a group of vitamins : B_1, the original anti-neuritic vitamin of Eijkmann, called *torulin* by Peters,[36] alkali-labile ; B_2, the anti-pellagra vitamin of Goldberger,[37] also called the anti-dermatitis vitamin, alkali-stable ; B_3, the thermo-labile pigeon factor of Williams-Waterman [38] ; B_4, the alkali-labile rat factor of Reader.[39]

Of these B_1 (or B_1 and B_2) is the most important and the one which has been studied in the case of milk. The identity of B_1 still remains in doubt, although very concentrated preparations have been obtained, e.g., Otake's oryzanin,[40] Jansen's crystalline [41] B_1, Windaus' preparation [42] and Peters' preparation.[43] The last-named preparation contains 470 pigeon units per milligram and is the most active preparation yet isolated. Van Veen [44] suggests the formula $C_{12}H_{20}O_2N_4S$, 2HCl for the hydrochloride.

In milk vitamin B shows little seasonal variation and is only slightly affected by heat treatment.[26] A variation in vitamin B content occurs in soured milk preparations, yoghourt showing a decrease but kefir and saya no change.

Lactoflavin, a fluorescent pigment, identical with hepato-flavin and similar to ovoflavin, has been isolated from whey (yield, 20 mg. per litre). The pigment is either vitamin B_2 itself or a very concentrated form of the vitamin.[118]

85. Vitamin C

A large amount of evidence seems to afford conclusive proof that Szent-Györgyi's hexuronic acid [32] (now termed *ascorbic acid*) is vitamin C, and it is of interest that the protective dose of the

pure vitamin is of a higher order than those for the other vitamins (500 γ).

The substance is highly reducing ; the reducing power of the protective dose from plant-extracts (1–1·5 ml. of lemon juice) has the same reducing power as the protective amount of the pure acid (0·5–1 mg.). The substance can be oxidised reversibly and irreversibly, and it is to the double function of oxidation and reduction in the reversible change that the acid probably owes its biological activity. Irreversible oxidation causes inactivation.

Vitamin C is present in fresh milk and does not appear to vary in amount seasonally.[33, 26] Normal milk contains 2·2 to 2·5 mg. of ascorbic acid per 100 ml., but the amount falls below this value in winter when roots and green succulent food are short. This vitamin has been found to be the one most easily destroyed in the treatment of milk, which is probably explained by the ease with which it undergoes oxidation. The destruction of the vitamin during the heat-treatment of milk is probably brought about by the irreversible oxidation mentioned above. When milk is drawn, it is charged with carbon dioxide, which is gradually driven off during the handling processes, and atmospheric oxygen is simultaneously absorbed. Heat-treatment, such as pasteurisation and sterilisation, increases the rate of oxidation of the vitamin, and possibly traces of heavy metals enter the milk during the processing and catalyse the oxidation.

Attempts have been made to determine the vitamin C content of milk by taking advantage of the reducing action of ascorbic acid, using as the oxidising agent an easily reducible dye, such as one of the indophenol dyes. Schlemmer, Bleyer and Cahmann [35] de-proteinise milk with a mixture of lead acetate and sodium sulphate, and titrate the filtrate with a 0·001 N solution of 2·6 dichloro-phenol-indophenol until the blue colour persists. They have found that exposing milk to the air destroys the reducing activity, but that brief boiling has only a small effect. Traces of copper and silver also destroy the reducing action, but salts of nickel, chromium and aluminium have no effect.

It is clear that the determination of the reducing action in such a manner requires rigid observance of certain conditions, especially those of the oxygen-concentration in the filtrate. The dye used is one which decolorises at a high oxidation-reduction potential, and either fresh milk or milk heavily contaminated with organisms will quickly reduce the dye. Inconsistencies in the results obtained by the titration-method may therefore arise from the fact that the dissolved oxygen as well as the dye is interacting with the reducing substance.

Vitamin E. This is present in milk in small quantities.[26] Factors in milk associated with reproduction and lactation have been found to be heat-labile. Thus the capacity of rats to gestate and rear a litter is reduced when they are fed on holder-pasteurised milk, and milk sterilised at 100° C. lowers the capacity to one generation. The influence of other dietary factors connected with fertility and lactation, especially vitamin B, have also to be considered.

Norris, Henser and Wilgus [107] have stated that milk contains a substance of the nature of a vitamin which prevents the development of a particular type of paralysis of the legs and feet of poultry, but Hart, Kletzein *et al.*[108] failed to confirm the presence of this factor in milk.

86. The Water-soluble Pigment, Lactochrome

Blythe [14] made the first attempt to isolate the water-soluble pigment of milk, which he called *lactochrome*. The pigment can be seen as the greenish-yellow colour of whey. This colour was formerly regarded as due to the urinary pigment, urobilin, but this has not been confirmed. Palmer [16] found the whey pigment to be closely related to *urochrome*, the more important of the urinary pigments. It appears possible to produce an identical substance from casein and peptone, and the name *protochrome*[15] has been suggested for the pigment.

Bleyer and Kallman [17] believe lactochrome to be an artificial product and that the true whey pigment is a protein-derivative occurring naturally in the peptone group in milk : the purest pigment fails to give any of the tests for protein other than those associated with the aromatic nucleus (xanthoproteic, Millon's and Ehrlich's aldehyde test), and since results by the Ehrlich diazo test for tyrosine and tryptophane are negative, the pigment is probably a compound of phenylalanine. Blythe and Palmer, and Cooledge, initially believed that the pigment as isolated was the pigment of whey.

The amount of pigment is of the order of 0·05 to 0·10 per cent. of the milk, or about 2 per cent. of the nitrogenous substances.

The pigment may be isolated from whey (rennet) by saturating with ammonium sulphate and extracting with alcohol, or by precipitating with acid mercuric nitrate and decomposing the precipitate in aqueous suspension with hydrogen sulphide ; or the pigment is dialysed from concentrated whey and extracted with alcohol from the concentrated diffusate, after saturating it with ammonium sulphate. Further purification is effected by con-

centration of aqueous solutions and extraction with alcohol-chloroform mixtures (1 : 2), evaporation, further extraction with a 1 : 8 mixture and evaporation of the solvent *in vacuo* ; or the pigment can be adsorbed on alumina gel, from which it is extracted by alcohol (or ammoniacal alcohol), or by lead-acetate precipitation and decomposing the lead salt with sulphuric acid.

Composition and Properties. The pigment contains the elements C, H, O and N, and free amino groups. It is a strong reducing agent, reducing ammoniacal silver nitrate in the cold. A number of heavy metal salts precipitate it, *e.g.*, silver nitrate, mercuric acetate and nitrate, basic lead acetate, copper salts, phospho-tungstic and phosphomolybdic acids. It forms humin easily on digestion with acid. Its solutions are easily bleached by light.

Lactochrome shows no absorption bands. " Active aldehyde " (acetaldehyde exposed to light for a considerable time) changes the yellow colour to orange, and shows a strong band around the green line (F) and another band appears later in the blue. Zinc chloride added at the first stage yields a brilliant green fluorescence. Urochrome behaves similarly.[18]

There is some correlation between the food and the lacto-chrome-content of the milk. Palmer and Cooledge [16] have found that lucerne hay and cereal grains cause a more intense yellow colour of the whey than when bleached timothy or clover hay is fed ; this may have been due either to a higher content of protein or to chlorophyll. It has been observed that a higher urochrome-content results from high-protein diets and diets containing green vegetable matter.[15] It has also been found that the breed of cow is of greater importance than the food. The following Lovibond yellow units represent the colour of a depth of 10 cm. of whey from milk from the following breeds of cows : Ayrshire, 4·8 ; Jersey, 3·5 ; Friesian, 2·4 ; Shorthorn, 2·2.

Kuhn and his associates [118] have isolated *lactoflavin*, $C_{17}H_{20}N_4O_6$, m.p. 276° C., in a yield of 20 mg. per litre from whey. This pigment accounts for the colour of whey and for most of the lactochrome. The pigment is similar to ovoflavin and identical with hepatoflavin ; it is either very concentrated vitamin B_2 or even the vitamin itself.

Milk from other species of mammals also contains lactochrome. It is present in human milk, and the concentration in the milk of the ewe may often exceed that in cow's milk. It is reasonable to assume that the nature of the pigment is the same, although this has not been established.

87. Cholesterol

This monohydric alcohol has the following structural formula :
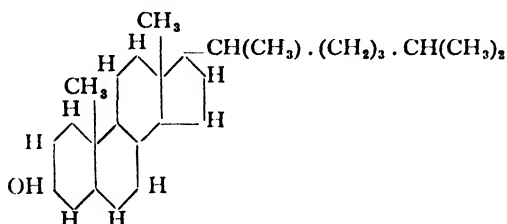
It occurs in milk fat as free cholesterol.   The amounts in milk as found by different workers are given in Table LXXVII.   The

TABLE LXXVII.   *Cholesterol-content of Milk (Percentages)*

	Before Saponification	After Saponification
Klostermann [46]	0·075	0·071
Boemer [47]	0·312	0·407
Kirsten [48]	0·36	0·43
Denis & Minot [54]	·0105–·0176 (15 samples)	

Nakanishi [49]	Mare ·012	Cow ·013	Goat ·014	Man ·016	Ewe ·023	Bitch ·055	Cat ·063	Rabbit ·109	Sow ·145

amount varies roughly with the fat content of milk of various species but the amount in cow's milk is variable.

A similarity in composition and properties of the sterol found in vegetable fats, *phytosterol*, has caused some confusion in the

TABLE LXXVIII.   *Melting-points of Mixtures of Cholesteryl and Phytosteryl Acetates (Jaeger)*

Cholesteryl Acetate Melt.-pt. 112·8° C.	Phytosteryl Acetate Melt.-pt. 129·2° C.	Melt.-pt. of Mixture
Per cent.	Per cent.	°C.
90	10	117
80	20	120·5
73·3	26·7	122·5
60	40	125
42·4	57·	129
20	80	129·1
10	90	129·2

chemistry of the two sterols. But these two isomers can be distinguished by taking advantage of their different crystalline forms, melting-points and optical rotations, and the melting-points of their acetates. This is of importance in the detection of vegetable fats in general and of adulteration of butter-fat with vegetable oils. Jaeger [53] has developed the phytosteryl acetate test as an index of such adulteration.

The melting-point of the first ·crop of crystals gives definite information as to the presence or absence of phytosterol. Phytosteryl acetate is less soluble in alcohol than the cholesteryl acetate, and hence, if the second recrystallisation is carried out, the melting-point should be higher than that of the first crop if phytosterol is present, whereas, if in the fifth to seventh recrystallisations the melting-point is below 115–116° C., the absence of phytosterol is certain.[52] The feeding of vegetable oils to cows (oil-cakes and meals) does not cause phytosterol to appear in milk-fat.

The total insolubility of cholesterol in the aqueous phase of milk in a way regulates the hydrophyllic nature of the lecithin of milk.

88. Phospholipins and Phosphoric Esters in Milk

The lecithin and kephalin of milk have already been dealt with to some extent (Section 28 b).

The fatty acids present in the lecithin of milk are a subject of some controversy. It is evident that Schulze's " distearyl lecithin " (1894) was the composition suggested when knowledge of lecithins in general was sparse. Sasaki and Hiratsuka [55] have found no unsaturated acid in milk lecithin, and state that the composition is myristo-lauro- and palmito-lauro-lecithin. Bischoff,[56] on the other hand, has found the composition to be stearo-oleo lecithin. The existence of butyro-oleo lecithin, as suggested by Gautier (cited by Mohr and Moos [57]), is doubtful.

The amount of phospholipin-phosphorus in milk, as determined by various investigators, shows wide variations. Mohr and Moos [57] have collected the analyses of a large number of investigators on various milk products, and the summary of their findings is given in Table LXXIX. References giving the values will be found in the paper cited. The table shows that either great fluctuations occur in the lipin-phosphorus of milk and its products, or that the methods of analysis of the different investigators have varied so much as to yield such large discrepancies between the results. As an example, the results of Rewald,[58] who has used methods similar to that used for blood analysis, namely, the alcohol-ether method of extraction, are always on the high side.

TABLE LXXIX. *Phospholipin-phosphorus in Various Milk Products (Mohr and Moos) (Percentages)*

Product	Range	No of Investi-gators Quoted
Whole milk (raw) . .	0·0010–0·0189	24
Skim milk . . .	0·0001–0·0186	12
Pasteurised milk . .	0·0008–0·0065	2
Whey	0·0002–0·0005	6
Cream	0·0025–0·0205	12
Butter-milk . . .	0·0009–0·0342	13
Butter	0·0005–0·0615	14
Butter-fat . . .	0·0000–0·0665	8
Dried milk . . .	0·0058–0·0250	2
Cheese	0·0021	1
Colostrum . . .	0·0007–0·0114	8
Colostrum-fat . .	0·0010–0·0012	2
Separator slime . .	0·0161–0·0231	2

Wiese, Nair and Fleming [27] have found from 0·018–0·020 per cent. of lipin-phosphorus in the fatty extract (Mojonnier method) of cream.

The partial breaking down of milk lecithin during pasteurisation has been observed by Supplee.[59] Fiori [60] has observed a similar breakdown, the rate of decomposition varying directly with temperature. Since there is no optimum temperature for the breakdown it is concluded that an enzyme is not involved in the reaction. Others report the stability of lecithin towards heat (see p. 72).

Ester-phosphorus Compounds. The lecithin and kephalin of milk are extractable by the alcohol-ether method, but are precipitated by trichloracetic acid solution with the proteins and fat. In the filtrate are found acid-soluble phosphoric esters (Kay [61]), which are subject to breakdown during the storage of milk by the native phosphatase of the milk. No diminution of ester-phosphorus is observed in holder-pasteurised milk ; from this it has been deduced that the enzyme is destroyed at a temperature not exceeding 63° C. The amount of ester-phosphorus ranges from 0·007 to 0·020 per cent. and appears to vary with breed of cow and with the fat-content of milk. Thus the following ranges have been found for the milk of different breeds : Friesians 0·0071–0·0107 ; Ayrshires, 0·0071–0·0123 ; Jersey, 0·0097–0·0201, per cent. The exact composition of the phosphoric ester present

in milk has not been investigated, but the phosphatase of milk is capable of hydrolysing added glucose monophosphate.

89. Traces of Heavy Metals in Milk

Milk contains traces of a considerable number of heavy metals. During the last few years the importance of these traces in nutrition has claimed much attention, especially in regard to the incidence of anæmia,[29] and incidentally the investigation of them has led to improvements in the micro-analysis of metals. The deficiency of iron in milk has long been regarded as one of

TABLE LXXX. *Iron-content of Cow's Milk (Various Investigators) (Parts per Million)*

Anselm [62]	0·62–0·84	Edelstein [69]	0·28–0·49
Friedjung [63]	0·84–1·82	Soxhlet [31]	0·18–0·84
Trunz [64]	0·22–0·36	Nottbohm [70]	0·21–0·19
Konig [65]	0·35–4·69	Lesne *et al.*[71]	0·95
Glikin [66]	56·8	Elvehjem [74]	0·35–0·36
Fendler [67]	2·8–8·4	Davies [75]	1·5–2·4
Langstein [68]	0·21–0·49	Macfarlane [76]	0·48–0·68

TABLE LXXXI. *Iron-content of Milk other than Cow's Milk (Different Investigators) (P.p.m.)*

Human	2·8 [30]	5·6 [72]	1·54–0·7 [73]	50·1 [46]	1·02 [31]	0·92 [71]
Goat	1·00 [71]					
Ass	0·83 [71]					

its drawbacks from the nutritional standpoint, since for the suckling it is the only source of that metal for the building up of hæmoglobin in the rapidly increasing volume of blood. The trace of copper present has, however, been found to be important in that it also takes part in the process of building up new red cells, and is associated with iron in warding off the onset of anæmic conditions.

Iron. Bunge [30] believed that there was a store of iron in the liver of the offspring upon which it drew for its development and that the iron in the food was of no account, but Soxhlet [31] showed that the amount present in human milk, for example 2·8 parts per million, more than sufficed to provide for the needs of the developing child, and that there was no need to call on this store of

iron in the liver. The iron-content of colostrum (cow) is higher (4 p.p.m.) than the average for cow's milk, so that these conditions, together with the high red-cell count of embryonic blood, tend to tide over for a time the possibility of the development of an anæmic condition in the newly born offspring.

As has been experienced in other directions, the different methods of analysis used by investigators has led to wide variations being found in the iron-content of milk (Table LXXX).

Both Trunz [64] and Nottbohm, and Dorr [70] have found that the iron-content of milk tends to rise towards the end of the lactation period : they have also found considerable individual variations in the iron-content of cow's milk. On the other hand, Camerer and Soldner [73] have found the iron-content of human milk to decrease with lactation, early milk containing 1·54, middle-period milk 1·40, and late-period milk 0·7 p.p.m. of iron. Samples of human and cow's milk on the same day were found to vary appreciably in iron-content. Davies (unpublished work) has found that the iron-content of milk of a typical herd of Shorthorn cows does not show any appreciable seasonal variation. Variations in iron-content have been found to be due to geographical variations in the source of the milk.[75]

Langstein [68] and Edelstein [69] have found the iron-content of milk to increase during keeping, and they believe this was due to solution from the utensils. Williams [77] and Davies [75, 78] have found that considerable contamination of milk and cream occurs during the handling and processing of milk, the amount of iron being sometimes doubled. The drying of milk by the roller process adds a considerable amount of iron to the product. Thus if the dried product is regarded as eight times the concentration of fluid milk in total-solid content, the iron-content should be from 10–16 p.p.m. Values roughly approximating these have been found for spray-dried milk; but the values for roller-dried milk are higher owing to contamination from the steel rolls. Some samples have been found to contain 35 p.p.m. This contamination may not be of much importance in pasteurised milk, and perhaps may be regarded as nutritionally beneficial, but the keeping quality of roller-dried milk is considerably lessened thereby owing to the heavy metal in high concentration catalysing the oxidation of fat.

(b) THE COPPER-CONTENT OF MILK. Milk contains traces of naturally-occurring copper (about 0·3 p.p.m.). The importance of traces of this metal as a supplement to iron in the regeneration of red blood-corpuscles in cases of anæmia has been investigated by Hart, Steenbock and associates.[79]

Different investigators have found widely varying ranges of copper-content in milk (Table LXXXII).

TABLE LXXXII. *Copper-content of milk (Different Investigators) (Parts per Million)*

Cow.	Raw Milk.			
	Supplee & Bellis [80]	0·52	Elvehjem & Lindow [84]	0·138–0·164
	Rice & Miscall [81]	0·50	Williams [77]	0·5
	Quam & Hellwig [82]	0·26–0·52	Davies [75]	0·15–0·65
	Gebhardt & Sommer [83]	0·30 (lowest)	Zoudek [85]	0·15–0·20
Human	Zoudek [85] . .	0·5–0·6		
Sheep	Quam & Hellwig [82]	0·45–0·50		
Goat	Quam & Hellwig [82]	0·19–0·25		

The copper-content of raw cow's milk is thus roughly a quarter of the iron-content. Variations in copper-content may be found in the milk of individual cows, but for herd milk the copper does not appear to fluctuate seasonally despite the changes associated with bringing in fresh calvers or the drafting out of cows from the herd (Davies[75]). There is evidence of variation according to the geographical source of production.[75]

The latest work shows that the values of the copper- and iron-contents of milk are lower than those found by earlier workers. Krauss and Washburn [113] report : Cu, 0·14–0·17, Fe, 0·40–0·53 p.p.m. and Remy [114] (German milk), Cu, 0·13 and Fe, 0·50 p.p.m.

Processing adds considerably to the copper-content since milk readily dissolves copper from detinned surfaces of plant.[75, 77, 78, 81, 83] From a large number of observations both on milk to which copper lactate has been added and by analysis of commercial samples, the minimum copper-content necessary to cause an " oily " or oxidised flavour to develop in cold storage for twenty-four hours has been found to be 1·5 p.p.m.[75, 78]

Metallic contamination of milk during processing has been extensively investigated and will be dealt with in later sections (Sections 149, 150).

(c) OTHER METALS. *Manganese.* Manganese occurs in very small traces in raw milk. Richards [86] has studied the nutritional significance of manganese ; she and Peterson and Skinner [87] give a comprehensive list of the manganese-contents of various foods. Table LXXXIII gives the manganese-content of milk.

TABLE LXXXIII. *Manganese-content of Milk (Parts per Million)*

Authority	Colostrum	Whole Milk	Milk Powder
Cow's Milk—			
Richards,[86] 1st milking	0·05–0·09	0·04–0·05	—
2nd ,,	0·04–0·06	—	—
3rd ,,	0·03–0·07	0·017–0·048 (average 0·028)	—
Peterson & Skinner [87] .	0·044	—	0·18
Sato & Murata [88] .	0·16–0·06 (5 days).	0·02–0·04	—
Mare's Milk . . .	—	0·03–0·04	—
Ewe's Milk . . .	—	0·05–0·09	—

There is agreement that colostrum contains more manganese than normal milk, the first sample after parturition being especially high. The milk of the ewe is higher in manganese than are cow's and mare's milk and tends to increase towards the end of lactation. Richards finds a tendency to variation in the manganese-content with the geographical source of production. There does not appear to be any appreciable loss or gain of manganese during the drying of milk.

Zinc. Traces of zinc are present in milk. Sato and Murata [89] give the following contents in various milks : Cow : colostrum, 13·57 ; second-third month, 2·35–2·12 ; end of lactation, 4·58. Human : three days *post partum*, 7·35 ; twenty-fifth day, 1·23 ; 360th day, 3·89. Ewe : colostrum, 13·78 ; seven months, 2·45–2·90 ; end of lactation, 3·5 (all figures in p.p.m.). The average for market milk is 3·3 p.p.m. The zinc-content of milk is thus of a relatively high order of magnitude.

The zinc of galvanised utensils is easily dissolved off by acid milk-products, such as whey. Burke, Woodson and Heller [90] report cases of poisoning of pigs fed on whey stored in zinc vessels.

Blumberg and Rask,[115] and Drea,[116] give a list of the elements which they detected spectrographically in milk ash. Both failed to detect Mn, Al and F, although these elements in Drea's product were present in the water and food of the animals. Büttner and Meiermeister,[117] however, find a range of 0·06–0·17 p.p.m. of Mn

in milk. The zinc-content of colostrum has been found to be three times that of milk (3·4–3·6 p.p.m.).

90. Other Elements

Iodine. Considerable attention has been devoted to iodine metabolism during the last ten years, and the importance of iodine in the development of the thyroid gland, which in its turn is of importance in regulating the development of the growing animal, has been demonstrated. The studies have been well advanced by improvements in the methods of determining small quantities of iodine, among which may be mentioned that of Fellenberg (described by Veil and Sturm [91]). Milk, according to the latest work, contains traces of iodine, although Forbes was unable to detect it in any of eighteen samples of milk. The amount of iodine in milk as found by various authorities is given in Table LXXXIV.

TABLE LXXXIV. *Iodine-content of Milk in γ per Litre*

	Raw Milk	Colostrum	Butter fat	Dried Milk
Cow's Milk				
Leitch & Henderson [93]	100	50	—	—
Hercus & Roberts [94]	20 (39)	—	—	96
McClendon [95]	—	—	4–78	142–162
Goat's Milk				
Leitch & Henderson [93]	120	—	—	—
Human Milk				
Hercus & Roberts [94]	43	—	—	—

Experimental Milk from Cows Fed on Foods containing Iodine (McClendon [95])

Feed : Cod-liver oil	36	7,320
Salt containing 10 per cent Iodide	378	158,000
Iodised oil	43	65,600
4–6 days after discontinuing salt	148	2,800

The iodine-content of butter-fat is very low, except in experimental milk, and that of blood is always higher than that of milk.

Scharrer [96] states that the iodine in milk is mostly in organic combination, only a small fraction being in inorganic form. The serum of milk contains most of the iodine ; the proteins contain a smaller and more variable quantity, and the fat little or none. When iodine is added to the diet it does not distribute itself uniformly between the dry matter of the fat and non-fatty-solid phases, but more goes to the non-fatty-solid fraction. The high iodine-content persists in milk for some days after ceasing to give a ration rich in iodine. Colostrum contains less iodine than the normal milk produced later, according to the figures of Leitch and Henderson, but Oertel [97] has found that the iodine is at a maximum in the colostrum of the goat and later falls to a minimum. Thyroidless goats were found to produce milk of very low iodine-content, which was reflected in the low vitality of the offspring fed on it.

The geographical mapping of regions according to the iodine-content of milk requires a very large number of samples before definite boundaries can be plotted. Aitken states that it is impossible to do so even from a very large number of results for fat and milk.

The milk produced by animals fed on iodine-rich foods contains only a trace of the iodine fed ; the production of the so-called " iodised milk " can best be simulated by the direct addition of small amounts of potassium iodide to milk.[109]

Fluorine. Traces of this element are said to occur in milk. This element enters into the composition of teeth, and according to Trebitsch [99] 0·29–0·59 per cent. of the dry substance of human teeth is fluorine. Traces of fluorine occur in the blood, and hence it is likely that some will appear in the milk. In the absence of information on the storage of fluorine in the body of the offspring it is reasonable to assume that the traces of fluorine necessary for dentition come from the maternal milk.

Mazé [100] has found that traces of fluorides and iodides have an effect on reproduction and lactation ; they appear to replace some of the factors lost during the heat-treatment of milk.

An excess of fluoride in the food has a detrimental effect on the teeth of animals of all ages.[101]

Silica. Pfyl [110] reports the finding of 2 p.p.m. of silica in milk, part of which possibly was derived from the glass of bottles in which the milk was sterilised. Ketmann [111] found a maximum value of 1·6 p.p.m. in a series of determinations on different milks which were variable. Strohecker, Vaubel and Breitweiser [112] found a range of 0·18–0·53 p.p.m. of SiO_2 in fresh milk, 8·48–9·80 p.p.m. in unsweetened condensed, and 26·4–30·4 p.p.m. in

dried milk (Krause process) (*i.e.*, 3·3–3·8 p.p.m. in the original milk). Added water naturally increases the SiO_2 content. It is not known to what extent glass-SiO_2 contributes to the above values. The SiO_2-content of the milk of other mammals has not been investigated.

91. The Gases of Milk

Milk contains the gases, carbon dioxide, oxygen and nitrogen, together with small traces of the rare gases. The growth of bacteria produces other gases, such as hydrogen and methane, in small quantities.

Van Slyke [102] has found that the average amount of carbon dioxide in milk in the udder is 10 per cent. by volume, which drops to 4 or 5 per cent. immediately after milking and to an average of 3 per cent. or less on standing for a few hours. Milk heated to pasteurisation temperature shows an average of 3 per cent. carbon dioxide. The substance is present as carbonic acid (H_2CO_3) and sodium bicarbonate.

The oxygen content of milk fresh from the udder is very low ; it is in this condition that milk shows its maximum reducing intensity. Methylene blue can be reduced within two hours by fresh milk straight from the udder. The measurement of oxidation-reduction potentials on such samples kept in an atmosphere of nitrogen bears this out very clearly.

Aeration, or letting the milk stand, reduces the CO_2-content, but atmospheric oxygen and nitrogen dissolve. Marshall [103] has investigated the composition of the gases of milk under different conditions. His results are given in Table LXXXV.

TABLE LXXXV. *The Composition of the Gases of Milk*

State of Milk	CO_2 %	O_2 %	N_2 %
Fresh from udder . .	81·50	2·42	16·54
After milking . . .	59·64	13·18	27·17
After aeration over glass . .	40·57	20·59	38·54
After aeration over tin . .	35·82	20·55	44·62
After aeration over copper .	42·33	17·26	40·42
After aeration through glass wool and copper sieves . . .	25·81	23·31	50·88

The expulsion of carbon dioxide is accompanied by a slight rise in density, and part of the Recknagel phenomenon is due to this circumstance. Table LXXXV shows that it is very difficult to eliminate the last traces of carbon dioxide.

Traces of hydrogen sulphide, derived from the decomposition of albumin, may occur in boiled milk.

92. Other Compounds

The traces of nitrogenous compounds in milk have already been described (Section 61).

Nitrates are not present in milk. Their presence in small traces has been used as the basis of the quantitative detection of added water, since practically all natural supplies of water contain a detectable amount of nitrate. The addition of small quantities of sodium nitrate to counteract the " turnipy " flavour of milk from cows fed on large quantities of that root naturally narrows down the application of the test.[104] Monier-Williams [105] describes a more sensitive test, based on Lerrigo's original method, in which diphenylbenzidine is substituted for diphenylamine, the latter requiring some nitrate for its oxidation before giving the test. Cows drenched with saltpetre do not show the presence of nitrate in their milk.

Indican is present in milk during certain pathological conditions.[106]

Orotic acid (uracyl 4 (6)-carboxylic acid) has also been found in traces in milk.

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PART III

THE PHYSICAL CHEMISTRY OF MILK

CHAPTER XII

GENERAL PHYSICAL PROPERTIES

93. Colour

THE white colour of milk is due to the scattering of reflected light by the contained ultramicroscopic particles—fat globules, colloidal calcium caseinate and calcium phosphate. The creamy colour of the milk of some breeds, *e.g.*, the Channel Island breeds, and of that of some individual cows, is due to the carotene in solution in the fat.

Separated milk has a blue tinge, which can be seen best when compared with the cream layer. The depth of blue varies with the amount of fat (or cream) remaining in the skim-milk layer, and the layer will appear more blue by watering the milk owing to the easier separation of the cream. Physiological disturbances in the cow render the milk more blue. Again, a relation has been noted between the feed of the cow and the blue colour.[1] Since different foods have a definite effect on the separation of the cream as exhibited by (*a*) depth of cream layer and (*b*) completeness of creaming, it is probable that the conditions governing cream-separation affect the nature of the blue colour given.

In pasteurisation, the *dead white* colour of milk may be enhanced through the destruction of the blue colour by processing. Holder pasteurisation at $143-145°$ F. preserves the colour.

94. Surface Tension

Various methods have been used for determining the surface tension of milk. Burri and Nussbaumer [2] have used the stalagmometric method of Traube, whilst Kobler [3] and Kreidl and Lenk [4] have used the capillary method with filter-paper. Burri and Nussbaumer have found that when milk is cooled to between $0°$ and $10°$ C. the surface tension reaches a minimum value which does not change appreciably on allowing the milk to warm slowly to $37°$ C. Kobler has found that the surface tension changes with rising of cream and with the precipitation of the casein. Kreidl and Lenk have observed that the higher the protein-content, the higher the surface tension, but that the degree of dispersion of the casein apparently has no effect, since mare's milk (large casein particles) and human milk (small casein particles) possess nearly the same surface tension. The filter-paper method is open to criticism in

that the adsorptive effect of the fibres plays a part and is apt to give false values. In the same way the semi-dynamic drop method does not give an exact picture of the state at the milk/air interface owing to the difference in the radius of curvature, so that a *static method*, such as the ring-torsion balance method, gives absolute results and is to be preferred.

Mohr and Brockmann,[5] using the ring method, have obtained lower results than by the stalagmometric method to the extent of 10 (whole milk), 7 (separated milk) and 3 (cream) dynes/cm. on values for the latter method of about 50, 52 and 45 dynes/cm. respectively. In the drop method the value varies according to the time of flow through the stalagmometer, the quicker the flow, the higher the surface tension.

The surface tension of milk is lowered by increases in the percentage of fat ; this occurs up to 40 per cent. fat content when determined by the drop method, but by the ring method the drop is very sharp up to 10 per cent., remaining practically constant up to 25 per cent., then showing a small rise up to 40 per cent. fat. This is probably due to the increased adsorption of the constituents responsible for the capillary action on the fat-globule surfaces, thus decreasing their concentration in the serum.

The processing of milk, such as holder- and flash-pasteurising, boiling or sterilising, has no appreciable effect on the surface tension ; ageing, freezing, and the addition of formalin also have no effect.

Increasing the amount of dissolved air, or replacing carbon dioxide by atmospheric gases, increases, whilst the development of acidity, by affecting the physical properties of calcium caseinate, decreases the surface tension. The precipitation of casein by rennet does not change the surface tension of the whey. With rise in temperature the surface tension decreases and the difference due to varying fat-content is not so marked at high temperatures (60° C.).

Homogenising causes a slight decrease when high pressures are used (1,500 lb. per sq. in.), but very little change occurs with pressures up to 1,000 lb. per sq. in.

The surface tension of the interface butter-fat/skim-milk is roughly 12 dynes/cm. ; that of butter-fat/water is roughly 23 dynes (both measured at 40° C.). By the stalagmometric method the former is slightly higher (14 dynes/cm.).

Separated milk churned for eighty minutes shows no change in surface tension. In the case of sweet cream a gradual lowering occurs in the churning process, until at the point where the cream

" breaks " very low values are found. The butter-milk has a lower surface tension than the original cream.

Table LXXXVI shows the range of surface tension values for different fat-contents and at different temperatures.

TABLE LXXXVI. *Surface Tension of Milk and Cream of Varying Fat-content at Different Temperatures (Dynes/cm.)* (*Mohr and Brockmann*)

Milk			Fat %	Ring Method	Drop Method
Skim	.	.	0·14	46 7	50 6
Whole	.	.	4 10	44 1	49 1
Cream.	.	.	15 0	43 4	48 3
,,	.	.	.	25·0	43·7	47·0
,,	.	.	.	38·0	44 1	46 9

Temperature deg. C	0	10	20	30	50	60	80	90
Skim	53 3	49·7	46 5	45 4	41 9	40 7	38 6	—
Whole	49 1	47 8	43·6	43 5	—	—	—	—
,,	—	45 9	43 5	43 5	42 2	41 2	—	—
Cream 10 % fat	—	48 6	46 4	44 8	42 7	41 9	—	—
20 % fat	—	46 8	44 8	43 2	41 6	41 6	—	—
Butter-fat	—	—	—	—	31 6	31 2	29 5	28 5

Water, 75·6 (0 C.), 73·5 (15° C.); olive oil, 33·5 (20° C.); saturated sodium oleate, 25 (20 C.).

Fat %	Raw		Dahlberg and Hening [6] Pasteurised	
	1st Day	3rd Day	1st Day	3rd Day
0	57 4	57 7	59 3	59 3
5	55·3	52·7	55·8	56 4
10	52·8	51·5	54·8	54 8
20	51·8	50·5	53·4	52·6
25	50·4	49 4	50·6	51·7
30	50·0	48 8	50·6	50·9
35	49·1	47 3	50·2	50 5

Dahlberg and Hening [6] state that the surface tension decreases slightly with ageing, whilst pasteurisation raises its value. Behrendt [7] states that fat has no influence on the value, but that

proteins and fatty acids are the determining factors. Kopac-
zewski [8] has found the surface tension of milk at $15°$ C. to be
52·8 dynes/cm. Considerable deviations from this value have
been obtained.

95. The Frothing of Milk

Freshly-drawn milk froths readily, *e.g.*, in the milking pail, by
stirring or pumping, or passing through a cream-separator. The
foam presents a technical difficulty and is a disadvantage during
pasteurisation, since the froth may be at $8–10°$ F. below the
pasteurising temperature.[9, 10] The difficulty has been over-
come by the use of tanks in which delivery from the bottom
ensures a rapid flow of foamless milk, the foam being trapped
in the tank, whilst the air in holder-pasteurisers may be steam-
heated. Engineering modifications that prevent foam-formation
have also been introduced.

Froth or foam consists of a gas phase dispersed as minute
bubbles in a liquid phase, an enormous area of gas surface being
produced. The aqueous films are of micronic or even sub-
micronic thickness. The gas (air) bubbles persist for some time
owing to the presence of milk colloids in a solid condition (the
Ramsden phenomenon).[11] The froth on skim-milk after cream-
separation contains an increased protein-content, but there is no
change in the content of inorganic matter. Rahn quotes the
following analysis of milk and milk foam (Table LXXXVII).

TABLE LXXXVII. *Comparison of Milk and Milk Foam (Rahn)
Percentage of Dry Matter*

	Strongly-frothing Milk		Ordinary Milk	
	Protein	Ash	Protein	Ash
Froth . . .	38·87	8·63	36·39	8·52
Milk . . .	35·60	8·58	34·72	8·57
Excess in froth .	+ 3·27	+0·05	+1·67	−0·05

When the froth on milk is undisturbed it gradually collapses,
and wrinkles of a delicate membrano-solid character lie on the
surface. Brouwer [12] claims to have proved the existence in milk
of peculiarly-shaped bodies which were originally membranes,
formed by the adsorption of protein on gas-bubbles, but remain
in the milk after the collapse of the bubbles.

SKIN-FORMATION. When the foam on the milk in bottles of
sterilised milk collapses and dries, the film assumes a " pear-drop "

formation on the neck. This formation can also be observed in isolated patches on the glass below the surface of the milk ; it can also be observed on some samples of milk which partially coagulate on heating. When milk is boiled with albumin, *e.g.*, in a baked egg-custard, pellicles of the same formation are found at the bottom of the vessel. This is due to adsorption and partial solidification of milk colloids at a gas/milk interface which under conditions of heating is not reversible. The escape of the gas deforms the pellicle on a vertical plane to a pear-drop shape. The skin which forms on the surface of milk when heated is probably formed in a similar manner from the colloids concerned in milk foam which are concentrated and denatured by heat.

The presence of a solute causing a lowering of the surface tension of an aqueous solution results in a concentration of the solid at the air/solution interface, which may even result in an " irreversible " precipitation of the solute (the Ramsden phenomenon).[13] In the case of milk, even evaporation *in vacuo* at room-temperature will cause the skin to appear ; normal heating above 50° C. also causes skin-formation. Semlritski [14] disproved the original idea that the skin was entirely albumin, and was able to remove a succession of skins amounting to 1·023 per cent. of the milk--an amount exceeding the albumin-content. Friese [15] obtained thirty such skins from a litre of milk at 100ᵛ C., and observed that the kind of container affected the number of skins obtained. The fat-content of the skin increases with time of standing. Müller [16] has found that 23 per cent. of the fat is removed in seven hours and that the skin contains, water 48·45, fat 41·43, protein 4·71, lactose 3·34, and ash 0·69 per cent. The protein consisted of *casein* only, with no albumin. The ash is mainly composed of calcium phosphate with some alkali phosphates.

ROPY MILK. Mattick [17] has investigated the formation of " ropes " of milk under certain favourable conditions of rate of flow, acidity and temperature, and has found that the " ropes " are modifications of Ramsden's " mechanical surface aggregates " of casein or casein plus albumin. The phenomenon occurs on the surface of coolers.

CAUSE OF FOAM FORMATION.[92] The formation of foam depends on two main conditions : (*a*) the lowering of the surface tension so as to allow the gathering and spreading of the active colloid into thin films, and (*b*) the films must be sufficiently elastic and tough to prevent the coalescence of the gas globules. A stable emulsion is thus formed when the surface tension of the liquid is not great enough to withdraw the film from between the globules

and when the stabilising agent has a great internal viscosity. The maximum viscosity obtainable need not be the optimum viscosity for the formation of the emulsion,[18] and the stability of the foam appears to depend on peculiar specific properties of the film, whilst minor properties concerned in the formation of stable foams are the thickness and vapour pressure of the films and the presence of finely-divided solid material at the interfaces.

In milk, the proteins, *e.g.*, calcium caseinate and albumin, are the agents causing the depression of surface tension. The addition of fat globules will further lower the surface tension, since the proteins surrounding the fat globules must be those which lower the interfacial tension to the greatest extent. The abrupt lowering of surface tension when milk is cooled, or on ageing, has been associated with the solidification of the fat.[2, 19] The attaining of an equilibrium is a slow process, due to the slow orientation of the adsorbed proteins in the shifting adsorption equilibrium.

The Effect of Temperature on Foaming. Between 20° and 30° C. the tendency of milk to foam is at a minimum. Below and above this range the tendency to foam increases. Temperatures above 30° C. cause a rapid increase in the foaming tendency and the stability of the foam is greater than that of the foam formed at temperatures below 20° C. Fat appears to be a stabiliser for the foam formed below 20° C., but that of skim milk above 30 C. is slightly more stable than the foam of either whole milk or low-fat cream.

Correlation of Foaming Tendency with Surface Tension. Although there is a general correlation between surface tension and the tendency to foam, the property alone does not account for the results obtained. This is evident from a comparison of the two properties with change of temperature. The lower surface tension at high temperatures allows for a greater concentration of protein at the air/liquid interface and a decrease in interfacial energy as well as a decrease of the forces exerted by the films. This explains the tendency to foam at higher temperatures, but not the minimum foaming tendency at 20–30° C. and the increase at temperatures below 20° C.

There is no doubt that the tendency to foam at lower temperatures is the result of a variation of those properties of solution associated with viscosity changes ; thus the films appear to be highly hydrated and possess a high vapour-pressure ; the films are thick and therefore drain rapidly. But drier and thinner films are formed at higher temperatures. Denaturation of the proteins further increases their stability.

The range 20-30° C. represents conditions in which hydration is practically nil and the stabilising factors have not come into play.

A large increase of fat stabilises the foam as well as increases the foaming, whilst the region of minimum foaming is extended to 40° C. The study of the action of fat is complicated by the variations due to the solidity of the fat, " piling-up " effect, and the treatment of the system.

EFFECT OF OTHER CONDITIONS ON MILK FOAM. The addition of o·5 per cent. peptone to milk alters the character of the foam. causing the bubbles to coalesce. This is due to the change in character of the films, which are solid in the air phase but re-dissolve in the liquid phase. The addition of gelatin to peptone-milk causes the displacement of the peptone by the gelatin to yield semi-solid films which slowly collapse.

Skim milk which has been churned at low temperature for 45 minutes does not froth when passed through a cream separator ; frothing is not prevented by cooling milk without previous churn-ing. Separated milk, cooled and churned, when mixed with cream, froths as usual when separated. The colloid is thus rendered inactive in whole milk by churning and the greater portion of the foam colloid is in the cream. Frothing is caused by churning skim milk, attended by an irreversible coagulation of the colloid, so that, on removing in this manner the cause of frothing, further agitation has no effect.

The foam on fresh milk is dull-looking with large bubbles, and differs appreciably from the fine, whipped egg-white froth of skim milk.[20]

96. The Whipping of Cream

Whipped cream is a special case of milk foam possessing remarkable stability. Cream is richer than milk in fat and pro-teins. The great viscosity of whipped cream is due to the cell-like structure of the foam with the walls stiffened by denatured protein and almost completely solidified fat. On heating, the fat melts, but some foam structure persists, due to the presence of solid protein.

Increases in the fat-content of cream reduce surface tension and enhance its foaming tendency, but these do not account for the wide variations in whipping properties observed. The stabi-lising action of the fat globules and their aggregates is the main factor involved, and Mohr [21] has shown that cream of the same origin and chemical analysis may possess physical differences affect-ing whipping qualities, according to the state of the fat globules (e.g., solid, liquid, homogenised or clumped). The duration of whipping influences the volume of whipped product, the volume

rising rapidly to a maximum (three minutes) and then falling to the original volume. As the cream stiffens the fat globules show distortion and clumping similar to that of the beginning of the churning process, and additional whipping above the point of maximum stiffness results in the formation of butter. The fat must be in a semi-solid condition in order to obtain the maximum " piling-up " effect. Individual fat globules do not pile up readily to form rigid structures and a slight churning or clumping effect is necessary. This is attained in both cases by lowering of temperature.

Babcock [22] and Dahlberg and Hening [5] have studied the influence of temperature on whipping properties and have found that the maximum capacity for whipping is obtained at temperatures closely approximating those at which clumping is most rapid. They have also found that an increase of fat-content gives increased stiffness and minimum drainage of the whipped cream.

Dahlberg and Hening have found that a good whipping cream yields a smaller volume of product than poor whipping cream. Increase in solids-not-fat content and ageing improve whipping capacity, and factors which generally increase the viscosity of cream (other than homogenisation) improve whipping properties. They state that the surface tension of milk and cream cannot be definitely related to whipping properties under all conditions, but that the general relationship of decreased surface tension with improved whipping properties is true for the normal product.

The various processes to which milk or cream may be subjected influence whipping properties through variations in the dispersibility and clumping capacity of the fat globules. Pasteurisation of cream reduces whipping capacity owing to the breaking up of fat clusters in the process and a partial destruction of the clumping capacity. Ageing of the cream from pasteurised milk will cause clumping and the attainment of a viscous condition. Ageing of cream generally is conducive to clumping and has an effect opposite to pasteurisation.

The effect of the temperature of the milk at the time of cream-separation is an important factor in governing the body of the product. When the fat globules are in a liquid condition a low-bodied cream, which increases but slightly in body on ageing, is obtained, but when the fat is in a semi-solid condition during separating, clumping is encouraged by the agitation and a cream of heavy body results.

The source of the cream is of importance. Large fat globules agglutinate or clump more readily than those of lesser dimensions, thus favouring structural formations to a greater degree. This

can easily be demonstrated by the difference in whipped creams from Jersey and Ayrshire cows.

The ability of fat globules to agglutinate is destroyed by homogenisation, and so the whipping property is markedly reduced, and this is more evident the higher the pressure of homogenisation. Clayton [23] explains this by stating that the newly-created enormous fat/liquid interface adsorbs the proteins to such a marked extent that insufficient protein remains in solution to be available for adsorption at the air/liquid interface created by whipping. The addition of gum tragacanth or gelatin after homogenisation restores the whipping properties.

Reid and Eccles [100] have observed the impairment of the whipping properties of cream by the addition of dry skim-milk powder, since the titratable acidity and viscosity are increased ; there is no effect on the surface tension. Dry skim milk also increases the viscosity and foaming of liquid milk. The substances contributory to foaming are contained in the group of " nitrogen extractives " (residual nitrogen) associated with milk protein.

The results of viscosity determinations on creams are measurements of structural properties of the system ; they are not a measure of true viscosity but of plasticity. The increase in the body of cream on homogenisation is due to the volume increase of the fat-phase through increased adsorption. The addition of sugar, while increasing the viscosity, decreases whipping quality. Viscogen (calcium saccharate) enhances aggregation and whipping quality owing to its being a protective colloid and causing the plasticity to increase, and possibly the formation of colloidal calcium phosphate partly explains its effects.[92]

The increase of cream acidity has practically no effect on whipping quality. The drying of cream destroys completely the whipping capacity because the protein is denatured in the drying process.

The failure of ice-cream mixes, made from butter and dried skim milk, to whip properly has been explained by Watts and Dahle [101] as due to the absence from the mix of the lecithin-protein complex which is present in cream. The addition of 0·5 per cent. of dried egg-yolk or sweet buttermilk is beneficial in restoring whipping properties.

97. Viscosity

The viscosity of a liquid—its internal friction or its resistance to shear, agitation or flow – is measurable in absolute units—the *poise*—which may be defined as the force required to produce a difference in the velocity of flow of a liquid of 1 cm. per second

when this force is exerted on 1 sq. cm. between two parallel planes each 1 sq. cm. in area and 1 cm. apart. Fluidity is the reciprocal of absolute viscosity. The viscosity is usually expressed in centipoises, the standard being that of water at 20° C. (= 1·005 centipoises).

Plasticity differs from viscosity in that in the latter the *shearing stress/rate of shear* is a constant, whilst it varies with the rate of shear in the case of plasticity, and an expenditure of energy is usually required before plastic flow commences.

The heterogeneous nature of milk demands that, apart from the true viscosity relationships, the colloidally dispersed components must be considered from the standpoint of fluidity and plasticity of suspensions. In milk the relationships are further complicated

TABLE LXXXVIII. *Effect of Temperature on the Fluidity of Milk*

Temperature °C	Viscosity of Milk	Fluidity of Milk	Fluidity of Water (approx.)
0	4·28	0·233	0·558
5	3·52	0 284	0·658
10	2·80	0·357	0·768
15	2·41	0·415	0·877
20	2·12	0 473	1·000
25	1·85	0·541	1·12
30	1·64	0·609	1·25

by the fact that variations in temperature will vary the volume of the suspended phase as well as the degree of dispersion of the fat phase. With respect to the fat, it must be borne in mind that below the melting-point temperature the properties of a plastic solid appear, and above this temperature the fluidity of the fat makes the system approach truer viscosity in behaviour.

The casein of milk (or calcium caseinate) contributes more to the viscosity of the fluid than does any of the other constituents.[24, 25] The fat contributes less than the casein but more than the albumin. The relationship fat (F), solids-not-fat (S.N.F.) and viscosity has been found by Taylor[26] to be S.N.F. = $\dfrac{1}{0·177}$ (Viscosity — 0·0665 F.). He finds values for a, 0·00723 and β, 0·000156 in Poisseuille's formula for connecting viscosity and temperature ($N_t = \dfrac{N_o}{1 + at + \beta t^2}$), where N_o and N_t are the

viscosities at o° and t° C. respectively. Milk heated to any temperature below 60° C. shows a decrease in viscosity on cooling to 20° C., whilst milk heated to 70° C. shows an increase in viscosity on cooling.

The values obtained by Soxhlet [27] for milk at various temperatures are given in Table LXXXVIII.

The values for water are also given on the basis of water at 20° having a fluidity of 1. It can be seen that the decrease in fluidity is not a linear function of temperature. Kobler [28] also, together with Soxhlet, has observed that water increases more rapidly in fluidity than milk with rise of temperature, and the former has also found that the agitation of milk has a marked effect. Weinlig [29] has confirmed the effect of heating and finds that heating milk to 60° C. decreases, whilst heating to 80° C. increases, the viscosity of the cooled product. Shaking and heating change the physical state of the colloidally-dispersed phases by either increasing or decreasing the degree of dispersion and thus causing variations in the fluidity values.

Large fat globules have been shown by Babcock [24] to increase viscosity, probably owing to the ease with which they agglutinate and form aggregates. The agglutination is more marked in cream in which structure is produced and friction is increased so that properties of plasticity come into play.

Wiegner [30] and Buglia [31] have found that the increased dispersion of fat, as by homogenisation, increases the viscosity due to the increased volume of the dispersed phase caused by adsorption on an enormously increased fat surface. Increasing the acidity of cream decreases the viscosity appreciably.

A method advocated by Dahlberg and Hening [102] for increasing the viscosity of cream consists in warming old cream to 80° -85 ° F., and cooling slowly to 40° F., the cooling of the last 30° taking about 2 hours ; this viscosity is temporarily lost on heating to 60° F., but gradually returns. Excessive fat-clumping does not appear to occur, but it is suggested that a greater hydration of the proteins takes place. The creaming properties of milk heated very rapidly to pasteurisation temperature and then rapidly cooled are not changed.[103]

No change in the viscosity of milk on adding lactic acid, or by natural souring, occurs until the titratable acidity reaches 0·45 per cent., after which there is a marked increase. The addition of rennet to milk first decreases the viscosity and then increases it rapidly until it curdles. Skim milk increases less in viscosity during cooling than whole milk.[104]

Heat-treatment of skim milk in which the fat phase is practic-

ally absent follows the same trend as that of whole milk and is probably due to a change in the equilibrium of the calcium caseinate system with separation of larger amounts of the suspended phase. In the concentration of skim milk, properties of plastic flow are exhibited by the product when 45 50 per cent. of total solids are present, but if preheated the product shows plastic properties when the content of solids is lower.[32] This may be due to increased concentration of the suspended phase through precipitation of colloidal calcium phosphate or increased hydration. Chorower [33] states that casein is hydrated to a greater extent after the calcium is split off in the heating process and that thickening is due to this cause.

The gelling of condensed milk in storage occurs more readily with milk heated to a high forewarming temperature than with low temperatures of forewarming.

98. The Separation of Cream

The separation of fat in the form of cream is possible because of the difference in density of the milk-fat (0·92 0·94, according to temperature) and the milk serum (over 1·03). van Dam and Sirks,[34] and van der Burg [35] have found that the rise of fat globules in milk obeys Stokes' law, which states that the velocity of ascent of the globules varies directly as the square of their radii.

(Stokes' equation is $V = \dfrac{2r^2(d - d_1)g}{9N}$, when

  V = constant velocity of dispersed phase (globules).
  r = radius of globule.
  d = density of the globule phase.
  d_1 = density of the medium (milk serum).
  g = gravity constant.
  N = viscosity of the medium.)

Hunziker [36] is of the opinion that the principal agent retarding the upward movement of the globules is the viscosity of the milk, which is largely due to constituents of a colloidal nature inherent in milk and cream. If the viscosity of milk were not greater than that of water, the fat globules would rise to the surface instantaneously in a similar manner to oil poured into water. Rahn [37] has shown that this view of creaming, and especially with regard to the viscosity, does not explain all the facts, for he has found that the viscosity of milk can be increased, but the rate of creaming instead of slowing down can be actually accelerated. Thus the addition of gelatin causes a quicker cream-rise, a thicker cream

layer, a looser cream of lower fat-content, a much more complete separation of the fat as cream, and also a skim milk of lower fat-content, in spite of the fact that the viscosity exceeds the maximum for normal milk (2·4). Increasing the viscosity to 5·6 even gives a better yield of cream than the untreated milk. The addition of colloids (gums tragacanth and arabic, peptones, albumin) accelerates creaming, but the increase of viscosity of milk by non-colloids like sugar delays creaming.

THE INHIBITED CREAMING OF HEATED MILK. When milk is heated above 63° C. the capacity to cream is partly destroyed. This was formerly explained as due to the loading of the fat globules with coagulated lactalbumin. But Rahn has found that the creaming property can be recovered by the addition of gelatin or other accelerating colloid after heating. By measuring the rate of ascent of fat globules in milk under various conditions it has been proved that the globules do not rise individually but clump together, and that such clumps possess greater buoyancy. The clumps contain about 50 per cent. of fat and their rates of rise are rapid enough to account for rapid creaming. Raw milk contains a large number of these clumps, whilst heated milk only contains a few, which explains the essential difference in creaming capacity, since heating has been found to destroy the clumping of fat globules.[29] The addition of accelerating colloids, such as gelatin and albumin, restores the clumping effect by forming an adsorbed colloidal envelope which admits of adhesion of the globules on colliding during their quasi-molecular movements. Rahn shows that the added colloid gives a looser cream of lower fat-content (Table LXXXIX).

The adhesiveness of the enveloping colloid is reduced by heat-

TABLE LXXXIX.   *Fat-Content (per cent.) of Accelerated Risen Cream (Rahn)*

Gelatin added %	Nil	0·35	0·70	1·05	1·40
Fat-content	33	30	27	25	23
Gum arabic %	Nil	1·18	2·36	—	—
Fat-content	27	26	25	—	—

ing. The fat globules then rise singly and a thicker cream layer results, but the time taken for creaming is considerably lengthened. The following table (Table XC) by Rahn [20] is of interest as illustrating the above principles.

TABLE XC. *Effect of Gelatin on the Creaming of Raw and Heated Milk*

Time of Cream Measurement	Raw Milk		Milk, Heated at 100° C for 10 mins	
	No Gelatin	1% Gelatin	No Gelatin	1% Gelatin
hrs	mm	mm	mm	mm
$\frac{1}{2}$	3	8	0	0
1	9·5	16	0	1
$2\frac{1}{2}$	11	19	0	3·5
4	13	21	2	5
18	16	28	3	16
48	18	29	5	21
Fat in cream . .	25·8	22·5	31·1	31·2
Fat in skim milk .	1·1	0·3	2·2	0·2
Total fat in cream .	g 4·50	g 6·50	g 1·65	g 6·55
Total fat in skim milk	2·56	0·66	5·39	0·46
Degree of creaming (%) . . .	65	91	24	93

The addition of such colloids as gelatin, starch, Irish moss and gum tragacanth to milk was found by van Dam and Sirks [34] to give a 15–25 per cent. increase in volume of cream, and clumping was postulated. They found no relation between creaming capacity and the contents of calcium and phosphoric acid of the milk ; and the addition of acid or alkali to fresh milk retarded creaming.

Palmer and Anderson state that during the creaming of milks of uniform fat-content the plasma colloids are more important than the fat globules. [38]   Calcium caseinate hinders satisfactory creaming, and this effect is increased by pasteurisation. The whey colloids, on the other hand, are effective promoters of cream-rising. " Both exhaustiveness of rise of fat and greater volume of cream are promoted by the truly hydrophilic colloids and depressed by colloids of hydrophobic properties." Holder-pasteurisation increases the effectiveness of the former but decreases the effectiveness of the latter. Palmer and Anderson thus do not agree with Rahn that clumping of the fat globules is the main factor involved in cream-rising.

Troy and Sharp [39] have published conclusive evidence that clumping of fat globules underlies creaming. They have found that the logarithms of the rate of rise, calculated according to Stokes' law, when plotted against the logarithms of the diameters of the globules give a straight-line graph. The rate of rise of single fat globules has been found to be in accord with Stokes' law. But from the calculated values creaming should occur more quickly at 25° C. than at 5° C.; the reverse, however, is true, so that the explanation is that the globules do not rise singly. By measurement, the rate of rise of clumps of fat globules is found to follow Stokes' law, and the rate of ascent is sufficient to account for the practical experience with regard to rate of creaming and temperature effects. The clumps are of a fragile and varied nature containing from a few to thousands of globules. The clumps contain milk plasma, and the densest clumps about 50 per cent. of fat, whereas the plasma around the clumps is relatively fat-free. The difference between deep and shallow layers of cream is that the former contain irregular stable clumps, whereas the latter contain compact spherical clumps and especially weak clusters. The fat-content of the shallow layer is naturally high. The setting of cream at higher temperatures weakens the clumping effect and permits closer packing, but a deep cream layer results from lower creaming temperatures.

Whittaker and his collaborators [40] have investigated the effect of pasteurisation on creaming. They have found that heating for thirty minutes at 61° C. results in an unchanged cream volume or even a slight increase, whilst heating for the same time at 63° C. causes about 8 per cent. decrease of cream volume, the effect being more marked with fresh than with old milk owing to the initially greater cream volume of the former. Absence of agitation during the holding period, and cooling below 7° C. after pasteurisation, favour a good cream volume. Burri [41] has also found that unheated milk creams more rapidly than heated milk. The boiling of milk very seriously reduces creaming. [20]

Cream as an emulsion requires further study, particularly with regard to the change operating above 61° C. and the effect of added colloids. The films around the globules, the adsorption of milk and added colloids on the fat-globule surface, and the effect of the state of the fat on the behaviour of the globule surface need further investigation.

99. The Mechanical Separation of Cream

Ordinary creaming of milk by gravity was the method of separating milk-fat used before the arrival of the cream separator.

Usually this was done either by allowing the cream to rise on the milk in (a) shallow, or (b) deep cans, and skimming off the cream or running off the skim from under the cream. Both types of creaming caused considerable loss of fat in the skim milk. Diluting milk with a third of its volume of water gives more rapid creaming, but the skim milk retains about 0·7 per cent. of fat.

For commercial practice, gravity-creaming is far too slow, and a constant-flow centrifugal separator is used to multiply the gravity effect mechanically to about 1,000 times and above. The separation of an emulsion of a fat in an aqueous medium is based on Stokes' law, and depends on the difference in specific gravity of the two phases (as expressed by the formula $g(d \quad d_1)$). The velocity of separation is influenced also by the centrifugal force exerted, the viscosity of the aqueous medium, the relation between the mass and surface area of the globules, and temperature. The importance of differences in specific gravity rapidly diminishes also when the diameter of the globule is sufficiently reduced, since the velocity of separation is proportional to the square of the diameter (r^2). The efficiency of separation therefore depends also on the length of time the emulsion is subjected to the centrifugal force.

The separator bowls are so constructed that (a) the formation of a vortex is prevented, (b) the distance the globules have to travel is of least magnitude, and (c) that both separated milk and cream leave the bowl as near the centre as possible, so that the minimum amount of kinetic energy is lost in the process. The bowls are in the main of two types, those with the inverted funnel-type discs and those with sectorial-arc discs. The milk is fed by gravity from a funnel with a constant-flow device to the base and centre of the bowl, and passes through slots in the base of a central shaft along gutters which extend over the walls of the hypothetical cream-cylinder in the bowl. It is thus delivered into a neutral zone without interfering with the cream wall, and is passed upwards into the separating discs where the separation takes place. Some of the smaller globules are carried onwards by the larger ones and the clumps when the cream moves inward to form the cream-wall around the distributing shaft and mounts upwards to the cream outlet. The skim milk moves outwards and upwards along the wall of the bowl to its discharge outlet.

Under the influence of centrifugal force the time required for a globule of radius r to travel through a given distance is proportional to r^j, and as the number of gallons treated in a separator per hour is inversely proportional to this time, it follows

that for any given radius of globule there is a critical value at which its velocity against the stream of milk is equal to the velocity of the stream itself, so that the globules of smaller radius pass out with the separated milk. The fat-content of the separated milk is proportional to the cube root of the square of the number of gallons per hour.[42]

Cream separators usually run at 6,000 r.p.m. The intensity of the centrifugal force exerted is proportional to the mass of the contents of the bowl, to the diameter of the bowl, and to the square of the speed of revolution. The ratio of volume of cream to that of separated milk depends on the relative distances of their respective outlets from the centre of the bowl. The cream outlet is in the form of an adjustable screw, which may be screwed in towards the central axis of the bowl for a smaller volume of high-fat cream or outwards for a larger volume of low-fat cream.

Cream separators are made in standard capacities, i.e., the quantity of milk which can be separated per hour depends on the flow and the centrifugal force. Any increase over the optimum capacity lowers the efficiency of the separator owing to a shorter period for the centrifugal force being brought to bear on the contents of the bowl, which means loss of butter-fat in the separated milk.

The temperature of cream separation is best at 29-35° C. (84 95° F.), below which the viscosity of the milk increases and the fat-globule separation is influenced by the greater resistance offered to separation. The cream is richer, since the milk flows at a slower rate through the machine, but the separated milk carries away more fat. The higher temperature is to be preferred, owing to the higher coefficient of expansion of fat over the water still further magnifying the term $g(d - d_1)$. The richer a milk is in butter-fat the richer the cream, and the greater the loss in the separated milk. This loss is proportional to the fat-content of the whole milk.

Sharp [43] has shown that the rate of movement of a fat globule through the milk plasma due to centrifugal force at any instant is given by the equation : —

$$v = \frac{0 \cdot 00244(d_p - d_f)\, r^2 n^2 k}{N}, \text{ where}$$

r == radius of the globule ; N = viscosity of the milk plasma ; d_p and d_f are the densities of plasma and fat respectively ; n == number of revolutions of the separator bowl per minute, and k = distance of the fat globule from the axis of rotation. If all

the factors in the equation independent of size of globules and speed of separator are covered by a constant k, then

$$k = \frac{0 \cdot 00244(d_p - d_f)}{N}.$$

The effective force tending to the separation of the fat globules depends on the magnitude of k and is more effective the greater the difference in the density of the phases and the lower the viscosity. Both factors are influenced by temperature. The most marked differences in phase densities, for instance, are between $5°$ C. and $35°$ C., and the viscosity decreases rapidly up to about $40°$ C., so that k increases rapidly up to the range 35–$40°$ C., after which it is not so pronounced.

Rahn concludes that globules of less than $1\,\mu$ diameter are not removed during separation, whereas those from 1–$2\,\mu$ are only slightly affected. Most of those of dimensions 2–$3\,\mu$ and practically all those above $3\,\mu$ are in the cream.

100. The Fat Globules of Milk

In fresh milk which has not been agitated the fat occurs as individual globules ranging in size from $0 \cdot 1\,\mu$ to $10\,\mu$, the average

TABLE XCI. *Average Size of Fat Globules in Milk from Cows of Different Breeds* [41]

Breed	Average Diameter of Globules			
	Van Slyke [44] Relative Size	Woll [44] μ	Gutzeit [47] μ	Schellenberger [48] μ
Jersey	956	4·05	3·50	2·95
Guernsey	717	3·71	—	—
Holstein	420	—	2·58	2·30
Ayrshire	421	—	—	—
Holderness	428	—	—	—
Devon	375	—	—	—
Shorthorn	—	3·46	2·76	—
Brown Swiss	—	—	—	2·33

approximating $3\,\mu$, whilst the number is of the order of 2 to 4×10^{12} globules per ml. of milk, but naturally depends on the size of the globules and on the fat-content of the milk. In normal milk of high-fat content there is a greater number of the larger globules than in normal milk of low-fat content. The size of the globules varies with different factors : (1) breed of cow ; the Channel

Island breeds give milk in which the globules are larger than in milk from other breeds ; (2) lactation period ; the size of globule decreases with advance in lactation ; (3) the type of foodstuff consumed ; small globules arise from dry feeds whilst succulent foods yield large globules ; (4) individuality of cow, and (5) health of animals. In considering any figures, therefore, these factors have to be borne in mind. Table XCI gives the average size of fat globules in milk according to the breed of the producing animal.

TABLE XCII. *Percentage of Total Fat in Groups of Globules of Various Sizes (Van Slyke)*

Breed	Average Diameters in μ					
	< 2·4	2·4–4·8	4·8–7·2	7·2–9·6	9·6–12	>12
Jersey . .	0·1	11·3	26·1	30·7	23·9	7·9
Guernsey .	0·1	11·3	33·2	29·7	25·7	—
Devon .	0·1	23·0	42·5	34·4	—	—
Holderness .	0·3	24·7	40·1	27·6	7·3	—
Ayrshire .	0·3	34·0	41·6	17·8	6·3	—
Holstein ⎱ Friesian ⎰ .	0·3	38·3	50·1	11·3	—	—

Table XCIII shows that the fat globules increase in number and decrease in size with advance of lactation except for a tendency for the reverse relationship to occur during the initial months.

The type of feed influences the size and number of the fat globules of milk. Woll [46, 49] has found that dry feeding affects

TABLE XCIII. *Average Relative Number of Globules during each Month of the Lactation-period (Van Slyke)*

Month of Lactation	Jersey	Guernsey	Holstein	Ayrshire	Holderness	Devon
1	49	61	—	62	—	68
2	53	46	53	66	67	61
3	45	52	67	70	68	99
4	86	76	132	85	87	77
5	56	64	95	93	79	152
6	76	70	66	94	104	144
7	62	103	94	117	111	186
8	80	89	123	140	94	241
9	83	120	132	168	116	225
10	103	104	168	162	106	211

the physical conditions in the fat globules in a specific manner, that is, in causing smaller globules to occur, but Gutzeit [47] does not confirm his findings. Hunziker,[50] on the other hand, has found that dry feeding produces small fat globules and that succulent feed causes large fat globules, as shown by pasture and silage feeding or the inclusion of oil-rich foods in the ration. The influence of such feeding on the unsaturation of the fat may have
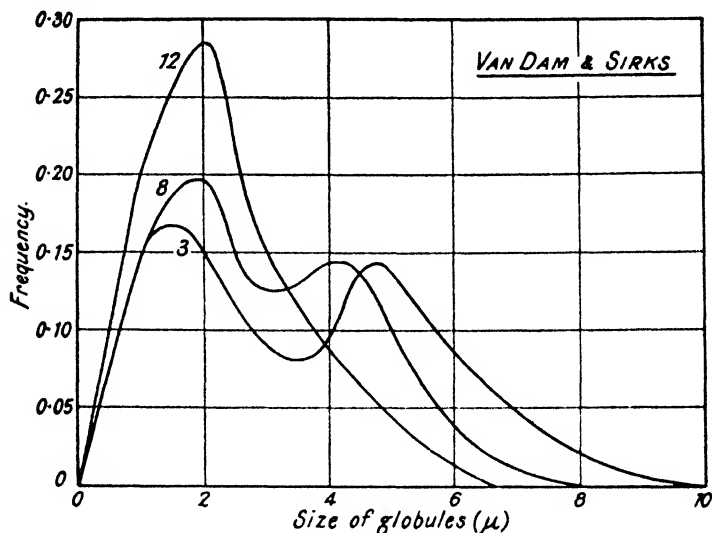
FIG. 14.—Frequency-distribution curves for the sizes of fat globules in milk at three stages in the lactation period. The figures on the curves denote weeks after calving.

a direct bearing on the size of the fat globule of milk : the more unsaturated the fat, the greater the size of fat globule.

The size-frequency distribution of fat globules in milk has been investigated by several workers.[52] Beckett,[51] in work on whole milk, separated and pasteurised cream, concludes that many large fat globules have been formed during the separating and pasteurising while the original distribution of small globules remains unchanged.

Van Dam and Sirks,[34] and Rahn,[53] have determined the distribution of the fat-content of milk according to numbers and sizes of globules (Table XCIV).

It may be observed that there is good agreement inasmuch as in the size-frequency curve the maximum is at about 2 μ, a size at which most of the volume of fat is also accounted for. Van Dam and Sirks find that the frequency curve for the various

sizes of globules in the milk from newly-calved cows shows two peaks (at $2\,\mu$ and $4 \cdot 5\,\mu$), and that these two peaks merge into one as the lactation-period progresses.

Lyons and O'Shea [105] have observed that the number of fat globules below $2\,\mu$ in diameter increases, and that of the globules above $4\,\mu$ decreases as the stage of lactation advances. An under-

TABLE XCIV. *Group-Distribution of Fat Globules in Milk* (Fig. 14)

Van Dam and Sirks Average Diameter of Globules	Relative Number in Groups	% of Total Number	Relative Amount of Fat in Each Group, % (calc.)
μ			
0·38	51	6·4	0·017
1·14	170	21·3	1·58
1·90	267	33·4	11·46
2·66	142	17·7	16·74
3·42	103	12·9	25·79
4·18	40	5·0	18·28
4·94	18	2·2	13·58
5·70	5	0·6	5·80
6·46	4	0·5	6·75
Rahn (Calculated)			
0–1	47·7	7·95	0·023
1–2	149·1	24·85	1·90
2–3	146·7	24·45	8·65
3–4	129·0	21·50	20·60
4–5	72·9	12·15	25·10
5–6	38·7	6·45	24·45
6–7	11·1	1·85	11·40
7–8	3·6	0·60	5·60
8–9	1·2	0·20	2·30
9–10	0·0	0·00	0·00

estimation of the fat in the Gerber method by $0 \cdot 1$ per cent. can occur with milk in advanced lactation, and spinning three times is advisable.

101. Change in the State of Aggregation of the Fat Globules

On agitating milk there is a tendency for the fat globules to adhere to one another to form aggregates (clumping) and the degree to which this occurs depends on the temperature, acidity, the fat-content and its degree of dispersion, the degree of agitation and the fluidity of the system, the latter three determining the

probability of collision of the globules. Temperature affects clumping owing to difference in the conditions on the fat-globule surface and the influence of the condition of the fat inside the globule on the properties of its surface. Agitation of milk or cream at temperatures above the melting-point of the fat promotes the formation of a relatively small number of large globules and may cause subdivision of globules.[54]

In clumping, each globule retains its structure although some distortion may occur. Clumping can occur over a wide range of temperature, although Rahn [53] finds that rise of temperature favours subdivision more than clumping and that above 63–65° C. there is no aggregation. Weinlig [29] is of the opinion that a time

TABLE XCV. *Relative Number of Fat Globules of Various Sizes and Relative Distribution of Fat in the Whole-Milk fractions (Rahn)*

Globule Size μ	Whole Milk, 3% Fat		Skim Milk, 0·13% Fat		Cream, 40·5% Fat	
	Relative No. of Globules	Fat distribution %	Relative No. of Globules	Fat Distribution %	Relative No. of Globules	Fat Distribution %
0–1 ⎫	—	0·002	—	0·002	—	0·009
1–2 ⎬	418	0·072	592	0·053	357	0·450
2–3 ⎭	—	0·390	—	0·042	—	2·000
3–9	182	2·540	8	0·033	231	23·570
>9	0	0·000	0	0·000	12	14·500
	600	3·000	600	0·130	600	40·520

factor is involved, and that 75° C. is the temperature at which aggregation ceases, although holder-pasteurisation temperature for 30 minutes will have the same effect. Below this temperature, clumping increases until it is at a maximum at 7–8° C.

Agitation of milk at the higher temperatures in preheaters, pumps or other equipment results in a higher degree of dispersion and smaller globules. Agitation at lower temperatures promotes clumping. The " packing " of the globules in cream promotes a greater degree of clumping than occurs in milk and the product contains a large number of large globules (over 9 μ). Table XCV (Rahn) shows the coalescence of globules during cream-separation.

102. Homogenisation of Milk and Cream

Homogenisation is the term applied to the artificial or mechanical subdivision of the fat-phase in an emulsion to a smaller and more

uniform size of globule. The practice is important in dairy work, since there is a demand for homogenised milk, whilst sterilised milk is wholly homogenised to avoid the formation of cream plugs in the narrow necks of the bottles used. The homogenisation of cream, especially coffee cream, is widely practised, whilst reconstituted cream is in essence a homogenised cream. The preparations of emulsions of various oils in separated milk for margarine manufacture is essentially homogenisation.

The usual methods of obtaining emulsions consist of (a) colloid mills, (b) high-speed rotors, and (c) the valve-pattern homogenisers. The first two types are not generally used for milk products, although in some processes for reconstituting milk and cream on a small scale the second method is employed. For large-scale

TABLE XCVI. *Size-Frequency Analysis of Fat Globules of Homogenised and Skim Milk. Influence of Temperature (Rahn)*

Diameter Range of Fat Globules μ	Percentage of Total Fat				
	Homogenised Whole Milk	Skim Milk	Homogenised at		
			20° C.	40° C.	65° C.
0–1	1·2	1·7	2·3	1·9	4·3
1–2	83·6	40·6	29·3	36·7	74·4
2–3	15·2	30·8	23·3	21·0	9·0
3–4	0	13·4	29·8	25·2	12·3
4–5	0	8·0	0	15·2	0
5–6	0	6·0	15·4	0	0

dairy work, the valve-pattern is almost universally used. It must be understood that homogenisation, technically speaking, is a relative term, and the force required increases enormously as the size of the globules diminishes.[55]

Homogenisation developed initially from the process of forcing the ingredients to be emulsified through fine orifices.[56, 57] Gaulin,[58] whose homogenisers are used extensively for milk work at the present day, first conceived the idea of homogenising already existing emulsions, such as milk, so as to obtain greater stability. He used a sheaf of capillary tubes with a spring-loaded concave valve-ball engaging against one end. A pressure of about 3,500 lb. per sq. in. was required to force milk (at 85° C.) through the tubes, whereby satisfactory homogenisation was achieved.

In the modern Gaulin homogeniser, the pressure is worked up by a duplex pump against an agate valve of the relief type, which

can be variably loaded up to 3,000 lb. per sq. in. When the valve is forced open in working, the milk is driven at enormous speed through the microscopically narrow clearance, so that the fat globules are comminuted to a degree depending on the pressure employed. The shearing action due to the great velocity with which the milk flows through the valve, and the atomising action due to the milk impinging on the walls of the valve, are the main causes of the subdivision of the fat globules. The greater the angle of impact the more pronounced is the atomising action ; thus the maximum effect is when the ground-surface is as nearly as possible at right angles to the adjacent wall.

Milk is always homogenised when warm, since the higher the temperature, the finer the homogenisation. Table XCVI gives the size-frequency analysis of homogenised milk (compared with skim milk) and the influence of temperature (Rahn [20, 53]).

The best test for homogenisation is to allow the samples to stand for a definite length of time and then to determine the fat at different levels. Very little evidence of creaming should be

TABLE XCVII.   *Fat-Contents of Various Strata of Homogenised Milks.   72 hours' standing (Sobbe [59])*

Sample	A (2 6% Fat)		B (3 3% Fat)	
	Raw	Homogenised	Raw	Homogenised
Fat in lower 50 ml. .	0·3	2·3	0·2	2·95
,, middle ,, .	1·4	2·5	0·6	3·20
,, upper ,, .	8·5	2·9	14·5	3·85

observed in well-homogenised milk. Table XCVII gives an example of such a test, creaming being allowed for 72 hours.

Separation by centrifuging has little effect on homogenised milk. Separated milk from the *raw* liquid contains only from 3·8 to 7·3 per cent. of the total fat, whereas the similar fraction from homogenised milk retains from 73 to 88 per cent.[60] Skim milk cannot be further homogenised and homogenised milk cannot be churned. Homogenised cream cannot be whipped unless a colloid like gum tragacanth is added to ensure a permanent foam. The fat globules show a pronounced Brownian movement and the surface tension decreases.   All these changes are due to the increased adsorption of caseinate on the fat globules, since the surface of the fat increases enormously. Homogenised milk and cream show a considerable increase in viscosity, which is another manifestation of protein adsorption. Thus Wiegner,[61] reducing the average

diameter of fat globules from 2·9 to 0·27 μ, calculated from viscosity measurements that the amount of casein adsorbed rose from 2·27 per cent. in the raw sample to 25·2 per cent. in the homogenised sample. (It was assumed that casein only was adsorbed and that the thickness of the adsorbed layer was 6·8 $\mu\mu$.) Briggs [62] has also demonstrated that adsorption is the main factor involved in emulsion-formation, both as regards stability and fluidity.

TABLE XCVIII. *Change in Diameter and Number of Fat Globules during the Homogenisation of Milk (Wiegner)*

| Sample | Fat % | Diameter of Fat Globules | | No of Fat Globules per 100 ml. | | Times Increase in Number | Increase in Surface Area | Viscosity Increase |
		Normal	Homogenised	Normal	Homogenised			
		μ	μ					
1	3·17	2·86	0·27	$2·87 \times 10^{11}$	$3·41 \times 10^{14}$	1190	1188	1·12
2	2·87	2·94	0·17	$2·40 \times 10^{11}$	$3·02 \times 10^{14}$	1290	1258	1·15

Wiegner reports no change in density and electrical conductivity, but a slight decrease in osmotic pressure after homogenisation. On the other hand Buglia [63] states that a slight rise in conductivity occurs.

The term " viscolising " is synonymous with " homogenising," especially in the treatment of cream. The practice of viscolising cream separated from milk and then mixing the viscolised cream with the separated fraction causes the formation of a greater volume of cream, naturally of lower fat-content. Homogenised

TABLE XCIX. *Cream Volume on Raw Milk and Milk made from Viscolised Cream and Skim Milk (Martin and Combs)* [64]

| | Temp. °C. | Per cent of Total Volume as Cream after Standing | | | | | | | | | | | |
		1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	8 hours	10 hours	12 hours	14 hours	16 hours	18 hours
Raw, 4% fat	50	10	11	11	11	11	11	12	12	12	13	13	14
	35	10	13	18	21	21	21	21	21	21	21	20	20
Pasteurised milk made from viscolised 40% cream to skim milk (4% fat)	50	10	25	26	26	26	25	25	24	24	23	22	22
	35	0	0	31	31	31	31	30	29	29	29	28	27

whole milk shows only a thin layer (about 2 per cent.). Table XCIX gives the depth of cream layer with time for raw milk and homogenised cream added to separated milk.

The skim milk from both types of milk has roughly the same fat-content, so that there is only a small tendency for the finest globules to remain in the serum. There is a decrease in size of clumps, but the smaller clumps contribute more towards a greater cream volume than would the large clumps. On pasteurising, the cream volume is still greater in the viscolised sample than in the unpasteurised sample. It is not the fat-content of the cream, but the volume of cream for viscolising, which is important.[65] Homogenisation sets up a structure of some sort among the fat clumps in cream, and the volume of this structure depends on the available space at the time of homogenisation, and this volume is preserved to a certain extent on dilution of the cream with skim milk. There is no doubt that homogenisation of cream, already rich in adsorbed milk proteins, further increases the protein adsorption on the greater fat/aqueous phase interface, and if the concentration is sufficiently high the adsorbed protein may be in a " precipitated " form, as is the case in foam structure. This structure will cream as a whole owing to its lower density when mixed with milk. No data are available concerning the gas-content, if any, of viscolised cream or milk.

Trout [106] has observed that homogenisation pressures of more than 1,500 lb. per sq. in. are unnecessary to prevent a cream-layer forming. Homogenisation has no effect on specific gravity, but increases the foaming tendency of raw milk and decreases that of pasteurised milk. A decrease in viscosity is observed after homogenising whole milk. If the cream from milk homogenised at 1,500 lb. per sq. in. is separated centrifugally, the loss of fat in the skim milk does not exceed 30 per cent. Homogenised cream churns more completely when ripened than when not. On freezing homogenised milk, there is very little separation or " oiling off " of the fat.

Hening [66] and Doan [67] have found marked clumping of fat globules in mixtures of viscolised cream and skim milk when the fat-content exceeds 10 per cent. The factors generally connected with clumping are (a) fat-content, (b) homogenising pressure, (c) heat-treatment of the plasma, and (d) acidity. Clumping begins when a pressure of 2,000 lb. is developed, and increases in a marked manner with higher pressures.

The heating of milk destroys the creaming capacity,[68, 69] and the effect is more pronounced if the milk plasma is heated. The effect of the heating of milk plasma on the degree of clumping of

subsequently homogenised mixtures has been investigated by Doan. Raw skim milk was divided into two parts, one of which was heated to 82° C. for ten minutes. Fresh cream containing 53·4 per cent. of fat was standardised to 12 per cent. fat, using the raw skim milk and also the heated skim milk. Two other mixtures standardised to 13 per cent. fat were also prepared. These mixtures were homogenised at 38° C. A reduction in fat-clumping in the two mixtures containing the heated plasma was observed.

The effect of change of acidity is slight, so that it can be concluded that fat-clumping is only slightly associated with the electric charge on the fat globules.

103. The " Feathering " of Cream

Some homogenised cream shows the undesirable property of precipitating or " feathering " on the top of hot coffee or tea. It has been found that increasing the pressure of homogenisation of milk reduces the time of coagulation at 120° C. Webb and Holm [70] have found that the thermal stability of homogenised cream depends mainly on its acidity. The effects of pressure in homogenising and the acidity work together in that the greater amount of adsorbed protein tends to coagulate at high acidity. Whittaker [71] observes that feathering is promoted by salt balance, by coffee which is concentrated, by prolonged extraction of the coffee grounds, by the use of small quantities of milk or cream in the coffee and the slow addition of such quantities.

Doan [72] states that fresh unhomogenised cream rarely feathers, and that acidity and homogenisation favour thermal coagulation, especially as the fat-clumping associated with the latter factor is favourable to precipitation. Homogenising so as to reduce fat-clumping reduces the tendency to feather, and it is recommended that cream of low acidity, pasteurised at 160–5° F. for thirty minutes, or flash-pasteurised at 175° F., be homogenised at the pasteurising temperature. If the two-stage homogeniser is used, it is recommended that the first stage should be at 1,000 lb. pressure, with the second valve at a lower pressure. The homogenisation is thus carried out in the first stage and the breaking up of the fat-clumps in the second. The addition of 0·025 -0·100 per cent. sodium bicarbonate or disodium phosphate (Na_2HPO_4) is recommended as a last resource. Preliminary observations on test samples of a batch should be made in order to determine the exact quantity to be added.

The two-stage homogenisers have come into general practice of late as a means of overcoming the drawbacks due to fat clumping. The first valve causes intense subdivision of the fat globules,

and can be worked up to a pressure of 5,000 lb. (although 1,000–1,500 lb. is the general practice for cream), and the second valve, which disperses the clumps, is worked at 1,000 lb. or less.[73] The second valve can also be set to regulate the viscosity of the homogenised product.[74]   A single valve with a breaker-ring attached enables the two operations to be carried out in one valve. (The Duo-Visco valve.[75])

104. Churning

If cream or milk is agitated at temperatures which favour the aggregation of fat globules, progressive growth of the clumps occurs, and when the point is reached where the ratio of the area of the clumps to their volume is relatively small, the emulsion breaks and the fat separates as an inverted emulsion. The progressive clump growth is accompanied by an increasing viscosity until a condition of pronounced rigidity is reached (cream " going to sleep "). The air beaten into the cream forms a foam which assists the resistance of the contents of the churn to agitation. When air is blown through milk, the fat is concentrated in the foam. This shows that the fat globules concentrate (and aggregate) in the air/liquid interface. The formation of a foam thus hastens aggregation, since the gas/liquid interface serves as a medium for the bringing together of the fat globules. Owing to the more pronounced effects of small radii of curvature, small air bubbles are more effective than the larger ones. The smaller bubbles are also more stable. When the aggregates join together, the globules which are sufficiently small are incorporated into the aggregate. During the agitation small water-globules are also formed. These are stabilised by protein and fat globules collected in the interfaces, and are also incorporated into the butter.

The composition and yield of butter and the ease of churning are influenced by a variety of factors in the cream, such as temperature, acidity, fat-content, size of fat globule and the viscosity, whilst the extent of fat-clumping [76] and the method of churning are also of importance. Babcock has shown that cream with large fat globules churns easiest, whilst Van Slyke [45] has found that milk from breeds giving large fat globules is the easiest to churn, and that churning is prolonged with advance of the lactation-period.

The lactic acid produced during the ripening of cream decreases the viscosity, and churning is easier. If the acidity is too high, casein is precipitated, and the butter will contain clots of casein which cannot be washed or worked out. Churning is usually

carried out at temperatures from 13–18° C., the lower tempera-
ture being used in warm weather to give the butter a better
consistency. The initial temperature of the cream and its fat-
content have, however, no effect on the size of the butter agglo-
merates formed. The freezing of cream at the usual temperature
before churning has been found greatly to accelerate churning,
probably because the adsorptive properties of the subsequent
almost solid fat globule are diminished.[77]

WATER-GLOBULES IN BUTTER. Numerous water-globules,
varying from 1·5 to 90 μ in diameter, can be observed in butter.
Boysen [78] gives the following distribution of globules in 1 ml. of
butter according to size (Table C) :—

TABLE C.   *Water-Globules in Butter (Boysen)*

Average Diameter μ	Number per ml.	Average Diameter μ	Number per ml.
1·9	12,905,700	40·0	57
4·0	230,490	50·0	25
7·5	61,469	60·0	12
12·5	10,740	70·0	8
20·0	628	80·0	5
30·0	139	90·9	4

Storch [80] states that a cubic millimetre of butter contains
2–15 million water-globules having diameters less than 10 μ,
and 4–6 thousands with diameters above 10 μ. Rahn and
Boysen [81] have found that butter contains 12–18 billions of water-
globules per gram, the majority, which account for 7 per cent.
of the moisture, being under 15 μ in diameter. Two per cent.
of the moisture is present as larger globules (15–100 μ in diameter),
and the remainder of the globules, which are over 100 μ in size,
account for the remaining 4 per cent. of the moisture.

King [79] grades the fat globules (100) which are also present in
butter from observations on ten samples as in Table CI,
page 268.

THE STRUCTURE OF BUTTER. There is general agreement that
butter is a solid system of a continuous fat-phase in which are
dispersed globules of fat, water and air, each type being stabilised
by an envelope of hydrated proteins.[82] The general emulsion
type is that of water-in-oil. The continuous medium is semi-
solid butter-fat.

The physical structure of butter must not be disregarded in
problems relating to the keeping quality of butter ; it is of

paramount importance, since the enormous surface of fat exposed at the air and water interfaces can alter and accentuate considerably the courses of deterioration experienced with the rendered or pure fat. King [83] has investigated the relationship between the structure of butter and the chemical changes in the fat. He concludes that chemical and bacteriological changes occur at the fat/water interfaces. The greater area in contact with an aqueous solution is that of buttermilk in which a greater amount of spoilage occurs than in contact with the wash water. Metals dissolved from equipment are also concentrated in the hydrated protein layer, so that spoilage from this cause is greatly enhanced in butter.[84]

THEORIES OF CHURNING. Opinions have greatly varied regarding the exact changes which occur when milk-fat is churned

TABLE CI. *Size-frequency of Fat Globules in Butter* (*King*)

Average Diameter μ	% of No. of Globules
2·6	8·45
3·9	49·55
5·2	33·80
6·5	6·95
7·8	0·85
9·1	0·275
10·4	0·125

into butter. Storch [85] has suggested the existence of a mucoid substance enveloping the fat globules (" slim-membran "), and that the process of churning consists in the rubbing off of this membrane, thus allowing the globules to coalesce. Fleischmann [86] has regarded the process as being due to the solidification of super-cooled fat-globules. This theory is untenable, since the fat-globules will solidify by mere cooling.

Two outstanding theories exist at present : (*a*) the foam theory, and (*b*) the phase-inversion theory.

(*a*) THE FOAM THEORY. The churning of cream causes the incorporation of air and foaming. The foam collapses suddenly, and the butter nuclei grow to macroscopic size. Before the cream collapses all the fat has been shown to be accumulated in the foam, whilst the serum is poor in fat. Cream from churned milk which has lost the property of foaming will not yield butter. Rahn [87] has proposed the theory that there is close packing of

the fat-globules in the froth *lamellæ* in the fat-rich foam of churned cream, finally leading to fat clumps. The protein in the *lamellæ* gradually assumes a solid character until further churning destroys the structure and the foam collapses. Later the fat globules gradually unite until they approach macroscopic size. Mohr and Brockmann [5] have found that there is a common origin of these two phenomena, namely, the precipitation (irreversible) of the foam-colloid and the coalescence of the de-stabilised fat globules after this precipitation. Rahn's theory holds for all conditions of the fat, semi-solid or liquid, and foam-formation and precipitation of the foam-colloid occurs at higher temperatures than 13–18° C. This is borne out by practical experience. A maximum limit of 34° C. is fixed by the mechanical breaking down of the newly-formed butter nuclei. For instance, churning at 50° C. for two hours completely homogenises the fat in new milk, and the cream will not separate when centrifuged.[88] Churning at low temperatures, when the fat globules are solid, does not yield butter owing to the non-cohesion of the globules, although there is a tendency to clump.[87] It is obvious that a liquid fat/serum interface must obtain. A full churn with no air-space will not yield butter even after two hours' churning.[20]

(*b*) THE PHASE INVERSION THEORY. Fischer and Hooker [89] consider milk as an emulsion of fat in hydrated protein, and the tendency to form butter is enhanced by the dehydration of the protein. " Since the hydrated colloids tend to collect in the surface layers between the fat particles and the aqueous phase of the cream, efforts are made to break these layers, and so to hasten coalescence of the fat droplets by churning. The combined efforts therefore bring about a progressive increase in the concentration of the oil with a decrease in the concentration of the hydrated colloid until the instability of the oil-in-hydrated-colloid emulsion becomes so great as to ' break ' and yield the hydrated colloid-in-fat emulsion which we call butter." Palmer [90] supports this theory from observations on the inversion of emulsion type by staining the fat globules with oil-soluble dyes before churning. By means of electrical conductivity measurements he has found that the resistance of cream during churning increases to a maximum, remains constant for thirty minutes, and then suddenly drops to its original value.[91] The point of maximum resistance is identified with the completion of the gradual inversion of the phases, whilst the release of the butter-milk following the " breaking " of the butter restores the conductivity.

Clayton [92] criticises this theory stating that true inversion of the emulsion type is not indicated in the formation of butter. He considers churned cream with its butter granules as a suspension of loose fatty granules within which the aqueous medium is enmeshed, and that the homogeneous mass known as butter is the result of the mechanical pressing together of the granules. Water-globules, owing to the incomplete separation of butter-milk, are entrained and the resemblance of butter to a water-in-oil emulsion is accidental.

The foam theory affords a better explanation for the loss in instability of milk or cream emulsions on churning, and why they " break," not invert, in the process.

FURTHER OBSERVATIONS ON THE CHURNING PROCESS. Pedersen [93] has washed cream with water to lower the protein-content before centrifuging, and then lowered the protein-content around the fat globules by bacterial action. Such cream had lost its property of churning into butter. Also an emulsion of *butter-fat* in skim milk, dispersed to a degree comparable with fresh milk, gives incomplete churning and a product resembling margarine.

Guthrie and Sharp [94] have found in their investigations on the mechanism of churning that decreasing the degree of dispersion (or hydration) of the casein of cream by adjustment of the acidity, *e.g.*, towards the iso-electric point, lessens the time of churning.

Wiese and Palmer [95] have studied the influence of the substances which are adsorbed on the surface of fat-globules on the capacity for churning of artificial butter-fat emulsions. Only the butter-milk emulsion gives normal butter. Casein emulsions can only be partly churned, lactalbumin-lactose gives the best churning, and lecithin emulsions give the best separation of cream. Fresh butter-milk thus contains the fat-globule stabiliser which is lost from the fat globules when the emulsion character is changed during churning.

The presence of individual fat globules in butter may be shown by allowing a thin film of butter to dry off at the edges to form a clear rim. In the rim the delicate outline of the envelopes of the fat globules, which are more or less spherical can be discerned. The work of King [78, 82] supports these findings.

OTHER METHODS OF OBTAINING BUTTER. Converting milk into froth by streams of gas concentrates the fat in the foam from which butter may be obtained by passing through a fine sieve.[96] Boiling cream *in vacuo* at low temperature is a modification of the same process.[97] The passage of cream and air simultaneously through long tubes of fine bore and sieving of

the resulting liquid is another modification of this principle of churning.

The fact that the principle of butter-making rests on the removal of the adsorbed protein-layer on the fat globules is further demonstrated by the following two methods of fat-separation : Alexander [98] facilitates the separation of butter-fat by heating milk under pressure so as to dissolve the casein. The addition of mineral acid, so that a pH of 3 is reached in the serum, turns the casein into the casein acid salt (*e.g.*, casein hydrochloride). The fat can then be removed by centrifuging.[99]

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CHAPTER XIII

PHYSICAL PROPERTIES (*continued*)

105. Electrical Conductivity

THE resistance offered by a regular cube of a conductor having sides 1 cm. long is called the *specific resistance* of the material and its reciprocal is the *specific conductance* or *conductivity*. In a pure solution the conductivity is a function of the ionic concentration, and this depends on the ionic dissociation. Physical conditions such as temperature and viscosity are of importance. The presence of colloidal particles lowers conductivity in that they obstruct, and consequently lower, the amount and rate of ionic migration. When the fat is removed from milk, there is an increase in conductivity undoubtedly due to the removal of obstructing fat globules from the paths of the ions,[1] and in part due to the greater concentration of salts in separated milk. Taylor has found that the conductivity is increased by 11·4 per cent. on removing the fat from milk (of 5 per cent. fat-content), whilst the addition of 5 per cent. of water to the skim milk lowers the conductivity only 3·6 per cent.

There is no relation between conductivity and the contents of ash and solids-not-fat.[1, 2] Coste and Shelborn[2] attribute from 49 to 78 per cent. of the conductivity to chloride ions. The dilution of milk lowers the conductivity but at a lesser rate than expected for dilution without dissociation. Ionic dissociation, therefore, occurs to some extent on adding water to milk.

Owing to the variation in composition of milk, and especially its chloride-content, the conductivity varies over a wide range. Koeppe[3] gives a range of 33·9 to 94·3 × 10⁻⁴ mhos. (average for normal milk, 45–48), Krenn[4] gives 35·1–105·1 × 10⁻⁴ mhos. (average 42) and Plucker[5] gives the range for normal milk as 39·3–56·3 × 10⁻⁴ mhos.

Krenn[52] working on a larger number of samples, further reports the conductivity of milk to range from 38·0–68·4 × 10⁻⁴ mhos., 91 per cent. of samples falling between 41 and 50 × 10⁻⁴ mhos.

The addition of neutralisers and ionisable preservatives naturally increases the conductivity of milk. Roeder[6] has studied the quan-

titative effect of adding to milk sodium carbonate, bicarbonate and phosphate, and traces of borates, benzoates and salicylates.

The increase in viscosity of cream during churning causes a decrease in the conductivity until the cream " breaks," when the original value is recovered.[7] The same effect is observed when air is incorporated into skim milk by churning.

The application of the conductivity method for detecting abnormal milk has been described by Krenn.[4] Milk with a conductivity above 54×10^{-4} mhos. may be considered abnormal.[8] In cases of high conductivity the refractivity of the calcium chloride serum falls well below the normal.

The conductivity of milk is lowest for human and equine milks (16–17×10^{-4} mhos.) and highest for sheep and goat's milk (56–60×10^{-4} mhos.). Milks high in lactose, or low in chloride, have low conductivity, and *vice versa*.[53]

106. Cataphoretic Behaviour of Milk. The Electric Charge on the Fat-Globules

The fat-globules of milk show cataphoretic behaviour in an electric field and the adsorbed casein layer has the effect of modifying the isoelectric point. Mohr and Brockmann[9] have found that the yield of butter-fat by migration to one plate in an electric field varies with the charge on the particle, which can be changed by altering the pH of the milk. The optimum yield of fat lies close to the isoelectric point of the globule (the point of no charge). In pure emulsion the isoelectric point of the fat particle is in a more acid region than for the particle in milk. This is attributed to adsorbed protein.

107. The Colour of Milk. Extinction Coefficient

The white colour of milk is due to the scattering of reflected light by the ultramicroscopic particles : fat-globules, colloidal calcium caseinate and phosphate. The creamy colour of the milk of some breeds, *e.g.*, the Channel Island breeds, and of that of some individual cows, is due to the carotene in the milk fat. The size of the fat globule has an effect on the colour of the milk or the cream, because larger fat-globules reflect a deeper yellow colour at an air/ or glass/milk interface than do smaller globules.

Separated milk has a blue tinge which is best seen by comparison with the overlying cream layer. The depth of the blue tinge is greatest when the separated-milk layer contains the least amount of fat ; the layer will appear more blue by watering milk, owing to a more thorough separation of the fat as cream. Since different

foods have different effects on the separation of the cream by gravity, as shown by (*a*) depth of cream layer and (*b*) completeness of creaming, it is probable that conditions which generally govern cream-separation affect the depth of blue in the separated milk.

The *dead white* colour of pasteurised milk is due to the destruction of the colour by high-temperature processing. The colour is not influenced by the holder method of pasteurisation.

The transmission of light through thin films of milk depends on the casein-content, and the fat phase has little effect.[10] The milk from individual cows shows considerable variation for similar casein contents, and it is probable that the state of dispersion of the casein causes the extinction coefficient to vary. Thus it is evident that the principle underlying the gauging of milk quality visually by comparing the white colour of a thin film of milk between a glass slide and a dark background with a standard set of whites, is totally misleading.

108. Refractivity of Milk and Milk Serum

The refraction of light by a solution is an additive function of the molecular concentration of the solute or solutes ; in solution each substance maintains its own refractivity, and the refractive index of a mixture is that of the total of the refractive indices of the substances plus that of the solvent. Density is a similar additive property and the specific refractive index (R_2) is related to density ; the relation is expressed by the Lorenz-Lorentz formula :

$$\text{Specific refractive index (or theoretical refraction constant)} = \frac{N^2 - 1}{d(N^2 + 2)}$$

where N equals the refractive index and *d* the density. This expression is independent of temperature, concentration, and the state of aggregation of the molecules.

The *refraction constants* for milk constituents have been calculated by Wiegner [11] as follows :

Lactose	0·20688	Serum proteins	0·21480
Citric acid	0·1922	Serum ash	0·1377
Water	0·20606		

The refraction constants for water, lactose, serum proteins and citric acid are close to one another, and since these substances constitute the bulk of the solutes in milk serum the addition of small amounts of water causes only a small variation in the refrac-

tion constant of the serum. Wiegner calculates the constant for
serum from that of the constituents thus :

Lactose	.	5·20 (per cent.)	× 0·20688	1·076
Protein	.	0·30 ,, ,,	× 0·21480	0·064
Ash .	.	0·55 ,, ,,	× 0·13770	0·076
Citric acid .		0·10 ,, ,,	× 0·19220	0·019
Water	.	93·85 ,, ,,	× 0·20606	19·339
		100	× R_2	20·574

from which $R_2 = 0.20574$.

The relation between total solids, density and the refraction
constant is given by the following expressions (Wiegner) :

$$\text{Total solids} = 245\cdot36 - \frac{N^2 + 2}{N^2 - 1} \cdot 50\cdot405$$

$$\text{or} = 245\cdot36 - 244\cdot92 \times \frac{1}{d_{4}^{20}}$$

Drost [12] has found the above formulæ to give values for total
solids accurate to within ± 0·20 per cent. Jorgensen [13] gives the
refractive index of milk as ranging from 1·3470 to 1·3515 ; that of
serum is lower (1·3430–1·3443). The higher soluble protein
content of colostrum gives it a greater refractive power.

Owing to the opacity of milk, the refractivity of the serum is
usually determined. The serum is prepared in a variety of ways,
namely, by coagulation of the casein by natural souring or acetic
acid, or by the precipitation of the total protein with copper sulphate
or hot calcium chloride. It is obvious that the sera obtained by
the different precipitants are of different composition and therefore
the results for each method are not comparable, but are comparable
for any one precipitant.

Precipitation with copper sulphate is the most favourable and
combines ease of manipulation with features least objectionable
on other grounds. The proportions of milk and copper sulphate
solution in general use are 4 parts by volume of milk (20 -40 ml.) to
1 of a copper solution containing 71·5 gm. of $CuSO_4.5H_2O$ per
litre. The latter solution is adjusted in strength to give a reading
of 36·0 at 20° C. by the immersion refractometer. The mixture
is well shaken and filtered, the refraction of the clear filtrate being
determined at 20° C.

The instrument used is the immersion refractometer in which
the prism dips into the solution contained in a bath of constant
temperature. Light reflected from a mirror falls on the solution/
prism interface, and the scale is moved until graze-incidence

with a clear-cut shadow-light boundary on the scale is obtained. The instrument is arbitrarily graduated from −5 to 105, corresponding to a refraction of 1·32539 to 1·36640 at 20° C. The refractive index (x) can be calculated from the reading (y) from the formula :

$$y = 2,683x - 3,561$$

The ranges of values obtained from differently prepared sera are : sour serum 38·0–40·0, calcium chloride serum 37·5–41·0, acetic acid serum 39–41, copper serum 37–39.[14] The readings, of course, are the dipping refractometer values, which are always used for this purpose. In the case of the copper serum, few samples exceed 39. Elsdon and Stubbs [15] state that of 1,000 samples of mixed milk, less than 10 gave readings below 37.

The determination of refractivity of the serum has been used as a means of detecting added water in milk. Elsdon and Stubbs (*loc. cit.*) state that the presence of 5 per cent. added water lowers the reading by one unit. The development of acidity increases the reading, and keeping for three days counteracts the decrease due to 5 per cent. of added water. Numerous difficulties arise in the carrying out of the test, namely, (*a*) the refractivity of the serum itself increases on keeping, (*b*) the development of acidity due to keeping of the milk for different lengths of time increases and then decreases the readings, (*c*) the addition of mineral acid increases and that of dilute alkali decreases the readings. Elsdon and Stubbs state that a refraction of less than 37 in most cases is accompanied by a solids-not-fat content of the milk of less than 8·5 per cent. In Tocher's work [16] on 676 samples, 10 gave readings less than 36·1, 37 less than 36·6. These figures are uncorrected for acidity.

For a full description of the test and research into the matter of age, acidity, technique, etc., the reader is referred to the cited paper by Elsdon and Stubbs.[15]

Elsdon [17] states that if the natural sour process is used and the refraction of the whey from the clot is determined, the souring must be carried out at uniform temperature, and corresponding conditions must be observed generally.

Beckel [18] states that the addition of 15 ml. of 17·5 per cent. copper sulphate solution to 30 ml. of milk gives a serum which shows the presence of added water more sensitively than does the calcium chloride serum.

109. The Density of Milk

The *true* or *absolute density* of matter is the mass of unit volume of the substance. *Relative density* is the ratio of the density of

a substance at a given temperature to the density of a standard substance (water) either at the same temperature or, for water, at 4° C., its point of maximum density. On correcting for air buoyancy in the latter case the number obtained is the *specific gravity* of the substance.

Density again is an additive property and is dependent on the amounts of dissolved and suspended matter. In milk the amounts of the constituents of the solids-not-fat vary over narrow ranges and consequently the variation in density of skim milk is of low magnitude. The greatest variation arises from the fat, and hence the variation in densities of whole milk and cream exhibits a wider range than for the fat-free serum.

The temperature of maximum density of milk is at − 0·3° C. (31·5° F.), but this temperature will naturally vary with the content of soluble and fat constituents.

The specific gravity of the constituents of milk are as follows :

Lactose :   1·666 ;   Protein :   1·346
Mineral matter :   5·50 ;   Fat : 0·9355–0·9448 (15° C.)[19]
Solids-not-fat (lactose : protein : ash − 13 : 9 : 2) : 1·616

The specific gravity of skim milk may vary from 1·032 to 1·0365 at 15° C. The density of skim milk at 50° C. varies from
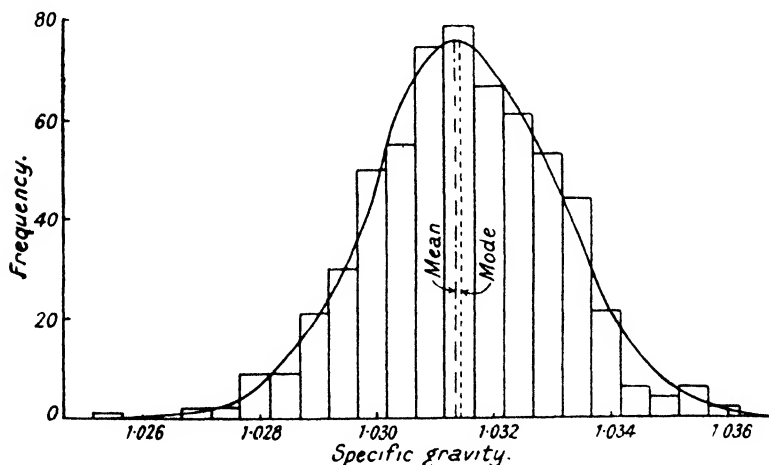
FIG. 15.—Specific gravity distribution. Tocher. 676 samples.

1·0203 to 1·0261, averaging 1·0233. The density of the fat at 50° C. varies from 0·89729 to 0·89897. The density of skim milk thus shows a maximum variation of only 0·56 whilst fat shows 1·30 per cent. variation.[22] The specific gravity of whole milk of

ordinary fat-content varies from 1·028 to 1·034 (average 1·032). Milk from individual cows may vary in specific gravity from 1·0135 to 1·0397. Tocher's distribution [16] for 676 samples is given in Figure 15.

The density of milk also varies with temperature. Whittaker, Sherman and Sharp [21] have found that the density of skim milk falls at a greater rate than that of water (heating from 5° to 80° C. and taking the density at each 5° interval). The lowering of density per 5° interval is also greatest at the higher temperatures (0·0026) than at lower temperatures (0·0010). The specific gravity, D_{milk}/D_{water}, at each temperature decreases gradually. Fleischmann [22] has also observed that the changes of volume of milk with temperature are greater than for water. Variations in hydration of protein may partly account for the difference. The fat also has an effect, since the thermal cubic expansion of milk rich in fat is greater than that of milk poor in fat.

The density of milk is at its lowest about an hour after being drawn. It is advisable not to take the gravity of milk when freshly drawn ; air-bubbles incorporated into the milk by the milking process will give too low a value. On standing for an hour the density slowly increases (Recknagel's phenomenon). This is attributed to (a) changes in the specific gravity of the fat due to cooling and partial solidification, (b) hydration of the protein, and (c) loss of carbon dioxide. Recknagel (v. Richmond,[20] p. 79) has found the rise in density to be regular, to be more rapid at low than high temperature and to amount to 0·001. Vieth has found the increase to be 0·0013, whilst Richmond (loc. cit.) has found it to vary from 0·0003–0·0015 (average 0·0006). Whatever the temperature of standing, the same value is reached finally. Richmond, from observations on the variation of the specific heat of milk on standing, concludes that most of this effect arises from the solidification of the fat. The effect does not vary seasonally but may vary with milk from individual cows or from one bulked sample to another.

Milk-fat is not of constant density. The following ranges of density at the stated temperatures have been found :

Fryer and Weston	.	.	0·936–0·942	15·5°/15·5° C.
Koestler [19]	.	.	0·9355–0·9448	15·5°/15·5° C.
Bell, Thorpe, Richmond	.	0·9094–0·9135	100°/100° F.
U.S. standard butter-fat	.	Not less than 0·905	40°/40° C.
Rahn [23]	.	.	0·8973–0·8986	50°/50° C.
Allen, Richmond	.	.	0·8655–0·8685	100°/15·5° C.

There is no information concerning a seasonal variation in butter-fat density or the relation between unsaturation of the fat and density. It is apparent that such ranges of density are apt to affect the densities of whole milk and cream, and are of great practical importance in determining the percentage of fat by weight in apparatus depending on volumetric calibrations.

110. The Relation between Specific Gravity and the Total-solids Content of Milk

It is clear that there is a relation between the specific gravity of milk and its percentage of fat and total solids. The specific gravity is raised by the solids-not-fat and lowered by the fat. This relation can be placed on a simple quantitative basis with a reasonable degree of accuracy.

Fleischmann [24] has calculated from the mixture law that

$$(1) \quad D = \frac{100 \, np}{np \, (100 - T) + nF + p \, (T - F)}$$

where D is the specific gravity of milk, n and p are the specific gravities of the solids-not-fat and fat respectively, F the fat-content and T the total-solids content. If p and n are constants, this equation would give the necessary relationship. The value of p may be taken as 0·93 and Fleischmann has found that n for a very large number of samples is 1·600734. Substituting these for p and n in (1) the expression

$$(2) \quad D = \frac{1,000}{1,000 - 3·75 \, (T - 1·2F)}$$

is obtained. If $1,000D - 1,000$ is G (the lactometer reading) (2) becomes

$$(3) \quad T = 0·2665 \frac{G}{D} + 1·2F.$$

Hehner and Richmond [20] (p. 88) have deduced the formula :

$$(4) \quad T = 0·254G + 1·164F$$

which is a more convenient form, but less correct, and is deviated from appreciably by milk samples differing from 1·032 in specific gravity. Richmond has later (p. 89) suggested a formula which was more scientifically correct :

$$(5) \quad T = 0·262 \frac{G}{D} + 1·17F.$$

From the exact analysis of a large number of milk samples this formula has been modified to

$$(6) \quad T = 0.2625 \frac{G}{D} + 1.2F.$$

Richmond has expressed this as a simpler formula :

$$(7) \quad T = \frac{G}{4} + \frac{6F}{5} + 0.14,$$

which holds within small limits for a range of D from 1.020 to 1.036. For average milks the formula : Solids-not-fat $= \frac{1}{4}(G + F)$ has been found by Fleischmann to hold approximately. This is deduced from his later expression

$$(8) \quad T = \frac{G}{4} + \frac{6F}{5} + 0.25 \quad \text{(Fleischmann)}.$$

Richmond [20] (p. 89) has evolved a slide rule (Milk Scale) to facilitate the calculation of the total-solid content from the lactometer reading $(1,000D - 1,000)$ and fat-content (or the fat-content from the gravity and total-solid content). For a description of the evolution and mechanism of the scale, the cited reference should be consulted.

Babcock [25] has also suggested the formulæ

Total solids $= G/4 + 1.2F$; solids-not-fat $= G/4 + 0.2F$

for calculating the total solids, etc., from the fat-content and the lactometer reading.

Van Slyke [25] (p. 223) suggests that Fleischmann's formula (1) may be used in the form :

$$n = \frac{T}{T - \dfrac{100 \times 0.3D - 100}{D}}.$$

to determine whether milk contains added water. Fleischmann has observed that n is remarkably constant at 1.6, and if the equation gives figures considerably below this, added water may be suspected.

Richmond [20] (p. 96) from his equation (6 above) deduces n to be 1.616 and p, 0.93. From the specific gravities of the constituents, and using Vieth's ratio (lactose : protein : ash $= 13 : 9 : 2$), the specific gravity of the solids-not-fat also works out to 1.616. The analysis of milk can be usefully checked by calculating the specific volume of the milk from the sum of the

specific volumes of the constituents according to the percentages obtained by analysis. Inverting the specific volume gives the specific gravity, which should approximate to 1·616.

Waal,[51] calculating from the analyses of 227 samples of Friesland milk, has found that the specific gravity of the dry solids-not-fat varies from 1·664 to 1·705, averaging 1·679 for morning milk, and from 1·603 to 1·648, averaging 1·625, for evening milk (average butter-fat content of 2·70 and 4·00 per cent. respectively). From a mathematical treatment of the data the following equation showing the most probable relation between F, G and T.S. has been evolved :

$$\text{T.S.} = 0.2285G + 1.3714F. \text{ (or T.S.} = 0.2285 (G + 6F))$$

that is, the total solid content is proportional to $G + 6F$. It will be observed that the factor attached to F (1·3714) is higher than in any of the above formulæ. Compared with Richmond's formula this equation gives approximately the same result with a fat-content of 4·6 per cent. and with Fleischmann's formula at a fat-content of 5·0 per cent. and with Moeslinger's formula $\left(\text{T.S.} = \dfrac{G + 5F}{4}\right)$ at a fat-content of 5·3 per cent., all these values being high for average milk (3·7 per cent.).

The " closeness of fit " of the above equations in practice depends on the constancy of the specific gravity of the fat and solids-not-fat, according to the average values taken from calculation. Seasonal variations, especially in the specific gravity of the fat, may cause an appreciable difference between calculated and determined values. The physical condition of the fat in milk may vary according to the temperature and length of time of keeping before analysis, which may be reflected in the specific gravity. Thus a solid fat would have a higher specific gravity than liquid or semi-solid fat, whilst seasonal variations of the unsaturation of the fat would show differences in the rates of solidification of fat when stored always under the same conditions. Bartlett, Golding and Wagstaff [26] have observed that the calculated figures for solids-not-fat agree better with the determined values in summer than in winter. In winter the calculated figure is generally higher than that determined by the gravimetric method. The calculated figures of the evening milk also are higher relative to the true solids-not-fat than those for morning milk. It has been found that when milk is kept at 40° F. the specific gravity increases one lactometer degree in fourteen hours, but when kept at 60° F. the increase is only 0·5 degree in the same length of time.

The difference between calculated and gravimetric values are also in part due to changes in gravity of the solids-not-fat, this being very marked when cows are in an advanced stage of lactation. Differences ranging from + 0·5 to − 0·4 per cent. are possible, and it is obvious that the above formulæ are of use for calculation only when cheapness is more important than accuracy.

111. Volume-changes with Temperature. Data for Calculation

In practice, the specific gravity of milk is either determined at 60° F. or is corrected to this temperature if determined at a temperature reasonably close to this arbitrary point. The lactometer in general use is graduated to give correct results at 60° F. If the gravity is determined at temperatures above or below 60° F. a close correction can be made by adding 0·1 for every degree above or, by subtracting 0·1 for every degree below 60° F. More exact corrections can be obtained by consulting tables.[20, 25] A means of correcting gravity for temperature is incorporated into the Richmond Milk Scale. The lactometer reading is placed against the 60° mark ; the figure against the actual temperature of the milk on the scale is the corrected lactometer reading.

The changes of volume with temperature have already been discussed generally in the section on Density (Section 109).

112. Specific Heat of Milk (Fig. 16)

Milk contains fat which melts or solidifies within the range of temperature in which the liquid state persists. Consequently milk shows a peculiar behaviour with regard to its specific heat at various temperatures, since the latent heat of solidification modifies the apparent specific heat in the range of temperature covering the zone of solidification (or of melting) of the fat when the temperature is lowered (or raised). It is obvious that the determination of the specific heat of milk is attended by some practical difficulties owing to the physical properties of the fat and fat globules. The thermal conductivity of fat is poor, and the state of division of the fat is such as to resist solidification for at least a sufficient length of time to annul the measurement of any fine changes of temperature in calorimetric work. Consequently a lag in attaining temperature equilibrium is experienced and approximate or apparent results only are obtainable. The theoretical temperature-specific heat curve should show a sharp peak at the melting-point of the fat, but in actual practice a " hump " spreading over a range of about 20 degrees (C.) is obtained. The height of this hump varies directly with the per-

centage of fat in the milk or cream. Whey and separated milk show straight lines similar to water but have lower specific heats,
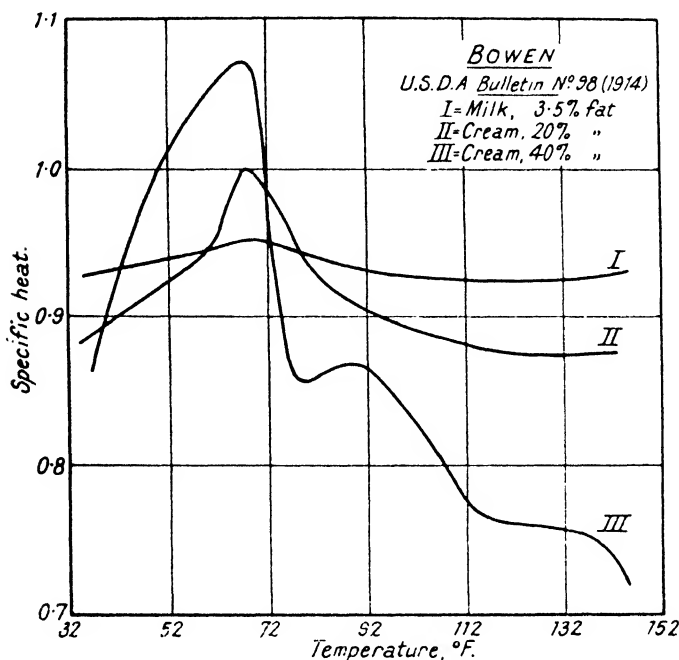
FIG. 16.—Curves showing the specific heats of milk and of cream containing 20 and 40 per cent. of fat.

separated milk (0·95) having a lower value than whey (0·98). The maximum of the hump is at 19° C.

TABLE CII. *Specific Heat of Milk and Cream at Various Temperatures*

Temperature (° C.)	0°	10°	20°	30°	40°	50°	60°
Whole milk . .	0·92	0·92	0·95	0·93	0·93	0·93	0·94
Cream 20% fat .	0·88	0·92	1·00	0·91	0·90	0·86	0·87
Cream 40% fat .	0·83	0·95	1·50	0·88	0·86	0·78	0·76
Cream 60% fat .	0·56	0·95	1·12	0·85	0·78	0·72	0·72
Whey . . .	0·97	0·97	0·97	0·97	0·97	0·97	0·97

Skim milk 0·95 ; Cheese 0·64 ; Butter 0·56.

Without taking into consideration the variations introduced through the latent heat of fusion of the fat, the specific heat of

milk is an additive property of the constituents. The following are the approximate specific heats of the constituents : fat 0·5, lactose 0·3, protein 0·5, ash 0·7, and water 1·0. The solutes and suspended materials will thus cause milk to have a lower specific heat than water, which, calculated from the mixture law, is 0·93. The actual value for the range 30–60° C. is 0·93.

Table CII gives the specific heat of milk and cream at various temperatures [27] (Fig. 16).

It can be deduced from the low specific heats of high-fat cream at the lower temperatures that the specific heat of solid butter-fat is lower than that of liquid butter-fat.

113. The Freezing-point and Boiling-point of Milk

The most constant physical property of milk is its *freezing-point*, that is, the temperature at which liquid milk is in equilibrium with solid ice. Ice separates from milk at −0·540 to −0·550° C. (or 31° F.). The constancy of the freezing-point of milk, or of the depression of the freezing-point of the water in milk, through the agency of dissolved molecules and the various ions in solution (Δ), means that milk has a constant osmotic pressure, irrespective of conditions obtaining during the production of milk, *e.g.*, season of year, intervals between milking, compositional quality, or breed of cow. The osmotic pressure of milk is closely similar to that of blood serum, but it must be understood that this may be pure chance.

A constancy of osmotic pressure means that a constant number of molecules plus ions is present per unit weight of solution and, as has been discussed previously, the composition of milk accommodates itself to suit this isotonic constancy. The value of the freezing-point determination as a means of differentiating genuine poor milk from milk to which water has been added cannot be overestimated. The freezing-point depression records a more theoretically additive property of milk than does specific gravity, where complications due to the fat enter, or refractivity, which only records the physical properties of some of the constituents, namely, those appearing in the serum.

Winter [28] first arrived at the conclusion that the sera of blood and milk have the same freezing temperature, and that this temperature was a physiological constant. Stoecklin [29] later found that milk was isotonic with blood, and that the freezing-point of the two fluids would range from −0·550 to −0·560° C. Various investigators give figures for the freezing-point of the serum of normal cows' blood ranging from −0·550 to −0·590° C., and Atkins [30] has predicted that the freezing-point of milk will

never fall below that of the blood of the animal producing the milk.

The osmotic pressure of milk, and hence the depression of the freezing-point, is due chiefly to the contained lactose and

TABLE CIII. *Osmotic Pressure of Average Milk (Coste and Shelbourne)*

Constituent	Percentage	Osmotic Pressure (atmos.)	Δ
Lactose . .	4·7	3·03	0·250^c
Alkali chlorides .	0·1	1·33	0·110^c
Other salts and ions	—	2·42	0·200°
Total . .		6·78	0·560°

TABLE CIV. *Range of Variation in the Freezing-Point of Milk*

Authority	Number of Samples	Range of Δ	Average Δ
Stoecklin * . .	2,500	0·545–0·565	—
Henderson and Meston *	? large	0·540–0·560	0·550
MacLaurin * . .	270	0·545–0·565	0·550
Winter * . .	49	0·540–0·570	0·555
Ducrose and Imbert * .	?	0·533–0·575	—
Gooren * . . .	20	None below 0·540	—
Hummelinck * . .	large	0·542–0·570	—
van Raalte * . .	155	0·540–0·570	—
Keister * . .	31	0·541–0·574	—
Stuber [32] . . .	262	0·530–0·562	0·546
Hortvet * . . .	75	0·534–0·562	0·548
Golding [44] . . .	91	0·543–0·564	0·550
Murphy [45] . . .	676	—	0·548
Buchanan and Lowman [46]	133	0·537–0·582	0·577
Stubbs [54] . . .	1,000	0·529–0·563	0·544
Denis-Nathan [55] . .	large	0·528–0·561	0·541
Krenn [52] . . .	712	0·513–0·582	0·545

* Reported by Elsdon and Stubbs, *J. Soc. Chem. Ind.*, 1931, **50**, 135T.

soluble salts. The fat has no effect, and the effect of the proteins is negligible or too small for cryoscopic measurement. Coste and Shelbourne [2] have suggested a distribution of the osmotic pressure in an average sample of milk (Table CIII).

Hortvet,[31] quoting Stoecklin (*loc. cit.*) states that "milk, freshly drawn, from any variety of cow whether of high or low breed, from whatever region of the country, from animals in stable or on pasture, whether poorly or substantially fed, whether drawn during a period near or remote from parturition, in winter or in summer, morning or evening, or whether the yield be scant or abundant, has a definite freezing-point which varies but little around $-0.550°C.$, although under various influences the chemical composition changes in enormous proportions."

The range of variation in freezing-point of milk as found by various workers is given in Table CIV.

The results given in Table CIV are subject to the criticism that the investigators have not used uniform methods for the determination of the freezing-point. Most are undoubtedly the results of painstaking work, but other workers have failed to take into account the essential factors which determine a freezing-point result, *e.g.*, the apparatus, thermometers, and methods of procedure.

The cryoscopes which have been used consist of apparatus of the Beckmann, Raoult, and the Dewar-flask types. Hortvet (*loc. cit.*) has described a standard apparatus of the last type in which the low temperature is obtained by forcing dry air through ether surrounding a glass tube containing the milk and thermometer. A control thermometer registers the temperature of the ether. Andrews [33] uses a simple apparatus of the Beckmann type in which the tube containing the milk is separated from the cooling mixture of ice and salt by an air-space. In this method the milk is partly frozen by immersion, with stirring, in a *separate* vessel containing ice and salt, the ice which has formed being subsequently almost completely melted by heat from the hand before the tube is placed in the cryoscope and the steady reading taken. Monier-Williams [34] employs a cryoscope of the Raoult principle, a current of air being drawn through ether in the apparatus to lower the temperature of the freezing-bath.

The thermometers may be of the Beckmann type or very sensitive thermometers of the ordinary type, graduated to $0.01°$, each degree covering from 7 to 10 cm. of stem. In every case, the thermometer is standardised by determining the freezing-point of freshly-boiled distilled water. Monier-Williams (*loc. cit.*) makes the reading more comparative by standardising with a solution of 9·495 grams of pure sucrose in 100 grams of water, a solution which freezes at $-0.5345°$ C. All thermometers are naturally subject to various defects natural to glass under such conditions, such as thermal changes in the glass, lag in the reading, effect

of exposed stem, and sticking of the thread. These difficulties are best overcome by the standard conditions of procedure in cryoscopic work (storing the thermometer at a low range of temperature, frequent determination of the freezing-point of the reference liquid, and tapping of the stem with a " cryohammer").

In ordinary cryoscopic practice, it is recommended that the solution should show a freezing-point not more than $0 \cdot 1°$ below that of the solvent, and that the amount of super-cooling should not exceed $0 \cdot 5$.[35] With milk it is naturally impossible to keep to these recommendations, since dilution of the milk would cause changes in the ionisation of the milk salts, which would not be comparable for samples of different compositional quality. Although it has been possible to get ice to separate out by only $0 \cdot 5°$ of super-cooling, it is to be feared that with some apparatus a higher degree of super-cooling is essential before the necessary nuclei of ice crystals will form. Monier-Williams (*loc. cit.*) has been able to carry out the determination with a relatively high temperature of cooling-bath and an initial super-cooling of $0 \cdot 5°$. Hortvet takes the temperature of the contact cooling-bath down to $-2 \cdot 5°$ C. and allows a maximum of $1 \cdot 2°$ of super-cooling.

CORRECTION FOR SUPER-COOLING. THE RAOULT CORRECTION. It is a matter of much controversy whether a correction for super-cooling should be brought in when determining the freezing-point of milk. Doubts exist as to the nature of the solid phase which separates, whether it is pure ice, or ice with entrained milk constituents. It must be understood that separation of a certain amount of ice in the case of milk ($\Delta = 0 \cdot 55°$) certainly calls for a correction if ordinary cryoscopic determinations (maximum $\Delta = 0 \cdot 1°$) call for such treatment.

The amount of ice separating for $1°$ of super-cooling can be calculated from the specific heat (C) of milk ($0 \cdot 92$) and the latent heat of solidification (L) (80). The *fraction* separating would be

$(C \times 1/L) = \dfrac{0 \cdot 92 \times 1}{80} = 0 \cdot 0115$. The concentration of the solution has thus been increased to $1 \cdot 0115$ and the corrected freezing-point depression (Δ_c) is the observed depression (Δ) multiplied by $1/1 \cdot 0115$ ($= 0 \cdot 989 \Delta$). (1).

Raoult's correction is calculated from the expression $K - \dfrac{\Delta - \Delta c}{\Delta c S}$

where S is the super-cooling in degrees and K is a constant which Hortvet states is $0 \cdot 017$ for milk. Using this figure for one degree super-cooling, the corrected depression (Δc) is $0 \cdot 983 \Delta$. (2).

Thus, for one degree of super-cooling, correction factor (1) entails the subtraction of $0 \cdot 006°$ and (2), $0 \cdot 009$ from the observed value.

The magnitude of these corrections must be considered in the light of (*a*) other errors entering into the determination, and (*b*) the point in view in the determination. If checking against water is frequently done, it is logical to assume that most of the other errors incidental to the method will almost cancel out. By using an almost isotonic sugar solution as standard the correction need not be applied in practice.

The value of the test enters into the determination of added water, which may be calculated from the usual mixture law:
$W = \dfrac{100\,(0.550 - \Delta)}{0.550}$, where W is the percentage of added water and Δ, the observed depression of the freezing-point. From this formula it can be deduced that 10 per cent. of added water lowers the depression by 0.05° and 5 per cent., 0.025°, values which are of much greater significance than the corrections mentioned above. It must be borne in mind that the value 0.550° used as the basis for calculation is the grand average for normal milk, and that samples may be obtained with Δ of 0.540. It is logical, therefore, to apply the correction here and return as genuine all samples showing a Δ of 0.530 and above. Thus all samples which may, when calculated on the basis of the grand average value of 0.550°, be considered to have 5 per cent. of added water (equivalent to a lowering of Δ by 0.025°) theoretically must be given the benefit of the doubt. It is true that if the Δ of the original milk is known, one can with certainty obtain evidence of 5 per cent. or even less of added water by the freezing-point method.

Stuber [32] states that super-cooling from 0.54° to 1.10° has no effect on the freezing-point.

EFFECT OF ACIDITY. The development of acidity in milk causes more ions to be in solution, and will give larger depressions of the freezing-point. The depression is increased at a rate approximately proportional to the increase in acidity, but the lactic acid produced is not the only factor involved. Saito [37] has found that on adding 0.92 per cent. lactic acid to milk the increase in Δ is 0.223°, whilst corresponding dilution of the acid with water gives only 0.197° depression. Keister [36] has observed an increase of 0.03° in the Δ for every 0.10 per cent. increase in acidity over the natural acidity of milk and has suggested a correction of −0.003° for every 0.01 over 0.15 per cent. acidity. It is, however, advisable to carry out the determination on sweet samples of milk only, since the corrected figure is at best only an approximation of the value for the sweet sample.

Gooren [38] has found that homogenisation, pasteurisation and

the sterilisation of milk lower the freezing-point. This has not been confirmed by Saito,[39] who boiled milk under reflux for two hours without a change being found in the freezing-point. Elsdon and Stubbs [40] have observed that no change in the freezing-point of milk is effected by pasteurisation or sterilisation.

The " *cryolac number* " for milk, or the quota of Δ due to lactose plus alkali chlorides multiplied by 10^3, has already been dealt with. (See Section 22.)

FROZEN MILK. In partly frozen milk, the frozen portion has a higher freezing-point and a lower solids-not-fat content than the liquid portion.[41] The fat tends to concentrate in the solid portion. The freezing of milk does not affect creaming ability after slow thawing. The creaming ability depends on the local rise of temperature if the milk is thawed quickly.[42] Cream has the same freezing-point as normal milk.

The physical effects of freezing milk and cream have been examined by Webb and Hall.[56] Slow freezing causes gradual precipitation of the caseinate system and immediate destruction of the fat emulsion. The heat stability is not affected unless the milk is held at $-18°$ C. for several months. Concentration, or increase in solids-not-fat content, and dissolved sugar lessen the destruction of the fat emulsion, and homogenisation retards slightly the separation of fat from low-fat cream. Freezing destroys the fat clumps formed in cream by homogenisation and restores the heat stability. Condensed pasteurised whole milk can be frozen without loss of body or flavour, and can be satisfactorily reconstituted after remaining four weeks in the frozen state.

Pure casein dispersions and milk serum can be prepared by freezing homogenised cream of 25 per cent. fat-content ; on slow thawing at temperatures below the melting-point of the fat, at clear serum separates ; the residue is washed with ice water to remove the serum. On warming the residue in water, the fat " oils off " and leaves a casein dispersion, which possesses all its original characteristics.

Evaporated milk with 26 per cent. total solids and 18 per cent. solids-not-fat would have a calculated freezing-point of roughly $-1.35°$. At $0°$ C. such milk is slightly super-saturated in respect to lactose. Sweetened condensed milk will freeze at temperatures below $-13°$ C., according to the amount of sucrose present. A eutectic point for cane sugar will be reached ($-12°$ C.) if crystallisation has begun.

THE BOILING-POINT OF MILK. Since the ebullioscopic constant for water is less than a third of its cryoscopic constant the

calculated elevation of the boiling-point of the water in milk, *ceteris paribus*, would be roughly 0·15°. Increases in the degree of ionisation of milk salts on heating to 100° would possibly increase this value, but no experimental information is available on this point. It has been stated [27] that the boiling-point of milk at 760 mm. atmospheric pressure is 100·55° C. (213° F.). When milk is concentrated by evaporation, the boiling-point rises by 0·5° C. (0·9° F.) for every doubling of the concentration.

114. Other Physical Properties

(*a*) ADHESION. The adhesion of milk to various surfaces increases as the temperature is lowered, and it is more difficult to clean by means of water a vessel which has contained cold milk than one which has contained warm milk.

(*b*) WETTING POWER. The wetting power of any liquid depends on its intrinsic nature and its relation to the composition and properties of the surface to be wetted, as well as on surface tension. Milk has a greater wetting power than water for most surfaces. Calcium caseinate is used commercially (*e.g.*, for insecticides and fungicides) as a " spreader " for sprays. Separated milk can be used for the same purpose.

(*c*) ABSORBING POWER FOR FOREIGN AROMAS. Milk and milk products possess a strong affinity for many substances, and will readily absorb gases, especially when warm. The absorption is mostly physical in nature, due undoubtedly to the large area of fat exposed. Volatile vapours such as petrol, paraffin, and lubricating oil fumes from power plant, ammonia from refrigerating plant, and food odours such as those of silage and decayed brassicaceous foods should not be allowed to come in contact with stored milk either in the cowshed or processing rooms. Dairy products should not be stored with other food products, as the food aromas will quickly be absorbed, neither should they be stored where other foods have left behind their particular aroma. The tainting of butter with an apple flavour by the transport of butter in ships' holds which had a previous cargo of apples is a case in point. Dairy products will easily take on the terpene flavour from various resinous types of wood with which the products may come into contact. The same precautions must be observed for varnished and waxed surfaces.

115. The Flavour of Milk

The effect of milk on the palate is to be regarded from two standpoints : (*a*) the balance of the sweetness of the sugar with

the saltiness, and (*b*) the balance of the fat with the protein to give a slight effect of " nuttiness."

The balance between sugar and salt is maintained over a fairly wide range of composition, namely, from 4·5 per cent. and above of the former and from 0·060 to 0·120 per cent. of chloride ion. Saltiness can be detected by the palate in samples containing 0·120 per cent. or more of chloride ion, whilst the saltiness of samples containing 0·150 per cent. is marked. Protein and fat " tone " down both effects. Whey from fresh milk is much sweeter than the original milk, but dried whey is more salty than dried milk.

The balance is naturally upset in abnormal milk owing to the complementary relationships of lactose and chloride-content ; sweetness decreases as saltiness increases. This effect is again more marked in the whey. The amount of ionic calcium is too small to detect any " liminess ", but cream, neutralised by milk of lime, gives a butter-milk of a limy taint, and neutralisation to an acidity below 0·25 per cent. is apt to give butter a limy flavour.

The slight nutty flavour is always present. Protein and fat give *body* to the flavour. The superior body of colostrum or molten ice-cream is partly due to the increased protein-content and to viscosity effects.[43]

Foreign flavours are accentuated in milk, especially if warm, when both senses of smell and taste can register the flavour simultaneously. The range of temperature for tasting is from 100–125° F. ; temperatures of 130° F. and above are apt to scald the palate. In routine tasting the sense of taste is disorganised before the sense of smell, and the former may thus be considered the more delicate. Quick recovery of tasting power may be effected by a small amount of acid fruit or fruit juice.

Freshly-drawn milk from any mammal possesses a faint odour of a natural scent peculiar to the animal. This is particularly true of the mare and sow. The " cowy " odour of cow's milk is variable, depending on the individual, season of the year, and the hygienic conditions of milking. The faint natural odour is evanescent and disappears on exposure of the milk to the atmosphere.

Food flavours in milk consist of (*a*) those which are absorbed from food aromas in the cowshed, *e.g.*, silage and decayed foods, and (*b*) those which impart a direct flavour to the milk when ingested by the animal. Plants containing essential oils impart the flavour of the volatile constituent to the milk, *e.g.*, chamomile,[47] mint, thyme, and garlic (wild onion). Babcock [48] submits

evidence of garlic odour and flavour in milk drawn one minute after feeding garlic, the intensity of the taint increasing until ten minutes after feeding. A half-pound of garlic consumed four hours before feeding gave an objectionable taint to the milk. The constituent responsible for the taint (possibly allyl sulphide) can be almost completely removed from milk by thorough aeration during processing.

Proctor [47] has found that taint of chamomile or mayweed arises from the consumption of the weed in mixtures of rye-grass and clover. Some species of chamomile give a stronger taint than others.

Cows on fresh pasture give milk with a more or less well-defined " grassy " flavour, due to the coumarin in the grass. A clovery flavour is observed in the milk of cows on clovery pasture, particularly on broad red clover. These taints are seasonal, and are not perceptible when the dried material is fed.

Traces of contaminating metals, particularly heavy metals, as salts, give an astringent taste to milk. This holds especially of copper, iron, and zinc. The proteins of milk seem to enhance the metallic flavour much in the same way as the taste of iron in blood is brought out by the blood proteins. A content of 3 p.p.m. of copper in milk gives a well-defined metallic taste. It needs a much higher amount of tin and aluminium to give a perceptible astringency (30 p.p.m. and over). (See Section 152.)

Oxidative taints, such as " mealiness," " oiliness," " tallowi-ness " or a " cappy," oily or rancid flavour, can occur in milk where conditions are favourable for the development of such flavours. These flavours are variously described thus according to the degree of development of the oxidised taint. Prolonged ultra-violet irradiation yields a similar taint.

A fishy taint in milk is of sporadic occurrence in this country, and on the Continent, when cows are fed on considerable quantities of sugar-beet tops and on beet molasses or molassed by-products. The taint is due to trimethylamine oxide, formed as a metabolic product of betaine, which is present in considerable quantities in the above feeding-stuffs. Trimethylamine oxide combines through its active oxygen with the double bond of the unsaturated acids of the fat, thus forming the compounds possessing the fishy flavour. The taint can be eliminated from butter by washing with weak organic acids, such as acetic or citric acid. Before the appearance of the taint a sickly, sweet or " beety " flavour is well defined.

For detailed treatment of the status of flavours in milk, the reviews by Leitch [49] and Roadhouse [50] are recommended.

Taints due to micro-organisms consist mainly of (*a*) acid, (*b*) proteolytic, and (*c*) butyric flavours. The time taken for these taints to develop, mostly the first, is used as a measure of the keeping quality of milk. The development of acidity occurs through the fermentation of lactose by lactic acid bacteria (the chemistry of lactic acid formation has already been discussed in Section 39). An acid taste can be detected in milk early in the fermentation process, but varies for different samples. Milk which coagulates on heating has a strong acid taste.

Proteolytic flavours occur in milk through the action of organisms capable of breaking up the proteins. This taint is evident when milk of low bacterial count " goes off," and is undoubtedly due to the predominance of the proteolytic over the acid-producing organisms. The milk usually clots through the action of the rennin produced by the organisms, whilst the clot may partly be digested later, or it may dissolve in the alkaline medium formed by the organisms.

A butyric flavour is accompanied by a bitter taste and a more or less definite butyric odour. The taint is due to lipolysis, and may be caused by the natural lipase of the milk, or by organisms which are favoured in their activity by the storage temperature and micro-organic distribution of the medium. The taint is prevalent in cream stored under " cold dairy " conditions, where natural ripening is checked by the low temperature (below 10° C.) of storage.

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CHAPTER XIV

THE PHYSICAL CHEMISTRY OF MILK.   ACID-BASE EQUILIBRIA

116. General Considerations

THE complexity of milk as a physico-chemical system can at once be realised when the properties of (a) the ampholytes—the proteins, especially caseinates—and (b) the inorganic salts of weak acids, calcium and magnesium citrates, phosphates and bicarbonates, of milk, are considered. The non-homogeneity of milk in this respect—the effect, for instance, of solid calcium phosphate—enhances the complexity.

The questions of hydrogen-ion concentration, buffer capacities, and the titration of milk at once arise. The practical aspects of the question are associated among others with rennet action and the behaviour of curd in cheesemaking and with the mechanism of rennet action, with the metabolism of bacteria in milk and in cheese-ripening, with the ripening of cream in butter-manufacture, with the stability of milk to heat, and with the changes in milk of importance, technically and nutritionally, which occur due to the influence of heat. Studies up to the present fall short in interpreting fully the physico-chemical behaviour of milk on a quantitative basis.

117. The Hydrogen-ion Concentration of Milk

Of outstanding importance is the hydrogen-ion concentration at which the buffer constituents are in equilibrium in fresh normal milk. There are many data concerning the average pH and range of pH of fresh milk (Table CV).

The pH of fresh normal milk, on the average, is thus approximately 6·6. The range given by Sharp and McInerney [15] includes the values for samples of colostrum, gargety milk and fresh milk covering a range of acidity (as per cent. of lactic acid—see Section 123) from 0·05 to 0·50 per cent. Most of their values for normal milk fall in the range of pH 6·4–6·6.

The conditions which may cause the pH to vary from its normal value are : (a) the loss of carbon dioxide after drawing, or by displacement with hydrogen in the hydrogen-electrode vessel ; (b) the freshness of the milk ; (c) the growth and type of organisms

which may have grown in the milk during storage ; and (d) the
condition of the udder.   Acid-producing bacteria will naturally

TABLE CV.   *The* pH *of Fresh Milk as Found by Various
Investigators*

Investigator	pH	Investigator	pH
Van Dam [1] . .	6·5–6·8	Clark [8] . .	6·6
Baker and Breed [2] .	6·5–6·6	Davidsohn [9] .	6·6
Van Slyke and Baker [3]	6·5–7·2	Taylor [10] . .	6·8
(Normal) . .	6·5–6·75	Duncombe [11] .	6·54
Allemann [4] . .	6·4–6·9	Mattick *et al.*[12] .	6·87
Milroy [5] . .	6·6–6·8	Golding *et al.*[13]	6·73
Lisk [6] . . .	6·4–6·6	Rice and Markley[14]	6·4–6·9
Schultze *et al.*[7] .	6·7–6·8		(average 6·6)
		Sharp and	
		McInerney [15] .	6·0 –7·73
		Nokayama [19] .	6·32–6·82

cause a change of the pH to the acid side, whilst alkali-producers
will change it to the alkaline side of the normal value.

Table CVI gives the ranges of pH of milk of mammals other
than the cow.

TABLE CVI.   *Range of* pH *Values of Milk from Various
Species*

Species	Investigator	pH
Human .	Clark [8]	7·0–7·6 (7·22) ; 7·1 [27]
,, .	Ohi [16]	6·9–7·1
,, .	Davidsohn [9] ·	6·6–7·2 (6·97)
Goat .	Schultze and Chandler [17]	6·4–6·7
Whale .	Takata [18]	6·67

Human milk is more alkaline than cow's milk and so also
probably are equine milks ; the milk of ruminants is usually about
pH 6·6, and that of carnivora on the more acid side, due to a
higher protein-content.

The relation between pH and titratable acidity, especially with
respect to the formation of lactic acid by bacteria, will be dealt
with later.   (Section 123.)

118. The Buffer Constituents of Milk. Buffer Index

Milk shows a considerable buffering action at the pH of normal samples. If milk is titrated with acid or alkali and a curve (the titration-curve) is drawn plotting the number of ml. of acid or alkali against the pH value, a smooth curve is obtained between pH 5·5 and 8·0 (Fig. 17). The slope of the curve at any point may be used to calculate the " buffer-index " ($d_B/d_{p\mathrm{H}}$) at that point. (The " buffer-index " [20] is the number of equivalents in grams of acid or alkali necessary to change the pH of one litre of buffer solution by one unit.) The index at pH 6·6 is
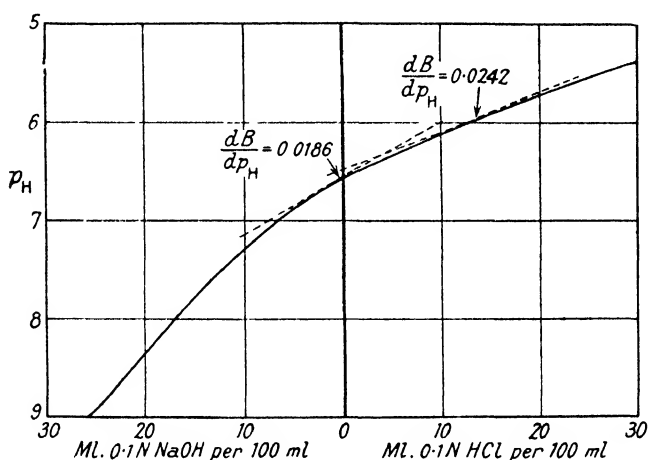
FIG. 17.—The titration-curve of normal milk.

0·0186 ; at pH 6·0 it is 0·0242, and at pH 8, 0·008. Buffer activity is greatest on the acid side of the normal pH value, and around a pH of 8·5 the change of pH with addition of alkali is abrupt. In this region, therefore, an indicator giving or changing its colour would give in milk, on adding alkali, a simulation of a titration to an end-point, like the titration of a weak acid in the same way. This affords an explanation of the use of phenolphthalein as an indicator in the so-called determination of acidity of fresh milk.

The titration curve represents the composite effects of the various buffers of milk over the pH-titration range. Clark [21] has tabulated the concentrations of the individual buffer systems operating over such a range in milk (Table CVII).

The minor constituents of milk do not play a significant part in buffering. The proteins, citrate, phosphate and bicarbonate, *i.e.*, the weak anions, are the principal buffers.

TABLE CVII. *Distribution of Chief Milk Components (Clark [21])*

	Grams per litre	Equivalents per litre	Mols. per litre	Mols. per litre in Homogeneous Solution	Equivalents of Base bound per litre, if Homogeneous	
K$_2$O . .	1·80	0·0382	—	—	—	—
Na$_2$O . .	0·72	0·0232	—	—	—	—
CaO . .	1·78	0·0635	—	—	—	—
MgO . .	0·30	0·0148	—	—	—	—
Total . .		0·1397	—	—	—	—
P$_2$O$_5$ . .	1·50	—	0·0211 (H$_3$PO$_4$)	0·0135 (H$_2$PO$_4$′)	—	—
				0·0076 (HPO$_4$″)	0·0135 0·0152	0·0633 (PO$_4$‴)
Citric . .	2·00	—	0·0104	0·0008 (cit″)	0·0016	—
				0·0096 (cit‴)	0·0288	0·0312 (cit‴)
Cl . .	1·00	—	0·0282	—	0·0282	0·0282
SO$_3$ . .	0·11	—	0·0014	—	0·0028	0·0028
Total CO$_2$.	—	—	0·0050	0·0034 (HCO$_3$′)	0·0034	0·0034
Casein .	28·0	—	—	—	0·0084	0·0084
Albumin .	7·2	—	—	—	0·0022	0·0022
Total . .	—	—	—	—	0·1041	0·1395

119. Data for the Buffering Constituents

Clark [21] has analysed the effects of the individual buffers of milk. The dissociation constants of the buffer acids of milk are given in Table CVIII.

TABLE CVIII. *Dissociation Constants of the Buffer Acids of Milk*

	pK_1	pK_2	pK_3
Citric acid . .	3·08	4·39	5·49
Phosphoric acid .	1·97	6·85	11·99
Carbonic acid .	6·2 ·	10·3	—

The approximate ionic ratios of the buffer radicals can be calculated from the equation connecting hydrogen ion concentrations

and dissociation constants ($pH = pK_a + \log. \frac{[salt]}{[acid]}$ [22]). These
are given in Table CIX.

TABLE CIX. *Ionic Ratios of the Buffer Radicals of Milk*

System	Value of Ratio at pH		System	Value of Ratio at pH	
	6 0	6 6		6 0	6 6
$\dfrac{[Citrate']}{[Citrate]}$	830	3,300	$\dfrac{[H_2PO_4']}{[H_3PO_4]}$	11,000	43,000
$\dfrac{[Citrate'']}{[Citrate']}$	41	160	$\dfrac{[HPO_4'']}{[H_2PO_4']}$	0·14	0·56
$\dfrac{[Citrate''']}{[Citrate'']}$	3 2	13	$\dfrac{[PO_4''']}{[HPO_4'']}$	1 × 10⁻¹	4 × 10⁻⁶
$\dfrac{[HCO_3']}{[Free\ H_2CO_3]}$	0 63	2 5	$\dfrac{[CO_3'']}{[HCO_3']}$	5 × 10	20 × 10

Clark then calculates the individual buffer indices of phosphate,
citrate and bicarbonate from the distribution values (Table CVII)
for pH of 6·0 and 6·6. The citrate values are determined from
the data of Hastings and Van Slyke.[23] The buffer indices are
given in Table CX.

TABLE CX. *Calculated Buffer Index of Milk. Inorganic
Buffer Components (Clark* [21])

System	Buffer Index	
	pH 6 0	pH 6 6
Phosphate . . .	0·0053	0·0112
Citrate . . .	0·0049	0·0018
Bicarbonate . . .	0·0027	0·0023
Total . . .	0·0129	0·0153

Milk proteins behave as weak acids at pH 6·6. It can be calcu-
lated (if all milk protein is considered as casein), from Loeb's [24] and
Palmer and Richardson's [25] curves that the buffer index of a

3·5 per cent. protein solution is 0·014 at pH 6·6 (Fig. 18). The total buffer index (pH 6·6) is therefore 0·029. The observed value for normal milk is 0·019. The discrepancy, according to Clark, is due to milk not being a homogeneous system and that the Ca salts establish a solid phase which necessitates a redistribution of the buffer values of all the component systems. Further evidence from the titration-curve of milk shows that this solid phase is some form of calcium phosphate. Thus at pH 8·5--9·0 the buffer effect is chiefly due to protein, since the buffer index values for citrate, carbonate and phosphate in this region approach
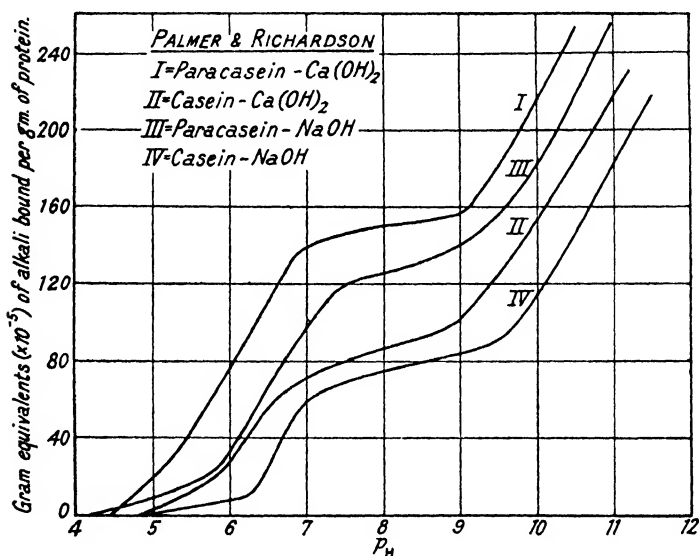
FIG. 18.—The binding-power of casein and paracasein for calcium and sodium hydroxides.

zero. At pH 5·0 the carbonate and protein systems have little effect, whilst the low concentration of citrate renders that system comparatively ineffective. The buffer action in this region is due to a phosphate system, and if this is so, this phosphate system functions less efficiently at higher pH values in proportion to the increase in efficiency in a more acid range.

Attempts have been made to gain an insight into the state of the calcium by determining the amount which can diffuse through membranes. The amount has been found to be roughly 40 per cent. of the total calcium. The solubility product of $Ca_3(PO_4)_2$ is 1×10^{-26} (horse-serum findings) and therefore $[PO_4''']$ would be 5×10^{-11} or 0·00011 molar at pH 6·6, which is not in harmony

with views that from 30 to 70 per cent. of the phosphate is in
solution. There is no doubt that the measure of diffusible
calcium is of comparative value only, since any abstraction of
calcium ions from the system within a membrane causes dis-
placement of the ionic equilibrium. The maintenance of this
equilibrium will cause more calcium to appear in the diffusate,
and total diffusible calcium would be a function of the diffusion
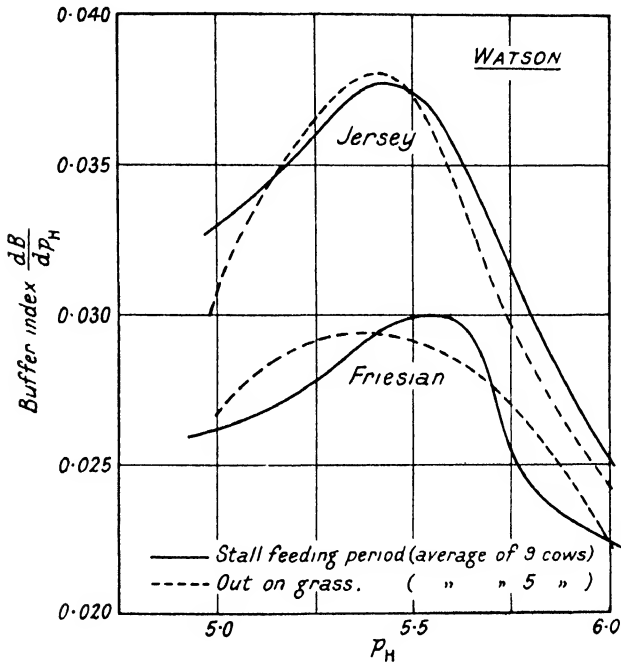
FIG. 19.—Buffer Curves of Jersey and Friesian milk.

time. The de-ionising of calcium by citrate and caseinate
undoubtedly has some bearing on the matter also.

Under heat-treatment milk undergoes a permanent change in
acid-base equilibrium. This is evidence that the equilibrium
which exists is not stable with respect to the distribution of the
significant components. The slow adjustment of the calcium-
phosphate system [26] is evidently concerned. The reaction,
$3 \ CaHPO_4 \rightarrow Ca_3(PO_4)_2 + H_3PO_4$, is displaced by the action
of heat, possibly due to the change in the adsorptive equilibrium
of the caseinate micelles. The same reaction has been suggested by
Van Slyke and Bosworth [28] to explain the fading of the pink colour
of phenolphthalein in milk at pH 8·3 during the titration of milk.

RANGE OF pH WHERE MILK HAS A MAXIMUM BUFFER VALUE. The point of maximum buffer value is at pH 5·5, although this changes with variations which have been found for the buffer values of herd milk from different breeds.[29] The range of maximum buffer value is from pH 5·1 to 5·8. The maximum is sharp for milk of high buffer values, but is flattened considerably for those of low values.

Watson [29] has found that Jersey milk has a markedly greater buffer capacity than Holstein milk in the pH zone of 5 to 6 (Fig. 19). The effect of grass feeding on the buffer value is negligible. It appears that the higher the solids-not-fat content, the higher the buffer value, and that dilution of a richer milk to the composition of a poorer sample brings the buffer value to the same order of magnitude. At this range of pH it is the phosphate system which is predominant, whilst the citrate system has a negligible effect.

Okulitch and Golding [49] report a monthly variation in the pH and buffer values of Ayrshire and Jersey milk ; Ayrshire milk varies less than Jersey. A change from indoor to outdoor conditions shows a definite lowering of the pH of the milk produced and an increase in the buffer value of Jersey milk.

Buchanan and Peterson [30] have shown that milk may be diluted with water up to 30 per cent. of its volume without appreciably changing the buffer value, whilst Van Slyke [20] states that the amounts of acid and alkali added in experimental work may be neglected if the maximum amounts do not exceed 50 per cent. of the original volume.

120. The Titration of Milk. Milk " Acidity "

As has already been pointed out, the so-called acidity of milk, which for pedagogic purposes is expressed as the percentage of lactic acid, is a measure of the quantity of alkali necessary to shift the pH of the various milk buffer systems from the original pH of about 6·6 to that of 8·3. The slope of the titration-curve at pH 8 denotes a low buffering capacity at this point, with the result that titrating to an end-point, with phenolphthalein as indicator, simulates the titration of a weak acid. Titration is carried out to a faint pink colour which fades rapidly owing to the slow adjustment of the phosphate system ; tricalcium phosphate is precipitated and free phosphoric acid is liberated ; on continuing the titration in the presence of calcium salts this reaction proceeds simultaneously. Dilution of the milk also displaces the end-point for the same reason.

The concentration of buffer constituents in milk naturally depends on the solids-not-fat content and therefore samples high in solids-not-fat are expected to have a greater titratable acidity than those low in this constituent. In this respect McInerney [31] has shown that milk from Jersey and Guernsey cows has a higher titratable acidity when fresh than that from Holstein cows. The solids-not-fat content has the same effect when addition of acid, as for the precipitation of casein at pH 4·6, is necessary. Thus Watson [29] has shown that Jersey milk has a markedly greater buffer capacity in the range of pH 5·0–6·6 than Holstein milk, and will therefore require more acid to shift the pH from that of normal milk to the isoelectric point of casein. Since milk shows its maximum buffer value in this range (pH 5·0–6·6), it is obvious that the differences due to variation in solids-not-fat content are greater in the acid than in the alkaline range.

121. The Effect of Dilution on the Titratable Acidity

Dilution of milk with water lowers the titratable acidity. Sommer and Minos [32] have found that dilution with an equal volume of water lowers the acidity by 0·0211 per cent., and with 9 volumes, 0·0623 per cent. In titrating solutions containing Ca and PO_4 in amounts representative of milk with alkali, $Ca_3(PO_4)_2$ separates, and if the same separation occurs in milk, the effect of dilution is to decrease the amount of tricalcium phosphate precipitating. There is no doubt also that a calcium to caseinate relationship is involved in the dilution effect. With diluted milk a lower pH has been observed for the phenolphthalein end-point, due possibly to the protein effect on the indicator. The comparatively more rapid fading of the colour observed with diluted milk is due to the quicker formation of $Ca_3(PO_4)_2$. The acidity of concentrated milk has been found to be higher than that calculated from the original milk and the ratio of concentration, in spite of loss of carbon dioxide. This is attributable to a reverse of the above process. Again, the neutralisation of milk or cream with lime does not lower the acidity to the calculated point since a greater Ca concentration causes a greater precipitation of $Ca_3(PO_4)_2$.

122. Titration in the Presence of Calcium De-ionising Reagents

The indefiniteness (fading) of the end-point in the titration of milk to phenolphthalein and its displacement by dilution can be overcome by the use of reagents capable of de-ionising the calcium. Van Slyke and Bosworth [28] have found that the addition of 2

per cent. of a saturated solution of neutral potassium oxalate removes the calcium ions, that the slow reaction

$$3 \; CaHPO_4 \rightarrow Ca_3(PO_4)_2 + H_3PO_4$$
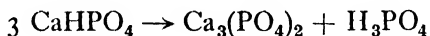
is prevented from occurring, and that the neutralisation does not pass beyond the stage of forming K_2HPO_4.. This gives a sharp end-point with no displacement on dilution. But the acidities determined in this way are found to be only about one-half those obtained in the absence of oxalate. The acidities also agree closely with the acidities of milk serum prepared by filtering milk through a Chamberland filter and subsequently oxalated. The residue of casein and colloidal calcium phosphate (and magnesium phosphate) on the filter is practically neutral to phenolphthalein, and thus does not contribute appreciably to the acidity of the milk. On examining large quantities of the colloid complex by high-speed centrifuging of milk, the above properties are confirmed and the complex is regarded as a mixture of neutral calcium caseinate and colloidal dicalcium phosphate ($CaHPO_4$).

Pyne and Ryan,[33] however, have observed that the addition of 2 per cent. of saturated oxalate is not always sufficient to remove entirely the disturbing calcium salts, and that diminishing acidities can be obtained by successively increasing the amount of oxalate, and, in a few cases, milk can be made definitely alkaline to phenolphthalein on adding 4 per cent. of oxalate. The agreement between the " oxalate " acidities of milk and cream also appear to have been fortuitous, as further additions of oxalate to the *milk* have shown that the interfering calcium ions have not been completely removed at the earlier stage. Pyne and Ryan quote figures for the acidity of milk and of its serum (in this case, rennet whey) for various percentages of added oxalate, the difference representing the contribution of the casein-calcium phosphate complex to the acidity. The contribution of the colloid complex gradually decreases after reaching 0·75 per cent. oxalate until, at a concentration of 2·50 per cent., the milk is alkaline to phenolphthalein. This decrease in acidity is attributed to the conversion of the colloidal calcium phosphate to the basic *alkali* phosphate, and the decrease is a measure of the amount of phosphate in this form in the milk. Van Slyke and Bosworth [34] have found considerable variations in the amounts of colloidal calcium phosphate in milk, and it appears that it is due to this factor, and not to the difference in the type of colloidal phosphate present, that the varied behaviour of different samples of milk with oxalate must be attributed. There is thus no

relation between the acidities of oxalated milk samples and those of unoxalated milk and serum.

123. The Relation between pH and the Titratable Acidity of Milk (Figure 20)

The hydrogen-ion concentration of milk is a property which is caused to change with change of titratable acidity, and there must

TABLE CXI. *Relationship Between pH and Titratable Acidity of Fresh Milk (Sharp and McInerney) (Figure 20)*

pH	Titratable Acidity	pH	Titratable Acidity
6·0	0·50	6·7	0·145
6·1	0·43	6·8	0·125
6·2	0·36	6·9	0·115
6·3	0·30	7·0	0·105
6·4	0·25	7·1	0·095
6·5	0·205	7·2	0·090
6·6	0·165	7·3	0·085
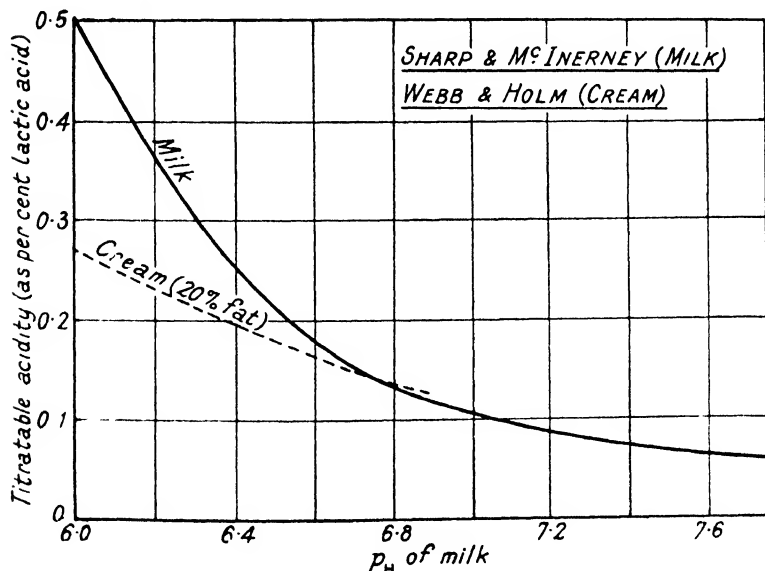
FIG. 20.—The relation between pH and the titratable acidity of milk (Sharp and McInerney), and of cream (Webb and Holm).

be, therefore, a close relationship between these two factors. Data to support this have been offered by Van Slyke and Baker,[3] Rice

and Markley,[14] and Sharp and McInerney.[15]   The last pair of workers have emphasised the existence of a distinctly different relationship between the two for fresh milk, as compared with milk in which the production of lactic acid by micro-organic fermentation has commenced.   Table CXI gives the pH of fresh milk and the corresponding titratable acidity expressed as lactic acid.

For a pH below 7·0, the value for fresh milk can be estimated from the titratable acidity with an error which is usually less than 0·1 pH.   Above a pH of 7·0, the abnormality of the samples is reflected in a greater deviation of the points from the projected smooth curve.   In this investigation milk was represented by all types of secretions, which explains the unusually wide variation of titratable acidities encountered.

Small differences in the pH and titratable acidity of milk have been observed by Caserio, varying with the metal of the vessel in which the milk is kept.   The changes in pH are greater when storage vessels of impure aluminium or tinned iron are used. The purity of aluminium must not be below 95 per cent.[50]

With respect to the relationship of pH to titratable acidity for milk in which lactic acid has formed, individual samples differ more or less in the amount of change in pH with a given amount of lactic acid formed as determined by titration, this undoubtedly being caused by a variable buffer-content.   Thus with milk of a high buffer-content a smaller shift in pH for a given amount of lactic acid produced, is observed.   The curves connecting the two factors commence on the curve for fresh milk and the slopes of the curves for each sample run fairly parallel.   The production of lactic acid is evident by the occurrence of a lower (more acid) pH than would be expected from the relationship for the two factors cited above for fresh milk.   It is thus clear that evidence of the production of lactic acid can be found if the pH is lower than that expected for the titratable acidity shown.

When soured samples of milk are partly neutralised, the increase in pH and lowering of acidity revert along the curve for sour milk.   As soon as real acidity develops in milk, the salt balance is changed and the addition of alkali does not readjust the buffer equilibria.

There is reason to believe that the artificial addition of lactic acid to fresh milk would give a relationship between pH and titratable acidity different from that by the " natural sour " method. In the latter method more time is allowed for the equilibrium of the buffer systems to be established, and the freeing of metallic radicals from combination with casein is effected more thoroughly,

so that when the isoelectric point is reached, the casein is completely kation-free. The relatively high ash-content of " grain-curd " casein, as against the low ash-content of " natural-sour " casein, can be explained in the above manner. Grimmer et al.[35] have found a progressive and regular decrease of the calcium-content of curd as the concentration of lactic acid increases in souring. They have correlated the "acidity effect" of developed lactic acid as follows : If $\sqrt{Ks}$ is the H' concentration of lactic acid of acidity x, and if y is the H' concentration of the milk and c a constant, then $y = cx^2\sqrt{Ks}$. The value for c was found to vary from 3·03–5·42 (average 4·3) $\times$ 10^{-6}.

124. Acidity of Milk or Cream not due to Lactic Acid

This type of acidity is mostly encountered in cream, and is due to liberation of the fatty acids of milk-fat by lipase action. Sharp and Tomasi [36] have found that lipase is active in cream at as low a temperature as $-$ 10° C., and that there is a greater development of this type of acidity in cream than in the raw milk.

125. Methods of Expressing the Acidity of Milk

Considerable variations occur in the methods of expressing the titratable acidity of milk. One of the most widely used methods is that of expressing the acidity as *percentage of lactic acid*. The calculation is avoided by using N/9 alkali for titration since the equivalent of lactic acid is 90 and every ml. of N/9 alkali used for 10 ml. of milk corresponds to 0·1 per cent. lactic acid. Instead of reporting the acidity as the percentage of lactic acid, the value is multiplied by 100 and termed " degrees of acidity." Thus, a milk, 10 ml. of which requires 1·8 ml. of N/9 alkali for neutralisation to phenolphthalein, has an acidity of 0·18 per cent. or 18° of acidity.

By using decinormal instead of N/9 alkali and calculating the acidity as the *number of ml. of* 0·1 *N alkali required to neutralise* 100 *ml. of milk,* another form of expressing the acidity is obtained This gives a higher number of degrees than the first method. Richmond and Huish [37] advocate the use of 0·1 N baryta and titrating to such an end-point that the colour obtained with phenolphthalein is equal to that produced by 1 drop of a 0·01 per cent. solution of rosaniline acetate in 96 per cent. alcohol. The use of a saturated solution of lime-water, as advocated by Storch,[37] is to be recommended. The strength of such a solution varies very slightly with temperature, and the only precaution needed

is to have excess of lime in the bottle ; the solution also should be decanted and not filtered for use. This solution is 0·0460 N at 15° C. (0·0475 N at 10° C. and 0·0445 at 20° C.) Degrees of acidity determined in this way are termed *Dornic* (D) degrees on the Continent.

The third method of expression is by Soxhlet-Henkel (S-H) degrees. This is the number of ml. of 0·25 N alkali required to neutralise 100 ml. of milk, and varies from 7 to 9 for normal milk. To convert S-H degrees to percentage of lactic acid it is necessary to multiply by 0·023.

Another convenience worthy of mention in testing milk for acidity is the use of the Babcock milk pipette where this method of fat-determination is used. This pipette delivers 17·6 ml. (18 grams) of milk, and on titration with *decinormal* alkali each ml. used is equivalent to 0·05 per cent. of lactic acid. If the pipette delivers a 9-gram sample, each ml. corresponds to 0·1 per cent. of lactic acid.

126. Variations in the Titratable Acidity of Milk

The titratable acidity of fresh milk varies from 0·10 to 0·22 with an average of 0·15 (Babcock) to 0·18 (Richmond) per cent. of lactic acid. As referred to above, this variation is due to the variation in the contents of protein and phosphate, which again are related to the solids-not-fat content of the milk. Alkaline or abnormal samples of milk (as detected by the brom-cresol purple, alizarin or brom-thymol blue test) will show low acidities (0·10 per cent.) when fresh, but such samples will rapidly develop lactic acidity on keeping.

It is obvious that whereas such a range of apparent acidity occurs for fresh milk, the detection of lactic acidity is difficult to interpret from the titration figures. The acidity test is used as an indication of the age of milk and of its care, and for sorting out batches which will stand processing ; and it is generally taken that a milk testing above 0·19 per cent. acidity contains developed lactic acid. Few samples of bulked milk in the *fresh* condition show as high an acidity as this, but samples have been met with, *e.g.*, Guernsey milk, which show 0·22 per cent. acidity.[31]

127. Tests for Developed Lactic Acidity

Certain tests have been advocated to detect small amounts of lactic acid in milk. The *boiling test* depends on the fact that normal milk coagulates on boiling when the acidity has risen to 0·26 per cent. This test can also be used for cream, and dilution with water after boiling assists in the detection of the flecks of curd.

The *alcohol test* depends on the fact that the addition of a 68 per cent. solution of alcohol in water to an equal bulk of milk will not coagulate fresh milk, but that at an acidity of o·21 per cent. and above, a coagulum of increasingly flocculent appearance is given as the acidity rises. The alcohol solution used is made up of 72 parts of 95 per cent. alcohol (rectified spirits) and 28 parts of water, equal volumes of milk and alcohol-mixture being used for the test. As will be seen later, coagulation is favoured by a high calcium- and magnesium-content but hindered

TABLE CXII. *The Alcohol-Alizarin Test (Morres)*

	Acidity %	Colour	Character of Coagulum
1	0·16	Lilac-red	Nil
2	0·18	Rose- or pale red	Nil to slight
3	0·20	Reddish-brown	Very fine particles
4	0·22	Brownish-red	Fine particles or flakes
5	0·25	Brown	Large or small flakes
6	0·27	Brownish-yellow*	Large flakes
7	0·31	Yellowish-brown	Large flakes (smell and taste)
8	0·36+	Yellow	Spontaneous coagulation
9	0·16–0·18	Dark brick-red	Cheesy flakes. Presence of rennet-forming bacteria
10	—	Violet	Fine flakes. Diseased udder

* Stage of resistance-to-boiling reached.

by citrates and phosphates, so that the coagulation tests are at best only rough methods.

The *alizarin-alcohol test* of Morres [38] consists in observing the colour and modifications which 3 ml. of milk undergo when shaken with 3 ml. of neutral alcohol of 68 per cent. strength saturated with alizarin (0·2 per cent.). Fresh milk of 0·16 0·17 per cent. acidity does not coagulate, and gives a lilac-rose colour ; at 0·18 to 0·19 per cent. acidity fine flakes are given, and at 0·32 per cent. the liquid is coagulated sufficiently for serum to separate, the colour being lemon yellow. If rennet is secreted by the bacteria in the milk, coagulation is given by the test without apparent change in acidity. Milk from diseased udders shows a violet colour, indicating alkalinity and unfitness for cheese-

making.   This test, which is a combination test for the capacity
and intensity factors of acidity, has been found useful for sorting
market milk into the three groups : (a) sound milk suitable for
processing or household use ; (b) suitable only for cheese-
making ; (c) abnormal milk, unsuitable for cheese-making or
household use.

Table CXII. gives the gradations of colour and appearance of
the coagulum with acidity, according to Morres.

Electrolytic Neutralisation of Milk.   When milk is made to
flow between several pairs of charged electrodes, its acidity
diminishes.   Gratz [51] states that the lactic acid is reduced to
acetaldehyde and later changed to carbon dioxide and water.
The acidity is more likely to be reduced by alkali hydroxides from
the electrolysis of alkali chlorides, the small amount of chlorine
entering into organic combination with the protein.   The milk so
treated is claimed to keep well and not to change in taste and
smell.

128. The Formol Titration of Milk (Proteins)

In the well-known Sørensen method [39] for the titration of
amino acids, polypeptides and proteins, neutralised formaldehyde
is added to the neutral solution and standard alkali is run in
until a pink colour is produced with phenolphthalein.   The
usual explanation of this phenomenon is that the amino acids
are neutral in aqueous solution because the basic NH_2 neutralises
the acidic COOH, and that the addition of formaldehyde destroys
the basic character of the NH_2-group, with the result that the
COOH is free to be titrated.   This is not quite correct, since
an amino-acid solution is neutral because the NH_2 and COOH
groups are not appreciably ionised in neutral solution.   The
COOH-group functions appreciably as an acid only at alkaline
pH, and the NH_2 as a base only when the solution is acid.   In
most amino acids the carboxyl groups are completely neutralised
at a pH of 11·75 ; when formaldehyde is added the neutralisa-
tion becomes complete at pH 8·7.[40]   Both from the raising of
the end-point and from the magnitude of the pH values resulting
from the addition of various quantities of alkali to mixtures of
amino acids and formalin, it is concluded that the Sørensen titra-
tion-method depends on the formation of methylene-imino acid
derivatives having dissociation constants (K_a) about one thousand
times greater than the constants for the original amino acids ;
the value of K_a for an amino acid is 2×10^{-10}, and for the sug-
gested methylene-imino acid, 2×10^{-7}.   Further, the methylene-
imino acid is stable only in the form of its alkali salt, since the free

methylene-imino acid tends to revert to the more feebly ionised amino acid.

The same principle holds for the free NH_2- and COOH-groups of proteins, and the method has been applied to the determinations of milk proteins owing to its ease, rapidity and reasonable accuracy. Steineggar,[41] who originally proposed the method, employed caustic soda, but Richmond [42] recommends strontium hydroxide as giving a sharper end-point and results higher by 10 per cent. than by the caustic-soda method. As in the determination of milk acidity, the addition of calcium ion (or of Sr˙˙ or Ba˙˙) brings about a greater precipitation of $Ca_3(PO_4)_2$ at the end-point, which means a greater amount of phosphoric acid to be neutralised (*vide* equation in Section 122). This explains the higher formol-titration values obtained by Richmond when strontia is used. Pyne [43] has shown that the formol-titration is considerably affected in different directions by the presence in milk of (*a*) soluble or easily reacting phosphates, and (*b*) colloidal phosphates. The former cause an increase, and the latter a decrease in the formaldehyde-value if alkaline-earth hydroxides are used for the titration. The effect of the colloidal phosphates seems to favour the view that calcium caseinate and the colloidal phosphate of milk are in some form of chemical combination. The disturbing effects of both classes of phosphates can be eliminated by de-ionising the calcium, such as by adding a soluble oxalate to the milk before carrying out the formaldehyde-titration.

Owing to the above disturbing effects, the various factors employed to calculate the protein from the formaldehyde value need revision. Steineggar and De Graaf and Schaap (*vide* Richmond,[37] p. 182), reporting the aldehyde figure in S-H degrees, use the factor 0·0777 (for 0·1 N alkali, 0·1942 ; for N alkali 1·942) to calculate the protein percentage. Richmond and Miller,[37] using strontia, have found the factor to be 1·99 on the average, the range for normal milk being from 1·81 to 2·26, Walker, using N/9 soda, has found the factor to be 1·6 for *casein*, which Richmond works out at 1·9 for total protein. Pyne (*loc. cit.*) using the oxalate method, finds the mean value of the factor to be 1·74. (Protein per 100 ml. of milk : formaldehyde titration value as ml. 1·0 N caustic soda per 100 ml. of milk.) On a weight-percentage basis, the factor is 1·70. This embraces all the nitrogen of the milk, of which roughly 94 per cent. is true protein nitrogen. The factor for *true protein* should be modified to 1·60.

Pyne (*loc. cit.*) has also shown that the ratio of casein N to

other N in milk decreases from 3·3 to 2·8 with advance of the lactation-period. (73·7 to 76·7 per cent. of the total nitrogen, values which agree closely with the average found by Davies [44] for normal milk (76·1 $\pm$ 0·6).) Pyne has also observed that 1 ml. 1·0 N NaOH is equivalent to 1·87 g. of casein per 100 ml. of milk, or 1·81 per 100 g. of milk. The casein content of normal milk may therefore be arrived at from the formaldehyde titration by multiplying the number of ml. of 1·0 N alkali required per

100 ml. by $\dfrac{1·74 \times 0·76}{1·032} = 1·28.$

Casein nitrogen, however, does not account for 76 per cent. of the total nitrogen in many samples, and Pyne suggests a general method of determining casein by interpreting the difference between the formaldehyde-titration value of whole milk and of the whey filtered from the milk after the precipitation of casein at pH 4·6 by acetic acid—sodium acetate buffer. Advancement of lactation, causing an increase in the formaldehyde-values of the whey protein, is the greatest factor rendering the above figure inapplicable for calculating casein from the total titration-value. The percentage of casein is thus equal to $\dfrac{1·87}{1·032} \times$ (difference in the formaldehyde-values of 10 ml. of milk and the corresponding quantity of acetic-acid whey). Much of the variation in the whey figures is due to the variation in composition of the non-protein nitrogen fraction.

For human milk, the aldehyde figure per 100 ml. $\times$ 1·34 gives the approximate protein-content.

129. Estimation of the Protein of Milk by pH Titrations

The main constituents of milk which act as buffers on the addition of hydrochloric acid are the proteins and the salts of citric and phosphoric acids. By constructing separate pH-acid combination curves for each of these constituents (making the abscissæ proportional to the percentage present) one can obtain by their summation a curve for the whole milk. This curve agrees excellently with the experimentally determined curve. Vieth's ratio states that protein : ash is 9 : 2, and Richmond [37] states that this proportion is " marvellously exact " (protein 37·8, ash 8·3 per cent. of the solids-not-fat). The inorganic phosphates and citrates form quite a definite proportion of the total ash. Likewise, the percentage of albumin in normal milk bears a definite (small) ratio to the percentage of casein, and the casein is responsible for the greater part of the buffer value.

Harris [45] therefore concludes that in moderately acid solutions the buffer value of normal cow's milk is directly proportional to the amount of protein present (as also to the phosphate and citrate).

Successive small amounts of 0·10 N HCl are run into a given volume of milk and the pH is determined after each addition. Three or four readings are sufficient, since the result of plotting pH against ml. of added acid is practically a linear relationship. The amount of acid needed to take the milk from one pH to another is directly proportional to the amount of protein present, and Harris (*loc. cit.*) states that the number of ml. 0·1 N HCl required to take the pH of 10 ml. of milk from 6·65 to 5·2 is numerically equal to the percentage of protein in the milk. It is claimed that this method can be used for the determination of milk protein to within 0·1 per cent.

The success of the method undoubtedly depends on the sample. Vieth's ratio, for instance, does not hold universally, especially with individual samples, and the method is not applicable for samples of milk in which the distribution of the buffer constituents varies considerably.

130. The Acidity of Human Milk

Very little information is available on the acidity of human milk, but what data exist show that it is a very variable property. Courant [46] states that " 100 c.c. has the same acidity as 3·6 c.c. of 0·1 N acid." Richmond [47] reports the following acidities (ml. 0·1 N per 100 ml. of milk) of 8 samples : 9·3, 5·5, 5·5, 4·7, 3·7, 3·7, 3·7, 2·8, *i.e.*, from 0·025 to 0·085 per cent. lactic acid. Richmond's data, reviewed by Partridge,[48] show that the acidity varies from 2·0 to 5·5 ml. 0·1 N alkali per 100 ml. Partridge also reports the acidities of 15 samples of human milk, 11 of which fall within this range of acidity. The samples showing high acidity are not further characterised, *i.e.*, neither age nor condition of the samples is given. All the above samples were low in protein and ash. Human milk thus shows only from a quarter to one-eighth of the apparent acidity of cows' milk.

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CHAPTER XV

THE COAGULATION OF MILK

131. Methods of Coagulating Milk

THE coagulation of any liquid physical system, such as an emulsion, entails the collapse of the disperse phase-protective colloid system. This results in the separation of the disperse phase alone or with the protective colloid sympathetically.

The natural coagulation of milk occurs either through the agency of the hydrolytic, coagulating enzyme, *rennin*, or by the development of lactic acid through the growth of lactose-fermenting organisms, or, to a small extent, through surface phenomena associated with foaming.

Total coagulation of milk by *heat* at temperatures below the boiling-point of water occurs only with colostrum, in which case the heat-coagulation of the globulin sympathetically brings down the casein (and fat). In the gradual transition of colostrum to normal milk, clotting by heat ceases when the combined *globulin plus albumin* falls below 0·9 per cent.

Partial coagulation of milk by heat at temperatures below 100° C. is shown by the formation of a " skin " on the surface of the milk. The same phenomenon can be observed at the milk/air interface in sterilised milk bottles (the dried foam forming a pear-drop shaped precipitate on the sides of such bottles where bubbles of gas have been liberated during the heat-treatment, and at the bottom of the dish in which an egg-custard has been cooked).

Milk-stone and milk-film formation on the hot metallic surfaces of processing plant, or at the bottom of pans in which milk has been boiled, is undoubtedly due to local heating of the liquid to temperatures above 100° C.

It requires heating at 100° C. for twelve hours to coagulate fresh milk ; at 130° C., the required time of heating is one hour, but milk will coagulate in about three minutes when heated at 150° C.[1] Individual milk samples vary considerably in their behaviour at these high temperatures, probably due to differences in solids-not-fat content, size of fat globules, and salt-balance. A decrease in time of heating and of temperature is observed as milk is evaporated. Holm, Deysher and Evans [2] have found

that milk evaporated to double its solids-not-fat content requires only ten minutes' heating at 131° C. for complete coagulation.

132. Physiological Considerations of Milk Coagulation

The capacity of casein to coagulate under the action of rennin is of the highest physiological importance.   Phosphoproteins are unique in their behaviour in this respect, from which it may be deduced that the phosphatic prosthetic nucleus, or phospho-serine grouping, is in some way involved in this reaction. The coagulation of casein with its entrained milk-fat has at least two explanations, namely, that volume is given to the young stomach by the curd, and that a slow flow of digested casein and finely-divided fat is available for the small intestine of the suckling. Where much globulin is present, as in colostrum, the character of the curd is modified to suit the requirement of the, as yet, inexperienced stomach, and a gradual change in the nature of the secretion to normal adapts that organ to the nature of the curd from the future main supply.   In maturer stomachs where *pepsin* takes the place of *rennin*, clotting can still be effected by this enzyme under the more acid conditions obtaining, in which case the proteins of milk are digested more quickly from a *casein acid salt* precipitate than from the *calcium paracaseinate* precipitated in the young stomach.   In the former case the calcium and phosphorus, which are more important to the young animal than to the adult, are also liberated slowly and are not as completely dissolved out from the precipitated proteins as under the more acid conditions of the latter case.

It can be said that no advantage other than the fineness of division and consequently greater ease of digestion of the curd of various fermented-milk beverages, such as *yoghourt*, *acidophilus* milk, acid butter-milk and *bulgaricus* milk accrues from the coagulated proteins in these preparations.   More important, perhaps, are the lactic acid and bacterial contents of such drinks.

133. Heat-coagulation of Milk Proteins

(*a*) THE MECHANISM OF COAGULATION.   Coagulation is essentially caused by the loss of suspension stability in an emulsion and heat is a factor which can cause it.   It is agreed in what is now classical work [3, 4, 5, 6] that there are two distinct stages involved in the heat coagulation of proteins, one chemical (denaturation) and one physical (agglutination).   The stability of protein solutions is a function of temperature and hydrogen ion concentration, and the rate of denaturation of individual soluble proteins is proportional to the nth power of the hydrogen ion concentration,

where n is the number of NH_2 or $COOH$ groups, whichever is functioning at the pH investigated.   The actual appearance of coagulation is due to neutralisation of the particle charge and the rate depends on the rate of denaturation.   Denaturation is irreversible and the main change appears to be an association of simple into larger molecules with less surface activity, possibly by mutual neutralisation of active groupings on the surface of the simpler molecules.   The specific properties of enzymic proteins,
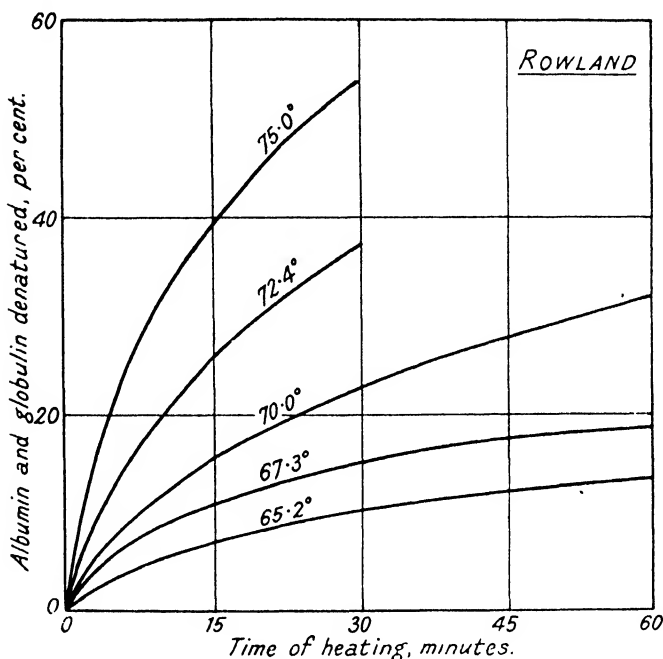
FIG. 21.—Rate of denaturation of the albumin *plus* globulin of milk
at various time-temperature combinations.

e.g., pepsin, are lost in the process of denaturation ; the protein need not coagulate.   Coagulation is association through loss of particle charge and is reversible.   This reversibility is necessarily connected with the hydration properties, and, with suitable treatment, much denatured milk protein may recover its suspension stability, a matter which is of importance in determining the so-called solubility of milk powders (see Section 178).

(*b*) MILK PROTEINS.   (i) *Albumin and Globulin.*   The many workers on the effect of temperature on the coagulation of milk proteins find varying results for the amounts coagulated at different temperatures. [7, 8, 9, 10, 11]

Rowland,[20] taking advantage of the co-precipitation of denatured lactalbumin and lactoglobulin at roughly the same isoelectric point as casein (pH 4·6), has investigated the heat-denaturation of these proteins in the same sample of milk for different lengths of time of heating at various temperatures from 63° C. to 80° C. It was found that an average of 10·4 per cent. of the soluble protein was denatured after heating for thirty minutes at 63° C., 28·0 per cent. for thirty minutes at 70° C., from 52·4 to 67·5 per cent. for thirty minutes at 75° C., and, in one case, 83·4 per cent. at 80° C. (Fig. 21). The amount of denaturation at any one temperature plotted against the time of heating gave smooth curves, but the rate of reaction did not decrease exponentially, this failure to comply with a monomolecular law being attributed to concurrent reactions (protein-splitting and change in the nature of the salt system). The relative increase in the rate of denaturation for each rise of 1° C. between 63 and 75° C. was found to be constant, the temperature-coefficient of the reaction being 1·5.

Svedberg [12] is of the opinion that general proteins, such as albumin and globulin, occur in the native state as molecules of low molecular weight, and that their isolation by precipitation involves a considerable degree of molecular association. It is quite conceivable that the influence of heat causing denaturation favours molecular association to that degree so that, at a critical temperature, the salt-content of the medium causes precipitation. A varying albumin-content in milk is known to be accompanied by a varying salt (NaCl) concentration which may have the tendency to allow the critical temperature to vary slightly with individual milk samples, although this may not be evident with samples drawn from bulked milk. Other factors involved would be the gaseous content and the effective surface and radii of curvature of the fat globules.

(ii) *Casein*. The coagulation by heat of the calcium-caseinate system of milk is a more complex problem than that of the general proteins. The stability of milk towards heat may be said to depend on this system and the precipitation by heat of the albumin does not influence the stability of the system nor affect the coagulation, which does occur at higher temperatures. These remarks, of course, apply to normal (non-colostral milk), for, as was pointed out in Section 131, the presence of 0·9 per cent. of general protein causes total heat-coagulation below 100° C. If the globulin, which is present in traces, in turn is stated to stabilise the colloidal caseinate complex, it can be deduced that its denaturation has little effect on the behaviour of that complex at high temperatures.

The influence of *surface phenomena* on heat-coagulation within the system may be summarised thus : The fat present in its natural state (unhomogenised) has no effect on time and temperature of coagulation, and the effect of homogenisation (up to 3,000 lb. per sq. in.) for milk of normal fat-content is small. As the fat-content increases, such as in 20–30 per cent. cream or in evaporated milk, the effect of homogenisation is more marked, especially when forewarmed to 60° C. before concentrating. As the solids-not-fat increase during evaporation, the necessary time and temperature for coagulation are lowered. The relation between temperature and time of coagulation for any one sample is logarithmic.

The partial coagulation of milk-protein at an air/liquid interface is a phenomenon of great interest in colloid chemistry (*cf.* Section 131), and has been extensively studied by Ramsden,[13] who asserts that, for this membrane-formation (involving an irreversible precipitation), a general system of water/dissolved colloid/gas is essential. Particles of the dissolved colloid pass out of the solution spontaneously to give rise to a delicate pellicle or membrane. The colloid lowers the surface tension of the liquid and passes into the surface-layer (adsorption), the process being irreversible, and definite mathematical relationships are followed.[14] Under the influence of heat the surface-coagulation in milk occurs to a detectable extent. A skin of considerable thickness can form in time on a plane milk-surface and the amount of precipitation is increased by the packing of fat globules in the upper layer through creaming. Solid tricalcium phosphate accounts almost completely for the ash of the coagulum. In the case of gas-globules adhering to the walls of the vessels in which milk is heated, the coagulum again possesses considerable thickness at the gas/liquid interface and assumes a " pear-drop " shape. Many of these can be observed on the sides of sterilised-milk bottles and at the foam-layer around the neck of the bottle.

This surface phenomenon has already been discussed in Sections 95 and 96 on the foaming of milk and the whipping of cream. Adsorption and partial heat-coagulation is more apparent at a gas/liquid than a liquid/liquid (water/butter-fat) interface, even though the average radius of curvature of the latter is of a very low order of magnitude compared with that of the former. The low adsorption at the fat/water interface is possibly due to the presence of a mutual constituent of these two phases in milk, the lecithin, which, acting as a go-between in the system and as a protective colloid in both phases, ensures the emulsion-stability at a lower degree of adsorption. A gradual decomposition of

lecithin occurs even at 62·5° C., which may be conducive to the occurrence (a) of a greater amount of coagulation at the surface of milk kept for a prolonged period of time at temperatures just below boiling-point, and (b) the easier coagulation of evaporated milk forewarmed to above 60° C. before concentration.

(iii) *Effect of Heat on the Calcium and Phosphorus of Milk.* Soldner [15] has shown that when milk is heated some of the calcium is precipitated as tricalcium phosphate. From 18 to 24 per cent. of the total calcium is thus thrown out of solution on boiling. Grosser [21] has found that 5·4 per cent. of the calcium in the ultra-filtrate of milk is precipitated on boiling. Orr,[22] and Magee and Harvey [23] state that about 26 per cent. of the calcium in fresh milk is in the soluble form, but that for pasteurised milk and boiled milk the fraction is lowered to 20 and 15 per cent. respectively. Daum [24] has found that the soluble Ca, Mg and P are lowered by heating for six minutes at 106–111° C. and that the process continues in the samples held at the ordinary temperatures. There is no change in the pH value or in the citric acid content. Mattick and Hallett [25] have found that on heating milk to 90·6–92·3° C., clotting with rennet, which requires a certain concentration of calcium ion, occurs only in the first half-hour, showing that gradual de-ionisation of calcium occurs in the heated sample on keeping.

Palmer [16] suggests that the precipitating effect of heat on the $CaHPO_4$ in colloidal form in milk is the cause of the partial fixation of the calcium. Again Sommer and Hart [26] favour the view that maximum heat-stability of milk is due to a critical balance *between the basic and acidic constituents, and that coagulation is* due to an excess either of calcium and magnesium or of citrate and phosphate.

(iv) *The Coagulation of Calcium Caseinate and of Evaporated Milk.* On boiling milk, a lowering of the degree of dispersion (or association) of the casein occurs. There is a measurable combination of calcium with casein when milk is boiled or when casein is boiled in a solution of calcium chloride ; this is accompanied by partial precipitation. This precipitation by heat is explained by the facts that casein is partly hydrolysed and that the amount of precipitation depends on the concentration of calcium. Casein boiled in acetate or phosphate buffers does not undergo partial heat precipitation.[107]

Howat and Wright,[108] in studying the cleavage of the phosphorus during the heat-coagulation of casein, found that when a neutral solution of calcium caseinate is heated at 120° C., the casein rapidly loses phosphorus and is partly degraded, and then

the partly dephosphorised product is coagulated ; the base-binding power of the product is less than that of the original casein, and calcium is also liberated in the heating process.

On prolonged boiling (twelve hours) the proteins become completely clotted.[1] On autoclaving milk, the time taken for complete coagulation decreases as the temperature of heating under pressure increases. Sommer and Hart [26] have found that the coagulation-temperature varies for different samples of milk from 130–150° C., also that the titratable acidity of the fresh sample bears no relation to the temperature of heat-coagulation and that the pH is not a determining factor. The main factor involved is the composition of the salts in milk, particularly the contained Ca, Mg, phosphate and citrate. They have observed that a marked optimum calcium-content confers optimum stability on the casein, and that the calcium-content of the casein complex is governed by the amounts of magnesium, citrate and phosphate present. The basic radicals produce effects antagonistic to the acidic radicals and an excess of either accelerates heat-coagulation. They were able to control the coagulation of evaporated milk by the regulated addition of sodium citrate or disodium phosphate.[27]

Rogers, Deysher and Evans [17] have found that the salt balance as determined analytically is no indication of the heat-stability of the evaporated product made from a normal sample. Holm and his associates,[18] studying the heat-coagulation of milk in relationship to its chemical composition over a lactation-period, could find no high degree of correlation for heat-stability either with *total solids*, fat, ash, Ca, Mg, P, citric acid or excess base contents, or with titratable acidity, pH, buffer capacity or stability to alcohol and to phosphate. But an increase in the stability is noted for both fresh and evaporated milk (18 per cent. of total solids) as the Ca + Mg content increases with advance of lactation. Webb and Holm [19] have attempted to stabilise (or de-stabilise) colloidal dispersions of calcium caseinate with various salt solutions, but they experienced a failure of the systems to respond in a regular manner. Again, forewarming, the addition of aluminium chloride and of small amounts of acid have been found either to increase or to decrease the heat-stability. In studying the effects of added salts, it is found that milks can be divided into two classes, one being stabilised by the addition of positive ions and the other of negative ions. They are of the opinion that the heat-stability of a concentrated milk cannot be judged from that of the original, as with samples of lower concentration of solids-not-fat the various electrolytes in the samples show considerable differences in effect

throughout their ranges of concentration. Benton and Albery,[28] concentrating on the effects of the acidic radicals, have found that citrate ion has a greater effect on the coagulation-temperature than slight variations in pH from that of normal milk (not below 6·58 or above 6·65). But both factors must be considered. There is an optimum combination of pH and salt-balance which results from several variable factors, and represents the colloidal peculiarity of individual samples, varying with different samples of milk.

Ramsdell, Johnson and Evans [29] have suggested a *phosphate test* for milk unstable to heat. This test, which, as mentioned above, is not applicable in all cases, depends on the combined action of added acid, salt, and heat. They have obtained data showing the relationship between heat-stability and the minimum concentration of phosphate necessary to produce initial coagulation in milk, and when the coagulation shows low heat-resistance. They suggest a " phosphate number " and, in practice, by excluding milks of low numbers, greater heat-stability is obtained in the bulked milk. They have observed remarkable constancy in herd milk. There is no relation between pH and the phosphate number.

When milk is heated, a drop in the titratable acidity is explained by the loss of carbon dioxide. Whittier and Benton [30] have observed first a drop and then a rise in titratable acidity on heating skim milk near the boiling-point. The pH is gradually lowered. This increase in acidity is explained by the formation of acids from certain constituents of the milk, notably the lactose, since the acidity increases at a rate which is proportional to the lactose-concentration and the time of heating. The rate of change of hydrogen-ion concentration during coagulation is lessened, possibly owing to buffer readjustments, whilst a distribution of the acid between whey and curd favours the latter, perhaps by adsorption.

Heating milk in sealed cans flocculates the milk in 8 hours at 110° C. or in 3·5 hours at 120° C., the resultant pH being 5·54. Complete curdling occurs after heating for 8·5 and 4 hours, the final pH values being 5·52 and 5·45 respectively.

From racemisation velocities, Wright [31] has concluded that the nature of the casein molecule is not affected by heating at 120° C. for thirty minutes.

In practice, the temperature of forewarming of milk before evaporation has a profound effect on the heat-stability of the evaporated product during the sterilisation process. Variations in the temperature and times of forewarming have been found by

Leighton and Deysher,[32] and Deysher, Webb and Holm [33] to affect the heat-stability. Improvements in stability were experienced with increases of temperature from 90° C. to 100° C., although the change above 95° C. was very small. Increases of temperature to 120° C. (ten minutes) were noted in some cases. With respect to the time of heating, thirty minutes at 95° C. improved the heat-stability, but at higher temperatures improvement was attained in a shorter time.

Variable, low fat-concentration, with or without homogenisation, affects heat-stability only slightly. But with higher fat-content minimum stability is more pronounced with a forewarming temperature of 60–70° C. for ten minutes, and the time of coagulation decreases with increase in fat-content. In addition the temperature of forewarming necessary for maximum stability is lowered. For 20 per cent. cream it is 80° C. as against 90–95° C. for 5 per cent. milk. The coagulation process is obviously dependent on a number of factors and the reactions involved are interdependent.

Webb [34] has found that the homogenisation of cream lowers its stability to heat but that a second homogenisation at a lower pressure (500 lb.) increases the stability.

Of considerable interest is the " feathering " of evaporated milk or of cream in coffee. Whittaker [35] attributes this precipitation to an incorrect salt balance, to a highly concentrated coffee, to a prolonged extraction of coffee grounds, to the use of a small quantity of milk, and to the slow rather than the quick addition of the milk. Doan [36] has observed that acidity and homogenisation favour the feathering of cream in coffee and advises the use of pasteurised sweet cream homogenised at 1,000 lb. with a lower pressure for the second stage. The addition of citrate, phosphate or bicarbonate (0·025–0·10 per cent.) will stabilise a cream subject to feathering. The mechanical destruction of fat-clumps by homogenisation increases the heat-stability of cream, the fat clumps being believed to act as nuclei for the separation of casein. It is advisable that cream should not be homogenised below forewarming temperatures unless preheating has been done at 170° F. for thirty minutes or at 180° F. flash.

During the thickening which precedes the coagulation, a release of calcium and phosphorus from the coagulum into the serum has been observed. Chorower [37] is of the opinion that the loss of calcium causes hydration of the casein, with the result that the volume of the suspended phase is considerably increased and causes the thickening. Increase in particle-size by aggregation to such a degree as to introduce plasticity-effects may also partly explain the thickening process.

The precipitation of the calcium and magnesium salts as phosphates and citrates with absorption of heat has been shown to occur during the coagulation of milk.[38, 39] The greater the stability of the milk, the slower does this precipitation occur. Artificially prepared milk serum behaves in a similar manner on boiling.

In the thickening of sweetened condensed milk during storage the best results have been obtained by forewarming near the boiling-point.[38] The temperatures of forewarming which will produce a sweetened condensed product that will also thicken seem to increase the hydration-capacity of the proteins and thus probably cause the gelling effect (*vide* the theory of Chorower).

Nichols and his co-workers [40] have found that the calcium caseinate in skim milk is dispersed in sizes ranging from molecular (5 mμ to 200 mμ), and that heating to 95° C. has no effect on this dispersion.

Boiling with soluble calcium salts lowers the pH of milk, and the casein is precipitated in the pII range 5·95–6·05. The precipitation occurs at the point of maximum adsorption of calcium.[41]

134. The Coagulation of Milk by Rennet.   General

One of the most important properties of milk is that of clotting or curdling by the action of *rennet*.   The firm gel which is formed entrains the fat and the insoluble salts, whilst the liquid portion (whey) contains the lactose and the soluble proteins and salts. The curd is elastic and, under the influence of increasing lactic acid content, contracts (syneresis) and allows most of the whey to escape.   The gelation occurs at a point not far removed from neutrality, so that the product is a favourable medium for bacterial growth, and is the basic material for the manufacture of the countless varieties of cheese of modern times.   By varying the manipulation and methods of ripening of the curd, a great range of properties and composition of cheese may be obtained.

It must be realised at once that rennet curd differs in properties from the curd obtained by the addition of mineral acids (*e.g.*, grain-curd casein) or of acetic and lactic acids (*e.g.*, natural-sour casein).   The latter type is precipitated at the isoelectric point of casein, and hence the protein is almost completely free from mineral matter.   Also in both types of coagulation, casein is the only protein precipitated.   The demand for a casein in which the high ash-content is of no significance, such as for the manufacture of casein plastics, has provided an outlet for a rennet casein made from fresh separated milk.   The fat-content of the dry

product (7–9 per cent. of moisture) is from 0·5–1·0 per cent. The high ash-content is conducive to a high viscosity in the working of the plastics. The ash-content is reduced when any appreciable degree of acidity has developed in the milk before " renneting." The value of rennet as a coagulant of sweet milk is therefore at once evident.

135. The Enzyme, Rennin

The class of enzyme capable of causing coagulation of milk is widely distributed in nature. It occurs in the gastric juice of all animals and in the digestive tract of birds, reptiles, fish, and some of the lower animals. It also occurs in the leaves and fruit of various plants,[42] and is present in some proteolytic bacteria.

The general term for the enzyme class is *chymase* or chymosin. That of mammals is termed *rennin*. The commercial preparation is derived from the inner lining of the true or fourth stomach of the young calf or lamb, and is called *rennet extract*. Pancreatic and intestinal rennins are also known ; it also occurs in various other animal organs.

An enzyme, *antirennet*, which inhibits the coagulating action of rennet, occurs in the blood serum of various animals, in urine and in human milk, but it is more active against rennet of animal origin than that of plant origin.[43] Wolgemuth [44] describes a method for the quantitative estimation of antirennet in serum.

Rennet is a protease in character, as can be deduced from its action on proteins other than casein, *e.g.*, legumin. Only traces of pepsin occur in the gastric juice of newly born animals, most of the enzyme being rennin, which is active at a very low hydrogen-ion concentration (pH 6·0–6·1) and acts only on a few proteins. During the development of the animal there is a gradual change into *pararennet* (rennin plus pepsin) and into normal pepsin, which acts as a weak rennet.[45] The rennet of the adult stomach is called pararennet (or parachymosin) for distinction between it and that of the calf's stomach. Michaelis and Rothstein [46] have found pararennet to be more easily destroyed than rennet under alkaline conditions (pH 6·8 and above). van Dam [47] has found that pararennet is more resistant to heat in a strongly acid medium (at 75° C.) than rennet.

Rennin has an optimum temperature of 45° C., but is also active at lower temperatures. The temperature of heat-inactivation depends on the reaction (since the enzyme is more resistant in alkaline solution) and on the concentration of the solution (less sensitive in higher concentrations). Dried rennet can

resist high temperatures, and is only slowly de-activated at 158° C. Arrhenius [48] reports a critical inactivation-temperature (at which the enzyme is half destroyed in one hour) of 46° C. for rennin, with a critical thermal increment of 90,000 calories. Tammann [49] has found that the inactivation follows a unimolecular law.

Rennin, like other intracellular proteases, acts best at its iso-electric point (pH 5·4).[50] Others [51, 52] give an optimum range of pH 6·0–6·5. The enzyme is quickly destroyed at pH 9·0.

It is generally agreed that rennin and pepsin are different enzymes.[53] Fenger suggests that pepsin is a factor in the secretion of rennin. Where pepsin is mixed with rennin, as in pararennet, Wolgemuth [44] was able to ascribe to the presence of pepsin a greater effect from the addition of calcium chloride ; but whereas in the case of rennet the product of the enzyme-concentration and the time of reaction is a constant, that of pararennet increases rapidly with increase in enzyme-concentration ; also with high dilution, the action of pararennet approaches zero.

Rennin is one of the most powerful enzymes known ; Fenger [54] has prepared a product of high purity which can coagulate 2,310,000 parts of milk.

Rennin obeys Segelcke and Storch's law in that the product of the time required for coagulation and the concentration of enzyme is a constant. The coagulation, however, is secon-dary to the changes produced by the enzyme, and it is therefore uncertain whether the primary action of the enzyme obeys the law.

Grimmer and Krüger,[61] however, state that the product of the concentration and the time of coagulation is not constant but is an exponential function of the enzyme-concentration. Grimmer and Rudzik [62] have also observed that the logarithmic curve followed by the product flattens with respect to its maximum as the pH decreases. Grimmer,[56] however, has observed that the dilution of rennet does not weaken the enzyme-action if the dilution is allowed for.

The process of clot-formation appears to be auto-catalytic ; that is, the coagulation seems to be accompanied by the forma-tion of new rennin. Thus Bauer and Herzfeld [55] have observed that coagulation proceeds along a narrow column of milk at a greater rate than can be accounted for by diffusion. This observation is contrary to the above law of rennin action. Christin and Virasoro [57] have observed that the law of rennin action does not hold when tested on a calcium caseinate-phosphate complex.

The actual nature of rennin has been studied by Fenger,[54]

who finds that it resembles a protein and can be salted out of solution by saturation with ammonium sulphate or common salt. It is, however, not coagulated in acid solution although its power is destroyed. The active enzyme appears to be an acid albumin, the acid being essential for its activity, and when the acid is broken off by hydrolysis, the enzyme becomes insoluble and inactive. Solubility and activity may be recovered by recoupling the acid. The active enzyme is highly dispersed and highly hydrated, and can diffuse through parchment, but not through collodion membranes. It is absorbable on proteins like gelatin and on animal charcoal. It is also irreversibly denatured through precipitation on the walls of a foam, and vigorous shaking de-activates the enzyme for this reason.[58]

Tauber and Kleiner [81] have isolated an extremely active (calf) rennin-preparation by fractional isoelectric precipitation. Its composition and characteristics show that it is a thioproteose. They have proved definitely that rennin and pepsin are distinct enzymes. Rennin is irreversibly inactivated by alkali, is easily soluble in dilute acids, is not coagulated by heat, and is dialysable. It gives protein colour-tests different from pepsin ; its isoelectric point is at pH 5·4, as against pH 2·75 for pepsin. Rennin has no peptic action at pH 2·0, whereas pepsin has a strong clotting action. Later,[82] they have shown that rennin is protein in nature, and is rapidly digested by pepsin, which is an easy method for separating the two enzymes.

Field and Noegerrath [59] have observed that the rennet of calves and goats shows a greater activity on the casein of the same species, indicating that a certain degree of specificity occurs in rennet from different mammalian sources. Although human milk does not clot readily with calf rennet,[60] better clotting may occur in the infant's stomach.

Tauber and Kleiner [109] have proved that rennin is a true protein, and not an enzyme adsorbed on a protein carrier ; rennin is inactivated by pepsin and trypsin but not by erepsin. Milk is not clotted by concentrated trypsin solutions, but only by con-centrations of o·1 per cent. or less ; after exposure for 10 minutes to solutions too concentrated to clot, the milk cannot be coagu-lated by active rennin preparations. This can be overcome by bringing the pH of the milk to the acid side so as to avoid hydro-lysis.

With pepsin, trypsin and rennin, the velocity of milk coagulation increases with the H-ion concentration. The addition of crystalline urease to milk inhibits the clotting action of rennin and pepsin. This is a property of the added enzyme, since boiling

the enzyme solution destroys its effect. Blood serum and the mucosa of the pig's stomach and of the fourth stomach of the calf contain inhibitors which act differently on rennin and pepsin. This is suggested as additional evidence that the two milk-clotting enzymes, rennin and pepsin, are distinct.[110]

136. Theories of Rennin Action

Many theories have been advanced to explain the action of rennin. The adsorption of the enzyme on the casein and the precipitation of the complex by bivalent ions has been suggested by Mellanby,[72] since the concentration of calcium ions necessary to produce coagulation is related to the amount of enzyme. It is not agreed whether rennin causes association of the casein to a less highly dispersed colloid, or if it peptises it to a more highly dispersed colloid. The latter view is supported by the statement that particles of calcium paracaseinate are smaller than those of the caseinate.[73] Wright [74] has suggested that the colloidal dispersion is altered in such a manner that precipitation by bivalent cations is facilitated.

Bang [75] has advanced the hypothesis that rennin changes the adsorption-affinity of casein for calcium, casein being the member of a series of compounds with the lowest affinity for calcium, and paracasein the member with the highest affinity. He concludes from his experiments that rennet is not a coagulating enzyme, since it does not alone produce curdling, for instance, of milk heated to 65° C. The final clotting is not true coagulation, but a reaction related to the precipitation of protein by neutral salts.

According to Schryver,[76] there are four components in the coagulating reaction : enzyme, calcium salts, colloids, and substances inhibiting coagulation. Thus when solutions of sodium cholate and calcium salts are mixed, warming produces a clot. The greater the concentration of salts which increase the surface tension of water, the shorter is the time required for clotting to occur, whilst salts which decrease the surface tension also decrease the time of coagulation up to a certain concentration, above which the time required for coagulation is increased or coagulation is even prevented.[77] Inhibition of coagulation is due to the adsorption of simple molecules by the complex colloids, thus preventing coalescence. In milk the adsorption of simple molecules causes suspensoid stability, and it is assumed that rennin clears the surface of the colloid from the adsorbed substances and allows coalescence to occur. Bile and bile salts, which are known to lower surface tension strongly, have been

shown by Clementi [78] to inhibit coagulation. The view that the adsorbed material is lactalbumin, and that this is destroyed by rennin previous to coalescence, has been advanced by Alexander [79]; but this theory is untenable in the light of the work of Palmer and Richardson [69] on the effect of gum arabic and gelatin, the presence of which favour clotting to a firmer gel. It has been proved also by Field [80] that there is no difference in the time taken for coagulation with rennet of milk diluted with milk serum and milk diluted with the same serum previously digested with rennet.

Of the chemical theories of rennin action, that of Hammarsten [68] has been supported by a considerable amount of experimental work. He suggests that rennin hydrolyses the calcium caseinate-phosphate complex into calcium paracaseinate (rich in calcium phosphate) and a whey poor in calcium. Careful examination of the chemical and physical properties of casein and paracasein has, however, revealed no difference between these two compounds. Their elementary composition, nitrogen-distribution and rates of racemisation in alkaline solution are identical. But, although they combine with the same amount of base when saturated (1 g. of each combines with 9×10^{-4} gram equivalents of calcium), the acid proteinates show marked differences. The mono- and dicalcium caseinates combine with $1 \cdot 1 \times 10^{-4}$ and $2 \cdot 2 \times 10^{-4}$ gram equivalents of calcium, and the corresponding paracaseinates combine with $2 \cdot 2 \times 10^{-4}$ and $4 \cdot 5 \times 10^{-4}$ gram equivalents of calcium, respectively. Thus the hypothesis is that one molecule of tetracalcium caseinate is hydrolysed by rennin to two of dicalcium paracaseinate, and that coagulation occurs through the paracaseinates being coagulated by a much smaller concentration of calcium than is required for the caseinates. Palmer and Richardson [69] stress the fact that the power to combine with a base is the main chemical difference between casein and paracasein as far as rennet-action is concerned. These proteins, however, do not dissociate as polyvalent acids, and the acid proteinates mentioned above are not true chemical entities. The base-binding curves of both proteins show only one calcium caseinate and one paracaseinate; the curves at about the pH of normal milk show the ratio between the calcium bound to casein and paracasein of $1 : 2$. It is possible also that the compounds at this pH (6·6) are only in the process of formation.

Palmer and Richardson (*loc. cit.*) conclude their observations thus: " It is obvious that rennin, acting on an incompletely formed calcium caseinate in colloidal dispersion at the pH of milk, converts it into a much less completely formed calcium

paracaseinate, the chemical binding capacity of which for both base and acid is permanently altered. The nature of the molecular rearrangement or surface change (since a substance in colloidal dispersion is altered) causing the increase in binding is not clear, but it is certainly not explained on the basis of simple molecular division or peptisation. Neither of these changes alone could increase the gram equivalents of acid or alkali bound per gram of protein. It is obvious that the instability of the highly unsaturated (with respect to base) paracaseinate, augmented by the higher temperatures employed in rennin clotting, is responsible for the greater sensitivity towards cations and explains its coagulation."

They are of the opinion that both colloidal reactions involved in the clotting process, namely, the action of rennin on the caseinates and the clotting of the paracaseinate, have to be taken together, and that these questions require answering : " (a) What is the exact character of the calcium caseinate ? (b) What is the nature of the change in this colloid causing its increased affinity for cations with a corresponding rise in sensitivity ? The nature of this change remains to be determined, but it cannot be a simple molecular division."

The question of the method by which minute amounts of colloidal enzyme can effect so profound a change in so short a time must at present remain on the unsatisfactory basis that it does so during a temporary adsorption on the surface of the particles of calcium caseinate.

" The second phase of the rennin phenomenon rests on much firmer colloidal grounds. The ultramicroscopic evidence and the accumulation of experimental observation definitely point to the conclusion that the rennet clot is an unstable, rapidly synerising gel of amorphous particles of calcium paracaseinate, each retaining its identity. The laws governing the formation of gels are identical with those summarised by Weiser for jellies formed by precipitation from a gel. The type of precipitation reaction is one involving the effect of valency of the precipitating ion . . . there is also a definite temperature range for the calcium paracaseinate gel. Temperature is also a factor in the rapidity of its syneresis. We have not yet determined the calcium-combining capacity of casein and paracasein at the optimum clotting temperature, but it is reasonable to suppose that the affinity for calcium increases with rise of temperature. This would account for the greater instability in the presence of calcium salts. The rule seems to be that the greater the calcium-combining capacity of the particles, the farther removed are they from saturation

with calcium, and the fewer calcium ions are required to neutralise their electric charge. This undoubtedly explains in part why sodium ions will clot the calcium paracaseinate formed in milk by rennin action."

The latest conceptions of the composition of casein have brought to the fore the consideration of the physico-chemical condensation theory of coagulation to explain both the nature of the coagulation process and the consistency of the curd. Beau [66] suggests that rennin-coagulation consists of a polymerisation or a physico-chemical condensation of casein and calcium phospho-caseinate. Calcium and phosphate act as plastifiers in the condensation.

The caseins in the milks of various mammals differ in respect of the distribution of the various fractions to which casein has been resolved ; these fractions show different behaviour towards specific rennins, and the casein-composition may differ in this respect in the milk of different individuals of the same species. Cherbuliez [83] has shown, for instance, that quicker coagulation with rennet occurs in milk which contains the higher proportion of the a_2 constituent of casein.

137. The Chemistry of the Clotting with Rennet

The casein of cows' milk exists chiefly as calcium caseinate. The clotted casein produced in rennet-coagulation is termed paracasein. It is generally accepted that the action of rennin on the caseinate occurs first and that the precipitation of calcium paracaseinate follows.

Hammarsten [63] first pointed out that rennin by itself does not bring about coagulation, but that clotting requires a certain concentration of calcium ions ; calcium deficiency or a low ionic-calcium concentration has been known to interfere with cheese-making [64]—the so-called slow-working cheese.

The part played by calcium has been differently interpreted by various investigators. [67] Thus it has been suggested that the free salts of calcium salt out the paracaseinate (Bang), or that paracaseinate is precipitated as its calcium salt (Ringer and Arthur, and Pages). van Dam is of the opinion that the colloidal calcium and the calcium associated with the casein cause the precipitation of paracasein. Calcium paracaseinates are insoluble in the presence of calcium salts, and are precipitated as soon as the rennin acts (Van Slyke and Bosworth).

The addition of calcium chloride to milk slightly increases the hydrogen-ion concentration and gives rennet an increased activity. The effect of Ca ions is additive to those of H ions, and the

consistency of the curd depends on the concentration of calcium ions.

Porcher [65] has found that with the same amount of rennin, different amounts of casein require proportionate amounts of calcium for coagulation. The effect of saturating casein solutions with carbon dioxide is not to interfere with rennin-coagulation, but a soft acid curd is given. Rennin cannot coagulate para-casein if calcium caseinate is also in solution with it.

It is obvious that the part played by calcium is not a simple one. Calcium caseinate and paracaseinate belong to the class of negatively charged suspensoid sols, and are stabilised by hydrogen ions in milk. The replacement of hydrogen ions by cations of a higher charge will induce precipitation at a rate proportional to the rate of ionic exchange. Paracasein, with its greater cationic binding-capacity, would be less saturated with respect to calcium ions than casein under the conditions existing in milk, and fewer calcium ions would be required to cause precipitation. Indeed, monovalent cations such as $K^{\cdot}$ and $Na^{\cdot}$ should assist the precipitation of the paracaseinate formed by rennin in normal milk. Hammarsten found that the common salt in rennet-extracts was sufficient to cause reprecipitation of calcium paracaseinate from solution when treated with these extracts.

The rôle of calcium phosphate is important in clot-formation, i.e., in the total gelling of the solution, as against partial precipitation, such as flaking. Hammarsten [68] has found that paracaseinate with calcium or sodium chloride and rennin gives flakes only, but that a clot is given with calcium phosphate, i.e., with the complex, calcium paracaseinate–calcium phosphate, as occurs naturally in rennin-treated milk. The phosphate may be retarding the rate of precipitation of the paracaseinate sufficiently to give a clot. Palmer and Richardson [69] have found that when gelatine is added, with paracaseinate alone, flaky precipitation occurs, but with the addition of colloidal calcium phosphate in gelatine a clot is obtained.

Casein, as a protective colloid, can stabilise a suspension, and such a suspension including insoluble calcium salts can be pre-cipitated by rennin ; the protective action of the casein is reduced to such an extent that the unstable colloid is precipitated. Marui [70] has observed that the decreased coagulability of casein with rennin due to boiling does not depend on the increased calcium-binding power of the casein. Univalent cations cannot bring about the same results in the coagulation as calcium, but all divalent and trivalent ions can. Porcher [71] has shown that the caseinate-phosphate complex is an association of two colloidal

systems, the caseinate acting as a protective colloid to phosphate, and that the loss of coagulability on heating is due to the greater heat-sensitiveness of the phosphate micelles. He also proved that by heating calcium caseinate to temperatures up to 100° C. a gradual alteration of the micelles occurred. By the addition of small amounts of phosphoric acid (H_3PO_4) to the heated solutions, calcium phosphate was formed in association with the caseinate. With increased heating, there was a greater tendency to restrict curd formation with rennin, showing that heating had altered the caseinate micelles.

138. The Influence of Various Factors on the Rennin Action and on the Coagulation

Although the nature of the change of casein to paracasein is at present obscure, it is known that under optimum conditions the rate of change due to the enzyme rennin follows the enzyme laws.

The effect of rennin is influenced by the temperature and the reaction of the milk. In both cases data have been obtained, not from the study of true rennin-action, but from observations on the conditions of optimum *clotting* ; optimum temperature conditions for rennin action, however, are probably the same as those for clotting (40–42° C. for calf rennet). Bostrom [84] has stated that rennin-activity does not occur at a pH more alkaline than 6·9, but, in the case of oxalated milk (when the pH is shifted to 7·0) treated with rennin and heated to destroy the enzyme, less additional calcium is needed for coagulation than if the enzyme had not been added. Rona and Gabbe [85] have found that the change from casein is complete only within the range of pH 6·0–6·4, and that clots on both sides of this range contain unchanged casein.

The rate of coagulation and the consistency of the curd are influenced by the previous temperature to which milk has been raised, the temperature used for coagulation, the pH, the casein- and calcium-contents, and the nature of other cations used in the coagulation.

For constant ratios of milk to rennin, the temperature at which most rapid coagulation occurs coincides with the optimum temperature for rennin-action, which for calf rennin is 40-42° C. On both sides of these temperatures, the coagulation rate decreases ; at 10–15° C. and 60–65° C. no coagulation occurs. The nature of the clot, however, differs with temperature, that at higher temperatures than the optimum being tough and stringy, whilst softer clots are obtained at the lower temperature.

The elasticity of the curd within the normal range of clotting is directly proportional to the temperature-increase, no maximum being shown.[86]

Palmer [87] sums up the fundamental factors determining temperature-effects as : (a) the rate at which paracasein becomes available for precipitation ; (b) the degree of instability of the calcium caseinate ; and (c) the rapidity of the neutralisation of the electric charges on the paracaseinate particles. With reference to (b), the paracaseinate at the pH of fresh milk is considerably removed from a stable compound, and if, as Bang [75] has stated, the calcium-combining capacity of paracasein increases with temperature, a corresponding decrease of the paracaseinate formed in milk is expected. With respect to (c), the concentration of the cations plays an important part, and since ionisation increases with rise of temperature, increased coagulability is partly explicable.

The rate of coagulation is retarded considerably, and the clot is very soft, when milk is boiled before renneting ; less pronounced effects are observed with pasteurised milk. The reasons usually given for these results are that (a) part of the calcium phosphate is precipitated in the heating process, and (b) calcium has become partly de-ionised, since the addition of soluble calcium salts will restore normal coagulability. The addition of colloidal calcium phosphate to milk from which soluble calcium salts have been dialysed out will not restore the coagulability of rennin,[88] and it has been suggested that the results are explained by the effect of heat on calcium caseinate. Michaelis and Marui [89] have found that, by subjecting casein solutions to high temperatures before the addition of calcium salts and rennin, the retarding of coagulation can be demonstrated, as with boiled milk, and that a different calcium-combining capacity of the casein after heating is indicated.

van Dam [90] first threw light on the relationship between hydrogen-ion concentration and rate of coagulability. Casein and paracasein have the same isoelectric point, so that the different coagulability of the latter in fresh milk is not due to one protein being nearer its isoelectric point than the other. Allemann [91] found that the optimum pH for most rapid coagulation was 5·35, but it has been shown later [92] that in the presence of calcium ions a definite zone lying between pH 6·0 and 6·4 and coinciding with the zone for the complete transformation of casein to paracasein gives the optimum clotting, whilst pH 5·5 and 7·0 were plainly outside the zone of coagulation. From the base-binding curves of casein and paracasein it can be seen that, at pH 6·0–6·4, calcium paracaseinate is relatively much nearer its

isoelectric point than calcium caseinate. The zone of maximum coagulability may be considered as the isoelectric zone of calcium paracaseinate in the presence of calcium ions.

Palmer [87] suggests this view, since it is in harmony with the theory of true rennin-action and with the results of experiments which prove that divalent cations displace the isoelectric point of electronegative amphoteric electrolytes towards neutrality.[93]

The character of the coagulum is partly determined by the hydrogen-ion concentration of the milk, and its rate of retraction and of freeing from whey depends also on the same factor. This

TABLE CXIII. *Relative Effectiveness of Cations (as Chlorides) in Accelerating Rennin-coagulation of Milk*

Salt concentration. Millimols per litre.	Order of effectiveness (Normal milk = 100 ; 50 = double time ; 200 = half the time)							
1	Ca 133	Zn 128	Ba 122	Sr 114	Li 106	Mg 66		
2	Ca 145	Zn 137	Ba = Sr 133	133	Li 106	Mg 71		
10	Ca 295	Ba 250	Sr = Al 227	227	Zn 149	Li 103	Mg 100	K 94 Na 87
20	Ca 526	Ba 400	Mg 270	Sr 266	Zn 175	Li 103	K 94	Na 81
100	Ba 526	Mg 475	Ca 345	Sr 179	Li 89	K 71	Na 70	Zn 37

is of great importance in the reproducibility of the time-table of cheese-making.

The concentration of the casein, associated with that of calcium and hydrogen ions, is also of importance since the dilution of milk delays coagulation by rennet, and the clotting is incomplete and soft. Partial restoration to normal behaviour by the addition of calcium ions seems to indicate that it is the dilution of the calcium ion which is the important factor, but it must be understood that the addition of calcium ions increases also the hydrogen-ion concentration. The concentration of the casein greatly influences the character of the clot.

Whereas calcium ions are those studied in rennet-coagulation,

any metallic ion can replace calcium in its effects. Loevenhart[94] found the following descending order of effectiveness of divalent ions in producing the coagulation of paracasein : Mn, Ni, Co, Fe, Ca, Ba, Sr, Mg, Be. Anions (Cl', SO_4'', NO_3') were without effect.

The effectiveness of various cations, superimposed on the calcium ions already present in milk, on accelerating rennin-coagulation has been examined by Lorcher.[95] Table CXIII gives the results of his findings.

Equimolecular concentrations of the various ions arrange themselves differently as the concentration increases. Thus Ca and Zn rise to a maximum and fall with the higher concentrations, whilst Ba and Mg show a continuous rise to the maximum studied. The alkali metals show no rise with increasing concentration, but generally depress the rate of rennin-coagulation of whole milk. The divalent ions are not equally effective in producing the same character of clot at equimolecular concentrations. Ca and Ba added to calcium-deionised milk give normal rennet-clotting and a firm clot, but Mg and Zn in equimolecular concentrations delay the time of clotting and produce a soft clot.

139. Curd-tension

It has already been recognised that the curd from the rennin-coagulation of milk varies in physical character according to such conditions as the concentration of calcium and hydrogen ions, the species of cation, and whether the milk has been heated or not. This difference probably depends partly on (a) the completeness of the change of casein into paracasein, and (b) the predominating casein-fraction in the casein-complex of the milk. The variation in curd-tension is of economic significance since it is the milk giving a soft flaky curd which is most suitable for infant-feeding, and the milk that behaves normally and gives a firm stringy curd which is most suitable for cheese-making.

The physical measurement of curd-tension has been attempted by Allemann and Schmid,[86] and Hill.[96] The latter works on fresh milk (100 ml.), which is placed in a jar containing a special curd knife. The knife possesses 10 radial arms (1 in. long), attached to a vertical rod, a loop at the end of which is attached to a delicate spring-balance. The coagulant is a pepsin-calcium chloride solution (three parts of a 0·6 per cent. solution of 1 to 3,000 dry scale pepsin to one part of a solution containing 378 grams of dry, granular neutral calcium chloride per litre of solution), 10 ml. of which are added. After mixing and keeping

at 35° C. for ten minutes, the spring-balance is attached to the knife and the knife drawn through the curd with a slow even tension. The reading on the balance is taken and the tare for the knife subtracted from it. This gives an arbitrary figure, which serves as a reliable comparative index of the curd-tension of the different types of milk met with. Average mixed milk shows from 50 to 90 grams of curd-tension.

The excess of calcium chloride in the above coagulating mixture retards coagulation and gives subnormal values for curd-tension. Miller,[111] therefore, suggests that a coagulant made by dissolving 0·45 gm. of pepsin in 100 ml. of 0·4 per cent. hydrochloric acid gives a truer picture ; the calcium chloride precipitant shows a greater divergence in curd-tension than the acid-pepsin coagulant.

Hill found less than 1 per cent. of the cows tested to give milk showing a curd-tension less than 20 grams, which is considered the most desirable standard for soft-curd milk. Cows giving soft-curd milk occur in all breeds, but the Friesian breed shows a preponderance of soft-curd cows ; soft-curd Ayrshires are very few. The presence of fat has a softening effect on the curd and separation of the fat hardens the curd. In general, however, milk with a high fat-content is more likely to give a hard curd. Prolonged heat-treatment softens the curd ; owing to this and to the final sterilisation process in its manufacture, evaporated milk gives a soft curd.

In the case of individual cows, the giving of soft-curd milk appears to be permanent, and has been observed in a few instances to be a hereditary characteristic. The curd-character of milk is fairly uniform throughout the lactation-period ; abnormalities enter at the beginning and at the end (increasing hardness). The food of the cow has no effect on curd-tension except with sudden changes in the ration.

The range of curd-tension experienced in the Hill test has extended from 15 to 200 grams when determined by the sub-merged-knife method.

Soft-curd milk is characterised by low casein, low total protein, low plasma solids and low ash-content. The curd-tension is a linear function of the casein-content and can be calculated from the casein nitrogen-content. The other constituents of milk have little effect.

Curd-tension is lowest in early summer and highest in early winter ; it is highest in the first four to six weeks of lactation ; the dilution of milk with water lowers the curd-tension in proportion to the casein-content. Owing to the dispersal of fat clusters by heating fat-rich milk, the curd-tension is increased. Homo-

genising reduces curd-tension, the optimal effect being obtained by heating to 180° F., cooling to 100° F., and homogenising at pressures not less than 2,000 lb. per sq. in. The addition of alkali to milk reduces curd-tension in proportion to the increase in pH. Udder infections decrease curd-tension, but a low curd-tension must not be taken as proof of udder infection.[112]

Caulfield and Martin [113] have found that the decrease in curd-tension due to homogenisation depends on the original curd-tension of the milk. Homogenising at 165° F. gives a greater lowering than at 120° or 145° F. The effect on curd-tension is permanent, no further lowering taking place with re-homogenisation. Holder-pasteurisation does not lower curd-tension, but heating to 180° F. has a marked lowering effect, as also has heating to boiling-point. Freezing increases curd hardness but viscolising at 3,000–5,000 lb. per sq. in. lowers it greatly. The removal of lecithin from the surface of fat globules by churning, so that it is absorbed on the casein network, converts whole milk of high, to one of low, curd-tension.[113]

The influence of curd-tension on cheese-making has been studied by Hill,[97] who finds that losses of fat and casein are higher for soft-curd than for hard-curd milk, and consequently a much higher yield of cheese (15 per cent.) is obtained with the latter. The soft-curd milk gives a higher rennet-test and about twice as long a coagulation-time as a hard-curd milk. The cooking-time of the curd is also more than twice as long for soft-curd milk, and thus the whey is run off at a higher acidity ; owing to this fact, the time taken for the subsequent handling of the curd is shortened. The final cheese also shows marked differences ; the soft-curd cheese ripens much faster, has an inferior texture and flavour, and is susceptible to " openness " on cutting.

Hill [98] has improved his test by using a special knife to cut down through the curd (the " Curd-o-Meter ") ; a similar instrument, claimed to possess advantages over the Hill method, has been suggested by Lauder and his associates.[99] Hill [98] has found that on the average goat's milk has a harder curd than cow's milk (100 grams, as against 70 grams tension for cow's milk) but that the same variations for cow's milk occur also in goat's milk (a range of curd-tension from 20 to 250 grams).

140. The Coagulation of Milk in Cheese-making

The rennet-coagulation of milk is carried out in the manufacture of nearly all types of cheese, and it is only in the manufacture of cream cheese, which is kept only from seven to ten days before consumption, that acid-coagulation by bacterial action is allowed

to take place. Seeing that cream for the manufacture of cream cheese may contain 45 to 60 per cent. of total solids, with only 2·5 per cent. of lactose and less than 1·5 per cent. of casein, it is imperative that the process of coagulation and the subsequent drainage of whey be slow ; this is best done by the addition of a small amount of a pure culture of lactic-acid bacteria. In such cheese the predominance of fat overweighs texture and the other differences in curd shown by lactic acid and rennet-coagulation. Such a cheese sours quickly and naturally has a short keeping-period.

All other cheese, such as soft cheese (unripened, mould-ripened or bacteria-ripened) and hard cheese (semi-hard, both ripened by moulds and by bacteria, and very hard, with and without gas-holes) are prepared from the curd obtained by the rennet-coagulation of milk. The many varieties of cheese, some only of local, others of world-wide fame, arise from different treatments of the milk, the rennet-curd, and in the ripening processes.

Hard and soft cheeses differ mostly in their moisture-content and in the composition of the protein degradation products after ripening. Hard cheese of 30 to 40 per cent. moisture-content ripens evenly throughout, and protein scission-products of nearly every molecular size occur, whereas with soft cheese of 40–55 per cent. moisture-content, not only is the ripe product more perishable, but more of the nitrogen is in a water-soluble form, and many more of the simpler molecules (amino acids and ammonia) are present. A simple definition of a ripe Cheddar cheese is one which contains a third of its contents as water, a third as protein, and a third as fat, with a third of the protein nitrogen in a water-soluble form. The main difference in the manufacturing technique is that curd for hard cheese-making is cut finer and scalded at a higher temperature. Very little grinding of the curd is done even for the semi-hard cheese, and with soft cheese not at all.

The temperature of coagulation in cheese-making usually lies between 28° and 35° C., soft cheese requiring a low and hard cheese a high temperature. The optimum temperature of coagulation is 41° C. The temperature governs the physical nature of the curd, whether jelly-like, firm or tough ; rise of temperature hardens and increases the elasticity of the curd.

The time for coagulation is relatively short for hard (twenty-five to forty-five minutes) and longer for soft cheese (one to two hours) ; but the first signs of coagulation appear long before the end of these periods ; the surface of the milk after coagulation will show pools of whey when the backs of the fingers are pressed

on the milk surface. Acidity is the major factor in determining the character of the curd and in the rate of whey-drainage later. The curd is cut with special knives into cubes of about $\frac{3}{4}$-in. side in hard-cheese manufacture. The curd is ready for cutting when the curd leaves the side of the vat cleanly on applying pressure. The gauging of the proper time of cutting is essential to guard against losses of fat in the whey and to ensure a uniform expected moisture-content in the curd at various stages. A thin pellicle at once forms around the piece of cut curd, and the outside layer has a lower moisture-content and a higher specific gravity than the inside. Later, after running off the whey, the pieces of curd have the property of welding together, although some air spaces are present. In the pressing stage the welding is carried far enough in the case of a fine curd to give a homogeneous mass (Cheddar), but with a coarse curd, such as in Cheshire cheese, the mass is not so homogeneous. The coherent properties of the curd occur most markedly with increase of acidity.

Since the acidity of the milk influences the amount of calcium ions, it also governs considerably the coagulating power of rennet. In cheese-making, acidity is determined by titration with alkali to phenolphthalein, the values being registered as percentages of lactic acid. Each step is controlled by the milk or drained whey being within a certain range of acidity. Trouble in cheese-making, such as a slow-working cheese, is encountered when the acidity does not increase at the expected rate. Such cheese, however, invariably catches up in the time-table of operations by being quick-working after the drainage of the whey.

Cheese is sometimes made from fresh sweet milk (soft and semi-hard), sometimes from milk of higher titratable acidity ($0 \cdot 2$ per cent. or thereabouts for hard cheese), which involves the use of a " starter." About 95 per cent. of the milk bacteria are entrained in the curd. On draining the whey, the lactose in the curd particles is quickly fermented to lactic acid, with the result that the whey draining off during the making of the curd increases rapidly in acidity with time and the whey from the presses is of high acidity. The operation of " cheddaring " is bound up with this increase in acidity, causing whey drainage and welding of the curd particles. In Cheddar cheese manufacture from milk of $0 \cdot 18 - 0 \cdot 20$ per cent. acidity, the whey at the time of cutting the curd has an acidity of about $0 \cdot 12$ per cent., at drawing $0 \cdot 13 - 0 \cdot 19$, at cheddaring $0 \cdot 24 - 0 \cdot 30$, and at salting and milling at $0 \cdot 70 - 1 \cdot 10$ per cent.

The scalding process necessary in the manufacture of hard and semi-hard cheese is done on the curd in its whey—cut for hard,

uncut for semi-hard. This checks the rennet-action, hastens the expulsion of whey from the curd, changes the curd-texture and the bacterial flora ; the curd in hard cheese becomes firm, tough and elastic.

The homogenisation of milk causes it to thicken with rennet more quickly than normal milk. Cheese from homogenised milk has a finer texture and holds more moisture than one made from normal milk. Owing to the fragility of the curd from homogenised milk, faults such as cracking and bad flavours develop in some types of cheese. The effect of clarification or centrifuging free from suspended material has no effect on losses of fat in the whey. The flavour and texture of hard cheese are slightly improved by this treatment.

PASTEURISED MILK AND RECONSTITUTED SPRAY-DRIED MILK FOR CHEESE-MAKING. Most Dominion Cheddar cheese is made from pasteurised milk. A comparatively mild-flavoured cheese results which differs considerably in texture from home-produced Cheddar cheese, being more " rubbery " and often suffering from the fault of openness, or the formation of fissures when the cheese is cut. It is possible that openness and texture are interdependent.

Owing to the slower working of cheese made from pasteurised milk, a higher acidity at renneting is used. This means a greater depletion of calcium and phosphorus from the curd by the more acid whey.[100, 101] Wode[102] has correlated weak body of cheese with a high calcium-content and *vice versâ*, and has found that the quantity of calcium dissolved from the curd increases with the acidity of the milk before setting. The quick separation of whey means less loss of calcium, since the total calcium dissolved by vat whey is greater than that by the much more acid press whey.

Moir[103] has observed that more protein remains in the rennet-curd from pasteurised milk than from raw milk, and that this is more pronounced with milk of low hygienic quality. The longer time required for the rennet-coagulation of heated milk is also associated with this decrease in whey nitrogen. Also the decrease in whey nitrogen is found to be much greater proportionally in acidified or naturally soured samples. This suggests that the decreased coagulability of heated milk can be partly explained by the flocculation of the soluble proteins on the casein micelles.

The presence of small amounts of albumin and globulin in curd gives a bad-flavoured cheese usually of poor texture on ripening ; the importance of excluding colostrum from milk is obvious. Moir has observed a bitter flavour in cheese made from heated milk, which is accentuated when high temperatures are used.

Spray-dried milk, after reconstitution, gives a product similar

to pasteurised milk, and can be manufactured into cheese. Dried milk has also been used to standardise the composition of milk for cheese-making. Milk standardised to a ratio of fat : solids-not-fat of 1 : 2·6–2·8 has been found to give satisfactory Cheddar cheese.[104]

The lessening of the coagulability of pasteurised milk has been overcome by the addition of soluble calcium salts before renneting. Thus the addition of 2 ml. of 25 per cent. calcium chloride, a quart of starter followed by 3 oz. of rennet per 100 gallons of milk at 32° C. gives good results ; the addition of hydrochloric acid in sufficient amount to raise the titratable acidity to 0·25 per cent., three quarts of starter and 2 oz. of rennet per 100 gallons of milk at 30° C. gives equally satisfactory results.[105] A greater yield of cheese and less loss of fat in the whey is experienced with pasteurised milk ; a cheese of higher moisture-content is also obtained. Cheese of a mild flavour results from pasteurised milk ; cheese from clean milk possesses a similar flavour.

The addition of calcium chloride to raw milk has been found to give more rapid curdling, a clearer whey, and a higher yield of cheese.[106] Neutralisation of acid milk, followed by pasteurisation, has been found unsatisfactory.

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PART IV

THE CHEMISTRY OF MILK PROCESSING

CHAPTER XVI

THE EFFECT OF HEAT ON MILK

141. Introduction

PROCESSING of milk in the wide sense consists in treating it to preserve its marketable value, to safeguard its value as a food, and to prepare non-perishable products from it. In the milk industry, however, the word " processing " has assumed the narrower sense of the treatment of fluid milk intended for liquid consumption, from the collecting depôt to the retail stage. These stages include the weighing and bulking, forewarming and (sometimes) clarifying, pasteurising and holding, cooling and bottling. The most important step is the pasteurising and holding process, and if this step is not efficient the whole aim of processing is frustrated.

The aim of pasteurising milk is to destroy by heat microorganisms which are pathogenic to man, and also to arrest the multiplication of organisms which, although harmless to man, may render milk unfit for human consumption. Incidentally, the keeping-quality is enhanced, and it is doubtful whether the costly process of pasteurisation is of more benefit to the processer by preserving the marketable value of his commodity and enabling him to offer a standard article of food at all seasons of the year, or to the consumer, who obtains a safe article of food that will keep sweet for a reasonable length of time at ordinary temperatures.

A certain amount of milk is also sterilised. This process is carried out by heating in bottles in tanks under pressure at temperatures above the boiling-point of milk. Narrow-necked bottles are always used, and in order to overcome the trouble due to cream plugs, which would occur with ordinary milk, homogenisation is carried out before the sterilisation process. There is also a local demand for homogenised pasteurised milk. Such milk naturally exhibits no cream line, whilst the mixing of the homogenised article with non-homogenised milk increases the cream line of the latter.

METHODS OF PASTEURISATION. (1) *Holder Pasteurisation.* The bulk of the milk offered for sale to urban populations is pasteurised by the " holder " method. In this method the milk is heated up to 62·5–65·0° C. (145–150° F.) and is held at this temperature

for thirty minutes, after which it is cooled, bottled and stored. This time-temperature combination is the officially recognised method of pasteurisation in this country, and licences for processing milk are given by local authorities acting under the regulations of the Ministry of Health for this method of pasteurisation only. Some steps, however, are being taken to pasteurise in bottle, a procedure which eliminates after-contamination of the milk. It may be pointed out that any milk may be treated in this manner and sold to the public, but it can only be designated as pasteurised milk when the plant is officially recognised.

(2) *Flash Pasteurisation*. In this process the milk is quickly raised to a temperature of 75° C. (165° F.), kept at this temperature for thirty seconds, and then quickly cooled. The heating may be carried out in continuous-flow heaters involving economy of heat by efficient heat-exchanging methods (*e.g.*, the A.P.V. heat-exchanging method), by passing into the heating-chamber as a fine spray (biorisation), or in the form of thin layers (stassanisation).

Stassano [1] has evaluated the process of pasteurising milk from the following criteria : (*a*) the imparting of a cooked flavour to the product ; (*b*) the degree of albumin coagulation ; (*c*) the increase in acidity in the first three to five hours' incubation ; (*d*) the maintenance of the original acidity of the milk after pasteurisation ; (*e*) the maintenance of the carbon-dioxide content ; (*f*) the extent to which the enzymes and vitamins are affected ; (*g*) the retention of the ability to coagulate on addition of rennet, and (*h*) the degree to which creaming ability is affected. The " flash " method of pasteurisation may be superior with regard to the above points. Other criteria, however, are the absence of metallic flavour or flavours due to fat autoxidation through the agency of traces of heavy metals, least pumping and agitation of the milk and, most important of all, the most effective destruction of micro-organisms. In Britain and on the American continent the " flash " method has fallen into disuse as a complete pasteurising procedure, but is often used as a means of prolonging the keeping-qualities of milk during transit to urban distributing plants. Of late, however, a considerable revival of interest in short-time high-temperature methods of pasteurisation has occurred. High-temperature pasteurisation is detrimental to cream-volume. Dahlberg [2] has observed that to preserve the cream-line, 71·1° C., for twenty seconds is the most severe combination of time and temperature which can be employed. Further, it has been established that although high-temperature pasteurisation is satisfactory for raw milk of low count, it is not safe enough for

raw milk of higher counts,[3] and a subsequent short period of holding at a lower temperature is suggested.

142. Superficial Effects of Heat on Milk

Milk tends to become slightly brown (caramelisation) on heating, this being apparent in " over-cooked " sterilised milk or re-sterilised evaporated milk. Wright [4] considers the browning to be due to the caramelisation of the lactose, but others think it is due to humin or melanin formation associated with protein breakdown in the presence of sugars. At holder-pasteurisation temperature, however, the effect is too small to be usually observed.

Pasteurised milk possesses a different odour from raw milk, probably owing to the formation of volatile substances, such as ammonia, hydrogen sulphide, mercaptans, and volatile phosphorus compounds, due to protein-breakdown ; the odour can be removed by aeration. The odour of milk heated to a higher temperature is more apparent, especially that of boiled milk. Sterilised milk possesses its own characteristic odour, which may be associated with the breakdown of fat.

The taste of boiled milk is produced only to a slight extent during pasteurisation but is easily discernible ; the responsible reagent is volatile and can be removed by aeration. Small quantities of acrolein, due to the breakdown of fats, have been suggested by Kieferle [5] as the cause of the taste. The taste increases with the temperature of heating, and a cooked, caramel taste is pronounced in milk kept at near its boiling-point for a considerable length of time. This applies especially to some samples of sterilised and evaporated milk.

Heating milk up to its boiling-point causes a skin to appear on the surface ; cream forming on such heated milk becomes associated with and adds to the quantity of skin formed ; the amount of fat which enters the " skin " in coffee milk is sufficient sometimes to cause the fat-content of the actual milk to fall below the legal standard. The skin may be present in milk heated at temperatures as low as $40°$ C.,[6] although it may be invisible to the eye. The composition of the skin varies with the amount of fat rising to the milk surface and may contain from 20 to 60 per cent. of its dry matter as fat, a small fraction of ash, which is mainly tricalcium phosphate, and the rest, denatured protein possessing the same properties as to isoelectric-point, etc., as casein. The physics of skin-formation is a modification of the Ramsden phenomenon, in which the concentrating of the proteins at the air/liquid interface causes an irreversible precipitation of a

portion of the proteins, both casein and albumin, an effect which is enhanced by evaporation from the surface layer. Where such precipitation occurs at the air/liquid interface of a bubble of air or gas in milk, the coagulum is soft. Such forms of coagulation are observed as pear-shaped objects on the surfaces of sterilised milk-bottles, or at the bottom of dishes in which egg-custards are made, and are prevented by the constant agitation of milk in processing plants. The formation of skin is, in practice, lessened by reducing evaporation from surfaces to a minimum.

Fresh milk foams considerably on boiling, and with this is associated the slight heat-coagulation on hot surfaces. Such coatings ("milk stone") have a serious effect on the efficiency of heat-exchange in such processes as pasteurising or vacuum-pan work. It has been found that preheating milk hinders considerably the occurrence of these drawbacks; thus, in condensing milk, forewarming avoids the coating of the heating coils with coagulated milk, losses by foaming into the condenser, and gives a product which will stand sterilisation at a higher temperature, or a sweetened condensed milk which will not readily thicken on ageing.

A form of precipitation in sterilised milk is that of a dark-coloured sandy deposit settling to a pool at the bottom, or a dark line on the side of the bottle. This fault is identical with "graininess" in evaporated milk. The sediment consists of calcium-citrate crystals together with some tricalcium phosphate, whilst a small amount of entrained iron salt gives a brown colour to the precipitate. The occurrence of this fault synchronises with the indoor-feeding period of the cows, when the citrate-content of milk is relatively high; the fault disappears when the citrate-content of milk decreases in spring and summer. The precipitation of calcium citrate is brought about by a prolonged heat-treatment of milk, and the crystals will not re-dissolve on cooling. The obvious remedy is to heat for as short a time as is compatible with the proper sterilisation of the milk (twenty to twenty-five minutes at 220° F.).

143. The Effect of Heat on the Individual Constituents of Milk

(a) LACTOSE. The caramelisation of lactose when heated alone in the dry state does not occur at temperatures below 145° C. But the heating of any sugar in the presence of protein or its degradation products, especially under acid conditions, produces browning (e.g., molasses, browning of hay heated in the stack). The browning of casein during the drying process is possibly due to this cause. Whittier and Benton [7] record indications of the

production of acid from lactose after the prolonged boiling of milk.

(*b*) FATS. The heating of milk does not appear to have any effect on the composition of milk-fat. The effect of heat on the physical condition of the fat globules is, however, considerable and of economic importance. As mentioned above, the loss of cream-volume is considerable with high-temperature heating, but the introduction of holder-pasteurisation at 143–145° F. followed by quick cooling has overcome this difficulty. Whittaker, Archibald, Shere and Clement [8] have recorded the following observations with reference to the heating of milk and effect on cream-volume : " Milk heated at 143° F. for thirty minutes showed practically no decrease in the cream-volume and in some cases an increase resulted. . . . Pasteurisation at 145–146° F. for thirty minutes reduced the cream-volume on an average by 8 per cent. with considerable variations above and below. . . .The tests showed that cooling milk to a low temperature after pasteurisation is necessary in order to obtain a good cream-volume."

Weigmann [9] has found that the ability of pasteurised milk to form a good cream-line depends on the temperature to which the milk is cooled after the holding process and the speed with which the cooling is effected. Immediate cooling to 41° F. (5° C.) is recommended. The property depends on the recovery of the semi-solid condition of the fat in the globule to give the maximum clumping effect.

Milk which is heated to a higher temperature is usually homogenised (*e.g.*, sterilised and evaporated milk). The occurrence of cream plugs in bottles or of churned fat in the tins is thus avoided.

(*c*) PROTEINS. The occurrence of two phases in protein coagulation—denaturation, the result of a chemical change in the protein molecule, and flocculation, the neutralisation of the electric charge on the molecule—has already been discussed (Section 133). The mechanism of denaturation is obscure, although many data have been obtained relating to the time-temperature denaturation of milk proteins. Kieferle and Gloetzl [10] heated large quantities of milk at holder-pasteurisation temperature, (63° C.), at 85° C., 100° C. and 115° C. for thirty minutes, the last temperature being obtained by autoclaving. A full distribution of the nitrogen before and after heating was determined (Table CXIV).

The albumin and albumose (proteose) fractions suffer a diminution on heating, and a small amount of protein decomposition appears to occur as well as coagulation. About 5 per cent. of

the albumin is coagulated at holder-pasteurisation temperature and 82 per cent. at sterilisation temperature. No diminution in the amount of diffusible nitrogenous substances was observed by Mattick and Hallett [11] on heating milk for thirty minutes at temperatures ranging from 40–90° C.

Table CXIV shows that there is no diminution in the figures for casein nitrogen on heating milk : the increases are due to

TABLE CXIV. *Distribution of Nitrogen in Milk heated to Various Temperatures (Kieferle and Gloetzl) (Mg. nitrogen per cent.)*

	Raw Milk	Heated (30 min.) at			
		63° C.	85° C.	100° C.	115° C.
Total N	540·4	537·8	537·3	540·5	537·2
Casein N	348·3	335·6	347·8	383·0	390·6
Albumin N	75·7	71·7	53·3	13·9	8·0
Albumose N	44·6	46·0	45·7	42·4	36·4
Peptone N	45·2	59·4	60·0	66·0	68·4
Total residual N	123·6	138·0	148·6	152·0	160·0
Amino N	4·12	4·17	4·39	5·45	5·53
Creatine N	3·39	3·58	3·56	4·03	3·73
Ammonia N	1·12	1·32	1·37	1·47	1·59
Urea N	13·80	14·38	15·83	15·93	17·41
Uric acid N	2·78	2·44	2·42	2·82	2·63
Residual N	31·12	32·56	35·00	37·80	41·12
Titratable acidity (S-H)	8·0	7·88	7·80	8·40	8·40
Sugar %	4·11	4·09	4·08	4·00	4·00

coagulated albumin now being reported as casein. Wright [4] has found that there is no change in the casein molecule on heating to 120° C. (248° F.) for thirty minutes. Casein, however, exists in milk as calcium caseinate and the physical properties of this complex, notably its coagulability with acids and rennet, are affected by heat-treatment. This has already been dealt with in Section 138.

(*d*) MINERAL CONSTITUENTS. The heating of milk causes the partial precipitation of the buffer salts. Many workers have investigated the action of heat on calcium salts. Thus, Soldner [12] observed the depletion of the calcium by its precipitation as tricalcium phosphate, this being confirmed later by a number of investigators (de Vries and Boekhout,[13] Purvis, Brehaut and M'Hattie,[14] and by Grosser [15]) who, however, did not obtain evidence that the precipitated calcium existed as $Ca_3(PO_4)_2$.

Diffloth [16] has also found that heating milk at 140° F. for thirty minutes decreases the soluble phosphates by about 26 per cent. Rupp [17] has found no difference in the phosphoric acid content of raw milk serum or that of the same milk heated at 155° F. for thirty minutes, but Milroy,[18] holding milk at just below its boiling-point for one hour, has observed a decrease in soluble calcium on filtering through paper. Palmer [19] has shown that calcium hydrogen phosphate ($CaHPO_4$), stabilised with gelatin, forms a precipitate on heating, and it is probable that the first action of heat on milk is to precipitate part of the colloidal calcium phosphate ; the work of Holt, La Mer, and Chown,[20] and of Hastings, Murray and Sendroy,[21] indicate the existence of a metastable state of supersaturation of the various forms of phosphates in milk. After heat-precipitation some of the soluble calcium and phosphorus would be taken out of solution to restore the equilibrium.

Leighton and Mudge [22] have heated milk in an autoclave at a temperature sufficiently high to cause coagulation, and they find that there is a marked heat-absorption at the same time as the appearance of a visible curd. A test carried out on an artificial serum containing the important inorganic radicals of milk with citrate, lactose and sucrose has indicated that the endothermic reaction is due to the precipitation of the phosphates and citrates of calcium and magnesium.

Bell [23] heated milk at various temperatures between 60° and 83° C. (140–180° F.) and prepared two kinds of ultrafiltrates : (a) by pressure filtration through a Pasteur-Chamberland candle, and (b) by passing through a Sharples centrifuge ; no difference in the calcium- and phosphate-contents of the ultrafiltrates of raw and heated milk could be detected by the first method of separation, thus confirming Diffloth's results, but by the centrifuge method there was observed a loss of soluble calcium of 0·4–9·8 per cent., and of soluble phosphorus of 0·8–9·5 per cent., according to the temperature of heating.

The amount of calcium present in the diffusible form has been found by Magee and Harvey [24] to be reduced from 26 per cent. in raw milk to 20 per cent. in the same milk pasteurised at 65° C. for thirty minutes, and to 15 per cent. when kept at the boiling-point for sixty minutes ; this was generally found to be the case also by Orr, Crichton, and their collaborators.[25]

The technique adopted by Mattick and Hallett [11] for determining diffusible calcium consists of dialysing for eighteen hours samples of milk, heated to various temperatures for thirty minutes, in standardised parchment capsules suspended in 3 per cent.

sodium chloride solution. Table CXV gives their results for diffusible calcium and phosphorus for the various temperatures. Definite indications have been observed that heating milk at temperatures of 145° F. and above causes a decrease in diffusible calcium, although no quantitative correlation of decrease with rise in temperature above 145° F. is interpretable, whilst the evidence for diffusible phosphorus is inconclusive even for temperatures of heating above 180° F.

The techniques used by Bell and by Mattick and Hallett were completely different in principle, which explains the conflicting

TABLE CXV. *Effect of Heating on the Diffusible Calcium and Phosphorus of Milk (Mattick and Hallett)*

Temperature of heating. °F	Diffusible Ca as % of total Ca			Diffusible P as % of total P		
	Raw	Heated	Differences	Raw	Heated	Differences
105–110	27·5	28·2	+ 0·7	38·4	38·9	+ 0·5
115–120	25·9	26·2	+ 0·3	34·9	30·9	− 4·0
125–129	27·0	26·1	− 0·9	31·6	33·3	+ 1·7
135–141	26·0	25·3	− 0·7	35·1	33·6	− 1·5
145–151	24·2	21·9	− 2·3	28·9	29·4	+ 0·5
155–160	28·1	24·8	− 3·3	32·0	33·0	+ 1·0
165–171	26·3	23·9	− 2·4	32·7	33·9	+ 1·2
175–178	30·6	26·9	− 3·7	35·2	31·4	− 3·8
185–189	24·5	21·2	− 3·3	31·2	28·8	− 2·4
195–198	25·3	21·8	− 3·5	28·7	25·7	− 3·0
205–209	29·8	26·8	− 3·0	33·6	33·0	− 0·6

results obtained from these two sets of experiments. Bell used the ultrafiltration method, which in the course of separation of the serum did not upset the equilibrium between colloidal and ionised calcium. Mattick and Hallett, on the other hand, on dialysing into a salt solution of roughly four times the osmotic pressure of milk, caused water to diffuse out of the milk in the capsule, thus concentrating the salts of the milk, whilst the diffusion of sodium ions into the milk would exchange in part with the calcium held in the caseinate-phosphate complex of the milk. The migration of calcium ions into the salt solution immediately displaces the equilibrium in the milk, and it is the accumulative migration in eighteen hours, if equilibrium has not set in by that time, which is the measure of the diffusible calcium. It may very well be that the differences in the diffusible calcium of raw and heated milk lie in the different degrees and rates of exchange of calcium for sodium in the colloidal micelles, coupled with a different physical

response (swelling) of the micelles to the increase of ionic (or salt) concentration in the milk during dialysis.

The importance of a uniform temperature for dialysis lies in the fact that ionic mobility is a function of temperature. Lampitt and Bushill [26] have used a method combining dialysis and ultra-filtration. They have found that heat-treatment (*e.g.*, comparing a solution of spray-dried milk with liquid separated milk) reduces the amount of inorganic phosphorus obtained by dialysis and ultrafiltration. They stress the importance of controlling dilution, acidity, and the previous treatment of the milk in determining diffusible calcium, which they carried out by the same technique as for dialysable phosphorus. From 25 to 36 per cent. of the total calcium of milk is found to be diffusible, that for a reconstituted, spray-dried milk and pasteurised milk being on the average less than for raw milk. The dilution of milk increases the amount of diffusible calcium.

The precipitation of tricalcium citrate by heat has been reported be several workers. The difficulty lies in accurately determining the citric-acid content of milk, and the results of Obermeier,[27] who found a loss of only 4 per cent. of the citric acid of milk by heating milk at 75·5° C. (168° F.) for fifteen minutes, are within the experimental error of the determination. Much higher temperatures of heating are required before crystals of tricalcium citrate in milk become visible to the naked eye. Too long a period of sterilisation of milk produced during the winter months can cause a silt of these crystals to deposit in the sterilised product. The crystals of tricalcium citrate are quite common as a sediment in evaporated milk which is sterilised in the tin at about 240-243° F. Some of the iron of the milk is also precipitated, giving a slight brown colour to the sediment. Matsuo [28] has found the soluble-iron content of both raw and boiled milk to be identical, although solubility in this statement must be taken to mean that it is not precipitated from milk in the heating process ; in any case only about 7 per cent. of heavy metals is in the ionic state in milk, the rest being in protein combination.

Magee and Glennie [29] report the loss of 20 per cent. of the iodine of milk in the holder-pasteurisation process ; the loss occurs as volatile substances containing iodine.

The increase in the amount of heavy metals in milk through the dissolving action of hot milk on metallic surfaces will be described later (see Section 149).

(*e*) ACIDITY. Heating milk drives away the free carbon dioxide and partly decomposes the bicarbonates, causing a small decrease of acidity as determined by titration to phenolphthalein.

Flash-pasteurisation has been found to decrease the carbon-dioxide content of milk from four volumes to 2 volumes per cent., but even on prolonged boiling all the carbon dioxide cannot be removed. The removal of carbon dioxide by heat has been stressed by some workers, who think that the amount present is of importance in the absorption of calcium and phosphorus in the alimentary tract ; but the importance does not appear to be borne out by facts.

Since the buffer index is changed by the precipitation of insoluble calcium salts, further heating of milk causes a slight increase in acidity. The mechanism of the change of titration-value is similar in principle to the changes obtained by dilution or precipitation of tricalcium phosphate at pH 8·0 8·5 (v. Sections 120–2). The slow adjustment of the calcium-phosphate equilibrium is evidently accelerated by heating.

144. The Effect of Heat on Milk Enzymes

When it is considered that the main object in the heat-treatment of milk is the killing of bacteria, and that this, in principle, amounts to an inactivation of the bacterial endoenzymes, it follows that the native enzymes of milk suffer inactivation to various degrees. Since the enzymes of milk, although present in small quantities, are capable of giving rise to slow progressive chemical changes in the fat, proteins, carbohydrates and minor constituents of milk, it is of importance to know at what temperatures they are inactivated, since if previous heat-treatment has not been sufficient to inhibit the greater part of their activity, they will be present in the products, viz., butter, condensed or dried milk, in sufficient amount to cause deterioration during storage.

Different enzymes have different coefficients of heat inactivation (K_c) and owing to this fact, to the length of the heating period and to the variation in the reaction of the medium, it is very difficult to define the distinctive temperature for any enzyme. Euler [30] has, in order to avoid confusion, defined the destructive temperature as " that temperatre at which the enzyme loses one-half of its activity when heated in an aqueous solution (free from substrate) for sixty minutes at a definite optimal hydrogen-ion concentration." In milk, however, there is a complex enzyme-protecting system associated with the soluble proteins, and undoubtedly the denaturation of lactalbumin by heat is a major factor in the partial destruction of milk enzymes by holder or flash pasteurisation. The inactivation of milk enzymes, however, has been found to occur at definite temperatures. Table CXVI gives the destructive temperatures as defined by

TABLE CXVI. *Inactivation of Milk Enzymes. Temperature Data*

Enzyme	Destructive temperature (° C.)	Destroyed in milk (° C.)
pase . . .	151° (dry)	80° (weakened at 60°)
roxidase . . .	69° [31]	72° (for 30 min.)
nylase . . .	57°	60–65° (for 60 min.)
ductase . . .	—	Above 80°
italase . . .	—	65–70° (for 30 min.)
iosphatase . .	—	62·5° (for 20 min.) [32]
alactase . . .	—	75–80°

Euler, together with data for inactivation of the enzymes in milk.

(*a*) LIPASE (see Section 71). This enzyme appears to occur in greater amounts in abnormal milk than in normal milk, and is concentrated in cream. Given the proper conditions for working, such as in milk where acid-producing organisms are inhibited, it produces butyric acid with an attendant bitter flavour, or in butter from unpasteurised cream it produces " butyric rancidity." The activity of the enzyme is considerably weakened by heating to 60° C., almost completely destroyed by flash-pasteurisation up to 71° C., and completely destroyed at 80° C.

(*b*) PEROXIDASE (see Section 71). A considerable amount of work has been done on the effect of heat on the inactivation of the peroxidase of milk, particularly in connection with the Storch reaction for heated milk. Table CXVII shows the inactivation temperature according to different authors. van Eck [34] has found that the destruction of the enzyme follows a mono-molecular law, and therefore the inactivation is a function of the time of heating. The actual peroxidase-content of milk has not been determined, and it is impossible in one test to determine both the temperature to which milk has been heated and the length of time of heating. Grimmer and Engle [35] state that peroxidase is closely related to lactalbumin and is probably bound to it. The inactivation of the enzyme at the temperature of complete coagulation of albumin is thus partly explained.

(*c*) AMYLASE. Koning [36] reports that 100 ml. of normal milk will hydrolyse 22·5 mg. of soluble starch in thirty minutes. The milk from diseased udders has a higher amylase content. Heating milk for sixty minutes at 60–65° C. inactivates the enzyme in milk and at 65–70° C. in colostrum. Gould,[37] in applying a modified " Rothenfusser test " for heated milk or for the presence

TABLE CXVII. *Inactivation Temperatures of the Peroxidase in Milk* [33]

Author	Reaction persists with heating to (° C.)	Destroyed at (° C)
Dupuoy . .	—	80°
Storch . .	75° for 2 min.	79–80°
Leffmann . .	76·5° (still active)	82°
Tjaden, Koske, Hartel.	Below 80°	Over 90° depending on time
Rullmann . .	30 min. at 70° C. (weak after 5 min.)	75° for 10 min. 69–70° for 60 min.
Schweitzer . .	About 60 min. at 65°	—
Utz . . .	71° for 75 min.	—
Koning . .	86° if heated quickly	73–74° if heated slowly
Neumann-Wender	—	83°
Seligmann . .	—	$\left\{\begin{array}{l}72° \text{ for } 15 \text{ min.} \\ 75° \text{ for } 5 \text{ min.} \\ 70° \text{ for } 1 \text{ min.}\end{array}\right.$
Butterberg . .	—	70° for 30 min.
Kastel and Porch .	—	$\left\{\begin{array}{l}70° \text{ for } 60 \text{ min.} \\ 75° \text{ for } 20 \text{ min.}\end{array}\right.$
Giffhorn . .	—	72° for 30 min.
van Eck . .	Depends on time of heating	—

of raw in pasteurised milk, assumes that all the amylase is destroyed at 60° C. in thirty minutes.

(*d*) REDUCTASE. This enzyme is not destroyed by heating milk at 65° C. for thirty minutes. Diccard and Rising [38] have shown that it is active up to the temperature of albumin-coagulation (72–80° C.). The Schardinger enzyme is destroyed by boiling milk.

(*e*) CATALASE. The amount of catalase in 100 ml. of fresh normal milk is, according to Koning,[35] sufficient to decompose 110 mg. of hydrogen peroxide in two hours. Heating causes a gradual inactivation, the temperature of heating having a greater effect than the time of heating ; but acidity exerts a preserving action during heating. Burstein and Frum [39] have found that complete inactivation occurs at 90–92° C. and heating for twenty to thirty minutes.

(*f*) PHOSPHATASE. Kay and Graham [32] find that a 96 per cent. inactivation of milk phosphatase occurs in about fifteen minutes at holder-pasteurisation temperature, five minutes at 67° C., three minutes at 70° C. and almost instantaneously at 75–80° C.

(*g*) GALACTASE is destroyed by heating milk to 75–80° C. ; in acid solution it is destroyed at 72° C.[40]

145. Tests for Heated Milk

Various tests have been devised to discover whether milk has been heat-treated or whether heat-treated milk contains raw milk. Some depend on tests for enzyme-destruction, whilst others depend on the destruction of the absorptive properties of the protein around the fat globules, and on the rising of the cream in milk.

A. REACTIONS DEPENDING ON ENZYME ACTION. (a) *Peroxidase. The Storch and other reactions.* The peroxidase of milk can liberate active oxygen from hydrogen peroxide ; the active oxygen in turn can oxidise various compounds, such as tincture of guaiacum and the class of compounds generally used as photographic developers, into coloured compounds. Advantage has been taken of this reaction of milk peroxidase, and of the destruction of the enzyme by heat, to differentiate between raw milk and milk which has been heated to a certain temperature.

Following the compulsory pasteurisation regulations in Denmark in 1898, it was necessary to provide a colour test which would ensure the fulfilment of the prescribed pasteurising regulations. Storch [41] then suggested his test in which the peroxidase of milk gives a grey-blue colour with *p*-phenylenediamine and hydrogen peroxide, and he believed it to be reliable for differentiating between milk which had been heated to 80° C. and above from milk which had not been heated to such a high temperature. Rothenfusser [42] used the hydrochloride of *p*-phenylenediamine and also a mixture of guaiacol and *p*-phenylenediamine. Benzidine has also been used with success, in which case a greenish-blue colour is given.

The guaiacum test has received considerable attention owing to the discovery that old solutions of guaiacum cause the colour to develop in milk without the addition of peroxide. Undoubtedly when tincture of guaiacum is stored for a considerable length of time exposed to light, a certain amount of peroxide is formed which, when broken up by peroxidase from any source, is sufficient to give the grey-blue colour. The colour is identical with that produced in the presence of added peroxide.[43, 44, 45, 46]

The time-temperature combinations required to inactivate peroxidase in milk, as found by various workers, have been given in the last section (Table CXVII). The thermal destruction of the enzyme evidently goes hand in hand with the denaturation of the lactalbumin.

Certain species produce milk without peroxidase. Thus although human colostrum contains the enzyme, the secretion

when the gland is in full flow has been found either to be free of the enzyme or to contain it only in traces. When the gland again becomes less active, it reappears [47] owing to the presence of leucocytes. Jolles [48] was so unsuccessful in obtaining positive reactions for human milk that he suggested the test to differentiate between human and cows' milk. Arakawa [49] has observed that there is no peroxidase in the milk of rabbits on a diet deficient in vitamin B. It may be taken that all ruminant milk at any rate contains the enzyme, since the producing animal does not suffer from vitamin B-deficiency.

The colour tests mentioned above are increased in intensity and rate of development by the use of sensitisers such as alkali borates,[50] tricresol,[51] or by pancreatic digests.[35] There is some evidence that the mechanism of the peroxidase reaction is bound up with the presence of traces of heavy metals, e.g., iron. It is known that traces of copper or iron, especially in the presence of protein, can set up a peroxide-peroxidase system; these systems can give the ordinary peroxidase reactions with the dissolved oxygen from the air, and van der Burg [52] has observed that traces of copper in milk give a reddish-grey colour which turns brown on rendering alkaline. The normal blue colour with hydrogen peroxide in milk turns red on making alkaline; the difference of colour when alkaline is, however, only apparent when a considerable amount of copper is present.

The Storch and other peroxidase tests cannot be applied for the detection of either holder-pasteurised milk or that pasteurised by the high temperature-short time method. Further, the test can be made positive in milk heated above 80° C. by the addition of a small quantity of raw milk.

(b) *Amylase.* Koning [53] has found that the amylase of cows' milk is inactivated by heating at 68° C. for forty-five minutes, and Giffhorn,[54] by heating for thirty minutes at 65° C. Gould,[55] however, claims that by his modified test he is able to detect milk heated below or above 60° C. for thirty minutes, and the presence of one per cent. of raw in pasteurised milk. A clear milk serum is obtained by centrifuging milk treated with basic lead acetate, hydrochloric acid and chloroform. Starch solution is added and the serum is incubated for four hours at 37° C. Samples of the solutions are added to a dilute I-KI solution. An intense blue colour shows that the milk has been heated above 60° C. and an orange colour indicates that the milk has been insufficiently pasteurised, or has been held for too short a time above 60° C.; a yellow colour denotes a raw milk or milk not heated above 60° C.

Orla-Jensen [56] reports that milk amylase is destroyed by heating for half an hour at 53° C. and that the starch-hydrolysis test (with KI and I) can be applied to detect weakly pasteurised milk, or milk momentarily heated to 70° C. The test is carried out on the whey by the addition of KI-I solution and some o·1 per cent. H_2O_2 solution, after allowing the milk to react on a series of quantities of starch solution.

(c) *Phosphatase*. The time-temperature combinations necessary to destroy the phosphatase in milk have already been given [32] (see last section). The determination of the phosphatase-activity of milk is recommended as a simple and valuable test for differentiating between raw and holder-pasteurised milk, and for evaluating the efficiency of commercial pasteurisation of milk by the holder method. The test is less sensitive than Gould's amylase test, since it can detect only 5 per cent. of raw in pasteurised milk with certainty. This applies to the quantitative test in which the rate of the hydrolysis of a standard glycerophosphate solution in a glycine buffer (pH 9·0) by the phosphatase is measured. A qualitative test, based on the increase in the depth of the coerulo-molybdate colour, after incubation for three hours, due to the hydrolysis of glycerophosphate by any phosphatase present, is able to differentiate between raw and properly pasteurised milk, and which, with slight modifications in the preparation of butter sera, can differentiate between butter made from raw or pasteurised cream.

This test has been improved (Kay and Graham [73]), using, as substrate, disodium phenylphosphate, which is hydrolysed by phosphatase about twice as fast as sodium β-glycerophosphate. Instead of determining the inorganic phosphate, the phenol, which is liberated, is determined using the Folin colorimetric phenol reagent. The colour produced is assessed either by a simple comparator, the Lovibond tintometer or a precision colorimeter.

The phosphatase test has been further developed and can now be applied as two separate tests. The shorter test (A) can be carried out in 30 min., and is used to determine whether milk has been heated or not, or for observing gross errors in the process of pasteurisation. Test B is used for determining small as well as large errors in processing, and is in most frequent use in control laboratories.

The reagents consist of (*a*) a buffer substrate (obtainable in tablets) dissolved in chloroform water (1 l.), containing sodium phenylphosphate (1·09 gm.) and sodium barbiturate (11·549 gm.); (*b*) a phenol reagent (Folin and Ciocalteau) made up of sodium

tungstate (100 gm.), sodium molybdate (25 gm.), hydrochloric acid (100 ml., sp. gr. 1·16), syrupy phosphoric acid (50 ml. of 85 per cent. in 700 ml. of water). The solution is gently refluxed for 10 hours and lithium sulphate (150 gm.), 50 ml. water and 4–6 drops of bromine added. After boiling for 15 minutes, the solution is cooled and filtered. This solution is diluted with 2 volumes of water before use ; (c) a 14 per cent. solution of anhydrous sodium carbonate.

Test A is carried out thus : To 10 ml. of (a) in a 25-ml. test-tube add 0·5 ml. of milk and incubate at 47 ± 2° C. for 10 minutes. Cool with cold water, add 4·5 ml. of (b) and filter after 3 minutes. To 10 ml. of filtrate are added immediately 2 ml. of (c) and the tube is placed in boiling water for 5 minutes and then filtered. Duplicate tests are made, and control tests, with 0·5 ml. added to both (a) and (b) together, are also run alongside. The colours formed in the carbonated liquid are compared with those of standard glasses.

Test B involves the use of the same volumes of milk and reagents as Test A, except that incubation in the presence of a few drops of chloroform is carried out at 37°–38° C. for 24 hours. The control test should be carried out either as in Test A, or the liquid should be filtered after adding (b) and kept in the refrigerator for 24 hours. The milk samples may be kept in a refrigerator for 24 hours, and controls need only be carried out on those which show a definite reaction after incubation for 24 hours.

If only a faint blue occurs in the four tubes of Test A, the milk has been heated. If a colour above 2·3 blue units is shown, the milk has been improperly pasteurised ; if above 6·0 B.U., the milk has probably not been heated at all. If the controls show more than a trace of blue colour, it is probable that a phenol-producing organism is present in the milk. If the colour in the incubated tubes comes very near to the standard tint, and more accurate information is required, Test B should be carried out.

In Test B, if the colour exceeds 2·3 B.U. the milk has been improperly pasteurised (either too low a temperature, too short a heating period, or admixture with raw milk). The admixture of 0·2 per cent. of raw milk to a correctly pasteurised milk gives a colour of 2·5 B.U. ; 0·5 per cent. of raw gives 3·7 and above. The colour of the filtrate from the control tests should not exceed 1·5 B.U. Raw milk gives more than 30 B.U. Test B is claimed to detect, (a) holding at 145° F. for 20 instead of 30 minutes, (b) holding at 1½° F. (i.e., 143·5° F.) instead of 145° F., and (c) 0·2 per cent. of raw milk in correctly pasteurised milk.

Anderson et al.[76] have made a critical study of the test. They

find the buffer substrate to be stable for months. They also find that milk samples can be stored for several days (up to 14) in a refrigerator without affecting the phosphatase test.

(d) *Other enzymes.* Schardinger's reaction is positive, even after heating milk to 68° C.[57] Koning [53] has also shown that the addition of lactose to sterilised or boiled milk restores the F. M. B. reaction. A small amount of ferrous sulphate solution also causes the reaction to be restored.[58] The addition of alkali also confers a sufficiently high reduction potential on lactose to reduce methylene blue. The test is thus of doubtful value for the detection of heated milk.

Catalase is fairly heat-resistant and consequently an examination of the time-temperature combinations for its inactivation in milk has not been fruitful in affording evidence of efficient low-temperature pasteurisation.

The amylase and phosphatase tests, therefore, appear to be the most useful for controlling the efficiency of low-temperature pasteurisation. It must be pointed out also that these two enzymes do not appear in the ordinary flora which would proliferate in pasteurised milk, whereas peroxidase and catalase would appear, in which case extra doubt as to the enzyme source is possible.

B. Tests Dependent on the Physical Properties of the Fat Globules. As has been shown in Section 98, the heating of milk changes some of the physical properties of the protective coating of the fat globules. After heating above 63° C., the property of cream volume is interfered with ; this is due to partial denaturation of the protein membrane of the globules. How far the denaturation of lactalbumin is bound up with this phenomenon is not known. Thus, holding milk at 60° C. is necessary to avoid precipitation of the albumin, but this is inadequate from the bactericidal point of view ; about 9 per cent. of the albumin is precipitated at 63° C. (thirty minutes' holding) and 10 per cent. at 68° (five minutes' holding). Again, holder-pasteurised milk shows very nearly the same cream volume as raw milk, but the creaming properties are altered by the holder process and, although not apparent in whole milk, can be demonstrated by dilution of the milk.

Again, a certain adsorptive property of the membrane surrounding the globules is partly lost during holder-pasteurisation. From the study of these two properties, two physical tests for the detection of holder-pasteurised milk have been elaborated.

(a) *The Orla-Jensen Creaming Test.*[59] This test depends on the fact that dilution of raw milk with water facilitates the rising of the cream, whereas dilution hinders it in pasteurised milk.

The milk to be tested, whole and diluted with an equal volume of water, is allowed to cream for two hours at 12–15° C. in a suitable vessel (Hoyberg butyrometers are convenient). The ratio (A) of the depth of the cream layer in the whole milk to twice the depth of that of the diluted milk is calculated. That for whole milk is always above unity, whilst that for milk heated for thirty minutes at 63° C. is less than unity. Heating above 63° C. causes the ratio to be still further depressed and, in some cases, heating at 64° C. causes the milk totally to lose its creaming properties. The importance of allowing natural creaming to occur for two hours only is stressed.

The test is unsatisfactory with milk containing large fat globules, such as Jersey milk. The amount of dilution with water is a factor in determining when the ratio A is unity. In the case of dilution with an equal volume of water, A was unity for milk heated at 62° C. for thirty minutes. But when three parts of milk were diluted with two of water, the temperature of heating to give A the value of unity was 63° C.

The creaming power of milk is associated with the globulin and not with the albumin content. Milk which has lost part of its creaming power by heat can recover it completely or partly by the addition of large or small amounts of the globulin fraction of milk, provided the milk has not been heated above 95° C.

(b) *The Schern-Gorli Test.*[60] This test utilises the fact that the fat globules of milk lose their adsorptive property for colloidal particles like charcoal, carmine, indigo, red blood corpuscles, or clay, on heating the milk above 58° C. A small quantity of the colloidal material is shaken vigorously with the milk and then allowed to stand ; a coloured ring forms in the cream layer of milk not heated above 58° C. Milk of high acidity, owing to rise of some curd when pasteurised, will give the test in two hours but boiled milk does not.[61]

In this test, the rise of carbon (or other finely divided material) varies directly with the rate of rise of fat globules, and indirectly with the adsorptive capacity of the globulin on the surface of the fat globules. Factors which influence the dispersion of the globulin, *e.g.*, shaking, change in pH or increase in the electrolyte-content, cause the adsorptive action either to decrease or disappear. The main reason why pasteurised milk does not give a positive test is the hydration of the globulin.[74]

(c) *The Rennet Test.* Owing to the prolongation of the time required for rennet-coagulation with increasing temperature of heat-treatment, efforts have been made to correlate the time of coagulation with rennet with the temperature of heating. Thus

Ladany [62] sets the times of coagulation of 50 ml. of milk with 2 ml. of rennet (1 : 100,000) at 35° C. as less than three minutes for raw milk, from three to ten minutes for pasteurised and over one hour for boiled milk. The value of this test is impaired by the fact that no cognisance is taken of the length of time of heating at any temperature, or of the increase in time of rennet-coagulation occurring in heated milk kept for a considerable time after the heating process.

146. Effects of Heat-treatment on the Physical Conditions in Milk

(a) PROPERTIES OF THE ACID-CURD FROM HEATED MILK. Zoller,[63] in his investigations on the manufacture of " grain-curd casein," found that when the casein was precipitated by acid from fresh skim milk heated to various temperatures, a variation in the character of the curd and in its behaviour on washing was obtained. He found that the curd from heated milk, on being washed with acidified water (pH 4.7), began to redissolve in the wash water ; the pH of the first washings also rose to 5·6. This was explained by the presence in the curd from pasteurised milk of precipitated alkaline-earth phosphates, which partly neutralised the acid in the wash water. Not only is the temperature of heating of the milk important, but also the period of heating since this increases the amount of phosphate precipitated and the amount of denaturation of the lactalbumin; the denatured albumin is coprecipitated with casein at pH 4·7 and modifies the gel-structure of the precipitate. Zoller notes the following results (Table CXVIII) for skim milk treated by the grain-curd

TABLE CXVIII. *Preheated Skim Milk and the Moisture-content of Grain-curd Casein after Drainage* (*Zoller*)

Skim milk	Temperature of pre-heating. ° C.	Duration of preheating Min.	Moisture-content of curd %
A	50	60	44·6
B	63	60	62·2
C	75	60	68·5
D	100	30	79·4
E	120	15	88·2
F	Control	0	40·3

Temperature of precipitation = 34° C.

method, draining the curd, washing once by decantation and draining the curd for thirty minutes.

Thus the higher the temperature of preheating, the greater is the water-content of the curd, which at the high temperatures approaches a gel in consistency. Milk, for instance, heated to 140° C. in a bomb, gives a curd gel, whilst solutions of pure casein in carbonate-free solutions of caustic soda and potash give a curd when heated to 118–135° C., the amount of precipitation depending on the time and temperature of heating. The pH changes to the acid side by 0·18 to 0·54 pH. The curd in this case is β-casein.[61]

The power of such curd to retain water is reversible, since the precipitation of the curd in a medium at the temperature of original pasteurisation results in a firm curd with an abnormal internal structure.

In studying the effect of temperature on the precipitation of casein from milk pasteurised at 63° C. for one hour, it has been found that the best results are given at 42·5° C., which is in contrast with the narrow range of 34–35° C., operating as the optimum for grain-curd precipitation from normal skim milk. The optimum temperature for precipitation is found to increase with the duration of pasteurisation, ranging from 40° C. for twenty minutes to 50° C. for one hundred minutes' heating. In the same way, with a constant pasteurising period of one hour, the optimum temperature of acid-precipitation rises as the pasteurisation temperature increases. Thus for pasteurising temperatures of 55°, 80° and 100° C. the temperatures for acid precipitation are 42·5°, 50°, and 52·5° C., respectively.

(b) RENNET CASEIN. Coagulation of rennet-casein from milk, pasteurised at 65° for sixty minutes at a pH of 6·2, causes (at 37° C.) the formation of a soft curd which needs digesting in the whey for thirty minutes at 60° C. in order to expel salts and water. A considerable amount of $CaHPO_4$ and $MgHPO_4$ enmeshed in the product gives it a high ash-content (5·8 per cent.). With normal milk, ten minutes' setting at 65° C. is usually sufficient.

With reference to the coagulation of cows' milk in the human stomach, Brennemann [65] has found that the coagulum, although independent of the volume ingested, is finer, flakier and more porous if diluted with water before drinking. Milk, pasteurised for twenty minutes at 68° C., gives a curd similar to that from raw milk, being only a little smaller in volume and softer.

(c) EFFECT OF HEAT ON THE DISTRIBUTION OF FAT GLOBULES AND CREAMING PROPERTIES. The processes of separation and subsequent pasteurisation of cream from raw milk cause some of the original fat globules to coalesce and thus change the size-distribution of the fat globules. Thus, Beckett [66] has found that the cream from milk, separated at 62° C. and subsequently pas-

teurised at 92° C., has after each process a larger proportion of the fat globules of a diameter greater than 8 microns, whilst the original milk contains no fat globules of this size.

In the creaming of milk of uniform fat-content the plasma colloids are of greatest importance. Palmer and Anderson,[67] when studying the effect of pasteurisation on creaming, showed that the whey colloids are effective promoters of cream-rising, that calcium caseinate hinders satisfactory creaming, and that pasteurisation increases the hindering action of the caseinate. " Both exhaustiveness of rise of fat and greater volume of cream are promoted by the more truly hydrophilic colloids and depressed by colloids with hydrophobic properties." Pasteurisation by the holder method increases the effectiveness of the former, but decreases the effectiveness of the latter.

The inhibited creaming of heated milk was formerly explained as due to the loading of the fat globules with precipitated protein (lactalbumin), but Rahn [68] found that the creaming of heated milk could be restored to normal by adding gelatin or other accelerating colloid after the heating process. By measuring the velocity of ascent of fat globules under different conditions, he proved that they do not rise singly but as clumps, which possess greater buoyancy. Whereas raw milk contains numbers of these clumps, heated milk contains only a few, the heating having destroyed them.[69] The essential difference in the mode of creaming is due to this lack of clumps or tendency to clump, and the addition of albumin, gelatin, etc. enhances clumping owing to the formation of an adsorbed colloidal envelope which permits the adhesion of globules as they collide. With added colloid a looser cream of lower fat-content is obtained. Heating with added colloid will again reduce the adhesiveness of the envelope, so that a greater proportion of the globules will rise singly to give a cream-layer of smaller volume and of higher fat-content.

A good cream-volume is favoured by the absence of agitation during the holding period and by cooling below 7° C. after pasteurisation. Further investigation is needed to elucidate the thermal changes which occur above 61° C. It is possible that partial destruction of lecithin at this temperature may to some extent explain the changed properties of the fat globule, since it is this material which acts as a hydrophilic stabiliser of the fat-phase in the aqueous phase.

The pasteurisation of cream is known to reduce its whipping capacity by breaking up the clumps of fat globules. Further heating of the cream completely destroys the whipping properties, and it is not possible to whip reconstituted cream made from

roller-dried milk powder and butter. On the other hand, the lower temperature of evaporation and the consequent lower degree of denaturation of plasma-proteins in spray-dried milk powder causes a reconstituted cream from that source to be whippable. The exact amount of denaturation of albumin necessary just to destroy the whipping properties of a cream has yet to be determined.

The fat globules disrupt to a greater extent in the homogenising process when the temperature of the milk is highest.[70] Milk, therefore, is homogenised at 55–60° C. At this temperature the larger fat globules (4–6 microns) are completely broken down and the product creams very little, and even centrifugal separation has little effect.

Parker and Spackman [71] have observed that pasteurisation at 63° C. for thirty minutes raises the freezing-point of milk by 0·01° C. The change in freezing-point, however, is usually too small to interfere with the interpretation of the results. Elsdon and Stubbs [72] report that the freezing-point of one sample of milk showed no detectable change when heated in an autoclave to 230° F.

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CHAPTER XVII

MILK AND METALS

147. Introduction

WHEN milk is processed it naturally comes into contact with a variety of metallic surfaces at various temperatures, and it is highly important to know not only what effect the milk has on the metallic surface but what effect the traces of contaminating metal have on the milk or on the by-products manufactured from it. The continuous contact of milk with metal in the parts of a processing plant slowly increases the amount of heavy metal in the milk, and this applies particularly to copper and iron.[1]

The following factors are concerned in the problem of what metals are best suited for dairy utensils and plant : (a) the metal must be serviceable and stand up to the ordinary wear and tear of everyday use ; (b) the metal should possess convenient fabricating properties such as malleability, capacity to be pressed, spun or cast into the desired shape ; it should solder or weld easily and should possess physical strength as thin sheeting ; (c) the metal should be a good conductor of heat ; this is of special importance in pasteurising and cooling processes ; (d) the surface of the metal should not be corroded by milk or its acid by-products ; it should resist the action of brine, soap and alkaline detergents, steam and hot water, and wear well under the continued effects of physical cleaning, e.g., by brushes and the abrasive powders present in detergents ; and (e) the metal, if it does not resist corrosion, should not shorten the keeping-quality of the food or by-product nor add to it traces of poisonous material, e.g., zinc, lead and antimony. From the engineering standpoint, both mechanically and technologically, plated copper has and still holds pride of place ; aluminium and the stainless steels are also extensively used, mostly for parts of plants where the unique heat-exchanging properties of copper do not need to be considered.

The appearance of plant also is important, and the use of untarnishable metal for vats, tanks, piping, etc. has to be considered. This introduces the problem of the effect of milk, its by-products (whey, sour milk, etc.), and the humid atmosphere of the dairy on various platings, such as nickel, tin, or chromium on steel and copper sheeting.

The storage of by-products and the transport and storage of

milk in large quantities require special consideration. This has been efficiently overcome by the use of aluminium, stainless-steel and glass-lined tanks.

148. The Effect of Milk on Metals. Corrosion

A very large amount of work has been carried out on the corrosive effect of milk on metallic surfaces, and the search for the ideal surface to come into contact with foods is still being carried on. The advances made lately in the variety of by-products made from milk and the better hygienic quality of milk and its by-products have demanded a closer examination of the problem of metallic corrosion, since the storage qualities of both milk and by-products have been found to be closely associated with contamination by heavy metals, especially where fatty foods are concerned. The significance of this will be dealt with in the next section.

Corrosion is a surface phenomenon and consists primarily and essentially of solution of a metal, or, in the case of an alloy, of one or more of its constituents, through contact with an electrolyte. Milk is an electrolyte and contains Na, K, Ca, Mg, Cl, PO_4, H and OH ions. The *acid* and *electrolytic* theories of corrosion, hitherto distinct and opposed, are combined and brought into agreement by the present-day conceptions of the mechanism of corrosion.[2] The acceptance of the electro-chemical ionic theory of corrosion enables all practical cases of corrosion to be divided into three types : auto-corrosion, contact-corrosion and externally-induced corrosion, the last type being that caused by the passage of an externally-generated current due to leaks, etc. from power circuits. Auto-corrosion occurs when a metal is in contact with an electrolyte, but not at the same time in contact with another conductor, and proceeds by the galvanic action set up by the physically and chemically heterogeneous structure of the commercial metal or alloy. Contact corrosion occurs when the metal is in contact with another conductor (metal or non-metal), both being immersed in an electrolyte, *e.g.*, soldered seams ; this sets up the galvanic action which is responsible for most corrosion troubles. Galvanic action is caused by differences in electrical potentials (or in solution pressures) of various metals. Thus when zinc is in contact with zinc sulphate solution, zinc ions pass into solution (electrolytic solution pressure), this action being balanced by the osmotic pressure of the positively-charged zinc ion, and at equilibrium these two pressures are equal. The metals have different solution pressures, those with the greater solution pressures being more electro-positive than those with the lesser.

The metals can thus be arranged in a series according to their electrical potentials, the following being the order of decreasing potential for those metals found in milk and those used for the construction of milk-processing plant : K, Na, Ca, Mg, Cr, Mn, Zn, (H), Al, Fe, Ni, Pb, Sb, Cu, Sn. Thus tin, having the lowest solution pressure, is least corrosive as plating for sheeting in dairy work; also any other metallic surface exposed with tin to milk must be anodic and therefore suffer corrosion while the kathodic tin is protected. It must be understood, however, that reversal of polarity can occur under certain conditions.

Most metallic surfaces with which milk comes into contact are frequently scrubbed and abraded, with the result that there is no tendency for rust to accumulate. If rust does form when the plant is idle, then the first runnings of the next batch of milk will dissolve it off and will be contaminated with iron and copper to a greater extent than the bulk of the milk. Under such conditions, therefore, it is doubtful whether Friend's colloid theory of corrosion [3] can be profitably applied to solution of metals by milk. But the action of the protein of milk is of interest in that it depresses the osmotic pressure of any heavy metallic ions in solution by the formation of metallic proteinates, which are very slightly ionised ; the solution pressure of the metal, however, remains constant and so corrosion is favoured. The amount of ionic copper in milk containing traces of copper is of a very low order of magnitude.[4] The amount of metal in the ionic form increases with increasing acidity of milk ; and for this reason it is not practicable to determine the copper-content of milk by means of concentration cells.

The *oxide, peroxide* and *biological* theories of corrosion are no longer regarded as tenable, but a resistant layer of oxide or the presence of a gaseous film has been suggested as an explanation of the passivity of metals to corrosion. The oldest of the theories— the oxide theory—assumes that the entire metallic surface is covered with a film of metallic oxide so thin—it is probably of molecular dimensions—that it does not interfere with the reflecting power of the surface. The gaseous-film theory attributes passivity to a film of gas similar to the metallic-oxide film. The physical theory assumes that the physical or electrical condition of the surface of the metal is so altered that it becomes less reactive chemically. Passivity is easily destroyed by contact with dilute acid, abrasion, galvanic action, and by heating in a reducing atmosphere. Milk usually causes a breakdown of the layer causing passivity ; it tends to have a low oxidation-reduction potential. Thus fresh milk straight from the udder has a consi-

derable reducing action which is overcome by solution of atmospheric oxygen. Later, when organisms grow, the tendency is to sweep up dissolved oxygen. This has the effect of destroying the oxide film and thus bringing the metal into the more active form ; also the regeneration of the film is hindered by the tendency for the concentration of dissolved oxygen to be decreased. Later still, when lactic acid is formed in small quantities, galvanic action on an active surface may be pronounced.

149. The Solubility of Metals in Milk

In ordinary dairy practice, the useful life of utensils and parts of processing plants is governed by wear and tear due to treatment in handling, and abrasion in cleaning with detergents and brushes. The amount of wear due to actual solution by liquids, such as hot water and milk, accounts for only a small quota of the loss of tin-plating from surfaces, and it is only when actual solution of the metal by milk is sufficient to appear either as a flavour in the milk or as the cause of " off " flavours in milk or its by-products that contamination with heavy metals assumes economic significance. To a lesser extent also must be considered the contamination with metals which do not give a metallic flavour or cause secondary reactions, but which act as cumulative poisons in the animal body. Although the effect of metals on milk will be treated in the next section, it must be understood that the whole progress of research into the milk-metal problem has been directed not so much to the subject of plant preservation, but to safeguarding the storage qualities of milk and its by-products. More attention has therefore been paid to those metals likely to initiate deteriorative changes in milk products.

COPPER. Owing to its mechanical and physical properties, plated copper still holds pride of place in dairy-plant manufacture. Unfortunately it requires only a small amount of copper to be dissolved by milk before oxidative changes of the fat are brought into action, a change which is handed on to all fat-containing by-products except cheese. A trace of copper has been found to be a virulent pro-oxidative catalyst to fat-autoxidation, and milk and its by-products suffer thereby owing to the development of oily, cardboard, or tallowy flavours during storage. (The mechanism of this change has already been described in Section 29e.)

Before the harmful effects of copper were realised, equipment made of copper and copper alloys was in extensive use in the dairy industry and a considerable amount of work was done on the solubility of copper in milk under various conditions. When it is realised that the majority of containers and apparatus in which

milk is heat-treated, *e.g.*, vacuum pans, hot wells, fore-warmers, pasteurisers, holders and coolers, are made of copper, plated copper or copper alloys, the menace to the quality of the dairy product is at once evident.

The solubility of copper in milk has been studied by Hunziker,[5] Miscall,[6] Quam,[7] Rice,[8] Davies,[1] and Gebhardt and Sommer.[9] The methods of investigation have differed somewhat, with the result that the data are not comparable, but some interesting facts have been revealed. Miscall,[6] and Rice and Miscall [8] exposed copper plates to the action of milk without agitation, but the results obtained were not comparable with those of Quam,[7] who
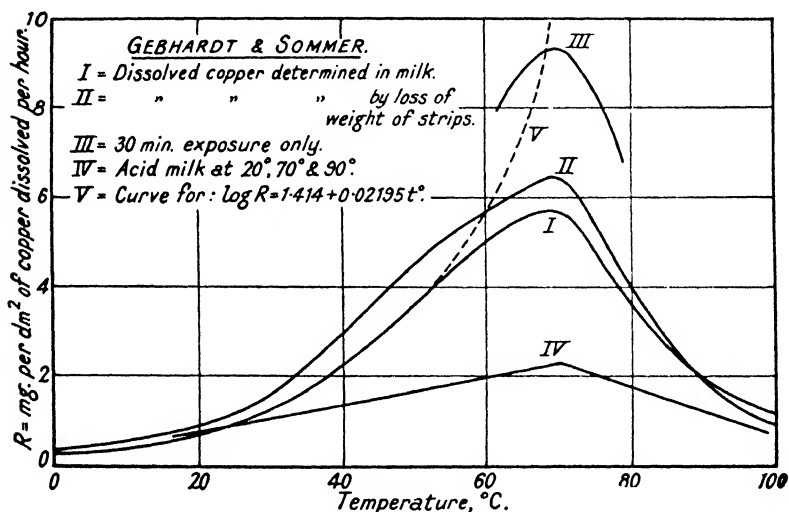
FIG. 22.—The solubility of copper in milk at various temperatures.

exposed copper to milk flowing to and fro over the test pieces, allowing the milk to have its full gaseous content, but with very little temperature control. Gebhardt and Sommer [9] overcame these objections by mechanical stirring in a thermostat.

The data of different investigators concerning the amounts of copper which dissolve in milk under certain conditions do not agree and are not comparable ; and this applies also to the temperature of maximum solution. Quam reports the temperature of maximum solubility as 85–90° C., but Miscall gives 66° C. for raw milk and 60° C. for pasteurised milk. Gebhardt and Sommer have found that temperature greatly affects the solution of copper, but that the maximum solubility is always 70° C. whether the time of exposure be thirty, sixty or 120 minutes. In milk of

high acidity the maximum is at the same temperature, but less copper is dissolved ; at boiling-temperature copper solubility is the same as at room-temperature.

The effect of various conditions on copper solubility may be summarised as follows : Small variations in acidity produce no perceptible effect on the solubility but higher acidity reduces the solubility, whether it is produced by added lactic acid or by biological agency. Neutralising the milk to an alkaline reaction does not perceptibly influence the solubility.

Copper appears to be less soluble in skim milk than in whole milk. Evacuation of milk before exposure to copper slightly decreases the amount of copper dissolved ; the bubbling of air through milk during exposure to copper slightly increases, but the bubbling of carbon dioxide markedly decreases the amount of copper dissolved. The effect of bubbling oxygen increases greatly the amount of copper dissolved, which is very pronounced at pasteurising temperature.

The salts in milk are not the most important factors in the solution of the metal but rather the oxygen exchanges and temperature.

With regard to the influence of temperature, the solubility increases at a logarithmic rate according to the temperature-reaction velocity equation : $\log R = a + bt$, up to 50° C., after which it falls off from the curve represented by that equation. The temperature of maximum solubility under all conditions is 70° C., so that chemical changes occurring in milk heated to this temperature for varying periods of time have no effect on copper solubility. The rate of solution of copper, however, becomes less as the time of exposure is increased. The decrease in solubility of copper at higher acidity is much more marked at high temperatures. (Figs. 22 and 23.)

Milk which has been boiled and cooled dissolves less copper than raw milk. Thus, in the experiments recorded by Gebhardt and Sommer [9] raw milk dissolved 16·25 mg. per litre at room-temperature, but milk previously boiled and cooled dissolved only 5·35 mg. and milk saturated with air only 5·45 mg. per litre. Milk preheated to 70° C. for one hour dissolved more copper than milk preheated to 60° C. ; the effect of preheating is bound up with the gaseous content of the milk.

The oxidation-reduction potential of milk remains constant to a temperature of 65° C., but when agitated with copper blades the potential increases rapidly up to 63° C. and is then constant to 75° C., after which it decreases rapidly. The presence of copper renders the potential more positive. Whereas the amount

of copper dissolved is related to the oxidation-reduction potential, the rise in potential may be an effect following on the activation of dissolved oxygen by copper (ion or proteinate). This phenomenon has been observed for copper only, and may occur to lesser extents with metals of the iron group, and certainly not with tin, zinc and aluminium.

The conclusion arrived at is that the solution of copper in milk is a case of metal corrosion in dilute acid solution.

The ratio of copper solubility in milk, when in equilibrium with oxygen, to that in equilibrium with air is 2·1 at room-temperature and about 3·0 at pasteurising temperature (62·5° C.).

The amount of copper dissolved per sq. dm. per hour is about 0·5 mg. in raw milk at room-temperature and about 6 mg. at pasteurising temperature.

These experiments were done on specially cleaned copper sheeting, no other conductor being in contact with the copper (auto-corrosion). In practice, it is certain that copper dissolves by galvanic corrosion, since other metals are present in contact with the same layer of milk, e.g., in the case of worn tin-plated copper. Davies [1] cites data for the amount of copper dissolved by milk when passing through a worn pasteurising plant. The increase in copper-content of the milk was, on the average, 0·25 mg. per litre. For a 1,000 gallon-per-hour plant this amounts to one gram of copper being dissolved from the various surfaces per hour, when the speed of solution has settled down to a uniform rate after the first half-hour of working the plant. The first 20 gallons, however, gain in copper-content from 0·5 to 0·75 mg. per litre, showing that bare surfaces of copper form a layer of rust during the time they are idle, this being dissolved away by the first flow of milk over the surfaces. It is advisable in such cases to reject the first 10 gallons or to use the milk for purposes where the effects of copper will not be apparent.

It must be understood that all parts showing exposed copper or manufactured from copper alloys add their quota of copper to the milk, e.g., brass taps, strainers, imperfectly tinned copper pipes, pumps, spreaders, coolers and milk-bottling machines. Davies (loc. cit.) describes a special type of bottling machine in which the volumes of milk to be delivered to the bottle were measured in half a metal cylinder, which rotated on a metal base until a hole in the cylinder engaged with a hole in the base for the delivery of the milk into the bottle. The metal-work was of copper alloy, and, during the abrading action of the rotation, sufficient copper was found to enter the milk to give it an oily flavour within sixteen hours. When the copper alloy was replaced

with stainless steel, all traces of milk taints due to the bottling machine disappeared.

Hunziker, Cordes and Nissen [5] immersed strips of copper and copper alloys in sweet and sour milk, and in sweet, sour and neutralised cream, for periods varying from five hours to five days, and observed the losses in weight of the strips and the state of the metallic surfaces after immersion. It was found that more copper dissolved in sweet milk in five hours at 145° F. (1·8 mg.) than in five days at 70° F. (1·1 mg.). Milk with a lactic acidity of 1·76 per cent. dissolved 4·6 mg. in five days, whilst butter-milk of 0·29 rising to 0·87 (70° F.) acidity dissolved 7·2 mg. Sweet or sour cream dissolved practically the same amount of copper at 70° F. in five days (4·0, 3·5 mg.), but sour neutralised cream dissolved only 1·0 mg. Acid milk cultures caused considerable losses of weight of tinned copper, monel metal, and nickel silver, the last-named losing weight considerably in all liquids under all conditions. These metals and alloys also tarnished severely. Of this group, tinned copper showed most resistance to loss of weight and to tarnishing.

Gebhardt and Sommer [9] state that copper dissolves in milk through the action of local elements in which the cathode consists of passive areas. They further studied the problem by substituting noble metal cathodes with copper anodes and measuring the current produced ; the amount of current was taken as a qualitative indication of the rate of solution under various conditions and the amount of copper dissolved could be calculated with fair approximation from the current. The method used was that of Toedt [10] for measuring residual currents of model elements which simulated the corrosion element of copper in practice. The effect of acidity was to decrease the current, and less copper was dissolved, whilst dissolved oxygen increased the current and the rate of solution of the copper. The current increased with temperature up to 70° C., and then decreased to the same value at 90° C. as at room-temperature. They state that the behaviour of other metals closely follows that of copper. They calculated from the residual-current measurements that the following amounts of metals dissolved per square decimetre per hour : german silver, 21·9 mg. ; solder-covered and tin-covered copper, 0·33–0·36 mg. ; monel metal, 25·8 mg. ; brass, 15·2 mg ; nickel dissolved at the rate of 3·63 mg./dm²./hr.

Hunziker (*loc. cit.*) also found considerable corrosion of copper and its alloys at the liquid/air interface.

ALUMINIUM. Owing to its lightness, cheapness, its purity and its mechanical properties, aluminium has of late been used

extensively for the manufacture of dairy plant, especially storage vats, frame castings, and utensils.

Hunziker found that aluminium and manganese-aluminium alloys resisted corrosion in cold organic acids, being slightly inferior to the chromium steels in this respect, but that considerable corrosion occurred in heated solutions. Aluminium-plated sheeting stood up to the attack of organic and dilute inorganic acids better than the pure metal. The amount of the metal dissolved by sweet and sour milk was of a very low order compared with that of all the other pure metals studied. The metal ranked with the chromium steels in its resistance to corrosion, and there was little evidence of tarnishing of the surface. Its superiority in this respect, even to tin and nickel products, suggests that the metal deserves favourable consideration. The metal is sometimes reported to pit badly when subject to liquids at high temperature, but this has not been borne out in practice where milk is concerned.

The physical state and purity of the metal are of great importance. Wrought aluminium, usually of high purity, withstands corrosion better than cast aluminium, which is less pure. Seligman [11] has observed that articles made from the sheet metal are unchanged after years of use, but cast fittings may give out in a shorter period of time, and the cause of the short life undoubtedly lies in the impurities providing the necessary elements for the formation of galvanic couples. Also contact of aluminium with other metals intensifies corrosion and pitting of the aluminium, the occurrence being usually highly localised. Hunziker states that aluminium rivets in nickel and the chromium steels become strongly corroded and pitted whilst those in tin are not affected.

Aluminium is soft and suffers damage under conditions of rough handling, and therefore robust construction is advisable. The alloys of aluminium—manganese and magnesium alloys (magnalium)—are much harder and can be fabricated in lighter grades.

The main drawback of the metal is its lack of resistance to alkalis, especially at hot-washing temperatures. Hunziker [12] found it to tarnish badly in sodium phosphate solution. The metal and its alloys are also seriously affected by lime and alkaline brines, but stand up well to all neutral brines.

Various magnesium-aluminium alloys have been tested by Davies (unpublished work) for their resistance to milk. The results show that for cold sweet milk the alloys are equal to the pure metal, but that considerable loss of weight and corrosion occur with acid products. Alkaline liquids cause high corrosion,

tarnishing and the formation of a crystalline scale ; after alkaline action the cleaned surfaces are also less resistant to milk on subsequent exposure to it.

The corrosion of aluminium by caustic alkalis can be avoided by treating the alkali with a small amount of sodium silicate. Thus 0·5 per cent. solution of sodium carbonate containing 0·05 per cent. sodium silicate gives no loss in weight when aluminium sheeting is immersed in it. Treatment with chromate of brines coming in contact with aluminium also prevents corrosion of the metal.

Aluminium bronze corrodes considerably in milk, especially at the air/liquid interface, and aluminium alloyed with copper, although improved in physical properties, is impracticable for dairy work.

THE STAINLESS STEELS. There are many alloys of steel and chromium, and of steel, nickel and chromium—the so-called " stainless steels "—which will not tarnish in a moist atmosphere. Their use in food plant, as well as in chemical engineering generally, constitutes a decided advance.

There are three broad classes of rustless and acid-resisting steels : (a) plain chromium steels containing 13–14 per cent. of chromium with a varying carbon-content ; a stainless iron containing 16-20 per cent. of chromium is also available ; and (b) a steel high in chromium, low in nickel (20 per cent. Cr, 2 per cent. Ni). Both of these types can be hardened and tempered and are magnetic at ordinary temperatures. These are *martensitic* steels and have well-marked carbide change-points so that high hardening-temperatures are necessary and the manipulation of the metal requires temperatures of 1,000-1,100° C.

(c) A steel high in chromium and nickel (18 per cent. Cr, 8 or 12–13 per cent. Ni). The higher content of nickel gives a softer and very ductile product suitable for deep press and cold work. These types of stainless steels belong to the *austenitic* class, which do not harden by quenching at high temperatures, and are non-magnetic. They require higher temperatures (1,150–1,200° C.) for manipulation. These steels have no carbide change-points and show a " solid solution " character. By the incorporation of 3 per cent. of molybdenum a special resistance to some organic acids (*e.g.*, acetic acid) at high concentrations and temperature is conferred on the steel. Special treatment of this type has overcome the intercrystalline corrosion difficulty met with in some stainless steels.

The plain chromium steels (Brown-Firth's FH and FG types) show excellent resistance to corrosive action by such substances

as milk, fruit juices and others met with in food-manufacture. The corrosion resistance increases generally with increasing hardness. The (b) (Firth Saitie Steel type) and the (c) class (Firth Staybrite F.S.T., F.D.P., D.D.Q., E.M.B., etc. types) are extremely resistant to corrosion and behave when in contact with most other metals in an electrolyte in a similar manner to the noble metals, and thus do not suffer from contact corrosion.

The thermal conductivity of the stainless steels is lower than that of ordinary steel. Thus, taking the thermal conductivity of silver as unity, that of class (a) is 0·043 -0·050, that of class (b) 0·046, and that of class (c) 0·033 (copper is 0·92 and german silver 0·14). This may be an undesirable feature in some cases, although for purposes of heat transmission, high thermal conductivity is desirable ; but it must be remembered that the thermal conductivity of the metal is often rendered of small importance owing to the formation of poorly conducting films on the surface of the material. Stainless steels are therefore much used for appliances through which heat can be transmitted without much loss of efficiency.

The surface finish of stainless steels may be variable. The surface may be de-scaled, dull-polished or bright-polished and the two sides of sheeting may be finished in a variety of combinations to suit requirements. The polished surfaces, which can attain a mirror-like brightness, should be washed with soapy water and a soft cloth ; coarse cloths, grit and metal polish cause scratching of the surface.

Hunziker [5] places chromium and chromium-nickel steels at the head of the list of metals suitable for use with milk. With the material available before 1928 (U.S.A. alloys, such as Ascaloy, Enduro and Super-ascaloy) he found that the chromium-nickel steels were on the whole superior to the chromium steels in resisting corrosion by milk products. He found, however, that they could not withstand succcessive exposure to steam, brine, and air, whilst the chromium steels gave a slight metallic flavour to acid milks. [12] North [13] and Quam [13] also rank the chromium-nickel steels as the most suitable for dairy plant, and chromium-steel cheese vats have been found quite satisfactory. Davies (unpublished work) has found that after the initial ageing process of a fresh Staybrite steel-surface in contact with milk, the amount of iron going into solution and the loss of weight of strips are negligible, and that after years of use utensils do not add a detectable amount of iron to the milk from the surfaces thus slightly worn by scrubbing and polishing. It is quite likely that, since Hunziker did his work, subsequent developments in the

manufacture of stainless steels have overcome the shortcomings of the earlier kinds.

The fabricating difficulties of stainless-steel equipment have largely disappeared through the introduction of types resistant to local physical changes due to heat-treatment in the welding process. Pressed seams are received with general disfavour in the dairy industry, because they entrap milk residues. Davies (unpublished work) has also studied the behaviour of seams sol-
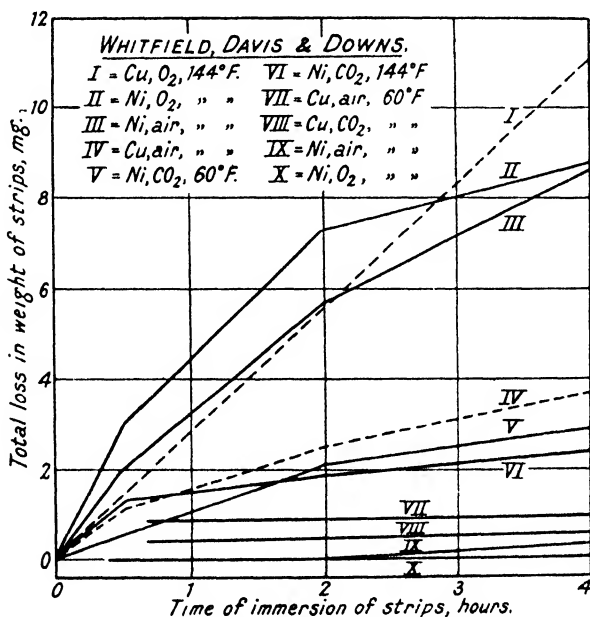
FIG. 23.—The solubilities of copper and nickel in milk under various conditions.

dered with lead-zinc in some of the original utensils turned out in chromium steel. The zinc of the solder rapidly dissolves leaving a *mud* of lead in a free condition in the seam, thus causing it to blacken. Such soldering is highly unsuitable. The riveting of handle-lugs on buckets with stainless-steel rivets has made such utensils more durable. It thus seems that the introduction of chromium-nickel steels into food technology has gone far to meet the need of an ideal metal surface for coming into contact with milk and its products.

NICKEL AND NICKEL ALLOYS. Nickel and its alloys are widely used in the manufacture of dairy plant and the behaviour of the metal towards milk and its products has been much studied. As

with copper, differences in the corrosion rates by milk appear in the findings of various workers. Thus the following weight losses in mg./dm^2./day were found by the investigators cited : 7 (Donauer [14]), 16 (Hunziker, Cordes and Nissen [5]), 281 (Quam [7]), 314 (Guthrie, Roadhouse and Richardson [15]), and 520 (Trebler et al.[16]). This variation drives home the need of testing metals intended for use in the dairy under all conditions of operation. La Que and Searle [17] state that nickel seems to be the only metal used in the dairy that is really protected by protective films, and that these films are formed under the following favourable conditions : a rise in temperature of the milk or the maintenance of a steady temperature in milk already heated ; a high rate of flow of milk and complete aeration or saturation of milk with air, the air acting physically as dissolved gas. A nickel surface, sufficiently preheated to show an iridescent film, suffered far less during a pasteurising run than similar specimens not so treated (a loss of 27 as against 532 mg./dm^2./day). It is also thought that a thin layer of precipitated protein on the metallic surface constitutes the protective coating in many cases, and that dissolved gases tending to be driven out of solution on contact with a hot metallic surface cause partial precipitation of milk protein at the gas bubble/milk interface, thus forming the layer of protein on the surface. Nickel has been found to corrode less at 35° F. in aerated than unaerated fresh milk, but with acid products oxygen increases corrosion at both low and pasteurising temperatures.

A rise in temperature increases the rate of corrosion of nickel, that at 145° F. being twice that at 35° F. The effect of agitation on the rate of corrosion depends on protective film formation ; other conditions being favourable, a high rate of flow is conducive to protective film formation and lessens corrosion, but otherwise an increased velocity increases corrosion. Acidity increases the corrosion of nickel only in fully aerated milk at low temperatures.

Nickel has the disadvantage that most of the other metals used in association with it in dairy plant are anodic with respect to it, and hence their rates of corrosion are increased many times. Thus, in a nickel vessel, aluminium paddles, lead-tin solder, and tin-plated copper corrode quickly in contact with milk. Nickel, on the other hand, is anodic to copper, silver solder, copper-nickel-zinc alloys, such as nickel silver, monel metal and stainless steels, and its rate of corrosion in contact with these metals and alloys is increased.

Alloys of chromium and nickel surpass nickel greatly in their resistance to corrosion in milk under all conditions of aeration, agitation, temperature and acidity. Nickel containing 14 per cent.

of chromium combines corrosion-resistance and surface-stainless-ness without losing its fabrication properties, and was experimented on originally for the specific purpose of milk-handling, the composition being based on the favourable results obtained by alloying steel with chromium and nickel. This alloy has the same heat conductivity as Staybrite steel but is slightly lower than that of the chromium steels (o·037, with silver as unity). The alloy welds more readily than pure nickel. It is resistant to alkaline detergents and hypochlorites ; it also stands up well against the action of brines.

Monel metal and nickel silver have proved disappointing in their resistance to corrosion and tarnishing, especially in contact with acid milk-products. These alloys also contain copper and cause either a metallic flavour or after-flavours (oiliness, etc.) due to the effect of small traces of dissolved metal on the fat of milk or its products.

TIN-PLATED STEEL. By far the greatest quantity of small ware used in dairy work, such as pails, jugs, measures, scoops, holders, cheese vats and moulds, curd knives, tanks and milk churns, are made from tinned steel. The gradual introduction of stainless steel utensils, cheese vats and bulk tanks may be said to diminish slightly the amount of tinware in use. Tin-plated steel has many advantages : it is comparatively cheap, easily fabricated and seamed, stands up well against corrosion by fresh milk, and wear through washing, steaming and rough handling. The average life of a milk churn in constant use is six years, during which it may require retinning and spinning once or twice. Milk comes in contact with most small tinware only for a comparatively short time, but the fact that storage of sweet milk in vats and churns, and of less valuable acid by-products, such as whey and buttermilk, in cheaper vessels, merits a consideration of the action of these liquids on tinned steel.

The tin-plating on steel differs from that on copper in that it is more porous, and there is more alloying of the tin with copper than with steel. It is also easier to obtain a clean metallic surface free from oxide and scale with copper than with the more reactive steel. The plating on steel-sheeting is never perfect and although a very close inspection is made for faulty sheets before boxing during manufacture, it is impossible to detect with the naked eye small imperfections in the plating which later act as nuclei for the steel to rust and throw up craters of detached plating. The life of the tin-plating on steel is thus materially shortened by the changes in the surface of the steel ; this is also evident when the tin has been abraded mechanically, and such bare patches of

iron will quickly increase in size due to rusting at the edges. The " spinning " of tin-plated steel also destroys the continuity of the tin-plate.

Hunziker has investigated the action of various liquids on tin-plated iron, using strips with the edges bare or tinned. In dilute acid solution, the loss in weight of such strips when half-immersed is about the same as with tin itself, the strips with bare edges showing more loss in most of the solutions. Heavy corrosion occurs with five days' immersion in one per cent. solutions of inorganic acids, acetic and lactic acids at ordinary temperatures (70° F.), whilst tarnishing of the tin-plating occurs with five hours' immersion in one per cent. lactic acid at 145° F. The strips with bare edges show more corrosion on the area outside the liquid than those with tinned edges.

With sweet and sour milk and cream, the loss in weight of partially immersed strips of tinned iron is again of the same magnitude generally as for tin itself, namely, about 7 mg./dm². in five days in sweet milk at 70° F., and 2 mg. /dm². in five hours in sweet milk at 145° F. ; the rates of corrosion are approximately 10mg./dm²./five days in milk of 1·76 per cent. lactic acidity (70° F.), 6mg./dm²./five days in sweet cream (70° F.), 14 mg./dm²./five days in sour cream (70° F.) of one per cent. acidity, and almost the same in five hours at 145° F. The strips with bare edges again show more loss in weight than the strips with the edges tinned. Slight corrosion of the immersed areas is observed with the acid milk and cream, tarnishing being more apparent with the bare-edged strips, thus showing that imperfect tinning leads to a greater amount of action on the tin-plating.

It is evident, therefore, that the resistance of tin-plated iron to corrosion depends on the quality of the tinning. Hunziker thinks that the greater loss in weight of tinned iron as compared with tinned copper strips is due to the greater porosity of the tin-coating on the iron.

Tin-plated metals are generally less resistant to action by alkaline washing liquids than tin itself, probably owing to electro-chemical effects. The plating also sometimes assumes a crystalline appearance similar to that of galvanised iron. North [18] has also observed that tin-plated steel shows much greater corrosion with alkaline detergents than chromium steels, nickel, and monel metal. The corrosion by alkaline substances, e.g., trisodium phosphate, can be greatly reduced by the inclusion of 0·025 per cent. of sodium chromate in the washing solution, tin-plate behaving very much like aluminium in this respect. The following concentrations of alkaline washing reagents have approximately the same effect on

tin-plated metals : 0·5 per cent. washing soda, 0·16 per cent. of trisodium phosphate, 0·5 per cent. of a mixture of 95 per cent. washing soda and 5 per cent. caustic soda. The importance of the choice of alkaline detergents for tinned surfaces is obvious, and the protective effect of small amounts of sodium chromate assists materially in solving the problem of preserving the tin-coating on such equipment. Kerr [33] has found that a ratio of 1 part of sodium sulphite to 10 of crystallised sodium carbonate in the detergent mixture (or 1 to 4 with soda ash or caustic soda) effectively prevents solution of tinplate with alkaline detergents.

Tinned iron corrodes heavily with solutions containing free chlorine, being inferior in this respect to tin and tinned copper. Alkaline brines generally cause heavy corrosion of tin-plate but chromate-treatment of all brines (neutral and alkaline, calcium and sodium) greatly lessens the amount of corrosion. Sodium silicate does not prevent the corrosion of tin-plate in brines.

IRON AND GALVANISED IRON. The use of cheaper vessels of iron or galvanised iron for holding large volumes of liquid milk by-products, such as whey and buttermilk, merits a discussion of the behaviour of such metals under those conditions. It can be said at once that these metals are inferior for the storage of milk and cream and are seldom used for that purpose. Hunziker has found that iron, galvanised iron and zinc lose weight severely in dilute inorganic and organic acids, in sweet and sour milk and in sour and sweet cream, whilst evidence of visible corrosion on surfaces either submerged in these liquids or in the air above these liquids is pronounced; tarnishing is also very marked. The loss in weight of partially immersed strips is so great, compared with other metals and alloys, and the ensuing flavour given to milk and cream so pronounced that it can only be concluded that this group is unfit for use in contact with dairy products. Tinned iron with areas of exposed iron is also unfit for the same purpose.

This group also suffers severe loss of weight in chemical sterilising reagents (hypochlorites) and in refrigerating brines. Hunziker, however, has found that these metals stand up to alkaline washing solutions better than tin-plated metals.

CHROMIUM-PLATING. Owing to the resistance of pure metallic chromium to corrosion, chromium-plated metal has not been neg-lected in the dairy industry. It is well-known, however, that chromium-plating in common with nickel-plating consists of electro-deposition of the metal, and that the metal is not alloyed into the hot metallic surface as in tin and zinc plating. The vulnerable point of chromium-plating thus rests in the union between the base

metal and the chromium, and the life of such a plating natur-
ally depends on the entirety of the thin plating. If the plating
is broken either by percussion, wear or abrasion, the sheeting
will corrode at once and the plating will curl or peel off. Chromium
as plating has therefore been found of inferior serviceability, and
it is evident that the non-corrosive properties of chromium can
best be exploited in its nickel or nickel-iron alloys.

LEAD, GLASS, GLASS ENAMEL-COATED METAL AND WOOD. Lead
is an easily fabricated metal and possibly could be used, when rein-
forced by wood, as a container of whey or other fluid milk by-pro-
ducts. Csizar [23] has found that the solution of lead by acid milk
depends on the acidity of the milk, the time of exposure, and the
quality of the lead surface. Thus a lustrous lead surface such as
would be obtained by etching with acid is not attacked by milk so
easily as a rough or a shiny surface, and possibly the solution of lead
from the latter surfaces is the result mainly of intercrystalline corro-
sion. An oxidised lead surface dissolves very rapidly. Increasing
acidity of the milk first increases and then decreases the solubility
of lead.

The lining of large-sized vessels, such as haulage tanks for road
or rail transport of milk, holding vessels and storage tanks, entails
the fusing of a glass or enamel layer on to an already fabricated
base ; a non-corrosive hygienic surface is then presented to the
milk. The durability of these vitreous surfaces is the major factor,
and this depends on their resistance to rough handling and to
changes in temperature, especially on round surfaces of small
radii of curvature. Other factors are the uniformity of thickness
of the vitreous layer and the resistance to chipping at seams
and openings. Such surfaces have proved generally satisfactory
on thick steel sheeting during the short experience of a few
years.

Wood is an important material for the fabrication of chemical
plant, and is extensively used in the dairy industry for structural
purposes and in direct contact with cream and butter in churns
and butter-workers. Owing to the difficulty of sterilising a wood
surface satisfactorily, milk should not be allowed to come in contact
with wood. For churning, wood surfaces must be " tempered "
so that butter does not adhere to them. This is done by scrubbing
all traces of fat off the wood, thus leaving the surface in a clean
hydrated condition, which will be wetted only by the aqueous-
phase of cream and by butter-milk. For long service only the
hard woods, such as oak and beech, are used for direct contact
with cream. Wood will withstand dilute acid conditions better
than most of the cheaper plated metals, and is therefore suitable

not only for the storage of large quantities of acid products but is very serviceable for the manufacture of casein.

Some coniferous timber has been found to confer its particular aroma on butter packed in boxes made from such timber species. This gives rise to the " wood taint " of butter. Wiley [26] has found that the wood taint of butter stored in boxes made from hoop pine (*Pinus radiata*) is due to an oil, which is volatile in steam but insoluble in water ; this oil accounts for the taste, but the odour is due to a water-soluble substance of a more volatile nature. Knotty wood contains o·5 per cent. and plain wood about o·05 per cent. of the substance which, when 1 to 10 p.p.m. are present, gives a taint to butter. Lining with moisture-proof cellophane, or spraying the inside of the boxes with alkali-caseinate solutions and subsequent hardening with formaldehyde, has been advised as preventive treatment, but it does not prevent tainting with strong-smelling pines.

150. Metallic Contamination of Milk By-products

(a) CONDENSED MILK. The manufacture of sweetened condensed and of evaporated milk brings milk at various temperatures and degrees of concentration into contact with metallic surfaces. For the metals used in condensing plant, ease of fabrication and maximal thermal properties are more important than the corrosive properties of the metallic surfaces under the action of the milk. Thus, the condensing process is carried out in a copper vacuum-pan where the milk comes into contact with a bright copper surface at about 55–60° C. Cases of metallic flavour and flavours due to reactions catalysed by the contaminating metals are therefore met with in the condensed products.

Thompson, Mears, Searle and La Que [19] have studied the problem of metallic contamination of milk during condensation determining the amount of copper dissolved during the whole process and the behaviour of other metals under the same conditions. Their method of testing was to expose discs of various metals and alloys on a Bakelite spindle at various points in the processing stages so that, although the test-pieces were exposed to the same conditions of temperature and agitation of the milk, they had no electrochemical influence on each other. The metals tested consisted of copper, tin, nickel, bronze, a nickel-chromium alloy (Ni 80, Cr 13, Fe 6), monel metal, soft solder, silver solder (Ag 60, Zn 15, Cu 25), chromium steel (17 per cent. Cr) and an 18–8 chromium-nickel steel. They found that corrosion, assessed by measurements of loss of weight and visible evidence of attack, was most active in the evaporators,

hot wells, drop tanks, preheaters and coolers, that is, at the stages where the liquid was longest in contact with metal at high temperature, or where the same liquid was in contact with metal at different temperatures. Copper was the least resistant to corrosion of all the metals which had good physical and fabricating properties ; the advantage of the other metals appeared to be greatest at the points where corrosion was most active, e.g., in the evaporators. Sweetened condensed milk was found to pick up 2·5 p.p.m., and evaporated milk 1·6 p.p.m. of copper during the evaporating process. A long period of exposure of the test-pieces to milk at high temperature caused a hard film to form on the metals, which was least protective on copper.

Rice and Miscall [8] have reported a copper-content of from 2·4 to 4·8 (average 3·7) p.p.m. of copper in 16 samples of sweetened condensed (10) and evaporated (6) milk ; these values include the natural copper-content and that picked up by the milk from parts other than the evaporator.

(b) DRIED MILK. The contamination of milk with iron during the roller-drying process is considerable. Whereas the arithmetic increase of iron due to drying gives a natural content of 12-16 p.p.m., some samples of the dried product have been found to contain up to 35 p.p.m. of iron. Very little increase above that taken up by the milk during its processing in the liquid state occurs in the iron-content of spray-dried milk. The high iron-content of the roller-dried product, although adding to milk what it lacks from the nutritional standpoint, may shorten the keeping quality owing to its catalysing effect on the oxidation of fat.

(c) CHEESE. During the manufacture of cheese almost the whole of the natural copper-content of milk enters the cheese, and it appears that the amount of leaching of copper from the curd, either by acid whey in the vat or by the very acid press whey, is small. Some Swiss cheese, e.g., Emmenthal, is made in copper vats. These are polished before fresh batches of milk are poured into them. A considerable amount of copper dissolves during the cheese-making operations, and Davies [20] has found 18 p.p.m. of copper in one sample of such cheese. It is doubtful, however, whether a comparatively large quantity of contaminating metal of this order is harmful to the flavour of the cheese ; no deterioration of fat can occur, since the amount of free oxygen inside a day-old ripening cheese is very small.

Particles of lead from lead ochre or soft solder cause black spots to appear in cheese owing to the formation of halos of lead sulphide.[24]

(d) CREAM AND BUTTER. Davies [4] has shown that metal

dissolved from surfaces of plant enters into protein combination, and that the metal proteinate is concentrated on the surface of the fat globules. Rice and Miscall [6] have found that the copper distributes itself between the fractions, cream and separated milk, during separation, roughly in proportion to the water-content of each fraction (*i.e.*, also in proportion to the curd nitrogen of each fraction). Davies, however, by a centrifugal method, which gave a cream of 70 per cent. fat, was able to demonstrate that the ratio of copper to curd nitrogen increased in the cream layer as the proportion of fat increased, thus showing that there was adsorption of complex metal proteinates on the surface of the fat globules. Further, this adsorption was found to persist when the cream was churned into butter, since there was a definite balance in the ratio copper : curd nitrogen in butter over that in the butter-milk ; this was also true for iron, whilst re-emulsification of such butter in metal-free separated milk caused the balance still to remain in favour of the butter after two to three successive emulsifications. It is clear, therefore, that the contamination of milk or cream by copper and iron through storage in rusty tinned-iron cans, or by processing neutralised cream, will cause a considerable amount of these metals to find their way into butter. It has been found for instance that, on a curd-nitrogen basis, about twice as much of the metals appears in the butter as in the butter-milk. With butter from sweet or neutralised cream this value is raised, but with cream which has been allowed to ripen naturally the ratio copper : curd-nitrogen in the butter and the butter-milk narrows.

Numerous investigators have shown that traces of contaminating heavy metals, especially copper, can cause oxidative deterioration of stored butter. Such " off " flavours as fishiness and tallowiness are developed. The onset of these taints is accelerated by a high acidity of the curd or serum of the butter, owing undoubtedly to the liberation of free fatty acids, especially oleic acid, with the result that the process of autoxidation, which occurs more rapidly with the free unsaturated fatty acids, causes the taints to appear in a shorter time than in butter with a neutral serum, *e.g.*, that from sweet cream. Davies (unpublished work) has repeatedly noticed that batches of Australasian butter which show these taints contain a greater amount of iron and copper (1 to 3 and 0·9 to 2·5 p.p.m., respectively) associated with a higher titratable acidity, than samples which have maintained their wholesome flavour during the same voyage.

Davies [21] has found that the metals commonly used in the manufacture of dairy plant and utensils can be divided into three

groups with respect to their catalytic action on the oxidative deterioration of butter. Copper stands alone in the first class as the most virulent pro-oxidative catalyst, being about ten times as active as the metals of a second group possessing the same action when present in butter in the same quantities. To this second class belong iron, nickel, cobalt, chromium and manganese. Aluminium and tin do not possess any pro-oxidative action even when incorporated into butter to the extent of 100 p.p.m., and these constitute the third group. Also the action of copper, even in amounts of only 2 to 5 p.p.m., is so virulent that the inter-mediate stage of fishiness is occasionally missed in test samples of butter, but with the iron group the fishy stage is well-marked before tallowiness and bleaching of the carotene set in.

151. The Mode of Combination of Traces of Heavy Metals in Dairy Products

There is considerable evidence that heavy metals form complexes with proteins and enter into an un-ionised condition. Vande-velde [22] has found that milk proteins are capable of combining with copper ions, removing almost all ionic metal from solution. Smythe and Schmidt [23] have found iron to behave in the same way. Under conditions of high protein concentration, therefore, it is evident that traces of heavy metals in milk will not exist as ions or in true solution, but that a distribution law greatly in favour of the protein-bound metal is followed.

A concentration of 30 p.p.m. of copper in milk, made by adding copper sulphate solution to milk, is sufficient to tint the milk a light green, but the colour disappears after an hour's standing. This is possibly due to the adsorption of the proteinate on the surface of the fat globules. Incidentally, this is the mini-mum amount of copper to yield a visible biuret reaction in milk on making alkaline with caustic soda solution. The formation of a green colour may be due to the copper forming highly coloured cupramino complexes, but Lieben and Lowe [25] have found that the precipitation of casein dissolved in potassium hydroxide by copper and silver salts quantitatively liberates an equivalent of potassium ion as shown by dialysis ; these metals are therefore regarded as not being in combination with the amino groups of casein. This, however, is not conclusive evidence, since it is unknown to what extent the copper co-ordination complex with casein has retained the original acidic properties of the casein, and possibly the formation of a co-ordination compound through the amino groups, especially when the sulphate is added, renders all the potassium dialysable. Vandevelde (loc. cit.) has also found

that a series of copper compounds with milk proteins can be prepared with an increasing copper-content.

It has been conclusively proved that the greater part of the heavy metal is in un-ionised combination with protein. The small fraction in the ionised state depends on the acidity of the milk. Davies [4] has investigated the amount of ionic metal by dialysis and potentiometric measurements. He has observed that with the acidity of milk increasing from 0·18 to 1·00 per cent. (lactic acid), the corresponding diffusible copper increases from 5·6 to 43·2 per cent. of the total copper, and for the same range of acidity the diffusible iron increases from 4·0 to 28·2 per cent. of the total iron. These results are, however, only comparative, since as soon as ionic metal diffuses from the milk the equilibrium between ionic and protein-bound metal is at once disturbed and tends to be maintained by bound metal going into the ionic form ; the above results therefore represent the cumulative effects of the reaction for the diffusion time (twenty-four hours) studied. The amount of diffusible metallic ions is found to vary directly with the acidity of the dialysate. The results obtained show that there is more metal in the ionic form in acid milk than in sweet milk.

The attempts at determining the concentration of the ionic form of heavy metals in milk by the use of concentration cells show that the concentration of ionic copper is of a low order, but that it increases with the acidity of the milk. A concentration of 1×10^{-1} N of copper in sweet milk (pH 6·6) shows a concentration of copper ion of $1·26 \times 10^{-10}$, $i.e.$, only $1·26 \times 10^{-6}$ is ionised. But at a concentration of 1×10^{-3}, copper in milk at pH 5·4 is ionised to the extent of $159·0 \times 10^{-6}$. Further experiments in which milk and acid and alkali were added to a solution of a copper salt have shown that the addition of small amounts of milk protein causes a large amount of the ionic metal to form compounds having a low degree of dissociation. By changing the pH to the alkaline side, the de-ionising power of unit weight of protein is increased, whilst increasing the acidity brings more ionic copper into solution, although in the presence of milk-protein the state of complete ionisation at high acidities is not recovered.

It remains to discuss which form of the metal is responsible for the catalysis of the autoxidation of the milk-fat. It does not seem likely to be the ionic form because the concentration of ionic copper in sweet milk is low. Most probably it is due to the organically-combined metal, since evidence in other fields of oxidation-reactions affords certain parallelisms. Thus the organically-combined iron in hæmoglobin and the copper in hæmocyanin are closely associated with the oxidative

reactions in blood ; the iron-cystine complexes are involved in the mechanism of oxygen exchanges in respiration and metabolism, whilst the oligodynamic action of traces of silver and copper is bound up with the effect of these metals when combined with bacterial protoplasm in activating dissolved oxygen to a concentration which is intolerable to organisms not containing sufficient catalase to destroy any peroxide which may be formed. The inhibiting action of traces of copper in milk on the acid-producing bacteria, and the consequent favouring of the growth of proteolytic organisms which contain catalase, is another example of this phenomenon. The investigation of the oxygen-exchanging properties of copper-casein complexes should prove fruitful in elucidating the activation of oxygen dissolved in milk and the subsequent oxidation of the fat to produce an oxidised flavour.

152. The Effect of Metals on Milk and its Products

Metals can affect milk in three ways : (a) by conferring a metallic flavour, (b) by catalysing the development of oxidised flavours, and (c) by adding to the milk metals that are cumulative, or other kinds of poisons.

(a) METALLIC FLAVOUR. A considerable number of heavy metals when dissolved in traces in milk can confer on it a metallic flavour. Donauer and Liedel [27] have investigated the amounts of metallic lactates necessary to impart a metallic taste to water and the amount of solution of the corresponding metals in milk at 140° F., for one hour. Table CXIX gives the results obtained.

TABLE CXIX. *Comparison of Metallic Lactates required to give a Metallic Taste, and Metal actually dissolved (Donauer, Liedel)*

Metal	Lactate Required to give Taste	Loss in mg. per dm.² at 140° F. for 1 hour (as Lactate)	Metallic Lactates Dissolved in Milk at 140° F. for 1 hour	Percentage of Amount Required to impart a Metallic Flavour
	p.p.m.		p p.m.	
Copper . .	4	7·4	2·4	61
Tin . .	50	14·6	4·9	10
Brass . .	7	6·6	2·2	31
Iron . .	15	61·7	20·6	136
Aluminium .	9	30·1	10·0	111
German silver .	6	7·1	2·4	40

The amounts of other metals which (as lactates) can impart a metallic taste *to water* are : aluminium bronze 6, bronze 6, monel

metal 10, nickel 14, nickel steel 14, zinc 19 p.p.m. As mentioned above, the casein of milk combines with added metal and, although no values are available of the amounts of metallic lactates necessary to give a detectable metallic flavour in milk, it is probable that amounts larger than the above will be necessary. The taste of the metallic salt in water is probably due to the ion ; it is unknown to what extent the proteinates depress the metallic taste, although blood which contains iron in organic combination tastes metallic. Hydrogen peroxide in very dilute solution also tastes metallic, and possibly the capacity of the ion or of the protein-metal complex to activate dissolved oxygen is partly responsible for the metallic flavour ; this may especially apply to silver and copper.

The values for the amount of metal dissolved (Table CXIX) are for one hour at 140° F., but these conditions are seldom reached in practice ; the figures however show that copper, tin and brass dissolve in smaller quantities than are necessary to impart a flavour, whilst iron, aluminium and zinc dissolve in excess of the amount required.

Hunziker [5] states generally that those metals which show definite corrosion have the most damaging effect on the flavour of the milk product. This refers particularly to iron, galvanised iron, copper, zinc, tinned iron, and nickel silver, the first three of which produce a marked metallic flavour in all milk products, and the last three in the majority of cases. The chromium-nickel steels, tin and properly tinned copper produce no flavouring effect. These are closely followed by aluminium products, nickel, monel metal and the chromium steels. Some cases of metallic flavour have been experienced with the last group, with the exception of the chromium steels.

(b) TRACES OF METALS CATALYSING THE DEVELOPMENT OF OXIDATIVE TAINTS. Although the amount of metals contaminating milk may be insufficient to impart a metallic flavour, lower concentrations of some are capable of causing the fat to be attacked by the dissolved oxygen of milk which they activate. Of all the metals, copper is by far the most virulent.

The copper-content of milk has already been dealt with in Section 89 (89 b). The natural copper-content of milk is variable, values ranging from 0·1 to 0·7 p.p.m. being reported by various investigators. Further, the general opinion of workers on the microchemistry of copper in milk is that these values are high and that the true copper-content is perhaps less than 0·1 p.p.m. The importance of the natural copper-content cannot be over-estimated, since it is customary to assess the possibility of an oxidised flavour developing in milk on the amount of contamination

during contact of the milk with plated copper and copper alloys. If small quantities of dissolved copper (*e.g.*, 0·4 p.p.m.) can cause an oxidised flavour to develop, it is reasonable to ask why the flavour does not develop in milk containing a natural copper-content of 0·7 p.p.m. The best explanation seems to be one that is based on a conception of all the conditions obtaining in milk that influence the development of the taint, namely, (*a*) the power of the micro-organisms partly to inhibit the autoxidation of the fat or other lipoid, which may be the reagent undergoing change, to give the taint ; no milk is completely free from the effects of bacteria ; acid-producers will tend to inhibit fat-oxidation but proteolytes will tend to favour it. (*b*) The seasonal variation in the amount and properties of natural inhibitors present in the milk. The latest work of Kende [28] points to the natural inhibitors being present in greatest quantity in milk from cows fed on fresh grass in spring and summer, stall-fed cows giving milk containing less of the inhibitors. This is in keeping with the general findings that winter-produced milk is more susceptible to oily flavour than summer milk, although the unsaturation of the fat of the latter (I.V. 36–42) is more pronounced than that of the fat of the former (I.V. 28–36). (*c*) The factors which activate the dissolved oxygen of milk, whether internal, such as traces of copper or copper plus iron, or external, such as sunlight, skyshine or diffused light, both working alone or together, appear to be of greatest importance in influencing the development of the taint. There is no doubt that *activation* of the dissolved oxygen is necessary, and that this activation is quickly carried out in the depth to which sunlight or strong artificial light can penetrate into milk, or by traces of copper salts distributed throughout the body of the milk. The increase in the oxidation-reduction potential, noticed by Gebhardt and Sommer [9] when small amounts of copper are dissolved in milk, is due to this fact. When milk coloured with methylene blue is held near a 100-watt lamp, the blue colour is immediately bleached on the surface ; this can be easily detected by slightly swirling the milk so that the unbleached milk displaces the bleached layer next to the glass. This is undoubtedly due to the dissolved oxygen in the layer next to the glass being activated by the chemically-active rays of light and immediately acting on the fat. The concentration of dissolved oxygen is thus reduced locally below the concentration at which leuco-methylene blue is oxidised by dissolved oxygen, and thus the reducing agents in the milk can bring about the reduction of the blue to its leuco form. That this can occur in pure butter-fat has been shown by Greenbank and Holm [29] in their optical method of measuring the susceptibility of fats to oxidation.

These activating factors are also cumulative ; when a case of oxidative taint occurs, it is undoubtedly the cumulative effects of traces of metallic catalysts together with catalysis by light, which leads to the development of a taint. It is difficult to assess quantitatively the part played by each, and it is only by analysing the milk for contaminating copper and iron that evidence of a reliable nature can be obtained. In all cases where taint has been observed, the copper-content of the milk has been found to have been increased during processing. It is true that in aseptically drawn milk the copper-content of milk as determined by analysis need only be from 0·2 to 0·5 p.p.m., or even less in some cases, to cause the taint to develop, but under commercial conditions with pasteurised milk, in which there is a considerable bacterial population. After processing, over 1·0 p.p.m. is necessary to give a taint within twenty-four hours. Davies [1] has found the lower limit to be 1·5 p.p.m. Certain samples of milk will give a taint with less and others with more copper ; this variation is due to the conditions enumerated above not being identical for each sample.

Dealing further with commercial milk, it can generally be taken that all metals other than copper cannot cause an oxidised taint to develop within the period that liquid milk, either raw or processed, is stored before consumption. Some metals, such as nickel and iron, can be made to cause the taint to develop in milk of low bacteriological count under laboratory conditions, but such conditions are seldom reproduced commercially, whilst with graded milk the conditions necessary for metallic contamination do not often exist to the same degree as in a commercial pasteurising plant.

When the storage properties of milk products are considered a different problem arises, since the time factor is of greater significance. The presence of traces of metals other than copper now has a significant effect on the deterioration of milk-fat. The contaminating iron of roller-dried milk has been found to shorten its keeping quality and to cause profound changes in the carotene and the vitamin-A-contents of the dried product. There is no doubt that traces of copper aid the deterioration also. It has been found that the moisture-content of the dried product exerts an effect on the course of the deterioration. With a relatively high moisture-content, a fishy flavour appears before the tallowy taint ; with the product of low moisture-content it is the tallowy flavour only which appears. It seems that where a considerable volume of moisture film occurs, the lecithin is dissolved out into the concentrated salt solution of this film and is oxidised, evolving

trimethylamine to yield a fishy flavour ; such conditions are not possible at lower moisture-contents. A parallel set of conditions is exhibited in butter, which can develop fishiness before the tallowy stage, whilst pure butter-fat will not show fishiness, although the lecithin is present in practically the same amounts as in butter.

In butter, traces of copper, iron, etc. can cause fishiness and then tallowiness to develop. Copper is the most virulent metal in bringing about these changes, whilst iron, nickel, cobalt, manganese and chromium give a well-defined lengthy fishy period before the period of tallowiness, which commences with bleaching of the carotene, sets in. Aluminium, zinc and tin do not cause these changes.[21]

(c) METALS AS CUMULATIVE POISONS. The amounts of heavy metals which enter milk and its products intended for human consumption are usually not large enough to be physiologically significant. One instance has been reported where pigs have been poisoned by the zinc which was dissolved from the surfaces of galvanised containers by acid butter-milk.[30] The solution of small quantities of copper may also be dangerous to swine.

The effect of small amounts of aluminium in human food is controversial, but certain maximum limits have been applied to foods like processed cheese. These limits are : Great Britain, Australia, 286 ; U.S.A., 300 ; some tropical countries (Dutch Colonies), 143 p.p.m.[31]

Although the amount of chromium which dissolves from chromium-plating or chromium steels is too small to be of any physiological significance, attention may be drawn to the use of potassium dichromate as a preservative of samples of milk for analysis. Certain workers are susceptible to weak solutions of dichromate and develop a form of dermatitis very quickly when such milk accidentally comes into contact with their hands ; most workers, however, are immune from such effects.

The accumulation of metals in the human body has been studied by Flinn and Inouye,[32] who find that 99 per cent. of ingested copper, tin and nickel is excreted in the fæces, and that 70 per cent. of aluminium is excreted in the fæces and 30 per cent. in the urine. There is thus practically no accumulation in the human body of the metals that may enter milk from dairy plant.

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CHAPTER XVIII

THE CHEMICAL TECHNOLOGY OF MILK CONDENSING

153. General

THE idea of commercially preserving milk by evaporating much of the water and preserving with sugar, or by sterilising, dates back to 1856, when Borden was granted patents in the United States and England for his vacuum-evaporation method, the principles of which still obtain in the modern methods of condensing milk. The process of preserving unsweetened evaporated milk was, however, introduced by Meyenburg in 1883, who sterilised the evaporated product by using steam under pressure and this method is still in use to-day.

Although the manufacture of both types of condensed milk involves the use of the same type of vacuum-pan and the appearance of practically the same *milk solids* in the final products, it is obvious that the evaporation of water from a concentrated sugar solution needs a longer time and consequently more heat units. Thus in evaporated milk, the final concentration of lactose is equivalent to that in a 15 per cent. aqueous solution, whilst in the final sweetened product the concentration of total sugar (sucrose and lactose) is about 57 per cent. The *sucrose ratio* in sweetened condensed milk (the percentage of sucrose × 100 divided by the sum of the percentages of sucrose and water) is roughly 61 per cent. The various *cocci* responsible for the defect in the sweetened condensed product known as thickening require a minimum sucrose ratio of 63·5 per cent. to become inactive.[1] The lactose ratio in sweetened condensed milk is 31 per cent., which is well above the normal solubility of α-lactose monohydrate in water at 16° C. (see Section 36). In the sweetened product, therefore, one of the main problems that has arisen is the controlling of the crystallisation of lactose to avoid the formation of crystals large enough to settle in the can and cause a gritty texture.

The main problem in the manufacture of the evaporated (unsweetened) product is the resistance of the condensed product to heat-coagulation at the high temperature of sterilisation, and this is controlled by carrying out various heat-stability tests on the raw milk, and by running a pilot sterilisation trial on samples from each batch after the addition of varying amounts of a stabiliser like

sodium bicarbonate or sodium citrate. The consistency of the sterilised product aimed at is that of a weak gel, which will prevent fat-separation during storage but will break up to a creamy fluid on shaking.

Other by-products of milk that are preserved by condensing are evaporated skim milk, both sweetened and unsweetened, semi-solid butter-milk and whey paste.

154. The Chemical Control of Evaporated and Sweetened Condensed Milk.   Raw Milk

Methods of payment for milk intended for manufacture vary so much in different countries that it is necessary to enumerate them. The most general method is payment by weight or volume (1 Imperial gallon = 10·3 lb. of milk) but other methods directly connected with the product manufactured, such as on the weight of fat supplied, are prevalent in cheese- and butter-producing districts. Denmark has advanced in the direction of payment on a quality basis, *i.e.*, on the weight of total milk-solids supplied. There is no evidence that a large-scale payment on the basis of the casein-content has ever been attempted, mainly perhaps owing to the difficulty of accurately determining that constituent. In countries importing much dairy produce, the price of the imported product has been taken as the basis of setting the price for its equivalent of raw milk. Thus, before the marketing of milk was reorganised in Great Britain, the price per gallon for manufacturing purposes was a penny less than the current price per lb. of imported Dominion Cheddar cheese. Competitive prices of milk for the fluid-milk market and the organisation of milk-marketing generally have caused this method to be discarded, with the result that better prices are now obtainable. The payment for milk on a quality (composition) basis shows no advantages in the long run over payment by weight. Payment for milk on the basis of hygienic quality, has, however, been carried out for a considerable time both by the official grading system and by private enterprise, and is likely to gain ground in the future.

155. The Quality of Fresh Milk

For making condensed milk, the good hygienic and compositional quality of the fresh milk is of the greatest importance, and the ignoring of this fact is bound sooner or later to cause a considerable loss in the marketable value of the condensed product as well as difficulties in manufacture. The composition of milk, on which the heat-coagulation point depends, is known

to vary with the health, period of lactation and the feeding of the cows, whilst the changes in milk due to bacterial fermentation depend on the care of the milk during and after the process of milking. Cows which are constitutionally diseased, or have diseased udders, produce milk that is abnormal in composition and reaction; in such cases the protein distribution and the balance of the mineral salts are affected, and both these factors are changed in the direction of lowering the heat-coagulation point of the evaporated product. The inclusion of late colostrum and of milk given at the end of lactation also cause a lowering of the heat-coagulation point.[2, 3] The effects of the food of the cow on the processing of the milk are not so clear-cut, but fortunately the effect of grass, the natural food of the cow, and that consumed generally at the period of greatest milk flow, is to improve the salt-balance and increase the resistance of evaporated milk to heat-coagulation.

Again, any condition tending to cause or accelerate lactic fermentation in milk lowers the heat-coagulation point, and organisms secreting rennin-like enzymes have the same effect. Proper cooling of milk on the farm and keeping in a cool condition until it reaches the factory minimise bacterial multiplication and increased acidity due to lactic fermentation. Although the heat-treatment of the milk before condensing (forewarming to 200° F.) destroys most of the vegetative forms of bacteria present, some of the residual live bacteria and the spores may persist in the viable form, even through the sterilising process, and give trouble in the final product. Elimination of high bacterial counts should take place at the source, and this entails proper hygienic conditions in milking, cooling and transport of the milk.

The milk should also be of a fairly uniform composition and quality. This is usually the case for bulk milk. The standard in Great Britain for condensed milk is 9 per cent. of fat and 22 per cent. of solids-not-fat. This gives a F : SNF ratio of 1 : 2·44, which nearly conforms to the ratio for the average composition of milk (F : SNF = 1 : 2·4) and allows for a slightly lower fat-content. As is well known, small consignments of bulk milk will vary a little from this ratio, and it is better that the ratio should be as near to the officially required ratio as possible, since this minimises the amount of adjustment with cream or separated milk necessary to standardise the milk before condensing. The milk supplied should also not contain less than the presumptive standards set for fat and solids-not-fat, since the evaporated product has to conform to a total-solid standard, although the ratio of fat to solids-not-fat of the poor quality milk might be correct. Also

milk low in solids-not-fat may be strongly suspected of possessing those factors generally responsible for a low heat-coagulation point (high salt-, albumin- and globulin-contents).

156. Inspection of Milk on Receiving at the Factory

Every churn or consignment of milk should be rigidly inspected at the receiving platform. An experienced platform hand can gauge with much accuracy, by the odour, temperature and the appearance of the surface of the milk (for churned fat globules), whether the churn contents will pass or not. Control at the delivery floor should also be frequently assisted by determinations of acidity, and by other tests enumerated below which have in view the suitability of the milk to withstand the sterilisation process.

Milk of high acidity generally has a low heat-coagulation point. But it must be understood that the *natural acidity* of milk, although calculated as lactic acid, is due to the presence of factors other than lactic acid. The natural acidity does not affect the heat-coagulation point. It has been found that it is the *developed acidity* which lowers the stability of milk towards heat, and since the acidity test gives the sum of the natural and developed acidity in milk, the titratable acidity is not an accurate index of its suitability for manufacturing purposes. This is further complicated by the possible variations in the natural acidity of milk, which in some cases lead one to believe that the milk possesses developed acidity. Generally, however, the acidity test is a useful method of control for bulked milk, and it must continue to be used on the receiving platform.

The natural acidity of bulk milk seldom exceeds 0·18 per cent., calculated as lactic acid, and generally no unnecessary hardship is inflicted by the rejection of samples testing above this value. On the technical side, the experience of the churn-opener tells him whether the churn contents have developed lactic acid or not, and the carrying out of the acidity test in doubtful or suspicious cases is valuable sorting evidence. The acidity test is a useful indication of the hygienic care taken in the handling of the milk and the efficiency of cooling, storage and transport; if these precautions are taken during the life of the milk before reaching the factory, there will be no causes of complaint relating to smell or acidity.

157. The Sediment Test

This test shows the amount of visible dirt in milk. Milk is forced or drawn under pressure through discs of absorbent cotton and the white, brown or black colour of the disc is used as a

rough index of the hygienic conditions observed during milking and handling. The filters can be dried and used as propaganda among the producers, or as a partial basis for the assessing of bonus payments for clean milk.

158. Tests for the Heat Stability of Milk

One of the important general properties of milk is its constancy of behaviour in certain processes ; but now and again certain drastic treatments to which it may be put bring out differences in behaviour that are of technical importance. As an example may be put forward the sterilisation of evaporated milk. This treatment is carried out on the evaporated product in its final container by heating with steam under pressure at 240–245° F. so as to destroy all forms of micro-organic life likely to spoil the product. During the sterilising process the optimum viscosity of product aimed at is that of a weak gel which can readily be broken up to a creamy liquid on shaking the tin ; but it is a rather delicate operation to strike the exact point. Consequently, a pilot sterilising-test is usually carried out with a batch of milk before the main sterilisation, using small and increasing quantities of a stabiliser (usually sodium citrate) to find out how much of it is necessary to give the optimum results for a given set of sterilising conditions. Some evaporated milks curdle completely in such conditions ; this still is one of the major difficulties confronting manufacturers.

Sorting tests for milk unstable to heat have been devised for the raw product and the value of such tests cannot be over-estimated ; their reliability has therefore been thoroughly tested. There are two main tests in use : the *alcohol-coagulation* and the *phosphate* tests. The reagents used accentuate in raw milk the physical changes due to the effect of heat on the evaporated product.

THE ALCOHOL-COAGULATION TEST. The principle of this test depends on the coagulation of milk under certain conditions of acidity or composition in alcohol of standard strength. All the factors which lower the heat-resistance of milk contribute to make the alcohol test positive. The test consists of mixing milk with an equal volume of 75 per cent. alcohol and observing whether any coagulation takes place, and, if so, the type of coagulation. If a visible coagulation is shown, the milk after concentration will become curdy during the sterilisation process. The test is easy to manipulate and gives clear-cut results. When carried out in a test-tube, coagulation is detected by traces of curd sticking to the walls after mixing by inversion, whilst no coagulation has taken place when the alcohol-milk mixture gives a clear glass

surface. The test on the platform can be simplified by using 2 ml. dippers and running in the alcohol from a burette or a pipette. There is also available a piece of apparatus which by means of a dipper measures out a volume of milk. The dipping arrangement is pivoted to a spindle, which acts also as a tap. On inverting the dipper around the pivot the volume of milk runs into a glass tube, and at the same time a definite volume of alcohol runs out of a reservoir through the opened tap. The test is then carried out by shaking and observing the alcohol-milk mixture. On inverting the dipper, the tube is emptied and the instrument is ready for carrying out the next test. (The " Bodo " milk tester.)

Dahlberg and Garner [1] have found that milk which gives the alcohol test coagulates more readily, when concentrated to evaporated milk, than alcohol-negative batches. Sommer and Binney [5] have enumerated the factors which cause the test to be positive, e.g., salt-balance, acidity, rennet-producing organisms, colostrum, and diseased cows and udders. An excess of calcium or magnesium, or of phosphates or citrates, lowers the heat-resistance of milk. Sommer and Binney have found that small increases in Ca and Mg will give a positive alcohol test, and that a high calcium-content in the ration of cows will cause milk to be produced giving a positive alcohol test ; but contrary to the reaction towards heat-coagulation, phosphates and citrates will not give a positive alcohol test and it is therefore concluded that it is the relative amounts of these four constituents present which are of importance, and that the effect of the Ca and Mg on the alcohol test is counteracted by the phosphate and citrate.

The alcohol test is a dependable index of the suitability of milk for evaporation from the point of view of salt-balance, since the disturbance in the salt-balance is usually confined to an excess of Ca and Mg.

It has been found that milk high in titratable acidity due to lactic-acid fermentation shows coagulation with alcohol. There is however no direct correlation between titratable acidity and alcohol-coagulation, owing possibly to the predominating influence of the salt-balance.

It must not be lost sight of that the physical conditions in the evaporated product differ from those in the original milk, and heat-coagulation of the original may not take place in the evaporated product. Also the results of the alcohol test are not always in satis-factory agreement with the heat-stability of the concentrated milk. Although heat-coagulation is influenced by salt-balance, Sommer [2] explains the difference as due to some salt being precipitated, whilst the albumin is partly coagulated and the hydrogen-ion

concentration is increased in the heating-process. The change in pH causes the secondary phosphate to change to primary. In view of such a complex readjustment of conditions, it is not surprising that there is no close agreement between the results of the coagulation by alcohol, heat and phosphate (see below).

The alcohol test covers most of the factors influencing heat-instability, but even then, the test alone is not enough to serve as the sole criterion, owing to the interfering actions which take place during the process of manufacture. It is useful in the sterilising process and can be put to constructive use to improve the quality of the milk supplied by various producers.

THE PHOSPHATE TEST. The addition of phosphate to milk has no effect on the alcohol test but does affect the heat-coagulation test for whole milk. Ramsdell, Johnson and Evans [6] have devised a test in which 2 ml. of milk are mixed with 0·2 ml. of 0·5 N KH_2PO_4 (68·1 grams per litre) and heated in boiling water for five minutes. The cooled mixture is examined for curd ; any coagulation indicates that the concentrated product is of low heat-stability. For factory routine, 10 ml. of milk may be measured with a dipper and 1 ml. of the solution may be run out of a burette.

In determining the actual concentration of phosphate solution necessary to produce initial coagulation of whole-milk samples, the idea of a " phosphate number " (or ten times the number of ml. of the phosphate solution required to coagulate 10 ml. of fresh milk immersed in boiling water for five minutes) has been advanced. The phosphate numbers of the samples examined by Ramsdell *et al.* vary from 12 to 32 ; this represents a considerable range of milks of different heat-stability and phosphate-resistance. Comparing the phosphate number with the time of coagulation of the concentrated milk during sterilisation, it has been found that those samples with a phosphate number of 20 or less coagulate in under four minutes, whilst those above 20 tend to increase in heat-stability. This applies to single herd-milk samples (Friesian and Channel Island breeds) and to composite milk samples. With milk of high phosphate numbers, the time of heating required for concentrated milk to coagulate is variable, so that a high phosphate number does not indicate a highly heat-resistant milk.

In the application of the phosphate test for grading milk, the elimination of the phosphate-positive milk often results in obtaining milk of higher stability to heat. A comparison between pH values and phosphate numbers fails to reveal any consistent relationship. Thus a range of samples with pH 6·4–6·7 was found to have the same phosphate number, and it was only when the

*p*H was distinctly abnormal, due to lactic-acid fermentation, that the phosphate numbers varied directly with the *p*H. The phosphate numbers of single herds are reasonably constant.

It has been found that the elimination of milk unstable to the phosphate test does not always produce a milk of higher stability towards heat. The trouble in sterilisation is usually seasonal, and the real purpose of the test is to help in the sorting out of those milks which impair the stability of the total composite at that time. The test is not intended as a quality test for the hygienic production of milk. The amount of phosphate may also require varying ; thus less should be used when the elimination of phosphate-positive milk does not improve the heat-stability of the concentrated bulk ; and the amount to add can only be ascertained by trial.

GENERAL CONSIDERATIONS OF THE ABOVE TESTS. It has been established that the heat-stability of an evaporated milk bears no relation to the stability of the fresh milk,[7] and that the heat-stability of a milk containing 18 per cent. of solids-not-fat cannot be predicted from the heat-stability of the original milk.[8] Holm, Webb and Deysher,[7] in an investigation of the relationship between heat-stability, the composition and properties of milk, have found no marked correlation of heat-stability with the above rapid tests or the acidity test. The salt-balance (defined as the ratio of the sum of the gram equivalents of Ca and Mg to that of citrate and phosphate) and the buffer intensity also show no correlation with heat-stability. The work, however, was done on the milk of individual cows. Webb and Holm [8] report the existence of two types of milk : (*a*) that stabilised by the addition of positively-charged electrolytes, and (*b*) that stabilised by the addition of negatively-charged electrolytes. In the lower concentrations of added electrolytes, milk is most sensitive to the bi-, tri- and quad-rivalent ions and H ions.

The conflicting results which have been obtained show that wide differences exist in the heat stability of the concentrated product at 136° C., and that our present knowledge of the subject is inadequate.

159. Standardising the Milk before Condensing

The milk after passing inspection is weighed, filtered, sampled and poured into a holding tank from which a sample is again taken for determination of fat and solids-not-fat. From the results obtained, cream or skim milk is added to bring the ratio of fat to solids-not-fat to the standard required for the final product. It is better to standardise the milk before processing and condensing,

since (*a*) addition of skim milk or cream to the concentrated product might bring in sources of faults, either bacterial or physical, (*b*) the analysis of the concentrated product can be accurately determined by hydrometers when the ratio of fat to solids-not-fat is standardised. In sweetened condensed milk, the amount of sugar added is calculated on the amount of total milk solids in the milk in which the F/SNF ratio must be standard. In full-cream evaporated milk, the final product must contain 9 per cent. of fat and 31 per cent. of total solids, *i.e.*, 22 per cent. of solids-not-fat. The F/SNF ratio is thus 1 : 2·44.

Shortage of fat can be overcome by adding cream of known analysis (fat and solids-not-fat), the amount to add being calculated from the mixture law. Surplus fat, on the other hand, is corrected for by adding skim milk. Tables and formulæ for standardising milk before condensing are given by Mojonnier and Troy.[9]

160. Forewarming and Condensing

The milk is next heated to near its boiling-point. This process serves many useful purposes : (*a*) it removes dissolved gases, decreases the amount of foam formed in the vacuum-pan in vacuo, and prevents the partial heat-coagulation or " burning " of the milk on the pipes and hot surfaces of the pan ; (*b*) it acts as an efficient pre-sterilisation process ; (*c*) it facilitates the solution of the sugar added to make the sweetened product ; (*d*) it has a marked effect in raising the heat-coagulation point of the evaporated milk in the sterilisation process. The process also influences and controls the tendency of sweetened condensed milk to thicken with age.

BURNING OF MILK ON HOT SURFACES. Cold milk coming into contact with a hot surface, bakes or burns ; the pipes which heat the milk in the vacuum-pan are charged with superheated steam, and milk burnt on the surface will give the product a burnt or caramelised flavour and cause it to contain brown particles. If the milk is hot when drawn into the pan, it will boil vigorously under reduced pressure and cause so much agitation, that there will be no danger of burning ; maximum speed of evaporation is thereby also maintained. There is no doubt that the natural and other gases dissolved in milk play a major part in this phenomenon and that heating expels most of them.

DESTRUCTION OF MICRO-ORGANIC LIFE. The forewarming process destroys disease-producing organisms, which might be passed on to the sweetened product. Other bacteria, moulds yeasts and enzymes which may bring about fermentative change,

in the final product, are almost completely destroyed in the process. The efficiency of the sterilisation of evaporated milk is higher after the forewarming process ; with the sweetened product, objectionable thickening due to biological agencies is avoided and the product remains wholesome for a longer time.

SOLUTION OF SUGAR.   The granulated sugar must be in complete solution before the milk is drawn into the pan, and this is facilitated by the high temperature of the milk. About 17 to 18 lb. of sugar for every 100 lb. of milk are added. The presence of undissolved sucrose crystals at the bottom of the tank when the last portions are drawn into the pan, may be avoided by the complete solution of sucrose in warm milk.

161. Effect of Temperature of Forewarming on Heat-stability of the Final Product and on the Properties of the Sweetened Product

It was stated above that the temperature of forewarming was generally near the boiling-point on the final product. Experimentation and practice have disclosed the effects of varying temperatures and times of forewarming.

SWEETENED CONDENSED MILK. Heating to near the boiling-point (190-210° F.) is more common than heating to holder-pasteurisation temperature (145° F.) ; in some cases, super-heating in closed vessels to temperatures above the boiling-point is practised. Technically, heating to near the boiling-point is preferable, since it efficiently destroys bacterial life and enables the sucrose to dissolve easily. But forewarming to such temperatures has been found greatly to intensify the defect of thickening with age. Thus Rogers, Deysher and Evans [10] report that " the tendency of the milk to thicken is greatly increased by the high temperature (95° C.) usually used in forewarming." Leighton and Deysher [11] have found that forewarming at 65° C. gives increased stability of the final product which is increased when the milk is heated to 75° C. At 85° C., however, a decrease in stability, which is more marked at 95° C., sets in. Increased stability follows heating to 110-120° C.

From the standpoint of thickening, therefore, it appears that heating either at 145° F. for thirty minutes or to temperatures *above* the boiling-point is preferable to the usual practice. As regards the killing of bacteria, flash-heating at the higher temperature is of the same degree of efficiency as the holder method, and the low-temperature method should yield satisfactory results ; Downs,[12] however, has found that heating to 145° F. generally results in the product developing a rancid flavour, which is

possibly due to the incomplete destruction of lipase at this temperature.

It seems that heating to temperatures around 230° F. would be advantageous, but it is unknown to what extent such treatment would interfere with the smoothness of the final product. The precipitation of Ca and Mg salts might give rise to nuclei upon which lactose crystals large enough to cause grittiness would grow.

EVAPORATED MILK. The temperature and time of forewarming have a considerable effect on the heat-coagulation point of evaporated milk. Evaporated milk, made from milk which has not been forewarmed, generally coagulates below its boiling-point, whilst forewarming at 95° C. raises the heat-coagulation point by 15 to 20° C. The effect of forewarming to different tempera-

TABLE CXX. *Effect of Temperature and Time of Forewarming on Coagulation in Sterilisation* (*Rogers, Deysher and Evans* [13])

Expt. No.	Forewarming temperature (deg. C.)	Forewarming time (mins.)	Coagulation temperature (deg. C.)
1	Not forewarmed	—	95
	95	10	125
	95	Not held	119
2	95	10	122
	95	20	125

tures for different periods on the heat-coagulation point is shown in Table CXX.

Leighton and Deysher [11] have found that heating milk to 65° C. causes the heat-stability to be lower than for milk which has not been forewarmed, and that heating to 95° C. gives a product of maximum heat-stability ; the heat-stability is decreased by heating to 110–120° C. No full explanation of the exact causes underlying this peculiar influence of the forewarming temperature on heat-stability is forthcoming. That forewarming lowers the content of soluble calcium is shown by rennet-coagulation experiments, so that it is quite likely that heat removes the excess of calcium and brings about a better balance between calcium and protein, thus stabilising the casein. The calcium is removed in the inorganic form. Excess of calcium undoubtedly combines with casein at the sterilisation-temperature, and coagulation occurs probably in the same manner as Ballowitz [14] finds for the addition of small amounts of calcium chloride to boiling milk,

i.e., at maximum absorption of calcium and the same concentration of calcium which will give normal rennin-coagulation with boiled milk.

Webb and Holm [8] have shown that the concentration of solids-not-fat is important in determining the effect of temperature and time of heating on heat-coagulation. Forewarming at high temperatures lowers the stability of samples of the higher concentration of solids-not-fat.

The effect of heat on lactalbumin is also of significance, since it is obviously better to precipitate or denature the albumin at lower temperatures than at the sterilisation-temperature, when coagulation of the albumin possibly also precipitates calcium caseinate or the whole casein-complex. The optimum temperature of forewarming at 95° C. may also be accounted for by the fact that denatured albumin at this temperature has reverted into solution in milk in the denatured form, whilst the denatured protein is in the form of a coagulum at lower temperatures ; the dehydration of the gel form of $CaHPO_4$ at temperatures above the boiling-point possibly brings about sympathetic coagulation of the calcium caseinate as a complex.

The use of the homogeniser for the evaporated product has somewhat simplified the coagulation problem. Whereas in non-homogenised milk the correct viscosity of the evaporated product had to be attained to prevent separation of fat during storage, with homogenisation a condition of sub-normal viscosity is sufficient, and the major problem lies in guarding against the formation of curdy milk.

When the seasonal trend in milk composition causes the evaporated product to have a low heat-resistance, the tendency should be corrected in the forewarming, namely, by heating at 95° C. for five to ten minutes. This is accentuated when a higher content of solids-not-fat is aimed at in the concentration. The quality of the finished product must not be ignored, since heating to a high temperature makes it become too brown ; this is another reason why heating at 95° C. for five to ten minutes is best.

162. Condensing and Striking

After standardisation, forewarming and the addition and solution of the sugar, the milk is ready to be drawn into the vacuum pan for condensing. The pan requires preliminary treatment before the milk is drawn in. It is steamed until a temperature of 180° F. or above is reached, and then sealed airtight, and the condenser and vacuum-pump are operated until about twenty inches of vacuum are shown on the gauge. The milk is sucked

into the pan slowly, and low-pressure steam is introduced into the coils of the pan as each set becomes submerged. Too high a pressure of steam (or too high a temperature), and heating the coils before they are covered with the milk, will cause burning of the product and result in a bad flavour and colour as well as impaired heat-exchange. The incoming milk is also laden with air, the sudden release of which at a higher temperature in the partial vacuum causes excessive frothing, which must be regulated by the " vacuum-break " in order to prevent entrainment losses. When the air has been removed the milk settles down to uniform boiling, after which the pressure of the steam is increased to the maximum suitable for use. This is from five to ten lb. pressure, but naturally varies with the efficiency of the heating-surfaces in the pan. All the coils should be covered, and the fresh milk then drawn in at a rate governed by that of evaporation.

The temperature of condensing must be low in order to preserve the natural flavour and colour of the fresh milk. This temperature lies between 130 and 145° F., corresponding to a vacuum of 24 to 27 inches of mercury. The temperature is readily controlled by the amount of water admitted to the condenser, since an increase in the flow of water will lower the temperature.

When the batch to be evaporated is all drawn into the pan, the concentration is nearly complete, and roughly ten to twenty minutes' further evaporation will give the product the desired density. The temperature, i.e., the steam-pressure in the jacket and coils, should be slowly reduced to avoid spoiling the quality of the increasingly viscous product.

When the desired degree of concentration is approached the batch is " struck," which is the term used to denote the sampling of the product previous to determining the composition either with the viscosimeter or by finding the density. Operations must be quick at this point, since under- or over-condensing may mean either too thin or too thick a milk. The pan-operator, by dint of long experience, can usually gauge with fair accuracy when the point for striking has been reached. The actual sampling is done by a device fitted to the waist of the pan and connected to it by two pipes. At the point of strike an upper valve is opened, when a small quantity of milk flows into the chamber of the device. The upper valve is closed after turning off the taps of the connecting pipes, and the sample run into a convenient vessel.

The degree of concentration can be most accurately gauged by a Beaumé hydrometer, but it must be remembered that with all the empirical methods used for this purpose the original milk must be accurately standardised for contents of fat, solids-not-fat and

sucrose. The temperature at which the reading is taken is also very important, and corrections to a standard scale must be made. Thus ordinary whole milk concentrated 2·5 times will show a value of about 33·5° B. at 60° F. or 32° B. at 120° F. Mojonnier and Troy [9] have determined accurately the Beaumé readings at different temperatures for sweetened condensed whole and skim milk as related to the composition of the condensed product. Readings of gravity should be made at a uniform standard temperaure or, failing this, 0·025° B. should be added for every 1° F. to correct for the difference in temperature between the sample and the standard temperature.

When the unsweetened product has nearly reached the proper concentration, it possesses the consistency of thin cream, and the sampling is easier to carry out than with sweetened condensed milk. The specific gravity at 60° F. varies with the ratio of concentration (and consequently with the contents of fat and solids-not-fat) from 1·050 to 1·075. Mojonnier and Troy [9] give the following specific gravity and Beaumé readings for a product containing 7·8 per cent. fat and 17·7 per cent. solids-not-fat : 1·0662 (9·0° B.) at 60° F. ; 1·0518 (7·14° B.) at 120° F. A difference of 0·1° B. is equivalent to about 0·30 per cent. of total solids. When the batch is struck, the gravity is determined at a uniform temperature (120° F.) by means of a Beaumé hydrometer registering from 5 to 15°, each degree being subdivided into tenths. The influence of temperature on the Beaumé reading varies with the range of temperature ; between 40° and 80° F. each degree Fahrenheit is equivalent to 0·025° B., between 80–110° F., ·034° B. ; and 110–140° F., ·039° B. The exact Beaumé reading must be taken, since a product of definite density and composition has to be manufactured, and the behaviour of the product in the sterilising process is greatly affected by the concentration. If the concentration of the sweetened product has been carried too far, the required standard of composition is reached by adding the calculated amount of water.

163. Superheating

With evaporated milk, it is sometimes customary to over-condense and subsequently superheat the milk in the pan with live steam until the product thickens ; the superheating may be carried out in another vessel after drawing the milk from the pan, in which case allowance must be made for the water added by the steam. The evaporated milk shows a thicker consistency in this process, the casein will be somewhat flaky, and lumps of curd will appear if the superheating is prolonged. The purpose of

superheating is to precipitate some of the casein and albumin in order to minimise the danger of getting too firm a curd in the sterilisation process. At the same time an increased viscosity hinders fat-separation. The process is not considered necessary for the quality of the product, but is used to prevent a curdy product being formed during sterilisation. In some factories the process is not carried out ; it is mostly used in the manufacture of plain condensed milk, which is not sterilised but marketed quickly after manufacture. During the superheating process, the same properties which affect the heat-coagulation point during sterilisation affect the behaviour during superheating. Thus milk with a low heat-coagulation point will become viscous, or form a " liver " at lower superheating temperatures, and will precipitate lumps of curd, or " crack," more quickly than milk coagulating at a higher temperature.

164. The Cooling of Sweetened Condensed Milk

The most important phase in the manufacture of the sweetened product is the cooling process, since quick cooling must be rapid in order to prevent superheating and thickening, and the texture of the product depends upon it. At the pan temperature ($130-140°$ F.) the lactose is present practically as a saturated solution ; cooled down to ordinary temperatures the lactose solution would thus be highly supersaturated.

Given the right conditions, therefore, the cooling process will cause the lactose to crystallise out, and this will proceed until the excess lactose at the saturation point has crystallised out. The rate of crystallisation does not proceed either uniformly or at the rate at which the milk is cooled. The crystal nuclei form slowly at first and then increase in number to a maximum at about $86°$ F., after which the rate decreases. The temperature of maximum crystallisation varies, however, with the concentration of total solids, the lactose-water ratio, and the viscosity. The size of the lactose crystals determines the texture of the product. If the crystals are very small, the texture is smooth ; if large, the texture is gritty and objectionable.

Success in obtaining a smooth texture lies in cooling the milk so that small crystals are formed. Therefore crystallisation has to be forced or accelerated at the point of maximum crystallisation by stirring the product at this temperature, after seeding with lactose powder or with a small quantity of a previous batch of sweetened condensed milk.

The solubility relationship of lactose in water has already been dealt with in Section 37. Initial, final and super-solubility curves

have been given, and the occurrence of labile and metastable areas has been described. The region of forced crystallisation occurs between the final and the super-solubility curves (Fig. 11, p. 103) and the curve of forced crystallisation is 10° C. above that of the super-solubility curve. The lactose-water ratio can be calculated from the composition of the original milk and the degree of concentration, or from the specific gravity, of the final product. Thus, assuming the original milk to have 4·8 per cent. of lactose concentrated 2·6 times, with 42 per cent. of sucrose in the final product, the lactose concentration is 12·5 parts in 100–42–31 = 27 parts of water, or 46 of lactose to 100 of water (*i.e.*, a 31·6 per cent. solution of lactose). The temperature of forced crystallisation for a condensed milk of the above composition is 31° C., or 10° C. above the super-solubility point (21° C.) for a corresponding strength of lactose solution. The importance of the manufacture of a product of standard composition is obvious. There is sufficient water in sweetened condensed milk to hold the added sucrose in solution, and the dissolved sucrose only slightly lowers the solubility of lactose. The range of temperature of forced crystallisation, which depends on the lactose-content of the product, is from 24° to 32° C. (75–90° F.). The duration of holding at this temperature, after cooling quickly from pan temperature, is from fifteen to twenty minutes. The seeding should be done at the temperature of forced crystallisation. The milk should subsequently be cooled to about 60–63° F.

The conditions at the forced crystallisation-period, in conjunction with the seeding, promote rapid crystallisation on a large number of crystal nuclei, and the low viscosity at this temperature enables the supersaturated lactose solution to diffuse easily towards the crystal centres. Supersaturation is thus quickly overcome and there is a sufficient number of crystals present to prevent excessive growth during the overcoming of supersaturation due to cooling to a lower temperature for storage.

The oldest and crudest but efficient way of cooling is the can-and-paddle method in which the milk is cooled in rotating cans immersed in a tank of water, the cans being provided with stationary fixed paddles to scrape the milk from the surfaces of the vessel. Other methods of cooling during constant-flow are the submerged-coil, the coil-vat and the internal-tube; these are described by Hunziker.[12]

165. The Sterilisation of Evaporated Milk

After leaving the vacuum-pan, evaporated milk goes through a preliminary treatment to make it more viscous so as to avoid the

separation of fat. The seasonal differences in the behaviour of milk towards heat in the superheating and sterilisation processes have made manufacturers adopt homogenisation to avoid fat-separation.

The milk has to be subjected to sufficient pressure to reduce the fat globules at least to one-third of their original size. A greater reduction in size may alter the physical properties of the casein or its concentration on the adsorbed layer of protein on the very minute fat globules, so that the heat-coagulation temperature is materially lowered, thus giving rise to difficulties during sterilisation. The homogenising pressures most suitable are from 2,500 to 3,000 lb. per square inch. The temperature of the milk during homogenisation is 120–130° F.

Superheating of evaporated milk before sterilisation may be accomplished as for plain condensed milk. Milk of a low heat-coagulation point may be heated to 180° F. and that of high coagulation-point to 200° F. ; this may be done before drawing from the pan or afterwards. The product is then cooled to 40-45° F., when it is ready for filling into tins. After sealing, the tins are ready for sterilisation, the aim of which is to destroy micro-organic life completely, and thus preserve the product. A subsidiary aim of the process is to increase the viscosity to such an extent that the fat is prevented from creaming out during storage, and from churning during transportation, the product thus attaining the creamy consistency that simulates richness. The viscosity aimed at should be a weak gel which can easily be broken up on shaking the tin. Too long a period of heating or too high a temperature will cause the product to darken. The use of excessive amounts of sodium bicarbonate as a casein-stabiliser accentuates this tendency.

The factors influencing heat-coagulation have already been dealt with in various sections (see Sections 133, (iv.), 158, 161). These may be divided into factors concerned with the raw milk and those concerned with the process of manufacture. The latter are there-fore under factory control, and include the time and method of forewarming, the ratio of concentration and the pressure of homo-genisation. The complete elimination of all the factors connected with raw milk would mean the rejection of all milk which would not conform to optimal behaviour in the sterilising process, and this would be impractical. It has been found that some of these factors can be removed by careful adjustment of the various processes preceding sterilisation. Acidity can be neutralised by alkali ; albumin can be precipitated by suitable times and tem-peratures of forewarming. In addition, the method of sterilisation

can be standardised so as to simplify the operation, to make the plant more efficient, and to reduce excessive spoilage of the finished product. This is done by standardising the properties affecting heat-coagulation by sodium bicarbonate as a casein-stabiliser. Sodium bicarbonate corrects the salt-balance ; it has also been found that other sodium salts, such as the phosphate (Na_2HPO_4) and citrate, are not only more dependable as casein-stabilisers but do not affect the colour and taste of the finished product.

The stability towards heat of a batch of evaporated milk can be determined by a pilot experiment ; if this determination is made on a series of tins to which small and increasing quantities of sodium bicarbonate have been added, it can be ascertained what amount of stabiliser is required to give the optimum viscosity of the product under the usual conditions of time and temperature of sterilisation. The apparatus required includes a pilot steriliser, a viscosimeter, a number of vent-hole tins, sodium bicarbonate solution, and the usual measuring and weighing apparatus. The Mojonnier method of evaporated milk control is specially adaptable for such work.

The bicarbonate solution used in the test is of 10 per cent. strength, and the tests in the series should contain added bicarbonate in amounts corresponding to 0, 1, 2, 3 . . . oz. of bicarbonate per 1,000 lb. of milk or for tins containing 6 oz., 0·1, 0·2, 0·3 . . . ml. of the sodium bicarbonate solution. The mixtures are made in cups, the contents of which are poured into vent-hole tins, which are carefully soldered, care being taken that no flux or solder enters the tins. The tins are placed in the steriliser, which is then sealed and heated slowly with steam under pressure so that a temperature of 240° F. is reached in twenty minutes. It is recommended that the tins be heated to 243° F. for fifteen minutes exactly. The steriliser is cooled with water so that a temperature of 75° F. is reached in five minutes. The tins are opened and the contents tested for viscosity by means of the Mojonnier-Doolittle viscosimeter.[9] This instrument consists of a ball, which is immersed in the fluid, fitted with a horizontal dial graduated in 360 degrees and suspended by a thin wire fixed to a support at the upper end ; the dial is turned one revolution and then quickly released. The dial will make one complete and part of a second revolution ; the reading at the position in which it stops before returning represents the viscosity of the sample in degrees of retardation.

(This value can be changed into centipoises by applying the equation $V = \dfrac{1{,}833R}{720-R} - 22$, where V = viscosity in centipoises

and R = degrees of retardation. Thus milk with a retardation of 180° would have a coefficient of viscosity of 589 centipoises. For comparison, glycerine has a viscosity of 830 centipoises at 20·3° C.)

The viscosity, or degrees of retardation, for evaporated milk of 7·8 per cent. fat and 25·5 per cent. total solids should be 150°, and that for 9 per cent. fat and 31 per cent. total solids should be about 200–210°. The tin showing the optimum viscosity is found, and sodium bicarbonate corresponding to the amount per 1,000 lb. determined and weighed out so as to treat the whole batch of evaporated milk. This is dissolved in boiling water and most of the carbon dioxide is driven off by a steam-hose. The solution is added in small quantities to the entire batch in the holding-tank and vigorously stirred for about twenty minutes. The batch is then ready to be filled into tins, which, when sealed, will be ready for sterilisation.

Treatment as above with sodium bicarbonate occasionally gives abnormal results, *i.e.*, the heat-coagulation point may be lowered and the viscosity increased after the addition of the bicarbonate. Hunziker [12] states that this is possibly due to a low calcium-content so that the addition of the carbonate intensifies the derangement of the calcium-casein balance. But such cases in herd milk are rare, and Sommer [2] has reported that troubles in condensing are never due to a deficiency of calcium in the milk. Hunziker further thinks that failure to respond to sodium bicarbonate arises from the calcium caseinate undergoing abnormal changes due to faulty handling at some stage in the manufacture, *e.g.*, improper fore-warming, excessive dilution with water, or addition of excessive bicarbonate.

THE USE OF SODIUM CITRATE AND SODIUM PHOSPHATE. The correcting effect of these salts resembles that of bicarbonate, but when the larger quantities of salt have to be used they are superior in that their effects are more regular and more dependable. Sodium bicarbonate is the salt of a weaker acid and has the double effect of balancing the excess of calcium and of changing the acid reaction. In large amounts it may replace calcium in casein combination and thus liberate calcium to impair further the cal-cium-casein system ; this results in a lower heat-coagulation point. Citrates and phosphates only balance the excess of calcium.

The bicarbonate tends to increase the caramelisation of the milk in the sterilisation process and to give a soapy flavour (compare the soapy flavour of butter from over-neutralised cream). Liber-ated carbon dioxide sometimes causes the ends of tins to bulge,

thus giving the consumer the impression that the contents are spoilt.

Determination of the amount of citrate or phosphate is carried out by a pilot sterilisation-test as described above for the bicarbonate. The evaporated product usually requires amounts of the dry salts ranging from 2 to 10 oz. per 1,000 lb. to balance the excess of calcium and to give a satisfactory viscosity.

Sterilisation of the tins may be carried out by the *batch* method or the *continuous* process. In the batch method the steps taken are : loading, heating, holding, cooling and unloading. The process is carried out in a large boiler-like autoclave in the inside of which is an arrangement for keeping the cans in motion to avoid local overheating, but the motion is stopped during the holding period or at intervals during the " coming up " period. The " coming up " time, *i.e.*, the time of heating until the tin reaches the sterilisation-temperature, should be between fifteen and twenty minutes and the holding time and temperature should not be less than fifteen minutes at 240–245° F. The tins in the steriliser are cooled to 70–80° F. by means of cold water. The cooling must be done quickly to avoid bulging of the ends of the tins, especially large-sized tins. Other aspects of the work include testing for " leakers," shaking the tins so as to render the weak gel into a creamy paste, and incubation at 70–80° F. for ten to thirty days to test for defects before marketing.

The old method of sterilisation by heating at temperatures lower than given above for three successive days, so as to kill the vegetative forms of bacteria, has been completely superseded by the high-temperature method of sterilisation.

166. Faults of Condensed Milk

SWEETENED CONDENSED MILK. The most common defects are (*a*) those due to physical conditions : sandy or gritty, settled, thickened and lumpy milk and " buttons " in milk ; (*b*) those due to micro-organisms : blown or fermented, rancid, putrid milk ; and (*c*) those due to miscellaneous factors : brown, metallic and tallowy milk.

The *gritty* or *rough* texture is due to the lactose crystals being so large as to be felt on the tongue and palate. The process of cooling and the forced crystallisation of lactose in order to get as many crystal nuclei as possible and to cause the concentration of dissolved lactose to reach that demanded by its final solubility curve quickly has already been described (Sections 37 and 164). The secret of obtaining a condensed product is intimately bound up with observing the conditions discussed therein ; by this

means the defect of sandiness can largely be avoided. *Settled* milk is due to heavy crystals settling by gravitation to the bottom or sides of the tin. The smaller the crystals, the slower will they form a deposit in the tin.

Thickening is one of the major defects of condensed milk. High viscosity is natural to sweetened condensed milk but is considered objectionable when increased to the degree that the milk will not flow out of the tin. The defect is common in milk manufactured in late spring and early summer, but will occur in any milk stored for a considerable period at temperatures above the ordinary, *e.g.*, in the tropics. The thickening is due either to physical or bacterial causes, or to both.

Rice and Downs [1] have found that certain species of bacteria are capable of causing rapid thickening of sweetened condensed milk. They find that bacterial thickening is due to the presence of cocci, which they were able to isolate from almost all samples of condensed milk bought on the open market. These cocci are easily destroyed at the forewarming temperature, and they concluded that contamination occurs at later stages in the manufacture. This was confirmed by searching the plant after the cooling process, when material from the cooling vat, wall and floor scrapings, etc., inoculated into condensed milk, produced thickening in fourteen days ; an almost pure culture of these cocci was isolated from the milk. With a sufficiently high sucrose ratio, however, these cocci are entirely checked and the thickening prevented. The thickening is due to the separation of a certain amount of lactic acid and a rennet-type of enzyme.[15]

Contained air, high acidity, high sucrose-concentration, high storage-temperature, increase in the ratio of concentration, high albumin-content, high forewarming and vacuum-pan temperatures favour thickening of sweetened condensed milk.

Lumpiness is due to poor quality of the fresh milk, unclean plant, milk from cows which have newly calved, acid flux in the tins, and dirty tins for canning. Rogers, Dahlberg and Evans [16] found that " *buttons* " are also caused by the growth of *Aspergillus repens* and other moulds.

Blown tins or " *bloats* " are due to considerable contamination of the condensed milk with specific types of micro-organisms, especially yeasts which can grow in high concentrations of sugar. The sources of these organisms may be the cowshed and dairy, unclean factory plant, exposure to air especially in the summer, the sugar, and the tin. The cause of the gaseous fermentation resulting in bloats has been extensively examined (see pp. 382–397 of Hunziker [12]). The yeast which is responsible resembles the

Torula spp.[17] Savage and Hunwicke [18] state that it is the quantity of available oxygen in the milk, together with the number of yeast cells, which determines the appearance of a bloat. Summer conditions are more favourable to inoculation from the air when tins are not hermetically tight, both of which may account for the occurrence of more blown tins in summer. Hunziker [12] (p. 389), in one epidemic of this defect, definitely traced the source to the sugar used, which was delivered by a chute into the hot well. The chute was insanitary owing to dirt and flying insects, and adding the sugar directly from the barrel prevented the occurrence of the defect. Damp sugar is also a sure means of causing bloats.

Various moulds and polluted water are frequently the cause of *rancid* condensed milk. Butyric rancidity may also develop through the incomplete destruction of the natural lipase of milk. Great care must be taken with the water supply, especially where milk residues are likely to lodge.

A *metallic* or *tallowy taste* may follow excessive contamination of the condensed product with heavy metals, especially copper. The vacuum-pan is made of copper ; the copper salts on an insanitary dome would dissolve in milk splashings and foam, thus imparting a metallic taste to the product. After-effects of the metallic contamination would be the development of an oily or tallowy flavour due to oxidation of the milk-fat. All copper surfaces with which milk comes in contact should either be kept bright or heavily tinned where possible.

The *browning* of sweetened condensed milk is due to caramel-isation of the sugar ; it is a defect which increases with time of storage, especially at moderately high temperatures, as in the tropics. The possibilities of humin-formation have not yet been investigated, and the slow hydrolysis of soluble protein in the presence of some invert sugar may explain the darkening of the colour with age.

167. Faults of Evaporated Milk

Evaporated milk may be grainy or curdy, churned, fermented, brown, or metallic. By far the most common and economically important defect is *curdiness*. This defect is due to an abnormally low heat-coagulation point of the condensed product, with the result that, despite all the precautions taken in the sorting of the original milk and the standardisation of the sterilising process, the milk cannot withstand the high temperature. The various factors involved and a description of the standardisation of the sterilising process have already been given (Sections 158 and 165).

Careful control of the sterilising process should minimise this defect.

A *gritty* sediment in evaporated milk tins has been found by Sato [19] to contain tricalcium phosphate, magnesium phosphate and calcium citrate. A high ratio of concentration will increase the amount of sediment. The higher the temperature of sterilisation, the greater the tendency for the gritty sediment to form. A high temperature of storage, *e.g.*, 68° F., instead of 45° F., favours the formation of a sediment.

Creaming, and subsequent *churning* during shaking in transportation, are due to inefficient homogenisation or too low a viscosity after sterilisation. Milk of high fat-content and containing large fat globules is particularly susceptible to this defect. The obvious remedy is to homogenise efficiently and obtain the proper viscosity during sterilisation.

A *bitter* flavour and *gaseous fermentation*, causing bloats, occur in evaporated milk as well as in the sweetened product. Hunziker[12] states that bitter flavour is the result of inefficient sterilisation and remarks on the perfect whiteness of the curd in such occurrences. Bulging cans are due to gaseous fermentation causing high pressure in the tins. The gases formed may be aromatic and pleasant, or fœtid and suggesting putrefaction. The organisms mostly responsible are members of the *B. coli* group and putrefactive bacteria such as *Plectridium novum* and *P. fœtidum*. Employing the highest sterilisation temperature or longest exposure to heat will largely avoid this defect. Blown tins may also be due to freezing, hydrogen gas, or to altitude.

Fishy flavour in evaporated milk has been found by Hammer [20] to be due to *Bacillus ichthyosmius*, which also causes an increase in acidity and some flakiness of the casein.

Evaporated milk should be of a rich creamy colour, but the effect of high temperature is to caramelise the milk to varying degrees, and the darkening is increased by excessive exposure to high temperature and by excess of sodium bicarbonate. The treatment of the milk during manufacture should be such as to avoid excessive darkening and to maintain a uniform colour in all batches.

168. Other Condensed Milk Products

PLAIN CONDENSED MILK. This may be whole, partly-skimmed, or skim milk condensed to three to four times its original concentration. The manufacturing process is the same as for evaporated milk, except that the sterilising process is omitted. The product is usually thickened by superheating, and is stored at low tempera-

tures (40° F.) for short periods before use. The control of the raw milk, forewarming and condensing processes follow closely those for evaporated milk. Owing to the higher ratio of concentration, the specific gravity of the skim milk is high (14–18° B. at 120° F.), but since the whole milk or part skim-milk product is concentrated usually only to ratios of 2–3 to 1, the Beaumé readings are from 9 to 11° B. at 120° F.

SEMI-SOLID OR CONDENSED BUTTER-MILK. The condensing of creamery butter-milk to a concentration ratio of 3 to 3·5 gives semi-solid butter-milk. The condensed product contains 27–28 per cent. of total solids, and since undiluted butter-milk contains about 9 per cent. or diluted butter-milk (containing the wash-water of the butter), 7·5 per cent., the concentration ratio is 3 to 4. The butter-milk is allowed to develop a high acidity because the material keeps better in the acid condition. The soured liquid is forewarmed by blowing live steam into it, and is then condensed in a vacuum-pan with a clean copper surface, although glass-lined pans would be more suitable. The product is viscous at the end of the concentration and does not require superheating. The condensed liquid is filled into barrels without cooling. It is used mostly as a feeding-stuff for pigs and poultry.

CONDENSED WHEY. The manufacture of condensed whey or *primost* is carried out by evaporation in a vacuum-pan. When sufficiently concentrated, the syrup is drawn off and allowed to crystallise in brick-shaped forms. The demand for the yellowish-brown gritty product is as yet very limited. A product made by a continuous concentrating process and having a concentration ratio of 15 to 1 crystallises out into solid blocks on cooling; it has an excellent flavour and other good dietetic properties.

169. Composition of Sweetened Condensed and Evaporated Milk. Presumptive Standards of Composition

The Public Health (Condensed Milk) Regulations, 1923, demand that all condensed milk shall contain not less than the appropriate percentage of milk-fat and milk solids as specified in Table CXXI.

LABELLING OF TINS. The full description of the condensed milk and the milk-equivalent of the condensed product have to be printed on the label. For the purposes of these Regulations, milk means " milk which contains not less than 12·4 per cent. of milk solids (including not less than 3·6 per cent. of milk-fat)," and skimmed milk means " milk which contains not less than 9 per cent. of milk solids other than milk-fat." It is forbidden to

TABLE CXXI. *Fat and Total Milk Solids in Condensed Milk.*
Standard Composition

Description of Condensed Milk	Milk fat %	Milk solids including fat %
1. Full cream, unsweetened	9·0	31·0
2. Full cream, sweetened .	9·0	31·0
3. Skimmed, unsweetened .	—	20·0
4. Skimmed, sweetened .	—	26·0

print on the label any instructions for diluting which will give a fluid containing less milk-fat or less milk solids than specified above.

The Federal (U.S.A.) standards are lower for the full-cream products (28 per cent. total milk solids, 8 per cent. milk-fat), the same for condensed skimmed milk, and higher for the sweetened skimmed product (28 per cent. of milk solids).

DETAILED COMPOSITION OF CONDENSED DAIRY PRODUCTS. The following Table (CXXII) gives the range of composition of condensed milk products found by various investigators :—

TABLE CXXII. *Analysis of Condensed Milk Products*

Product	Milk solids %	Fat %	Protein %	Lactose %	Ash %	Water %	Sucrose %
Condensed whole milk, sweetened	29·4–36·6	8·0–11·5	7·1–9·5	11·3–15·2	1·7–2·2	21·7–32·2	37·6–43·4
Evaporated whole milk .	21·1–32·0	7·3–10·5	5·8–8·5	8·6–11·9	1·1–1 7	68 0–78 9	—
Condensed butter-milk .	26·5–31·8	1·0–2·3	9·0–10·8	9·8–12·0	1·7–2·2	69·0–73·5	—
Whey paste .	54·0–66·0	—	11·7	33·0	9·4	34–46	—

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CHAPTER XIX

DRIED MILK PRODUCTS

170. General Considerations

(*a*) DEVELOPMENT OF THE INDUSTRY. The development of the dried milk industry has run parallel with that of condensed milk, since the difference between the two products lies only in the degree of concentration. Inventors of the methods of manufacture of one product undoubtedly contemplated the possibilities of the manufacture of the other type, but owing to the increasing demands for the condensed product and the difficulties of getting capital invested in milk-drying developments, progress in the latter was relatively slow. The early methods of drying were relatively crude and the properties of the product, especially its solubility, were disappointing.

The first attempts at milk-drying were made about the middle of the 19th century, when Grimwade secured his British Patent in 1855 for a process which was the forerunner of the " dough-drying " system. Milk containing added alkali carbonate was evaporated with constant stirring in a jacketed pan until a doughy product was obtained ; cane sugar was then added, and after pressing between rollers into thin ribbons, the product was dried and powdered. The sugar caused the product at the end of the drying process to become granular, and so facilitated the final drying.

Later, the manufacture of dried malted milk was developed (1883–7) by Horlick, involving the same principles as are used in the manufacture of the present-day article.

Further progress in the drying of milk without the addition of foreign ingredients began about the last decade of the 19th century, when the first true milk-powder was manufactured. Since that time progress has been swift and has been reflected both in the growth of the annual output and in the quality of the product.

(*b*) DEFINITIONS. Milk powder, dried milk (dehydrated or desiccated milk, milk flour or flakes) may be made from whole milk, partly or completely skimmed milk, from milk to which more milk-fat, sugar, salts or alkalis have been added. The milk may be evaporated either in vacuo or at atmospheric pressure.

The fat-content of the milk defines the name of the product on the market. Thus a milk containing not less than 26 per cent. of fat is designated *Dried Milk* or *Whole Milk Powder*. When the product contains 50 per cent. or more of fat, the material is *Dried Cream* or *Cream Powder*. When the fat-percentage falls below 26 per cent., the product is termed *Partly Skimmed Milk Powder*, whilst that from skim milk is *Dried Skim Milk* or *Skim-milk Powder*. Butter-milk and whey are dried to form *Dried Butter-milk* and *Dried Whey*, respectively.

171. Methods of Drying

(*a*) PARTIAL CONCENTRATION BY FREEZING. By cooling milk slowly with constant stirring it is possible to separate much of the water in milk as fine crystals, which can be separated in a crystal centrifuge. The thick paste containing the milk solids can then be dried with a considerably smaller consumption of heat per lb. of solids than would be required for the original whole milk. The efficiency of the process is, however, influenced by (*a*) loss of milk solids in the ice crystals, (*b*) the separation of lactose after 2·5–3 times concentration of the " mother liquor," and (*c*) the non-destruction and proliferation of micro-organic growth in the process before drying. There is no evidence that the method has progressed beyond the experimental stage.

(*b*) DRYING BY THE USE OF HEAT. These methods comprise the dough-drying, film- (or drum-) drying, belt-drying and the spray-drying systems ; they are the only methods which have been applied commercially.

The Dough-drying System. The earlier and cruder processes belong to this group and consist in condensing the milk with proper agitation either in open pans, vacuum pans, or in vats by blowing hot air through the milk until a dough is formed. The paste, spread as a thin layer, is dried either in vacuo, or in tunnels, by heated air to give dry slabs, which are then ground to a fine powder. (Wimmer and Campbell processes).

The Film, Roller-, or Drum-drying System. This method consists in drying a film of milk on revolving drums or rollers heated by hot water or steam under pressure ; suitably-located knives or scrapers remove the dried film from the rolls. The film of milk (whole or concentrated) may be squirted on to the rolls and spread by a " spreader," or by having a short sector of the roll dipping in hot milk ; where twin rollers are used, the milk may be fed to the V aperture between the two closely-set drums. The speed of the rolls is regulated so that the film is dry by the time the scraper is reached. The dried film falls into a

trough, from which it is removed by a worm-conveyor to a grinder and bolter.

Improvements in the method have consisted largely in improving the quality and solubility of the product by perfecting the manner of applying the milk to the rolls, and in regulating temperatures and roller speeds, consistent with efficiency of drying, without overheating the product. The main problems have centred around the economical evaporation of the large quantity of water present in milk, and avoiding the denaturing of the protein by excessive baking of the product.

In this direction the pre-condensing of milk in a vacuum-pan or in a continuous concentrator, which is a rapid and economical method of evaporation, and the drying of the product by either the roller- or the spray-drying process are slowly gaining ground.

Roller-drying systems may be divided into two classes : drying at atmospheric pressure or in vacuo. Most roller-driers work at atmospheric pressure. They suffer from the disadvantage of working at relatively high temperatures, which impair the " solubility " of the product. Loss of solubility, of course, is not objectionable where the dried product is used in the dry state, such as for the baking, confectionery and stock-feeding trades, or where the constituents are not in complete solution in the original liquid, such as in butter-milk ; but where reconstitution is required or where the product is intended for human consumption, solubility is an important factor and atmospheric roller-drying is then disadvantageous.

The drying can be effected at lower temperatures by enclosing the roll or rolls in a vacuum-chamber and working at a reduced pressure. Greater differences of temperature between the milk and the heating medium cause more rapid heat-transmission and therefore more rapid evaporation and drying, and this, together with the lower temperature to which the milk is exposed, helps to maintain the solubility of the product. The lowering of the temperature of the rolls can still further protect the reconstituting properties of the dried product.

172. Roller-drying Processes

THE JUST-HATMAKER PROCESS. This process, a modification of the original Just process, is carried out in equipment consisting of two closely-adjusted twin rollers which are steam-heated, a milk-distribution tank with adjustable outlets over the cylinders, scrapers or knives which remove the film of dried milk from the rolls, and receptacles for the dried material. The original Just patent (U.S.P. 712,545, 1902) also suggests the addition of small

quantities of lime and calcium chloride or of sodium calcium citrate to reduce the acidity of the milk, and of alkaline hypochlorite to preserve the fat of the dry product. The addition of neutralisers and of protein stabilisers was considered necessary to minimise the lowering of the solubility of the product by the high temperature of heating. The temperature of the heating-surface of the rolls lies between 212° and 270° F.

The twin rolls rotate in opposite directions while a quantity of milk is kept constantly boiling in the V aperture between the rolls ; a uniform layer of milk forms on the rolls as they rotate, and their speed ensures that the film is dry when the knives are reached. Steam condenses inside the rolls, the condensed water being intermittently blown out through valve-traps.

The *James Bell* milk-drier is similar to the Just type and is used extensively in Australia. It is provided with a compressor-attachment, which takes the place of the steam traps ordinarily used. This attachment consists of a pump which causes a continuous return of steam under pressure from the rolls to the boilers ; it is claimed to ensure greater uniformity of temperature to the rolls and thus gives a more uniform product, as well as to increase the capacity of the machine and hence give more economical working.

The *Mynot-Plumey* milk-drier consists of one heated roller (30 in. diameter) which is fed by another but smaller unheated roller directly underneath it and dipping in the milk. The thickness of the film on the heated drum can be regulated by adjusting the distance between the two rollers. The heated drum is kept at 92°–94° C. The capacity of the machine is naturally much less than that of the twin-roller machines operating at higher temperatures. Pre-condensed milk is required, but the product is of high solubility. Other machines involving the same principles are the Gabler-Saliter and the Kunick.

The *Gothman Process* (U.S.P. 834,516, 1906) involves the use of a spirally-corrugated, conical heated drum, working against a similarly corrugated surface in close contact with the corrugations on the cone. The milk is fed in at the wide end of the cone and is worked gradually to the thin end, from which the dry powder is brushed off. The temperature of the heating surface is that of boiling water but may be between 212° and 270° F.

173. Vacuum Roller-driers

The most important vacuum roller-drying processes are the Passburg, Ekenberg, Govers, and Bufflovak driers.

In the *Passburg process* (of German origin) a steam-heated drum

(104–131° F.) dips into milk drawn into the vacuum-chamber by the vacuum and maintained at a constant level. The drum is housed in a vacuum-chamber and fitted with a scraper which discharges the dried milk into an evacuated receiver. Water vapour is let out through condensers outside the drying apparatus. The capacity of the machine is from 350 to 400 gallons per hour.

In the *Ekenburg* process, which is also carried out in vacuo, a drum with concave ends is used ; the milk is first sprayed on the concave ends where it is pre-condensed and withdrawn from the vacuum chamber by a pump ; it is then sprayed on the periphery where it is dried and removed by scrapers. The dried product is removed by an arrangement of air locks so that the vacuum is not broken, further dried at 90° F. until the lactose crystallises, and is then ground and packed.

The *Govers* process comprises the use of twin-rollers fed and worked as in the Just process. The rollers are, however, installed in a vacuum-chamber which is maintained at such a reduced pressure that the milk boils at about 157° F. Pre-condensing occurs in the V aperture between the rollers, and a thin film is dried as the rollers revolve. This film is scraped off the rollers into receptacles which can deliver the material into the outside without breaking the vacuum.

The Bufflovak Drum-drier consists of a single steam-heated roller turning in a vacuum-chamber ; milk in a reservoir at the base of the chamber is pumped continuously into a shallow pan beneath the roller, and by a slight pressure on the pan, a film of milk is formed on the roller. The greatest possible amount of surface of the roller is used for drying the film, which is scraped off by a knife ; the dried product is collected in a receiver, which is emptied by a spiral conveyor leading to two receptacles that are used alternately so as to preserve the vacuum.

A *Vacuum Spray-film Drier*, made by the Chemical and Vacuum Machinery Co. (Buffalo, N.Y.), is a single roller working in vacuo on to which a spray of milk is fed by a threaded roller revolving in a reservoir of milk. Milk is carried on the threads and sprayed by centrifugal force on to the surface of the heated roller. This spray builds up a film of uniform thickness, which is further adjusted by a deflector. The surface of the roller is at 200–203° F. The process is continuous.

The same company has manufactured a *combined vacuum evaporator and drum-drier* in which the milk is pre-condensed in a concentrator of the rapid-circulation evaporator type integral with a vacuum-chamber containing a steam-heated drum similar to the Bufflovak type. The evaporator consists of a series of tubes

which contain the liquid to be condensed, with steam on the outside. Milk is evaporated to the desired concentration in these tubes and the drying drum set in motion. The boiling milk in the tubes acquires a sufficient upward velocity for a film to form on the drum, the thickness being regulated by a spreader, while the surplus milk falls back through a down-take into the reservoir in the evaporator. The processes of condensing and drying are then maintained continuously, and the dried milk is discharged automatically.

The *Sahara Milk-drying Machine* is a batch machine consisting of two drums, one inside the other, the outer forming a jacket for the inner. Spray pipes in the jacket carry the heating medium—steam or water—on to the outer surface of the inner drum. The inner drum is kept evacuated and a stream of milk is drawn in and sprayed on the walls. As the drums revolve the film dries and is scraped off and pulverised by a layer of steel balls inside the drum.

174. Spray-drying Processes

This system of milk-drying consists of producing a fine spray of milk in the presence of a current of hot air. The hot air dries the atomised milk particles, while the dry product separates out. The process was not originally devised for milk; its later application to that liquid has caused considerable developments and improvements in its use, which have resulted in a high operating efficiency and quality of product.

Various methods for the spray-drying of liquid dairy products differ chiefly in such details as the concentration of the fluid product before drying, method of forming the spray, method of preventing loss of milk dust, and the rapidity of the removal of the dried product from the drying-chamber. It is obvious that more economical and rapid drying would occur with pre-condensed milk, even if the condensing were carried out in a vacuum-pan ; the hot gases escaping from the drying-chamber through a preliminary spray of milk can also cause some pre-condensing.

The spray can be formed either by stationary or by revolving spray nozzles, or by centrifugal force, where the milk falling on rapidly revolving discs is broken up into minute droplets. Cones of spray can be formed by discs or bowls running at high speed and fed with milk as a thin film.

Recovery of milk dust has been improved by allowing the hot air first to pass through the driest particles and finally through the wettest spray, or even a preliminary milk spray. In this way

a combination of preheating and pre-condensing is attained ; special dust-extractors are costly.

The product of spray-drying was usually not removed from the hot chamber until the day's run was over ; this condition impaired the quality and also the physical and nutritive value of the product. Improvements in this direction are the mechanical carriers that remove the powder as soon as formed, or the provision of chambers with false-bottoms.

The first patent for drying fluid mixtures by the spray process was the Percy process (1872), which was first successfully applied to the drying of milk by Stauf (1901). The milk was atomised around the sides of a central chamber, up which the hot air was admitted ; the dried powder was carried over to large side-chambers in which the dried powder was gathered in hoppers. The McLachlan process (1905) entailed the use of one drying-chamber only, the milk entering as a spray from the top and descending through heated air ; the dried product was collected at the base of the chamber and was discharged through a sliding door. A similar principle was embraced by the Merrel-Gere process for the drying of previously condensed milk. In the Rogers process, the milk is sprayed in from the top, and hot air enters from near the bottom of the chamber. Pre-condensed milk (140° F.) and an air temperature of 180–200° F. are used. The Gray-Jensen process (1913) incorporates a number of improvements connected with the treatment of the liquid milk, viz., minimising loss of the dried product by entrainment, very low moisture-content of the product, preservation of reconstituting properties, freedom from contamination of the final product coupled with maximum economy of operation.

The Krause spray-drying process (D.R.P. 297,388, 1912) uses a centrifugal method for atomising the milk instead of the usual nozzle method. The milk is fed by gravity on to a rapidly revolving disc in the centre of the heating-chamber and atomised in its flight from the periphery of the disc. A current of hot air is drawn through this spray and desiccates it, most of the powder falling to the floor, from which it is continuously removed.

In the Dick process (1919), the milk is atomised by centrifugal force. By the suitable location of the intake and exit of the heated air, the drying chamber may be regarded as made up of four zones : (*a*) the spraying and evaporating zone at the top, (*b*) the dead air zone, (*c*) the final drying zone, and (*d*) the cooling and collecting zone at the base of the chamber.

In all the above processes provision is made for recovery of the fine powder carried away by the hot air. A detailed description

of the processes can be obtained in Hunziker's " Condensed Milk and Milk Powder " (La Grange, Illinois, 1926, Chap. 28).

In addition, a belt-drying process (*e.g.*, the Scott process) has lately been introduced.

175. Management of the Milk for Drying Purposes

In the roller process, the greater portion of the milk is applied to the rolls in the raw untreated state. Where pre-condensed milk is dried, it is applied to the rolls at the condensing temperature, and both condensing and drying constitute a continuous process. In spray-drying processes, requirements of heat economy and of getting the fullest amount of evaporation demand that the milk be preheated to 140-160° F. The temperature of preheating is directly related to the solubility of the dried product, which is preserved by not heating above 160° F. This temperature should also not be exceeded in the pre-condensing process.

ADVANTAGES OF PRE-CONDENSING. The pre-condensing of milk is more advantageous in the spray-drying process, because not only is heat more economically utilised in the vacuum-pan, but also heat applied as heated air is less completely utilised and requires more power for circulation. The principle of carrying out the greater portion of the evaporation by more economical methods means less labour, fuel and time, and therefore an increased capacity in the drying process. The same advantages hold for roller-dried milk to a lesser degree.

The product from pre-condensed milk is more compact and packs into a smaller space than that from unheated milk, which is fine and fluffy. Regulation of the fineness of the spray can, however, influence the nature of the dried product. With a coarse spray, for instance, the dried product is more granular. Also the superheating of milk to 212° F. minimises the bulkiness of the powder, but of course is detrimental to its solubility. The nature of the powder influences the amount of material lost by entrainment in the air current, and therefore maximum recovery is facilitated by pre-condensing. In all cases a dust-collector is necessary and the recovery is then influenced by the efficiency of such an apparatus. Entrainment-losses in the roller processes are possible only during the vacuum-evaporation before drying.

Pre-condensing does not appear to affect appreciably the solubility of the product, although the fine powder from untreated milk may take a longer time to dissolve than a more compact and granular powder ; speed of solubility, however, is of no significance and it is quite possible for the dried product from whole milk to

be more soluble than that from pre-condensed milk. Provided that the temperature used in the pre-treatment of the milk has not exceeded 160° F., previous condensing of milk before drying has no effect on the solubility of the product.

176. Composition and Properties of Milk Powder

The composition of dried milk will naturally vary with the composition of the original milk and the moisture-content. A rough analysis can be obtained by multiplying the numbers of the average percentage composition of whole milk by 8, or of skim milk by 11.

The moisture-content of any dried milk product should be as low as reasonably possible. That of the spray-dried product should not exceed 5, and that of the roller-dried product, 8 per cent. The average moisture-contents of samples fresh from the process are 2·5 and 5·5 per cent. respectively. Dried milk is hygroscopic and samples for moisture-determinations should not have been previously exposed to the air ; the same can be said concerning the storage of the dried material.

The following table (CXXIII) gives the range of composition of various dried milk products :—

TABLE CXXIII. *Range of Composition of Dried Milk Products (Percentages)*

Material	Water	Fat	Protein	Sugar	Ash
Whole-milk powder .	1·4–6·4	25·0–29·2	24·6–32·1	31·4–37·9	5·6–6·2
Part skim-milk powder .	2·1–8·3	13·0–22·0	25·7–38·4	34·7–48·9	5·7–7·3
Skim-milk powder .	1·0–7·4	1·0– 2·6	33·3–37·7	45·6–52·2	7·9–8·2
Cream powder	0·6–1·0	50·0–72·0	11·1–19·2	14·7–25·5	2·4–4·2

The ash of whole-milk powder (6·0 per cent.) contains roughly 1·4 per cent. of common salt, 1·5 per cent. of CaO, and 1·8 per cent. of P_2O_5. The minimum copper and iron contents are roughly 4 and 12 p.p.m. respectively, but these values are greatly exceeded in the dried product made from milk pre-condensed in copper vacuum-pans, and in roller-dried milk. Drying on rolls adds a considerable amount of iron to the dried product, some samples showing 30-35 p.p.m.

There is no doubt that the low moisture-content of milk powder

is directly connected with the hydration of the lactose. The spray process is by far the most efficient desiccating process. The temperature to which the roller-dried product is heated before reaching the knife can cause a considerable amount of the lactose to appear temporarily in the unhydrated form, which at a lower temperature crystallises out as the lactose monohydrate, thus desiccating the product still further. This is especially noticeable in the case of roller-dried whey. In a spray-dried product of low moisture-content, the lactose is mostly non-crystalline (and unhydrated), with the result that it requires the addition of moisture before crystallisation sets in and thus to liberate the fat which it envelops in a form extractable by solvents.[1]

177. The " Solubility " of Milk Powders

The term *solubility* of milk powders is loosely applied to define the amount of recovery of suspension-stability when the dried product is reconstituted in water to the extent of the total-solids content of the original milk. It may be said at the outset that loss of solubility is vested in the denaturation by heat of the protein constituents, and that this denaturation depends on the time and temperature of heating of the original, pre-condensed, or the final dry product.

The solubility of dried milk is an important factor in the trade. Dried milk is one of the few straight dairy products of good hygienic quality offered for sale, and its reconstituting properties for the preparation of a substitute for whole milk for table purposes or for infant-food are of great practical importance ; some of the improvements in the methods of manufacture, especially those of the spray-dried product, have had this end in view.

The factors affecting the solubility of dried milk are those which tend to alter the physical properties and behaviour of the casein and lactalbumin. These factors are bound up *inter alia* with the quality and acidity of the raw milk, the process of manufacture, the moisture-content and the age and conditions of storage of the dried product. The earlier methods of manufacture involved the use of protein-stabilisers, such as alkalis and citrates, but progress has ensured the exclusion of foreign materials other than sucrose during manufacture, and the ideal is to manufacture a product of high solubility containing milk solids only.

ACIDITY. Milk of high acidity due to lactic acid fermentation gives a product of decreased solubility ; the acidity is intensified in the dried material and the heat of drying accelerates the action of the acid on the calcium caseinate-phosphate complex. The denaturation of lactalbumin is also intensified in an acid medium,

owing to a higher concentration of hydrogen and other cations. Milk with developed acidity rapidly develops more acidity; fresh milk containing proteolytic organisms gives a product of lower solubility, owing to the action of the rennin produced by such bacteria.

TEMPERATURE OF HEATING. The greatest effect on solubility is due to the temperature to which the liquid milk and the dried product, either or both, are exposed during the drying processes. The effect of heat on the denaturation of milk proteins and on the precipitation of calcium and magnesium salts has already been discussed (Section 143c and d).

Wright [2] has investigated the effect (a) of liquid or moist heating, and (b) of dry heating on the solubility of milk-powder. The insolubility caused by liquid heating is due to the denaturation of the milk proteins, particularly the casein, and the insoluble form of protein is produced by the ordinary steps of protein coagulation, namely, denaturation followed by flocculation. This type of coagulation is exemplified in the manufacture of evaporated milk. The denaturation process appears to be associated with both concentration of milk solids and temperature. It is possible that the steps which are taken to reduce the susceptibility to coagulation of evaporated milk, e.g., the alcohol test, and the process of forewarming before condensing, may be used in controlling the solubility of milk powders.

Wright has examined the inter-relationship of the temperature and the time of heating and the influence of concentration of milk solids on the solubility of the protein of milk powders. Holm, Deysher and Evans [3] have shown that a logarithmic relationship exists between temperature and time of heating, and that increasing concentrations of milk solids lower the coagulation-temperature. The experiments carried out by Wright have brought out the profound influence of the above factors on protein solubility. At temperatures near 100° C., protein insolubility in a product containing 60-90 per cent. of " total solids " may be induced by heating for periods ranging from a few seconds to a fraction of a second. The extreme difficulty of producing an atmospheric roller-dried milk powder of a high degree of solubility is at once apparent, because during drying, the heated milk solids are bound to pass through these ranges of concentration and temperature. The time-temperature relationships are found to be linear over a wide range of temperature and concentration of milk solids.

Heating in the dry state at 100° C. causes a definite alteration in the solubility of the casein, especially when reconstituted in

cold water, but the solubility is recovered by reconstituting in hot water. Heating at temperatures above 100° C. gives a progressive increase in the insolubility of the casein, the degree of insolubility and time of heating being directly proportional. If the logarithms of the times of heating necessary to make the casein 50 per cent. insoluble are plotted against the temperature of heating (degrees C.), the points fall on a straight line ; the reaction velocity is increased 6·32 times for every 10° C. rise in temperature of heating.

The increased insolubility resulting from dry heating is almost entirely associated with the change in properties of the casein. Wright states that the change is physical, *i.e.*, it is a dehydrating effect caused by the removal of the water of imbibition of the casein particles. The restoration of solubility in hot water seems to confirm this view. Wright has also dehydrated dry milk powder with absolute alcohol ; 45 per cent. only of the casein is then soluble in cold water but the whole of the casein returns into solution in hot water.

Samples of roller-dried milk show differential solubilities according to the temperature of reconstitution. This appears to be associated with the period of dry heating in the latter stages of the roller-drying process ; it may be observed also in spray-dried powders which, although they are not overheated in the actual desiccating process, may be overheated either by too high a temperature of the hot air or by prolonged exposure at the bottom of the drying chamber.

178. The Influence of the Temperature of Reconstitution on the Solubility of Milk Powders (Fig. 24)

The methods used for determining the solubility of milk powders vary greatly in technique and temperature of reconstitution. Marquardt [4] treats the sample with successive amounts of water at 50–55° C. and weighs the insoluble residue. Supplee [5] stirs the powder for ten minutes in water at 65° C., whilst Hunziker [5] gives results from reconstituting for ten minutes at 25° C. and for five minutes at 100° C. The two types of protein-insolubility mentioned above, viz., that due to the heat-denatured protein and the dehydrated dry protein, were at that time not recognised. In view of this new knowledge, Howat and Wright [6] have investigated the solubilities of an artificially-heated milk powder and of commercial milk powders. A spray-dried milk powder containing 2 per cent. of its protein in the insoluble form was heated at 100–105° C. for six hours, and the percentage of insoluble protein was determined at various

owing to a higher concentration of hydrogen and other cations. Milk with developed acidity rapidly develops more acidity ; fresh milk containing proteolytic organisms gives a product of lower solubility, owing to the action of the rennin produced by such bacteria.

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temperatures of reconstitution up to 100° C. About 60 per cent. of the protein was insoluble at 20° C., but the solubility increased progressively with increasing temperature of reconstitution until at 100° C. the whole of the protein was soluble. With a constant time of heating, the percentage of protein rendered soluble during reconstitution is directly proportional to the temperature of reconstitution.

In the case of the commercially dried samples, each roller-dried
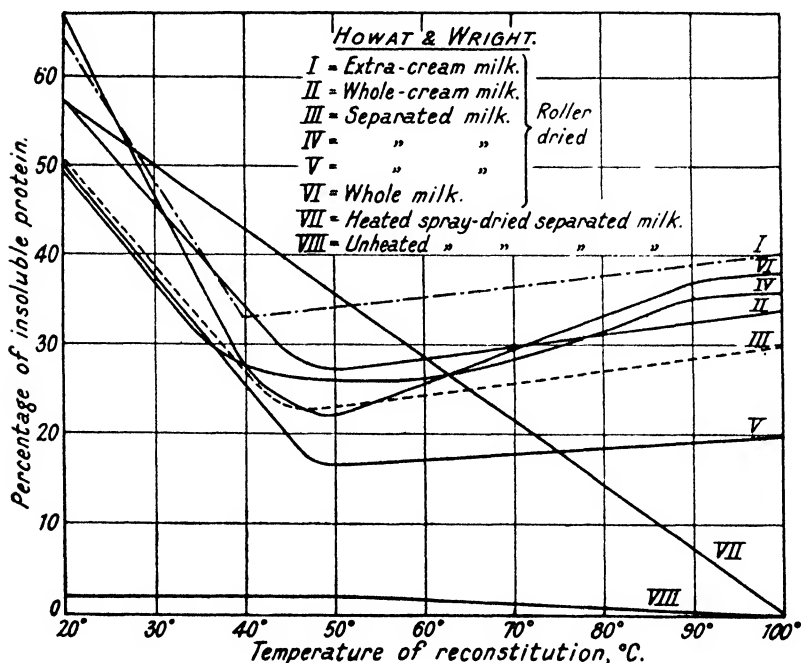
FIG. 24.—The influence of temperature of reconstitution on the solubility of the protein of milk powders.

sample showed an increase in solubility when reconstituted at temperatures between 20° and 50° C., due to the samples containing protein which was rendered insoluble by dry heating. Between 50° and 100° C. these samples showed a decrease in solubility, which might be attributed to the precipitation of albumin. The roller-dried samples already contain much insoluble casein which, at the temperatures of albumin-denaturation, might be *mutually precipitated* with the albumin. Where the casein is almost completely soluble, as in the spray-dried product, the casein might induce *mutual stability* of the albumin at its denaturing

temperatures. The percentage of fat also has the general effect of depressing the solubility, possibly due to the effect of heat on the localised concentration of the protein on the surface of the fat globules.

Howat and Wright, on the basis of the above findings, suggest that, owing to the possibility of denaturation at temperatures above 60° C., temperatures above this should be avoided in carrying out the solubility test. A milk powder should be reconstituted at the two temperatures, 20° C. and 50° C., since the values obtained at these temperatures will afford evidence of the extent of protein-insolubility induced during manufacture by moist heating and by dry heating, respectively.

179. The Influence of Physical Factors and of Ageing on Solubility

The solubility of both roller- and spray-dried milk usually decreases with the time of storage ; this decrease is most marked in samples of high moisture-content ; moisture or the dissolved substances in the moisture evidently cause the protein partly to lose its property of re-emulsification. Since milk powder is hygroscopic, the material can take up moisture when exposed to the air during storage ; this moisture has the same effect of rendering the protein insoluble. Supplee and Belliss [7] were able to increase the moisture-content of milk powder from 2 to 11 per cent. by circulating moist air through the powder. This process rendered the protein completely insoluble, whereas the circulation of dry air in a similar manner had no effect on the solubility of the protein. They set the upper limit of moisture-content for no loss of protein solubility as 3 per cent. ; samples of 4 5 per cent. moisture-content showed loss of protein-solubility in 12 months ; samples of 3–5 per cent. moisture-content, showing little loss of protein-solubility in 12 months, became completely insoluble when the moisture-content was raised to 7 per cent. A gradual rise from 4 to 5 per cent. moisture-content during 12 months' storage showed less insolubility than samples of 5 per cent. moisture-content.

Supplee [8] has found that the capacity of milk powder to absorb moisture is influenced by excessive heating and by the fat-content. The absorptive capacity decreases with increase in fat-content and a correspondingly lower content of protein. Lampitt and Bushill,[1] in their study of the amount of " free fat " in milk powders, have found considerable differences between the structures of the particles of (a) roller-dried and (b) spray-dried milk powders. It appears that the lactose of spray-dried powder is in the amorphous form, and that when the powder is allowed to absorb

moisture either from a humid atmosphere or from 96 per cent. alcohol, the lactose crystallises (β-anhydrous $\rightarrow$ α-monohydrate) thus freeing the fat from its impermeable coating and enabling it to be dissolved out by organic solvents. A fine milk powder on absorbing water first becomes clammy and in time forms a hard dry powder. This condition is associated with a fairly regular moisture-content for powders of equal fat-content, and the minimum content of moisture at which all the fat is extractable by solvents by this procedure has been termed the *critical moisture-content*. This value naturally varies according to the fat-content, owing to a correspondingly variable lactose-content. Thus a powder containing 20 per cent. of fat has a critical moisture-content of roughly 10 per cent., that of 28 per cent. fat about 9, and that of 32 per cent. fat about 8 per cent. The critical moisture-content varies directly with the content of solids-not-fat. In the roller-drying of whey, the material is scraped off the rollers as a gummy mass which, on cooling for a few minutes in the trough, crystallises out into a dry hard mass ; this phenomenon is of value in yielding a product which is reasonably dry and easy to grind.

The freeing of the fat by allowing milk powder to absorb moisture is not instantaneous but requires from seven to twelve hours. The age of a milk powder is also of importance, since fresh samples have a higher critical moisture-content than older samples, and the time of exposure to a humid atmosphere necessary for aged samples is considerably less.

It is obvious that absorbed moisture is used up for the crystallisation of α-lactose monohydrate from the amorphous β-form, and that although this reaction takes time, all " free " water in the powder is undoubtedly utilised for this purpose. It is equally clear that the factors responsible for the acidity of milk, even when fresh, are very highly concentrated in any residual water, and that a slight trace of developed lactic acid would be present as a strong solution in the moisture of the powder. Fouassier [9] has suggested that protein-insolubility is induced in stored milk powders of high moisture-content by the high concentration of the " natural acidity " of the fresh milk in the slightly moist powder. Howat and Wright,[6] deducing from the drift of the pH of concentrated milk from 6·6 in the whole milk to 5·97 for a milk containing 65 per cent. total solids, point out that, as further concentration proceeds, the pH still changes to the more acid side and to the isoelectric point of casein, at which the protein is more sensitive to heat-coagulation than it is in whole milk.

It is probably not the total moisture-content of a slightly moist

milk powder which is of importance, but the distribution of the moisture into that in lactose hydrate and that holding soluble material in solution as a film.   Lampitt and Bushill (*loc. cit.*) note that all powders retain tenaciously the last 4–5 per cent. of moisture, even with vacuum-drying at 100° C.   A further investigation into the nature of the insolubility developing with age in order to ascertain the amounts of (*a*) permanent insolubility due to denaturation, and (*b*) recoverable insolubility due to dehydration, would be profitable.   .

DISSOLVING CAPACITY OF MILK POWDERS. Of practical significance is the behaviour of a milk powder when it first comes into contact with the water used for reconstitution. A fine spray-dried powder may form dry or wet lumps, but the more granular roller-dried product will be more completely wetted and dissolved with less difficulty.   Fine particles of the former may contain minute bubbles which prevent instantaneous wetting, whilst rapid solution would cause the formation of an outer coating of concentrated milk solution through which more water for dissolving the inner layers would diffuse very slowly. With spray-dried powders, the nature of the product can be controlled by regulating the size of the orifice of the spray-nozzle and the pressure applied to the milk.   High pressure through a small orifice gives a fine, flaky powder, whereas a large orifice and low pressure gives a more granular product, which does not tend to cake in the reconstituting process. The spraying of a pre-condensed milk also gives a product more granular than that from whole milk similarly treated.

180. The Keeping Quality of Milk Powders

One of the main problems of the dried-milk industry is the preservation of the flavour of the dried product for a reasonable period of time before consumption. The development of stale, fishy, tallowy or rancid flavours was common in the early days, but better methods of manufacture, packing and storage have considerably improved the keeping quality of the products. Whole-milk powder is more susceptible to taint, but skim-milk powder (1–2 per cent. fat) can also develop taints when the storage conditions are unsuitable.

BACTERIAL SPOILAGE.   Milk powders of low moisture-content (5 per cent. and under) provide a poor medium for bacterial proliferation and action ; if powders are stored so that they are protected from dampness, spoilage from bacterial action will not occur.   In powders of excessive moisture-content, due to improper drying or exposure to the air, lumpiness, mould growth and

bacterial spoilage may appear in time. There is no relation between the bacterial count of the original milk and that of the final powder. The bacterial count of roller-dried milk powder is very low, but re-contamination occurs before packing ; spray-dried powder made from pre-condensed milk shows much larger counts, and the spray-dried product from whole milk shows the largest count of all.[10] The count decreases rapidly for the first few months of storage and then may decrease more slowly or remain constant ; the moisture-content of the powder does not appear to have any effect on the count, as the counts in high- and low-moisture powders are similar.[11] The type and number of living bacteria in dried milk are an index of its purity and of the amount of contamination during manufacture. The roller-drying process is capable of destroying all non-sporing bacteria, but the spores of spore-bearing organisms are not destroyed ; the roller-process also destroys or renders avirulent *M. tuberculosis* of both human and bovine origin.[12]

ENZYME ACTIVITY.   Just as with bacterial action, the previous treatment of the milk before drying by the spray-process governs the survival of enzymatic activity in the dry product. Roller-dried milk is free from lipolytic and peroxidase activity, but spray-dried milk powder, especially that from whole milk, possesses consider-able peroxidase activity in spite of the temperature to which it has been exposed.[13] Lipolytic activity may also be found in such powders, and in those from roller-processes associated with vacuum-drying, if the temperature of the milk has been kept low to preserve the solubility of the product. Milk which has developed a certain degree of acidity before drying was found by Fleming and Nair [14] to give the dried product a disagreeable acid flavour. The introduction, by Supplee, of lipases into a roller-dried powder caused butyric rancidity to develop in a few weeks.[15] Lipase action is evident only in powders containing milk-fat. If the temperature of the milk is kept down in the manufacturing process so as to give a product of maximum solubility, the possible development of butyric rancidity has always to be faced. There is always a certain percentage of milk from more or less diseased udders in market milk, and it is only by examination of the milk on the platform, or in the producer's cowshed, that this trouble can be effectively overcome.

181. Chemical or Autoxidative Taints

The most widely-distributed fault of long-stored dried milk is that due to autoxidation of the fat —" *tallowiness.*" The mechanism of the causative reactions is identical with that of the development

of tallowiness in butter and other commercial fatty foods, and all the factors, *e.g.*, acidity, moisture-content, light, metallic contamination, and heat, play a similar part in the deterioration of the fat of milk powder. The mechanism of the development of tallowiness has been dealt with fully in Section 29. The reaction is bound up with the oxidation of the unsaturated acids of butter-fat at the double bond, although the amount of reaction which need occur before an oxidative taint can be detected organoleptically is exceedingly small. Thus there need be no lowering of the iodine value of butter-fat before tallowiness is apparent,[16] although such a lowering may be detectable in advanced fat-oxidation. Rather may it be said that tallowiness is the result of the oxidation of unsaturated acids (oleic acid), with subsequent splitting to form aldehydes and aldehydic acids which possess a tallowy odour and flavour.[17]

Since tallowiness is produced by oxidation, it is obvious that atmospheric oxygen is a component of the reaction. The exposure of milk powder to air, or in non-airtight containers, has been found to produce tallowiness rapidly. On the other hand, storage in a closely-packed condition in airtight containers has delayed the onset of tallowiness and improved the keeping quality.

AIR. The complete exclusion of air from a dry powder is, however, impossible, and either gaseous displacement or evacuation will not free the powder from the oxygen entrapped in air-cells within the structure of the powder-granule. Palmer and Dahle [17] have found that granules of spray-dried powder contain an air-cell in their centres, so that the fat is exposed to oxygen on both sides. They have confirmed Coutts' [18] observation that since roller-dried powder does not contain air-cells, it possesses better keeping-qualities than the spray-dried material. The rate of development of tallowiness has also been found proportional to the size of the air-cell. Thus powders made by the centrifugal (Dick) process show a lower keeping quality than spray-dried powders, owing to the presence of a larger air-cell in their particles. A homogenising action in pressure-spraying, may, however, offset this effect, since a greater area of fat is exposed to the action of the air. The milk in these processes is evidently dried in the form of a fine foam, and no practical method seems to have been evolved to destroy the air-cell structure of the particles.

Holm and Greenbank [16] stored milk powder in an atmosphere of carbon dioxide, but found no difference in the time necessary for tallowiness to be detectable, although the intensity of oxidation was lowered. It must be understood, however, that (*a*) dissolved oxygen in the film of moisture cannot be removed by displacement,

and that this form of oxygen is quite as active as gaseous oxygen, and (*b*) the development of a detectable degree of tallowiness requires very small amounts of oxygen only; indeed, sufficient oxygen for the subsequent development of tallowiness might have become associated with the oleic-acid radical during its contact with hot air in the drying process, and the appearance of the taint in detectable amount might be the result of the next steps in the oxidation process. If a powder contains a fat of low free fatty-acid content, it should keep well in vacuum-storage. Supplee [15] has found that the displacement of air by an inert gas is uncertain and that complete removal of oxygen is necessary.

LIGHT AND HEAT. The factors necessary to activate or ionise molecular oxygen, before oxidation can occur, naturally accelerate the development of tallowiness. Thus exposure to *light* causes milk-fat, alone or in a dairy-product free from a biological oxygen demand, rapidly to become tallowy. Therefore freedom from prolonged exposure to sunlight or strong artificial light during manufacture or storage is essential to obtain maximum keeping properties. *Heat* also accelerates the development of tallowiness; milk powder stored at 0° C. will maintain its normal flavour for prolonged periods but when stored at temperatures above 20° C. will become tallowy in a few months. Thus Dahle and Palmer [19] found that the difference between storage at 4° C. and 20° C. was not very great, but that rapid deterioration and discoloration occurred during storage at 37° C. This occurred irrespective of the type of container—glass, metal or cardboard. Supplee [15] found a marked difference in the keeping quality of milk powder when stored at 0° C. and 20° C. Storage at 0° C. caused a powder to keep sweet for at least eighteen months; storage at 11° C. caused tallowiness to develop in twelve to thirteen months, whilst storage at 25° C. caused the taint to appear in five to six months. The intensity of tallowiness was also more marked at the higher temperatures, and by subsequent storage at a lower temperature the onset of the taint could be arrested. There is no doubt also that the exposure to hot air of spray-dried powder at the bottom of the drying-chamber shortens the induction period to autoxidation, and the more recent practice of removing the powder as soon as it is dried may be the reason why spray powder keeps better now.

MOISTURE-CONTENT. Tallowiness most readily develops in milk powder of very low moisture-content (under 2 per cent.).[16] The moisture relationship of the reactions involved differs in principle from the phenomena associated with the other forms of deterioration mentioned above. Thus high moisture-content

favours the onset of staleness and increase of insolubility. The latter reactions, however, are associated with the protein, but tallowiness arises from deterioration of the fat.

Holm and Greenbank [16] place the optimum moisture-content for preventing the development of tallowiness (so far as that defect can be controlled by humidity) at 1·88 per cent. for spray-dried and 3·00 per cent. for roller-dried powders. At moisture-contents above 4 per cent., a fishy flavour and odour develop. Increase in the moisture-content probably prevents the formation of the intermediate compounds (aldehydes, etc.) which give rise to a tallowy flavour. Supplee [15] considers that, with excessive moisture-content, the tallowy condition is masked to such a degree as to be undetectable. The findings of Dahle and Palmer,[13] that an increase in moisture-content accelerates the development of tallowiness, may be due to their method of humidifying the powder, namely, by the oxidising action of the humid air to which they exposed their powders.

The presence of 2·0 per cent. moisture in milk powder ensures that all the lactose present has just sufficient water to form the monohydrate, although the physical condition of the lactose-film on each particle precludes crystallisation. There is thus no free-water film in such a dry powder. An increase in the moisture-content therefore would start the formation of water-films, which would act as solvents for lactose and the salts of the milk, and consequently would dissolve some of the lecithin from the fatty phase. Autoxidative action would thus take place in the aqueous film, and the lecithin, which relatively to the fat proper is an antioxygen, would be oxidised first, and in the case of moisture-contents of 4 per cent. and above would cause (a) protection of the fat proper from autoxidation, and (b) fishiness to appear through the hydrolytic oxidation of the choline residue of the lecithin. This explanation fits in with the observations of Holm and Greenbank.[16] The lecithin apparently cannot act as an anti-oxygen unless a film of water is present ; thus pure dry butter-fat cannot be induced to give a fishy flavour, whereas butter does give such a flavour before tallowiness sets in. The autoxidation of dry milk in powders of low moisture-content is a direct attack on the fat-molecules, the oxygen being activated by the formation of organic peroxides, such as occur in the determination of the oxygen-absorption curve of pure dry fat. Lampitt and Bushill [1] have found that a continuous glaze of lactose covers about 80–90 per cent. of the fat in spray-dried powder. This glaze is evidently permeable to oxygen, and its continuity is broken only when the critical moisture-content is reached on humidifying the powder.

The development of tallowiness is less marked when the powders contain the normal amount of moisture (4 per cent.). This is possibly due to better conditions for the natural anti-oxygens of butter-fat to come into play.

FAT-CONTENT. The incidence of tallowiness in skim-milk powder, containing 1–2 per cent. of fat, is much smaller than in whole-milk powders. However, it does not necessarily follow that the higher the percentage of fat the more susceptible is the powder to become tallowy. Dried cream, for instance, usually possesses good keeping-quality. Supplee [15] found that a powder of 5–6 per cent. fat-content was tallowy in four months, one of 13 per cent. in seven months, one of 26 per cent. in thirteen months, whilst a powder made from thin cream, containing 50–55 per cent. of fat, kept satisfactorily for fifteen to eighteen months. This was explained on the basis of the exposed area of a unit amount of fat. The exposed area per unit weight of fat is greater in low-fat than in high-fat powders, and therefore the steps in the reactions necessary to produce tallowiness are reached more quickly. Holm, Greenbank and Deysher, [20] however, contradict Supplee's findings in that they have observed perceptible decreases in keeping-quality with increased fat-content, especially in the higher ranges.

ACIDITY OF THE MILK AND ACID VALUE OF THE FAT. Free fatty acids present in a fat shorten the induction period to autoxidation and accelerate the subsequent oxidation. Indeed, acidity may be taken as playing a greater part in the autoxidative process than any other factor, owing to the fact that much of the free acid formed in milk fat is oleic acid, which is more reactive than oleic esters. If the original milk has developed a small amount of lactic acidity, this acid would be concentrated in the dry powder and would largely be dissolved in the fatty phase in a similar manner to the entrance of lactic acid into the fat of cheese. Although the pH of, say, dried milk powder is not known, it is suspected to be more to the acid side than in whole milk or even in condensed milk. Howat and Wright [21] have observed the trend of pH to the acid side with increase in concentration of the solids in milk, and the extrapolated value for the linear relationship of hydrogen-ion concentration with milk-solid concentration gives a pH of 5·8 for 96 per cent. solids, which is near to the average value for a ripened Cheddar cheese. The concentrated " natural acidity," as suggested by Fouassier, [9] may also be operating in the slow hydrolysis of the fatty esters.

Roller-dried milk is not so susceptible to tallowiness as the spray-dried product. This may be due to a less effective area per unit weight of fat, and to the possible volatilisation of acids during

the drying process. The roller-dried material usually contains more moisture, and there is no lactose glaze around the fat. Evidently antioxygenic conditions are also more favourable in the roller-dried powder.

CONTAMINATION WITH HEAVY METALS. The pro-oxygenic effect of traces of heavy metals, such as iron and copper, operates in dried milk as much as it does in milk and butter. The concentration of the heavy metals is considerably increased in the drying process, but possibly owing to different moisture-conditions their catalytic effect is not multiplied proportionately. The metals nevertheless show the same order of potency in initiating and catalysing the development of tallowiness, copper being the most powerful of all the metals generally used for the manufacture of dairy-plant. This subject has been treated in Section 158b.

Supplee [15] has investigated the effect of the copper-content of whole milk on the development of tallowiness in the dried product. He found that whole milk containing from 0·7 to 1·0 p.p.m. of copper when dried became tallowy in ten months, whilst milk containing 3 to 7 p.p.m. caused tallowiness to develop in the dried product in five to seven months ; in each case the powder made from milk containing its natural copper-content was free from tallowiness for over twelve months. The action of iron was much less pronounced, but the amount of contamination with iron, e.g., from the cast-iron or steel rolls, is much greater than with copper. It appears that the metallic surfaces with which milk comes into momentary contact during its pre-treatment before drying contribute very little to the metallic contamination, but with containers and vacuum-pans in which the milk is held for a considerable period of time, the nature of the metallic surface is important. The copper surfaces of the vacuum-pan are of importance to the keeping-quality of the product made from pre-condensed milk ; these should be kept clean and bright, especially the dome. It may even be advisable to separate the cream and condense only the separated milk, to which the cream may be added before drying. This will partly overcome the shortening effect of traces of copper on the induction period to oxidation of the butter-fat. Powders from roller-driers in intermittent use are more heavily contaminated with iron than those in constant use. The iron-content of many samples of such powders is from 20 to 35 p.p.m., thus showing a doubling of the iron-content during the drying operation. The practice of incorporating iron salts in dried milk also shortens the life of the powder.

OTHER FACTORS. Holm, Greenbank and Deysher [20] found that

drying pre-condensed milk gave a product which was superior in keeping-quality, possibly owing to the acid-removing effect of the heat-treatment during evaporation. These workers also found that the homogenisation of the milk improved the keeping-quality of the powder ; this is contrary to the expected result that the increase in the size of the fat-surface enhances the possibilities of oxidation.

Milk powders keep better when packed in containers which exclude air and moisture. Humid air increases insolubility, whilst free ventilation with air causes tallowiness to occur sooner. Tin-plate or paraffined-wood containers are satisfactory for storage in bulk ; faced ground-wood packages are unsatisfactory ; unlacquered tin-plate containers, even for small amounts, are advisable. Dried milk is especially prone to infestation with insects and to attack by vermin, so that the choice of a suitable container is important.

The presence of sucrose in dried milk delays the onset of staleness and tallowiness, possibly owing to the protective effect of a layer of sugar around the dried-milk particles and the granular nature of the product. Such a product is also more resistant to humidity-changes and is less hygroscopic.

182. Other Dried Milk By-products

DRIED BUTTER-MILK. Butter-milk has been dried by both roller- and spray-drying processes, but the latter method has been abandoned owing to mechanical difficulties due to clogging of spray-nozzles and to the variable total-solid content of the product from creameries. The use of the dried material for poultry and animal feeding allows more drastic treatment in the drying process, and no attempt is made to preserve its solubility. The liquid is always roller-dried at atmospheric pressure, although some progress has been made of late in drying the pre-condensed liquid, the volume usually being reduced in a vacuum-pan to a quarter of the original. The product is ground, sifted, and packed in bags, barrels or tin-containers for market.

Dried butter-milk keeps well because its moisture-content is low (6–9 per cent.) and its content of lactic acid is high (5–6 per cent.). The powder is hygroscopic, and when stored in bags in a damp atmosphere is liable to become lumpy and mouldy. The composition of dried butter-milk, according to Hunziker [5] is given in Table CXXIV.

The fat-content is variable and depends on the fat-losses in the butter-milk. The amount of lactic acid naturally depends on the acidity of the cream before churning ; butter-milk from

TABLE CXXIV. *Composition of Dried Butter-milk, Dried Whey and Whey Paste*

	Dried butter-milk (Hunziker)			Dried whey		Whey paste
	A	B	C	A	B	A
Moisture	7·50	6·00	9·25	11·21	7·76	45·92
Ether extract	6·50	7·23	3·40	—	1·39	—
Protein	36·24	36·10	33·50	12·62	12·62	11·69
Lactose	35·50	35·28	40·55	} 68·52	70·53	33·03
Lactic acid	6·00	6·70	4·95			
Ash	8·25	8·69	8·35	7·65	7·70	9·36
CaO	—	—	—	1·018	—	1·917
P_2O_5	—	—	—	1·667	—	1·523

neutralised cream will contain a higher ash-content, and most of the lactic acid will be present in the dried product as sodium (or calcium) lactate.

DRIED WHEY. There are many possible commercial outlets for dried whey. Thus it may be used as a source of lactose for making either whole or dried cow's milk similar in composition to human milk, and so utilised for infant feeding. It may be used in the confectionery and baking industries, and also as animal food. The chief problem in drying whey is the economic one of securing a reasonable profit over and above the capital and current cost of drying a liquid which contains roughly only 7 per cent. of total solids.

It has long been the custom for Swiss cheese factories to evaporate the sweet whey, obtained as a by-product in the manufacture of some Swiss cheese, until a profitable yield of crude lactose is obtained on cooling. This crude product is sold to a central refinery. The mother liquor contains a sufficiently high content of solids to be profitably dried by the roller- or spray-process, preferably the latter.

Acid whey from the manufacture of cheese from acid-drained curd is a potentially valuable source of food material, but in the past, apart from the fraction used for pig-feeding, most of the whey has been disposed of as waste. Owing to the regulations in this country regarding the pollution of river waters by dairy waste, its disposal may incur considerable expenditure in the erection and upkeep of aeration and settling tanks and filter-beds. Additional difficulties in the handling of whey are connected with its bulk and its perishable nature, so that partial condensing or drying would have to be carried out at the cheese factory.

Considerable progress has been made in manufacturing lactose from dried whey and in the preparation of a marketable commodity from the mother liquor. Successful attempts to dry whey itself have also been made. Golding[22] and Stead initially attempted the drying of whey and of a mixture of whey and separated milk by the twin-roller drier at atmospheric pressure, and were able to produce a satisfactory product. Further progress was made by the same workers in the installation of a preheater and a blower, whereby hot air from the rolls was economically used to evaporate part of the water from thin films of whey flowing down over a large surface above the rolls. By this means the volume of whey dried per hour was considerably increased and the product was satisfactory. The drying of whey on rolls is accompanied by a certain amount of caramelisation of the lactose. The use of more concentrated whey, partial neutralisation, and mixing the whey with separated milk, give a better coloured product. The whey comes off the rolls as a gummy solid, which crystallises to a hard cake on cooling. This material is easily ground to a creamy powder, which has rather a " sandy " effect when tasted. The colour naturally depends on the amount of caramelisation during drying, but what appears to be a very brown product coming off the rolls grinds to a powder of better colour. The inclusion of separated milk naturally improves the colour. The dried cake tends to clog the stones or beaters on grinding.

The powder is hygroscopic but, owing to its high lactose-content, and to the fact that the lactose has crystallised as the monohydrate, the moisture-content, determined by the oven-drying method, is higher than for other dried-milk products ; it amounts to 8–12 per cent. The ground material keeps well when properly packed in paper-lined barrels, but the unground cake tends to develop moulds quickly. Table CXXIV gives the composition of two samples of dried whey.[26]

Both dried whey and dried whey *plus* separated milk have been used in bread-making and have been found to give improved loaf-size and better-textured bread. The taste and appearance are similar to those of ordinary milk bread, but the keeping quality is slightly inferior.

183. Malted-milk Powder

This is the product obtained by mixing whole milk with the mash of barley malt and wheat-flour, allowing the enzymes of the malt-extract to hydrolyse the starch, and then drying the product. Certain salts, *e.g.*, common salt and sodium bicarbonate, may also

be present. The product should be of low moisture-content (3·5 per cent) and contain about 7·5 per cent of milk-fat. A pound of malted milk thus contains the solids of 2·2 lb. of fresh milk. The product has always been well received by the medical profession and the public, and the industry has made great progress since its inception by William Horlick in 1887. The manufacture of malted milk comprises two main phases : (a) preparation of the malt-extract, and (b) mixing of the wheat-flour and malt-extract and allowing dextrinisation and maltose-formation to occur, separation of the spent barley residues, addition of whole or condensed milk, and final drying of the product.

The barley may be malted in the factory, but it is advantageous to buy the malt from approved maltsters, as this insures a uniform material made from graded barley under controlled conditions which comply with the rigid standards required for the brewing industries. One of the main constituents of malted milk is maltose, and since malt is much dearer than wheat-flour, and contains more diastase than is needed to convert the barley starch to maltose, advantage is taken of the excess of enzyme to hydrolyse the cheaper source of starch in wheat-flour. The malt flavour, however, is associated only with barley malt and the preparation from wheat-starch is weak in malt flavour. A cheaper source of starch would perhaps be maize, but wheat is the richer in protein and mineral matter and its starch-cells disintegrate more readily on boiling with water.

The starch of the wheat-flour is rendered more " soluble " by boiling with water, after which it is cooled and mixed with roller-crushed malt. It is most desirable that the end-products of starch hydrolysis should be maltose and only a little dextrin. This is accomplished by keeping the mixture at 45° C. for thirty minutes, then raising the temperature at the rate of 1° C. per minute to 70° C. (taking twenty-five minutes) and maintaining this temperature for one hour. The mashing process is complete when all the starch has been hydrolysed to maltose and dextrin, and usually takes from two to three hours. In this process the malt flavour enters from the barley husk.

The barley husks are allowed to settle and the liquid portion is removed. Sometimes this liquid is filtered free of the suspended protein-material, which is of considerable nutritive value but apt to cause a sediment in the reconstituted malted milk. Filtering undoubtedly gives a final product which is completely soluble and assimilable ; insoluble protein can be obtained more cheaply than as an ingredient of malted milk, but the balance of the nutrients in the filtered product is not so good, too much sugar being present.

The mash extract is then mixed with whole milk, condensed and dried. The ratio of the two liquids is such that 1 lb. of the dried product contains the equivalent of 2·2 lb. of whole milk, which usually means that the mixture contains 40–45 per cent. milk and 55–60 per cent. mash. Both liquids may be condensed separately to about a third of their bulk before mixing. The condensing and drying is done in a vacuum-pan provided with a mechanical stirrer, or by drying-rolls working in vacuo. The product must not be exposed to temperatures above 130–140° F. during the process, owing to the risk of injuring the value of some of the ingredients and the flavour. Towards the end of the drying the material becomes very viscous and finally dries to a brittle, porous cake. The product from roller-driers is the usual papery sheet, which breaks up into shreds, whilst the spray-dried product is light and flaky. Grinding and bagging is done in a dry room, into which " conditioned " air enters through ammonia-expansion coils.

The composition of some samples of malted milk, as collected by Hunziker,[24] are given in Table CXXV.

TABLE CXXV. *Composition of Malted Milk*

Source	Moisture %	Fat %	Ash %	Crude protein %	Casein %	Lactose %	Maltose %	Dextrin %
Horlick's [23] 1908.	3·63	8·36	3·70	12·94	—	—	71·37	—
Horlick's [24] 1923.	2·15	8·10	3·96	14·80	6·44	10·04	43·40	—
Borden's [23] 1908.	5·42	6·14	3·17	13·38	—	—	71·66	—
Borden's [24] 1923.	2·00	8·95	2·77	14·11	7·40	11·81	47·60	—
Thompson's [24] 1920.	4·04	7·12	3·80	10·00	—	—	74·69	—
Thompson's [24] 1925.	2·15	8·10	3·85	12·15	—	—	72·95	—
Coor's [24] 1923	2·06	8·97	3·84	14·91	9·25	15·71	38·40	—
A.D.S.[23]	5·93	6·75	3·08	14·06	—	—	70·05	—

The casein values given in Table CXXV are rather high, and it is doubtful if the milk protein has been satisfactorily separated. The ratio lactose-maltose-dextrin appears to be roughly 1 : 4 : 2. The fibre-content, which is a true indication of whether the mash has been filtered or not, may vary in the unfiltered mash products from 0·05 to 0·45 per cent.

Malted-milk powder keeps excellently and almost indefinitely in any climate, provided it is not exposed to humid atmospheres. The fat does not become tallowy, and it appears that the coating of gluten, dextrin and sugars completely excludes air from acting on the fat globules. The method of manufacture, namely, the mashing and the subsequent mixing, is intimately bound up with the keeping-quality, since the mere mixture of the ingredients gives a product which soon develops tallowiness ; the physical nature of the powder, therefore, must favour its good keeping-quality. The powder is very hygroscopic and therefore must be packed in moisture-proof containers. In humid atmospheres it turns gummy but does not lose its flavour.

Malted milk is sometimes sold as tablets which are more resistant to humidity effects ; they may be flavoured with chocolate or other flavouring material. Chocolate-covered bars are also sold. It has also found an outlet in the bakery and the sweetstuff trade.

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PART V

THE NUTRITIONAL VALUE OF MILK

CHAPTER XX

NUTRITIONAL VALUE OF THE MAJOR CONSTITUENTS OF MILK

184. Introductory

MILK is the last medium of contact between mother and offspring. It must be, therefore, the vehicle of a number of nutritional factors essential to the life and growth of the offspring for periods of weeks or months until these can be provided from outside sources. The complexity of the nutritional value of milk is at once obvious, if only from the fact that, for the time being at any rate, milk must be a complete food. It is not surprising, therefore, that nutritional chemistry has largely centred around the production and composition of milk, and that our newer knowledge of nutrition, which has developed during the last twenty years, began with the study of the growth-promoting factors in small quantities of milk added to synthetic diets. As is well known, this study by Hopkins,[1] and afterwards by Osborne and Mendel,[2] led to the discovery of the vitamins, which revolutionised the earlier conceptions of the physiology of nutrition. The progress subsequently made in the chemistry of the vitamins has been outstanding ; the chemical constitution of the most important vitamins has been elucidated and some have been synthesised, but the exact mechanism of their physiological behaviour is still obscure. It seems as if more attention has been paid to the determination of the amounts of the vitamins in foods, their destruction by various agencies, their concentration in, and ultimately their separation in pure form from, various sources and their general physiological behaviour, than to the actual mechanism of their action ; this, however, has not been lost sight of.

The first step was to recognise the presence of two classes of vitamins in milk, one being fat-soluble, the other water-soluble, and the need of both these classes to support health and growth in young animals. Later the vitamins were subdivided according to their specific physiological properties. It need not be emphasised that the quantitative aspect of each of the vitamins of milk has been extensively investigated.

Other aspects of the nutritional value of milk are connected with the functions of colostrum. Colostrum, the secretion given

by the mammal immediately before and after parturition, is a liquid containing a much higher protein-content than milk, the chief protein being lactoglobulin. Various workers have regarded this protein as the carrier of antibodies or other immunising factors from the mother to the offspring, although in some species colostrum is not essential, since these factors have already been transferred to the embryo *in utero*, owing to the nature of the placental barrier.

The calorific value of milk, especially of dairy products, must also not be lost sight of in the light of our newer knowledge of nutrition. It seems as if the nutritional factors which are present in small quantity in milk are more important when growth and health are concerned, whereas the major nutrients—fat, protein and lactose—have to be considered as are other foods, *i.e.*, from the energy-producing standpoint and the general requirements of the diets of adult animals. The subject has been extensively investigated with regard to the chemistry and composition of milk and its nutritive properties and with special reference to the nutritive properties of some particular constituents of milk.

185. The Functions of Colostrum

The composition of colostrum and its change of composition to that of normal milk has already been dealt with (Section 14). Colostrum has an important function in the nutrition of the newly-born offspring. Up to 1922, the material was considered as a laxative agent only and as nature's provision for clearing the alimentary tract of deleterious matter. Howe [3] found, however, that although colostrum had no laxative effect, it did not delay fæcal excretion as milk did.

The globulin of colostrum is identical with serum globulin.[4, 5] Colostral fat shows the same characteristics as milk fat but generally contains more capryllic and capric acids.[6] The fat of the first colostrum contains nine times as much carotene, eight times as much vitamin A and twice as much vitamin D as normal butter fat. The amounts of these constituents decrease regularly until normal milk is secreted.[7]

It was realised by Famulener [8] that colostrum was responsible for transferring passive immunisation from the blood of the mother to that of the offspring and of passing over maternal antibodies to the offspring. Hæmolysins were transferred rapidly to the blood of newly-born kids after suckling colostrum. The amount of these hæmolysins in colostrum varies with the globulin content. Similar results were found for the transfer of bacterial agglutinins to the blood of the offspring.[9]

Howe [10] has elucidated the problem of globulin distribution in the blood of newly-born calves. Working on three calves, he found no euglobulin in their blood at birth but that the fraction pseudoglobulin II only was present. One calf fed colostrum at eleven hours old and another at twenty-six hours old showed all globulin fractions in its blood (euglobulin and pseudoglobulins I and II) six hours later. The amounts of euglobulin and pseudo-globulin I decreased somewhat later although colostrum was still being fed. The rates at which these proteins entered blood when normal milk was fed was much slower and did not reach the same temporary high level as when colostrum was fed.

In infant's blood, only the euglobulin is absent and the human placenta does not bar the entrance of pseudoglobulin I into the embryonic blood.[11] The feeding of human colostrum immediately causes the appearance of euglobulin.

There is no doubt that the concentrated globulin solution in colostrum carries antibodies from the maternal blood which can be transferred to the blood of the offspring during the short time that these protective proteins can be absorbed unchanged from the alimentary tract.

Later work [12] showed that colostrum supplied antibodies to counteract pathogenic bacteria in the intestinal tract, and that calves fed on colostrum survived more frequently than those not given colostrum. Pasteurisation of colostrum only slightly decreased its protective powers. When deprived of these protective powers, intestinal bacteria invade various organs of the body and septicæmia results unless the resistance of the calf is great. The feeding of colostrum is accompanied by albuminuria, all three globulin fractions being excreted, whilst the fæces contain large proportions of unchanged globulin.

Kuttner and Ratner [13] later established the effect of the placental barrier in various species on the entry of these protective factors, either into the embryo *in utero* or through the colostrum. The intimate association of the various globulin fractions with the protective factors brings into prominence the significance of the fractions themselves through the permeability of the placentae of mammals of various species to both globulin and the protective factor. Thus, in species of mammals with epithelio-chorial placentae, colostrum plays a more important part than in species with hæmo-chorial placentae. Species of the former type are the cow, goat, horse and sheep, and of the latter, guinea-pig, rabbit and man. The blood serum of human infants contains as much pseudoglobulins I and II as that of adults. The feeding of human colostrum makes up therefore for the deficiency of euglobulin ;

human colostrum, however, is secreted about two hours after parturition, and nursing has to be stimulated.

186. Quantitative Nutritional Aspects

The economy of nutrition is based on a knowledge of the composition of the foods entering into a diet, their digestibility, their energy values, and the energy requirements of an individual at rest or at various forms of work or production ; the calorie value only of the ration enters into this conception, and the quantity factor is measured by the calorie value without considering the qualitative differences of the component nutrients. This was the earlier conception of quantitative nutrition, but progress was later made in the study of the quality of food constituents, particularly that of the proteins. The simple proximate analysis of a food does not throw light on its physiological effects, and it is only by prolonged feeding experiments that these can be elucidated. It was known in 1905 [14] that the animal body could not be maintained indefinitely on the only constituents of food known then—proteins, fats, carbohydrates and mineral matter. A supplement was necessary and this supplement was supplied by *milk*. This was the starting-point of vitamin chemistry : but before this vast subject is treated, other considerations need discussion first.

187. Protein Investigations

The structure of proteins was elucidated by Emil Fischer and the analysis of this class of compounds has provided a fertile field of research. These analyses provide evidence that there are pronounced differences in the number, amount and the relative proportions of the amino acids which are condensed to form the protein molecules. Thus the first two proteins to be prepared in pure form were casein and gelatin, and by certain reactions it was established that the latter was deficient in tyrosine, tryptophane and cystine. Gelatin is thus an incomplete protein, and one limiting factor in its utilisation as a food protein is that it cannot supply these amino acids for the building up of animal protein. The distribution of the amino acids is another limiting factor in that the protein fed may contain either too much or too little of certain amino acids for the building of animal protein. It is obvious that the excess of the other amino acids cannot be utilised in the specific and profitable direction of animal-protein synthesis but has to be eliminated after being metabolised in a similar manner to a cheaper fatty acid or carbohydrate.

The next steps consisted of (*a*) the analytical determination of

the twenty or fewer different amino acids contained in food (and muscle) proteins, and (b) ascertaining which of the amino acids are essential in a diet. Gelatin, an incomplete protein, was made a satisfactory material by the addition of tyrosine, cystine and tryptophane ; these amino acids, therefore, were essential for growth. Lysine has been demonstrated to be essential both for growth and maintenance. Proline is another essential amino acid. Glycine, the simplest amino acid, can be synthesised in the animal body, and is thus not an essential amino acid in food. Ackroyd and Hopkins [15] found that if arginine is present in the diet, histidine could be omitted, and *vice versâ*. Tyrosine and phenylalanine were also claimed to be similarly interchangeable. Rose,[16] however, found that histidine could render a diet, free from both arginine and histidine, complete, but that the addition of arginine to such a diet did not cause the same effect. Histidine is therefore an essential amino acid and arginine is not. However, owing to the difficulty of obtaining diets completely free from any special amino acid, the safer course is to regard *all* amino acids other than glycine as essential.

The completeness of milk proteins in this respect has therefore to be discussed both with regard to the building up of new protein in the rapidly-growing offspring (the manufacture of muscle-, blood- and enzyme-protein) and the elaboration of protein to replace " wear and tear " in the adult. (See p. 467.)

188. The Energy Value of Foods

Foods not only replace the waste produced by the activity of the body and provide the necessary material for growth and production, but also supply the materials for energy production. These processes are oxidative in nature and are measured (a) by the amount of oxygen consumed and carbon dioxide evolved (respiratory studies), and (b) by the amount of heat evolved (calorimetric studies). Initially it was believed that the energy of foods was derived from the burning of carbon and hydrogen only, protein being regarded as a material of paramount importance. Liebig (1842) showed, however, that it was proteins, fats and carbohydrates which underwent metabolic changes. Determination of the amount of protein utilised by the body and the occurrence of a nitrogen balance in the body were studied later by Voit.

The next steps consisted of (a) the calorimetric determination of the energy values of the food constituents, fat, protein and carbohydrate, and (b) the analyses of foods into these constituents. Rubner initiated the first step and was able, by supplementing his work with respiration studies, to prove that the heat output in

the body was quantitative. Henneberg initiated rough methods for the proximate analyses of foods, and adduced data for evaluating foods according to their components and for determining their calorific values. The feeding standards of Lehmann, Grouven and Wolff followed on these conceptions, and it was not until the comparative slaughter methods of Lawes and Gilbert and the balance and the respiration-chamber methods of Kellner, that the concepts of " *gross digestible* " and " *nett digestible* " energies of feeding-stuffs were advanced. The animal was unable to use all the calories of a food ; some of the food was not digested and was voided in the fæces. The gross value obtained from a food was the calorific value of the digestible portions of each component ; some energy was used for the digestion of these available components, thus leaving a major portion of the calorific value in reserve for fattening, work, or other form of production. Kellner evaluated these in terms of " starch," *i.e.*, the gross and nett digestible energies from 100 lb. of a foodstuff were given in terms of lb. of starch (starch equivalent). This conception has been a valuable instrument in calculating rations for both maintenance and production. The amount of fat which could be laid on by a fattening animal could also be calculated from the amounts of digestible nutrients by making use of *value numbers* (V) for different feeding-stuffs (the value number is the percentage of the calculated fat-producing power actually converted into fat).

METABOLISABLE ENERGY. While Kellner was studying the quantitative aspect of fat-production, Armsby studied the production of heat and the storage of definite quantities of heat in an animal. This was done by determining the quantity of heat given out from the consumption of certain quantities of foodstuffs, an animal calorimeter being used. Armsby determined the *heat-producing values* of the constituents for the animal, the total for a food being its *metabolisable energy*. The digestible nutrients supplied the following numbers of large calories (C) per grm.: protein, 4·7 ; fat, 8·8 ; and carbohydrate, 3·76, or as therms (1,000 calories per lb.) 2·13, 4·00 and 1·71 respectively. Since 1 lb. of starch yields 1·71 therms, the starch equivalent can be calculated from the total metabolisable energy by dividing by 1·71.

Refinements in this direction consisted of the division of the heat energy into *thermic energy* (the proportion of the total energy transformed into *heat* in the process of digestion) and *dynamic energy* (the proportion of the total energy which could be transformed into fat, growth, milk or work). Heat appearing in the body due to the stimulating effect of the products of digestion on the rate of chemical change in the body complicated matters,

especially when protein (or even digested protein) was fed, and this increase of heat production was termed the *specific dynamic action* of the food given. Both Kellner and Armsby, working by entirely different methods, found that only a certain proportion of the energy of any feeding-stuff can be used by the animal for productive purposes, since some is always transformed into heat during the processes of digestion and absorption.

189. Nutritive Ratio and Protein Equivalent

The *nutritive ratio* of a foodstuff is an indication of its relative richness in protein, and is calculated by dividing the sum of the starch equivalents of the digestible fat, carbohydrates and fibre by the amount of digestible protein. Another useful method of expression is the *protein equivalent*, or the number of lb. of digestible protein in 100 lb. of the foodstuff. Various forms of production (work, milk, fattening) require definite nutritive ratios for economic results. Thus the ratio for pig-fattening is 7 : 1, for the maintenance of dairy cows about 9 : 1 (6·5 lb. of starch equivalent containing 0·7 lb. of digestible protein) ; for milk-production, per gallon, 2·8 : 1 (2¼ lb. of starch equivalent containing 0·6 lb. of digestible protein).

(For a more detailed discussion of the chemistry of feeding, the reader should consult Wood's " Animal Nutrition "; Kellner, " The Scientific Feeding of Farm Animals "; Armsby, " The Nutrition of Farm Animals.")

190. The Energy Value of Milk

The high water-content of milk necessarily gives it a low energy value per lb. Lusk gives the value as 68·3 calories (C) per 100 grams, or 310 calories per lb. (0·31 therms). The fat accounts for roughly 50 per cent., the proteins for 21 per cent. and the lactose for 29 per cent. of the total calorific value. A milk of average composition—3·7 per cent. fat, 4·9 per cent. lactose and 3·1 per cent. protein, yields a gross energy of 72·48 per 100 grams, but since only 90·7 per cent. of the total heat energy is absorbed, the nett energy is 65·7 calories. A higher fat-content than the above will increase the value considerably. The concentration of half the energy value of milk into less than 4 per cent. of its volume is significant.

The starch equivalent of milk of average composition is 16 ; the protein equivalent is 3 and the nutritive ratio 5·3.

The energy value of milk is naturally much influenced by the fat-content. Haecker [17] arranged the results of analyses of 543 samples of milk according to fat-content, and found the total

energy per pound to rise from 253 calories for 2·5 per cent. fat to 492 calories for 7·0 per cent. fat. Black and Voris [18] arranged the analyses of 134 samples of milk from individual cows into 10 classes according to fat-content, and found the energy-content per 100 g. to rise from 58·6 calories for milk of 2·7 per cent. fat to 82·5 calories for milk of 4·5 per cent. fat. The calculated mean energy value was 68·0 calories. The energy value could be correlated with the amounts of total solids, solids-not-fat, protein, calcium, magnesium and phosphorus in the milk. The proposal of Kahlenberg and Voris [19] to use the fat-content of milk as a basis for estimating its approximate average composition appears to be sound for the estimation of the energy value.

Owing to the higher content of total solids, colostrum shows high energy values when sampled immediately *post partum*, and decreases in calorie value as the solids-content approaches that of normal milk. Langstein, Rott and Edelstein [20] found that the energy values of human colostrum of good quality were 148, 118, 81, 73, 70, 68 calories per 100 ml. for the six successive days after childbirth.

The following table (CXXVI) gives the energy value of various milk products :—

Table CXXVI. *Energy Value of Milk Products (Calories per pound)*

Product	Energy Value	Product	Energy Value
Whole milk . .	310	Butter . . .	3410
Skim milk . .	165	Cheese (Cheddar) .	1950
Cream (20% fat) .	900	Cheese (Swiss) .	1950
Butter-milk . .	160	Cheese (soft) . .	540
Condensed milk		Whey . . .	115
(sweetened) . .	1480	Kephir (2 % fat) .	220
Evaporated milk .	645	Koumiss (2% fat) .	200
Skim-milk powder .	1640		
Whole-milk powder .	2300		

Adult human beings require the equivalent of from 25 to 45 calories (C) per kilogram of body-weight per day of energy expenditure ; the total amount required naturally varies with the size and activity of the individual. An average man of 60 kg. body-weight expends 2,400 calories, which are present in 7 pints of milk. Obviously milk is unsuitable as the sole source of food for adults from this standpoint alone, but, on the other hand, its value as one of the fundamental constituents of a well-chosen diet

cannot be over-emphasised. Table CXXVI also brings out the high calorific value of concentrated dairy products, which may be used to supplement or form part of a mixed diet.

In the economy of milk-production in the cow, the food energy required to produce 1 lb. of milk (4 per cent. fat) is 336 calories ; this will give a gain of fat in a fattening bullock of an equivalent of 252 calories. This has been used as a basis for computing rations for milk-production ; in such calculations the fat-content of the milk and the lowering of the digestibility of the rations by about 5 per cent. in well-fed cows have to be taken into account.

191. Nutritive Value of the other Components of Milk

(a) LACTOSE. The hydrolysis of lactose gives glucose and galactose ; the presence of a lactase is necessary for enzymatic hydrolysis to the hexoses. This class of enzyme does not occur in baker's or brewer's yeast, but is present in the mixture of enzymes which cause alcoholic fermentation of milk, e.g., in the preparation of koumiss and kephir. Milk itself contains a trace of lactase. The enzyme is, however, present in the intestine of the infant (Plimmer [21]) at birth and in some species of animals before birth in considerable amounts ; the lactose is thus rapidly converted into simpler sugars and absorbed. The economy of utilisation of the high lactose-content of human and equine milks is at once evident.

In adults, lactose passes the ileo-cæcal valve in a form substantially unchanged and forms a favourable medium in the large intestine for the growth of non-putrefactive bacteria e.g., Bacillus acidophilus. Lactose-therapy in the direction of maintaining this type of organism in the large intestine has been the subject of considerable interest and investigation.

Lactose also behaves in a peculiar way in that it increases in some obscure manner the adsorption of calcium and phosphorus from the intestines, and their retention in the body; under certain conditions the effect of lactose in mobilising the calcifying elements in this manner may be as great as the bone-calcifying vitamin D. When sufficient amounts of lactose are added to rachitogenic diets, definite antirachitic effects are exhibited in the chicken [48] and the rat.[49] It is probable that this effect is due to increased acidity in the intestinal tract, and better absorption of Ca and P due to fermentation of the unabsorbed sugar. Adults generally utilise lactose poorly ; from 40 to 50 per cent. of ingested lactose may be lost to the rat as a source of energy and growth. For instance, part of the rarer incidence and severity of rickets in infants reared on human milk, as against those

reared on cow's milk, may be due to the higher lactose-content of human milk, although cow's milk is richer in calcium and phosphorus.

Lactose accounts for about 30 per cent. of the energy-value of milk and is in a readily assimilable form. Lactose is slightly laxative.

It has been found that the inclusion of large amounts of lactose in the experimental diets of rats causes the formation of cataract, the onset of symptoms being roughly parallel to the level of dietary lactose.[50] Galactose produces the same effect. When fed lactose, rats are hyperglæmic and the mechanism of cataract formation may be the same as with diabetic cataract. When lactose is fed in milk, to which it contributes about 40 per cent. of the nutritive value, no such developments occur. Rats have been kept for at least five generations on a diet consisting of milk supplemented with Fe, Cu and Mn without showing any lens degeneration.[51]

Animals receiving lactose have been shown to contain less fat than those on a similar amount of sucrose, and to live longer.[53] Rats fed on a diet containing 20 per cent. lactose showed a higher content of cerebrosides in their brains than rats on a sucrose diet.[54]

(b) MILK-FAT. The unique composition of milk-fat has already been described (Section 26). The gradation of the molecular weights of the fatty acids present in the fat as esters is physiologically important because each acid formed in the progressive break-down of the highest member—stearic acid—is present in significant amount. The lower and water-soluble fatty acids occur in considerable quantity. When saponified in the small intestine of the infant, therefore, the organism has the advantage of having to deal with small molecules in part, the larger molecules being in part excreted in the faeces as calcium soaps, thus tending to bring the Ca : P ratio to an economic level. The composition of the fat of the milk of bovine species and of ruminants in general is very similar, but it varies considerably with the species of other mammals. Although this may be associated with such factors as the stage of development of the embryo at birth, the amounts of other milk constituents and the nature of the diet of the species, no strict nutritional significance can be ascribed to these differences.

The fat is present in milk as minute fat globules, the size and distribution of which vary in cow's milk with breed, stage of lactation, and amount ; the size of the globule is generally also a species characteristic. The precipitation of casein in the young stomach through the agency of rennin also brings down the fat.

There is thus no tendency of the fat to coalesce into drops of macro-size through churning or partial peptisation of the casein; on the other hand, the fat is led into the small intestine and made available to tryptic digestion in its original form, and there is thus no danger of large drops of fat causing intestinal troubles, such as diarrhœa. Indeed, owing to its finely-divided form some fat may be digested in the stomach.

Milk-fat contains lecithin and some cholesterol. Of greatest importance is the fact that the fat-soluble vitamins are carried by it; these will be dealt with later.

On the average, about 50 per cent. of the energy-value of milk is vested in the fat. When it is realised that fat is a separate phase, exerts no osmotic pressure, and accounts only for 3·7 per cent. of milk, the economy of nature in its secretion is unique.

(c) PROTEINS OF MILK. The description and composition of the various proteins of milk have already been discussed (Section 45, Table XLIV). The most important is the specific milk protein, the phospho-protein, *casein*. Its properties and composition are very similar to the phosphoprotein of the hen's egg, vitellin. Since both these proteins are associated with the development of either the embryo or the newly-born, their nutritive properties must be unique and have much in common. They carry phosphorus in organic combination, and consist of very large molecules of a size representing large multiples of the general proteins—albumin and globulin—in their native state. Casein is insoluble at its isoelectric point and can be precipitated by rennet, which is the chief enzyme of the stomach of the newly-born animal. The physiological significance of clotting with rennin is (a) to give volume and activity to the stomach so as to encourage muscular action and secretion, and (b) to entrap the fat and dole it out to the animal in small quantities in its original state of division. The encouragement of gastric secretion is undoubtedly part of the mechanism set in action for making the hæmoglobin required by the rapidly increasing blood volume of the young animal (the *hæmopoietic* factor of gastric juice, the absence of which is partly responsible for pernicious anæmia).

Casein accounts for 2·5–3·0 per cent. of milk. The next important protein, lactalbumin, is present to the extent of about 0·35–0·40 per cent. (Casein plus lactalbumin account for 93·5 per cent. of the protein of normal milk.) Human milk contains only 0·5–1·5 per cent. of casein and about 0·3 per cent. of lactalbumin (a fifth of the casein). Thus human milk contains a much greater proportion of lactalbumin.

Both proteins are biologically complete, *i.e.*, when fed singly

in conjunction with an otherwise adequate diet, each gives satis-factory growth and reproduction in test animals. Casein possesses one disadvantage, in that its content of the sulphur-containing amino acid, cystine, is low compared with the amounts of the other essential amino acids. Lactalbumin and lacto-globulin contain sufficient amounts of this amino acid to make up for the unsatisfactory amount in casein. Casein, however, contains another sulphur-containing amino acid, methionine (2 per cent.). Lysine occurs in both proteins in considerable quantities. His-tidine and tryptophane are also present in sufficient quantities. Tryptophane is usually prepared by the tryptic digestion of casein. Where abnormally large quantities of non-protein nitrogen occur in milk, as in some cases of low solids-not-fat, this amino acid is also present to a greater degree in an uncombined state. Lactoglobulin, a protein identical with serum globulin, is present in milk in about half the quantity of the albumin. The amino-acid distribution of this protein is almost identical with that of casein, and one might be led to believe from a superficial examination of the amino-acid content that casein is elaborated from globulin. There is a greater proportion of lactoglobulin in human than in cow's milk. Bauer and Engel [22] found that albumin and globulin are more closely related serologically than is either with casein, but globulin is more closely related to casein than is albumin.

The significance of the proteins of colostrum, notably the globulin, has been discussed (Section 185). The colostral period may be regarded as the transition stage for the infant from that period when it was nourished directly by the blood of the mother to the subsequent period when it must depend on its own alimen-tary tract for its nutrients. The proteins of blood and whey are almost identical, the only difference being the method of adminis-tration. During the period of feeding with colostrum, the infant can absorb some of the proteins practically unchanged, whereas after this period is over the proteins must be broken down and the products absorbed. The transference of immunity factors points in this direction.

The " biological value " of milk protein is high. Protein is used for maintenance and growth, and it is difficult to separate these two requirements in feeding experiments owing to the qualitative differences between the amino-acid requirements in each case. The biological value may be deduced either from the nitrogen-balance,[30] from the growth obtained per unit weight consumed,[31] or by the more comprehensive method of McCollum, Simmonds and Parsons,[32] all of which methods include observa-

tions on growth, the maternal functions, the appearance of the characteristics of senility and the well-being of succeeding genera- tions. Fairbanks and Mitchell [54] report a true digestibility of 95 and a biological value of 90 for raw milk proteins when fed at a nitrogen level of 8 per cent. of the diet. (The reader is referred to Mitchell and Hamilton, " The Biochemistry of the Amino Acids," Reinhold Publ. Corpn., New York, 1929, Chap. 10, for a full discussion of the subject.)

Drummond [33] found casein slightly inferior to the proteins of fish and of beef-muscle, when fed at a 10 per cent. level. Osborne, Mendel and Ferry [31] demonstrated the superiority of lactalbumin over casein in promoting growth, although Sure [34] believed the former to be an incomplete protein, probably due to the intake of the protein being insufficient for successful nutrition. Edestin has been found superior to casein for maintenance requirements, but Osborne and Mendel [35] found that casein was superior for growth. In nitrogen-balance studies, where the retention of protein nitrogen (as percentage of nitrogen fed) under strictly controlled conditions was used as the measure of biological value, Mitchell and Carman [36] found the proteins of milk to have a value of 83 as against 93 for the proteins of the hen's egg, 83 for those of egg white, 74 for those of pork muscle, and 67 for those of wheat.

Milk proteins act as valuable supplementary proteins to cereal proteins both for the human species and animals (notably pigs). Hart and Steenbock [37] found the biological value of the mixed protein of a maize and milk ration for pigs (maize 3, milk 1) to be 80, the same mixture for the rat being 76. Later, these workers examined the effect of different mixtures of maize and milk on the retention of nitrogen in the pig with the view of arriving at the optimum supplementary relation between the two classes of protein. As the proportion of milk was increased from 21 to 29 per cent., a marked rise in the retention of nitrogen was noticed, this being due largely to the marked improvement in the digestibility of the nitrogen in the ration. If maize had been fed alone, the biological value of the protein would have been about 60 per cent. Assuming the efficiency of 100 per cent. utilisation of milk protein, a ration containing 30 per cent. of milk protein would have the value of 72. Actually the value was 87, the difference being due to the supplementary relation between the proteins of the two foods. Oat proteins are similarly supplemented by milk proteins. Further work of the same nature on rats by Mitchell [38] has clearly demonstrated that the deficiencies of amino acids in maize proteins are supplemented by the excess of amino acids in milk

proteins. When milk and white flour were mixed so that a third of the total protein was milk protein, the gain in weight of rats fed on the diet showed three times the gain in weight per gram of protein consumed.[39]

This supplemental value is of the greatest importance in human nutrition. Milled cereals account for about two-fifths of the average human dietary ; the importance of milk (and animal) proteins appears to reside as much in the extent to which they improve the utilisation of inferior cereal proteins as in their own nutritive excellence.

Hoobler,[40] and Hart and Humphrey [41] have shown that milk proteins are superior for the production of human and cow's milk, respectively.

(d) THE MINERAL CONSTITUENTS OF MILK. The inorganic constituents of milk supply an essential part of the diet for building up the skeleton, for the development of soft tissues of the body, for co-enzyme action generally, and for the maintenance of the osmotic pressure and neutrality of the body fluids. Milk is the most satisfactory source of these elements. The rate of growth during the early period of infancy is rapid, and milk is the only source of the inorganic constituents ; the demand for a complete, well-balanced and available source of the necessary inorganic substances is therefore great.

Milk is the best source of calcium ; with the exception of green vegetable material (notably leguminous plants) all other foods are relatively poor sources of calcium. The retention of calcium by the animal body is also greater when milk is fed than when an equal amount of calcium is given as green material. Babies fed on cow's milk absorb and retain more calcium and phosphorus than those reared on human milk. With growing children also, Sherman and Hawley [23] have shown that the optimum storage of calcium occurs when the daily diet of the child contains at least two pints of milk. Calcium is also usually low in the diets of adults when cheap carbohydrates of cereal origin form their chief ingredient ; the supplementing of such diets with milk has constantly been advocated. The phosphorus-balance in all cases closely follows the calcium-balance. The close association of the vitamin D of milk with the calcium and phosphorus is mostly responsible for this high efficiency of mineral metabolism and will be discussed later.

Nutritional Anæmia. It is agreed that it is only during the early stages of life that milk is a complete food. Milk as the sole source of nutrients after the suckling period causes the onset of varying degrees of nutritional anæmia. This condition arises

from the deficiency of milk in both iron and copper, although the evidence for this statement is based on experiments with animals which usually reach the adult stage quickly or grow very rapidly, so that as yet its clinical application to the human species cannot be put forward.

The iron-content of cow's milk is o·0002 per cent. and the copper-content, o·00005 per cent. Both metals are held to be necessary for maintaining the hæmoglobin-content of the blood at its normal level.[24]

In the case of the suckling, Bunge [25] believed that the liver was a store of iron which was drawn upon for development and that the iron in the milk was of no account ; but Soxhlet [26] showed that the amount in human milk (2·8 p.p.m.) more than sufficed to provide for the needs of the developing child, and thus there was no necessity to call upon the store in the liver. The iron-content of cow's colostrum is higher (4 p.p.m.) than in milk, so that these conditions, together with the high red-cell count of embryonic blood, tend to tide over for a time the possibility of the development of an anæmic condition in the newly-born offspring. Bunge showed that the absolute quantity of iron in the animal body remained constant during the suckling period, so that with increasing body-weight iron was relatively highest at birth and reached its minimum at the end of the suckling period. When the body-weight of the infant reaches about three times that at birth (usually in eleven to fourteen months) in cases of natural feeding, or a little earlier where modified cow's milk has been used, the diet should be selected with a view to increasing the iron-content while still maintaining the necessary calcium and phosphorus.

The presence of iron and copper is now generally recognised as being necessary to ward off anæmia or to restore the normal blood picture. Krauss [27] and Orten, Underhill and Lewis [28] have lately confirmed this, but Mitchell [29] has found it possible to cure anæmia in rats on a milk diet by the addition of iron alone, whilst the addition of copper causes a more rapid recovery and eventually a higher level of hæmoglobin. It is believed that copper-free lactose can replace copper in the cure of anæmia,[42] since the disease can be produced by an exclusive diet of cow's milk but not by one of human milk, probably owing to its richness in lactose.

The food of the producing animal evidently plays an important part ; the milk from properly fed animals is claimed to be a complete food,[43] and when fed exclusively brings about normal growth and a normal blood picture in the albino rat ; winter-feed milk, however, is valueless as regards its anti-anæmic properties,

and heavy milkers, even when properly fed, give inferior milk. In contradiction to this, Krauss [44] has observed that the development of anæmia is unaffected by season or by change from dry food to fresh grass and that the above findings could not be confirmed.

Manganese. Rats maintained on an exclusive milk-iron-copper diet can be maintained in an apparently normal condition for a very long time ; there is reason to believe, however, that iron and copper are not the only metals in which milk is deficient. The addition of traces of *manganese* to a milk-copper-iron diet enhances growth chiefly because it stimulates appetite.[45] The addition of energy to the diet in the form of manganese-free sucrose also markedly improves growth and, in female rats, causes the resumption of an almost normal ovulatory rhythm which is upset on the milk-iron-copper diet.[46] The deficiency in milk and the rôle of manganese in nutrition have been demonstrated by Daniels and Everson.[55] Exceedingly rapid growths in rats and pigs have been obtained on an exclusive diet of milk supplemented with iron, copper and manganese, and in the case of pigs, cod-liver oil. The variation in the manganese-content of milk is geographical.

Iodine. The iodine-content of milk depends on the stage of lactation, season, the locality in which the milk is produced, and the treatment of the milk. The presence of varying amounts of iodine in artificial food renders the mapping of geographical areas useless. Summer milk is higher in iodine-content (145 γ per litre) than winter milk (about 65 γ per litre). Feeding cattle on foodstuffs of marine origin, or in districts near the seaboard, gives milk of high iodine-content, and feeding of small quantities of potassium iodide gives highly iodised milk. Vigorous boiling of milk may reduce the iodine-content by as much as 20 per cent. · It is easier to add iodine (as iodide) directly to milk than to add it to the food of the cow when a deficiency is suspected or when iodised milk is required.

Processed Milk. The contents of copper and iron, and those of the heavy metals generally, increase with every step in the processing of milk. The manufacture of milk powder by the roller process and of condensed milk in copper vacuum-pans adds considerably to the amounts of iron and copper, respectively, in the products. Pasteurisation, boiling, or evaporation may cause a slight precipitation of calcium phosphate, but the amount lost in this manner is very small ; since cow's milk is at least four times richer in calcium than is human milk, the loss to the infant is inappreciable. Willard and Blunt [47] found that evaporated

milk was quite as good a source of the inorganic elements essential for human nutrition as pasteurised milk.

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Osborne and Mendel [3] simultaneously observed that the decline could be turned to active and healthy growth by the addition of butter-fat. With more careful refining of the synthetic diet, the latter workers [4] found later that the absence of butter-fat from the food not only caused a rapid loss of weight but gave rise to a pathological condition of the eye, in which invasion by bacteria induced a lesion which has been named xerophthalmia. The factor in butter-fat which prevented this condition was termed vitamin A. Later investigations have shown that the eye trouble is a late manifestation of a condition which is far more serious and advanced in the epithelial tissues of the body. Xerophthalmia has been observed in children [5] who have not had access to milk, eggs, butter, green vegetables or cod-liver oil. In the early part of the World War all Danish butter was exported and the families subsisted on margarine ; during this time many cases of this disease were reported. After exportation of butter was reduced in 1917–19, the eye trouble disappeared.

The precursor of vitamin A is *β-carotene*. The relation between carotene and the vitamin has been investigated by Moore,[6] Capper,[7] Euler and Karrer,[8] Kuhn and Lederer [9] and Drummond, Ahmad and Morton,[10] who found that the hydrocarbon ($C_{40}H_{56}$) was the precursor of the vitamin and that the conversion occurred in the liver. The conversion of carotene into the vitamin was found not to be quantitative ; the liver was found to control the concentration of the vitamin throughout the rest of the body and the amount entering into milk.

Later work by Kuhn *et al.* has shown that α- and γ-carotene possess only half the biological activity of the β-form.

The vitamin is destroyed by prolonged exposure to air and by heating in air or oxygen (autoxidation). When the fat in which it is contained autoxidises, the vitamin is destroyed. In common with other fat-soluble vitamins it is separated completely from milk or cream by churning out the fat.[11] No appreciable loss of the vitamin occurs in the usual processes (pasteurisation, condensing and drying) to which milk is subjected.

The physical estimation of carotene in butter presents some difficulty as it is uncertain whether the pigment obeys Beer's law strictly. If, for more exact work, the pigment is determined in the unsaponifiable residue, carotene is less stable than the vitamin A also present. There is also the difficulty of determining the distribution of the various forms of carotene in butter-fat, which can only be done by a chromatographic examination, a method which can easily lead to fallacious results.

VITAMIN D. This vitamin is the *antirachitic factor*, or the

factor which is essential for the laying down of calcium salts (phosphate and carbonate) in the joints of growing young, thereby preventing or healing *rickets*. In general, milk very seldom contains sufficient vitamin D to prevent rickets in babies. Some assays of human milk have revealed its total absence ; [12] cow's milk contains small amounts and the general practice of diluting cow's milk with water naturally lessens the amount. It is, however, practicable to increase the vitamin D of cow's milk and human milk by feeding cod-liver oil or irradiated yeast or other dietary means, and by ultra-violet irradiation. The amount in cow's milk also depends on the degree of insolation of the producing animal, summer milk being much more potent in the factor than winter milk. Irradiation of the cow or of the milk with ultra-violet light increases greatly the vitamin-content. Steenbock *et al.*,[13] however, did not find irradiation of the cow to increase the amount of the factor in milk.

Vitamin D is fairly stable towards heat, and milk can be pasteurised or boiled without injuring it. Thus, commercial evaporated milk is as potent as the corresponding raw milk.

Average human milk contains very little vitamin D, yet breast-fed children develop rickets less frequently and with less severity than those on other types of milk. This probably depends on the high lactose-content and the favourable distribution of mineral salts in human milk. From the point of view of infant-feeding, no milk, either human or cow's, can supply sufficient vitamin D to the rapidly growing infant to allow for perfect bone-development. Intake of cod-liver oil, egg-yolk, or exposure to direct sunlight should be begun as early in life as possible. The isolation of vitamin D in pure form, or in a concentrated form, from irradiated yeast and its addition to milk, provide the bases for methods of quantitatively making good the deficiencies of milk in this vitamin.

Vitamin D is fat-soluble and is retained completely in butter, when churned from milk or cream. Skim milk is therefore devoid of it.

VITAMIN E. This, the third fat-soluble vitamin, is completely removed from milk in the cream or in butter and so is absent from skim milk.[14] The investigation of this factor is in the experimental stage only ; it is necessary for reproduction in the albino rat, but its value for the human species has not yet been proved. The amount in cow's milk is very small even when the ration contains ample proportions of green material like lucerne. No tests for it have been made in human milk. It is stable to heat and oxidation.

VITAMIN B. This vitamin has been subdivided into (*a*) B_1, the

heat-labile, antineuritic (antiberi-beri) of Eijkman ;[15] (b) B$_2$, the heat-stable, pellagra-preventive (Goldberger, 1926),[16] which prevents dermatitis in rats, (vitamin G in America) ; B$_3$ (Williams and Waterman, 1927-8),[17] a heat-labile factor necessary for the full normal nutrition of the pigeon (found in yeast and whole wheat) ; B$_4$, the heat- and alkali-labile factor of Reader [18] (in yeast) ; B$_5$, the factor of Carter, Kinnersley and Peters,[19] necessary for weight-maintenance in pigeons. The whole group forms the *vitamin B complex ;* only fractions B$_1$ and B$_2$ are of importance in milk. This vitamin is not plentiful in milk ; cow's milk is about one and a half times richer in it than human milk, and weight for weight, has about a fiftieth of the potency of dried brewer's yeast. It is water-soluble and is therefore present in skim milk.

The presence of this vitamin in milk is determined by its occurrence in the maternal diet, and since it is not stored in the body its absence in the food is quickly reflected in the quality of the milk. The vitamin B of human milk varies inversely with the quantity of milk secreted ; some other source of it must frequently be supplied to infants.

VITAMIN C. This is the water-soluble, *antiscorbutic factor*, lately discovered to be *ascorbic acid*. The fresh milk of many species has been found to be rather a poor source of this vitamin. The amount in summer milk may be greater than that in winter milk, but the fluctuation is never great. The amount of ascorbic acid as determined by titration of the protein-free serum with 2 : 6 dichlorophenol indophenol amounts to 2·2 2·5 mg. per 100 ml. in normal milk. The latest work shows that the level of ascorbic acid in milk is independent of the season or the ration.[95]

The vitamin is quickly destroyed by heat, oxidation, direct sunlight, and by ultra-violet irradiation, and processed milk is deficient in it. Pasteurisation by the holder method destroys it almost completely, and the amount in milk powders depends on the method of manufacture, spray-dried milk powder containing less than the product from the roller process.

A study of the effect of light on the ascorbic-acid content of milk shows that at first the oxidation is largely reversible (formation of dehydroascorbic acid), but longer exposures bring about an irreversible oxidation.[96] Light starts a chain reaction which goes on in the dark. The reduction of the reversibly oxidised form is necessary before determining the total ascorbic acid ; this can be done by bubbling with hydrogen sulphide.

Adults as well as children should obtain vitamin C from other

sources, such as fresh fruit and vegetables, which are rich in the vitamin but usually comparatively cheap in price.

194. The Vitamins in Detail.   Conditions Influencing the Vitamin-content of Milk

For a detailed description of the vitamins of milk, the Special Report of the Medical Research Council,[20] the Report of the White House Conference on Child Health and Protection,[21] and a review of the latest work (Kon [22]) should be consulted.

A. INFLUENCE OF DIET ON VITAMIN-CONTENT. It may be stated generally that changes in the vitamin-content of the diet of the producing animal influence directly the concentration of

TABLE CXXVII.   *Vitamin Potency of Butter* [90]

	International units per gm	
	Vitamin A	Vitamin D
Kon and Booth [89] (1934) . . .	40-85	—
Danish workers (1934) . . .	5-14	—
Coward and Morgan [91] (1935) . .	26-200	0·4-4·0
Treichler, Grimes and Fraps [92] (1935) .	1 70	—
Bechtel and Hoppert [88] (1936) . .	—	1·0
Campion, Henry and Kon [93] (1936) .	—	0·15-0·88
Kon [94] (1936)	—	0·7 (summer)
Morgan and Pritchard [90] (1937) . .	5-38·6	0·06-0·99

vitamins in the milk rather than the amount of milk secreted. Inadequacy of vitamins in the diet manifests itself in the vitamin-quality of the milk probably more rapidly than inadequacy of any other dietary factor.   Mammals appear to be little able to store the vitamins for any long period, and the provision of vitamins in her daily milk-supply exhausts the body stores of the lactating female with comparative rapidity. The variable results obtained in vitamin assays of cow's milk are undoubtedly due to the variable vitamin-content of the cow's diet. Thus Hopkins obtained good growth in rats on a synthetic diet by adding only 2 ml. of milk daily, whilst Osborne and Mendel working with a similar diet were unable to obtain successful growth when even 16 ml. of milk were given.   Kennedy and Dutcher,[28] pursuing this theme, investigated the milk from cows (*a*) on a

typical winter ration known to be deficient in vitamins A and B, and (b) on a ration generously supplemented with rich sources of these vitamins. Rats receiving their vitamin A from the butter of ration (a) failed to grow normally when the butter consisted of 20 per cent. of their diet, but a 5 per cent. level of butter from milk on ration (b) was sufficient to produce satisfactory growth. Fifteen ml. of milk of ration (a) was needed to maintain stationary weights, whereas the rats on milk from ration (b) grew satis- factorily on 10 ml.

Luce [24] has demonstrated also that the growth-promoting value of milk depends principally on the cow's diet. Depleting the source of vitamin A in the diet of the cow for three months caused the growth value of the milk to fall to a very low level, which persisted for another five months when the cow was on free range in summer. Golding, Soames and Zilva [11] showed that the addition of kale to the diet of the cow increased the vitamin A content of the milk, and the addition of cod-liver oil increased the contents of both vitamins A and D.

McCollum and Simmonds [25] investigated the vitamin-content of rat's milk by finding the ability of the mother to rear its young after being fed on (a) a diet deficient in both vitamins A and B, (b) a diet deficient in vitamin B, but the vitamin A was derived from a butter source, and (c) a diet deficient in vitamin A but containing plenty of vitamin B as an alcoholic extract of wheat germ. All three diets were failures, and it was only when diets (b) and (c) were combined, that is, when sufficient of both vitamins were given, that the mother was able to rear the litter successfully. These vitamins therefore cannot be synthesised in the animal body and have to be supplied in the diet of the mother in order that they may appear in the milk.

With the ruminant, however, the case is different and it is now generally accepted that the vitamin B-complex may be formed in the rumen of the cow by bacterial synthesis. It is not surprising therefore that the concentration of the vitamin B-complex in cow's milk remains constant throughout the year. Neither breed, season, nor feeding has any effect on the B_1 content of milk,[26] whilst feeding of 300-1,500 grm. daily of yeast rich in vitamin B_1 does not raise the level of the factor in milk.[27, 28] A slight increase in the B_2 factor has been observed when cows have access to pasture,[29] and an insignificant rise when they are fed on yeast.[30] Milk from a high-protein ration is slightly richer in the factor than that from a low-protein diet. Investigations, covering two years, by Kon,[31] have also shown that the food of the cow has no noticeable effect on the amount of the complex in milk.

Milk is an inferior source of the antiscorbutic factor and the amount it contains depends not only on the food of the cow but on the later treatment of the milk. There is close agreement in the findings of various workers that the vitamin C-content of the food affects that of the milk. Hart, Steenbock and Ellis [32] found that 50 ml. of the milk of cows on summer pasture afforded complete protection to guinea-pigs against scurvy, whilst 100 ml. of milk from dry feed and 75 ml. of milk from cows on dry feed plus silage were required to supply the same amount of protection. Dutcher and his co-workers [33] found that 20 ml. of summer milk was superior in nutritive value and in antiscorbutic capacity to 60 ml. of winter milk. Hess, Unger and Supplee [34] fed guinea-pigs with the equivalent of 80 ml. of fluid milk in addition to oats and bran, and found that those fed on milk from dry-fodder cows developed scurvy within twenty-one days and died in fifty-six days, whereas those animals fed on milk from pasture-fed cows lived at least 120 days.

Certain experiments, however, have shown that no greater protection against scurvy in guinea-pigs was given by milk from cows fed on rations adequately supplied with vitamin C than when milk from cows rationed on dry foods was given.[35] The work of Kieferle and Eisenrich,[36] who found that winter milk contained more vitamin C than summer milk produced on the same farm, must also be mentioned.

The influence of the food on the vitamin D content of milk has been examined both in lactation studies on rat's and on cow's milk, using rats for assay purposes. Korenchevsky [37] found that if the mother (rat) received a diet containing cod-liver oil during pregnancy and lactation, with an adequate amount of calcium and phosphorus, the offspring were rendered resistant to rickets; this was attributed to the fact that the antirachitic factor passed from the diet to the milk of the female, was stored in the tissues of the suckling young and served to protect them when they were later placed upon a rachitogenic diet. Hess [38] could not find it possible, however, to render rats completely refractory to rickets by supplementing the diet of the mother; on the other hand, the resistance to rickets may be broken down by an inadequacy of the antirachitic vitamin in the diet of the mother. The fact that susceptibility to rickets can be induced by inadequacy of the vitamin in the food of the mother does not mean that the young can be rendered resistant by fortifying nutrition during the ante-natal period.

McCollum and his co-workers [39] obtained results similar to those of Hess, except that they found that vitamin D did enter

the milk of the rat when cod-liver oil was fed. They found that the feeding of cod-liver oil to female rats before mating, during pregnancy, and for the first two weeks of lactation, caused the sucklings to be protected to a certain degree from rickets when fed on a rachitogenic diet. The sucklings of females fed on a good-quality diet but low in vitamin D showed no such protection against rickets when so tested.

With respect to conditions of environment and food of the cow on the antirachitic potency of the milk, Boas and Chick [40] found that milk from (a) cows fed for six months on dry fodder in a dark stall, and (b) cows fed for two months on green fodder in a dark stall, when fed to rats, caused defective calcium-retention when compared with cod-liver oil, whereas the milk from cows after two months on summer pasture when fed in a similar manner showed no difference from the ration containing cod-liver oil. Luce [24] elaborated the last-quoted work and kept a cow out of doors on a diet deficient in vitamin D ; for the second period dry fodder was given in the dark ; in the third period green fodder was given in the dark, and in the fourth the cow was placed on pasture. At the beginning, when the cow had been out on pasture, doses of 2 ml. of the milk showed antirachitic properties and a fall in this factor was not noticed until the cow had been fed for four and a half months on a diet deficient in vitamin D (in which time also the vitamin-content had reached its minimum). There was a slight improvement in the antirachitic value when green fodder was fed in the dark, and the original value was gained on letting the cow out to grass. When dry food had been substituted for green grass for two months, the milk still contained anti-rachitic properties to a considerable degree.

The vitamin D-content of milk thus depends on the diet of the cow and possibly on the amount of sunlight to which the animal is exposed ; the pasture-fed cow gives milk of a definite and high antirachitic potency, whereas the same cow, fed indoors in the dark, yields a milk which is much inferior in antirachitic value. The addition of foods containing vitamin D, such as green leafy foods, cod-liver oil or irradiated yeast, increases markedly the antirachitic value of the milk thus produced.

There is little evidence of any relationship between the vitamin E-content of the fodder and of the milk, although Evans [41] states that " there is definite evidence of the higher E content given by cattle with access to fresh alfalfa (lucerne) pasturage." [11] Milk at its best is a poor source of vitamin E, since sterility occurs in rats when as much as 9 per cent. of butter-fat is included in the diet.

B. THE TREATMENT OF MILK. EFFECT ON VITAMIN-CONTENT.

(*a*) *The Irradiation of Milk. Vitaminised Milk.* The vitamin D-content of milk, whether in the liquid or dry state, can be increased many times by ultra-violet irradiation ; such milk has been used prophylactically in protecting children from rickets, and claims have been advanced that it also cures rickets. Thus Cowell [42] and Daniels, Pyle and Brooks [43] showed that children could be cured from rickets by giving them daily a pint of milk which had been irradiated for twenty minutes. Irradiation in the presence of oxygen (dissolved air or oxygen) for a considerable time imparts an unpleasant odour and taste to milk (irradiated taint), which Schultz [44] has ascribed to the oxidation of proteins, but which is probably due to fat-peroxides ; the vitamin A may also be partly destroyed in the process and vitamin C completely destroyed. Various forms of apparatus are now used in which the irradiation is carried on out of contact with air or on milk saturated with carbon dioxide in an atmosphere of that gas. Such milk is free from taste and smell and is prophylactic and curative to rickets.[45]

The ergosterol-content of milk is subject to considerable variations, and it is not surprising that Coward [46] found a great variation in the antirachitic potency of various samples of milk after irradiation for periods up to thirty seconds. Such irradiation increased the antirachitic value from five to fifty times ; it was estimated that, with efficient irradiation, three pints of milk were equivalent in antirachitic value to one teaspoonful of cod-liver oil.

The attempts which have been made to alter the vitamin-content of milk by suitable feeding, or by the treatment of fluid or dried milk, have been numerous. The increase in the antirachitic value of milk has of late been most successfully attacked and the treatment is now carried out commercially (Supplee,[47] Hess [48, 49]). These investigators irradiated milk (1.2 per cent. fat) as a thin film, using carbon arcs of the flaming type for a period not exceeding sixteen seconds. It was found that to produce the maximum concentration of vitamin D, $2\frac{1}{2}$ million ergs per ml. of milk have to be supplied as radiant energy. In this manner milk was uniformly made to possess twelve times the amount of vitamin D originally present, or 50 Steenbock units per quart (1 Steenbock unit $= 2.7$ International vitamin D units). Such milk, when carried through a series of processes, such as pasteurisation, boiling or drying, was still clinically active.

The biochemical changes occurring in milk after irradiation, and in butter made from the milk, have been investigated by Anderson and Triebold,[50] who found that very slight changes occurred after eight times the normal period of irradiation ; and

so they concluded that no change would be noticeable after fifteen seconds' irradiation. A slight destruction of vitamin C was observed by Supplee and Dow [51] after a few seconds' exposure; no change was observed in the vitamin C content of dried milk. The vitamin A content of milk was not affected by irradiation up to forty-eight seconds (6.78×10^6 ergs per ml. of milk).[52]

The feeding of irradiated yeast to cows as a means of increasing the vitamin D content of the milk has been used extensively and the technique of the method has been worked out in detail by Hess,[27, 53] Thomas and MacLeod,[54] and Frey.[55] It
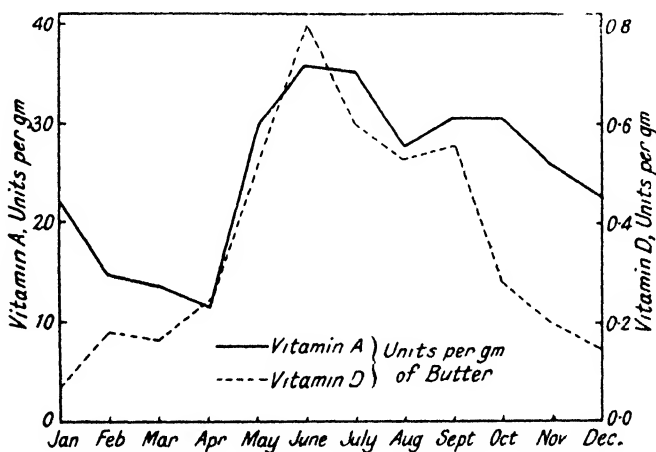
FIG. 25.—Monthly variations in the vitamins A- and D-contents of butter. (Morgan and Pritchard.[90])

has been found that about 60,000 Steenbock units of vitamin D have to be fed daily to the cow in order to give milk of the required clinical potency, namely, 150–160 Steenbock units per quart.[53] The amount of yeast required varies according to the milk yield; heavy milkers are more efficient in transferring the vitamin from the food to the milk than are poor milkers. Russell has found that it requires three weeks from the commencement of yeast-feeding for the milk to attain maximum potency, after which the concentration is maintained. About 2·3 per cent. of the vitamin of the yeast enters the milk and 30 per cent. is excreted in the fæces.[27] About 70 per cent. of the vitamin D fed can be demonstrated in the blood in the first and second hours after feeding, but the concentration in the blood soon drops rapidly, the vitamin probably undergoing destruction in the blood stream. Commercially, moderate amounts of irradiated yeast are fed and

these have no effect on the chemical composition of milk, but when fed in large quantities, such as ten times the usual dosage, a slight rise in the Ca : P ratio and in the ash-content has been observed.[27]

In feeding to the cow still more concentrated forms of vitamin D, such as irradiated ergosterol, Hess and his co-workers [53] and Thomas and MacLeod [54] found that it was necessary to feed three times as many Steenbock units as ergosterol than as yeast (both irradiated) in order to produce milk having equivalent potency for rats. The feeding of irradiated ergosterol is not economical at the present price, and the same applies to concentrates of cod-liver oil, prepared by Zucker's method.[56] It was necessary to feed 60,000 rat units in this form to produce an elevenfold increase in the vitamin D content of milk (from 2·8 to 30 Steenbock units per quart). The direct addition of irradiated ergosterol to milk in order to increase its antirachitic potency has been advocated, and Zucker [57] has described a method of incorporating his concentrate into milk. This method has been applied practically in the U.S.A. and a dairy has been licensed for the sale of " vitamin D-concentrate milk."

Irradiation of the udder and the underside of the cow has been found to affect the milk so that it affords a good protection and cure for rickets in infants.[58]

The feeding to cows of 2 lb. daily of cacao shell, a by-product of chocolate manufacture, has been found by Kon and Henry [103] to raise the vitamin D-content of milk from winter to summer level.

(b) *Irradiated and Vitaminised Milk for Infants.* To cure or prevent rickets from developing in infants, Hess [53] found that 1½ pints per day of milk from cows fed either 60,000 Steenbock units of vitamin D as irradiated yeast, or 200,000 units as irradiated ergosterol, were necessary. Milk obtained from feeding half these levels of vitamin D was not so effective. Clinical experience thus supports the findings on rats, viz., that three times the amount of vitamin D must be fed as irradiated ergosterol than as irradiated yeast. Wyman and Butler [59] found that milk from irradiated yeast-fed cows (160 Steenbock units per quart) was highly active in curing rickets in four cases when given at the rate of a quart per day. Five minutes' boiling did not decrease its antirachitic value. One pint per day of milk containing irradiated ergosterol (100 S. units per quart) has been found to be slowly curative for rachitic infants.[56] Irradiated pasteurised milk fed to a group of about 100 babies, many of whom were negroes, who are particularly susceptible to rickets, was found by Supplee *et al.*,[47] and

Hess and Lewis,[49] to give good results when 750–1,000 ml., *i.e.*, not more than 50 Steenbock units, were given daily. Hess and Lewis claimed that this treatment was at least as effective as " yeast milk " containing 160 Steenbock units per quart.

Hess and Lewis [60] have found that the following numbers of Steenbock units of the various sources of vitamin D are required to cure X-ray rickets : irradiated milk 35–42, yeast milk 70, cod-liver oil 250, irradiated ergosterol as viosterol 600–800 units. This confirmed their previous findings that irradiated milk has about twice the potency of yeast milk although their rat potencies are equal. The number of rat units contained in a fortified milk is no guide of its clinical value ; the source of the vitamin D addition must be prescribed.

Yeast milk has been given to lactating mothers and to expectant mothers from about the sixth month of pregnancy. To one group, one quart of milk (160 Steenbock units) was given daily and to another group, one quart of ordinary milk only. The sixteen babies of the first group were observed from two months onwards and compared with twenty-six babies whose mothers were in the second group. The incidence of rickets was found to be much smaller in the first than in the second group, although many of the control cases were given some form of antirachitic treatment.

Some misunderstandings as to the clinical effectiveness of various milks fortified with vitamin D, and other antirachitic agents of apparently the same rat unitage, have been cleared up by applying more exact experimental conditions of testing. For instance, in carefully conducted experiments on infants, Gerstenberger *et al.*[97] and Wyman *et al.*[98] found no difference in the curative action of irradiated milk and of milk produced by cows given irradiated yeast, when both milks contained the same number of rat units. The milks supplied 110 international units (I.U.) per day in one case and 110–160 in the other. The present position is that pediatricians are less ready than they were to suggest wide differences in the response of the infant to equal numbers of rat units from different sources. Hess and Lewis's [60] findings, however, appear to have been further supported by Drake *et al.*,[99] who found that 94·5 I.U. in the form of irradiated milk were as efficacious as 2,160 I.U. as irradiated ergosterol. The response, however, seems not to depend on the level of the intake of ergosterol, since 270 units were as effective as 2,160.

Ergosterol dissolved in milk is more effective than when dissolved in oil.[100] Supplee [101] suggests that lactalbumin plays a part ; increased absorption in the intestinal tract may also account

for better utilisation ; there is also the suggestion that the action of vitamin D is enhanced by the presence of vitamin A.[102]

(c) *Heat Treatment of Milk. Effect on the Vitamins.* The influence of heat on the nutritive value of milk has been discussed at length by Lane-Claypon.[60] She refers to many authorities who claim no proof of inferiority of heated or pasteurised, when compared with raw, milk. Since then, more information on the effect of heat on the vitamins has accumulated.

Of the vitamins of milk, vitamin C is the most sensitive to the combined effects of heat and oxidation. Schwartze, Murphy and Cox [61] showed that the loss of the vitamin varied with the metal with which the milk came into contact during pasteurisation, this being 20-40 per cent. with aluminium, slightly greater with tinned copper, and from 80-90 per cent. with copper. All methods of pasteurising (and of heating) milk were found by Kieferle and Eisenrich [36] to be detrimental to the antiscorbutic potency of milk, but flash methods were definitely less harmful than holder methods. These tests were proved both by animal-feeding and chemical methods. Chemical tests on similar milks have yielded similar results. Simple boiling is accompanied by the least destruction of the vitamin especially by boiling in aluminium or glass vessels.

The *sterilisation* of milk, *i.e.*, heating at temperatures above 100° C., causes a rapid destruction of vitamin C. Autoclaved milk (at 120° C. for ten to sixty minutes) has been used with impunity to form part of the basal ration of guinea-pigs when testing for antiscorbutics.

The vitamin A-content of milk does not appear to be affected by pasteurisation and whatever loss has been observed by some investigators is insignificant. Stassanised milk shows a smaller loss of vitamin A than holder-pasteurised milk, the loss being again insignificant.

Vitamin D is resistant to heat-treatment.[62] No difference has been found in the antirachitic value of butter churned from either raw or pasteurised milk from the same source. On the point whether the other factor in the rachitogenic condition, the calcium, is sub-optimal, Ellis and Mitchell [63] found that the calcium of raw milk is used more efficiently than that of pasteurised milk even in the presence of sufficient vitamin D (98 per cent. utilisation for raw, 92 per cent. for pasteurised milk). This lowering of the calcium utilisation is difficult to explain, as the slight change which the salts of calcium and phosphorus suffer during the process can hardly be supposed to alter the value of the calcium for growth. The above authors suspect that a vitamin-like factor is responsible ;

also that vitamin C, the only vitamin impaired by pasteurisation, may possibly play a part in the nutritive economy of the rat. (It is generally thought that the rat does not need vitamin C at any stage in its life.) Catel [64] found that kids thrive better on boiled than on raw cow's milk.

(d) *Drying of Milk. Effect on the Vitamins.* What has been said about heated milk applies also to dried milk so far as the action of heat is concerned. The time of exposure of milk to 140° C. in the roller-process is relatively short, and is probably insufficient to lower the vitamin-content appreciably. In the spray-process, however, the spray of milk is exposed to a stream of hot air at 115° C. and the dry powder settles at the bottom where it is exposed to a temperature above 100° C. until it is removed for packing. The spray-process by which cold milk is atomised and distributed into a large stream of air heated only to a moderate temperature has much to commend it, for vitamin-preservation at least. If the dry powder is removed from the floor of the drying-chamber as soon as it has settled, very little diminution in vitamin-content should be expected.

The spray-process does not appear to damage the water-soluble B vitamins.[65] The fat-soluble vitamins in the product, especially vitamin A, will oxidise slowly if stored for an appreciable length of time.

Owing to the variable amounts of vitamin C in the original milks before drying, investigations on the fate of this factor in the drying process have yielded conflicting results. The low antiscorbutic value of milk is still further lowered in the drying process.[66] Guinea-pigs which had developed scurvy could not be cured with any amount of dried milk which they could consume ; a monkey which had developed scurvy on dried milk was cured by the same amount of scalded fresh milk. The dried milk had roughly half the antiscorbutic value of the raw milk in these experiments (Barnes and Hume [66]). They also obtained indications that the antiscorbutic value of milk rose when cows went on to pasture.

The roller-process of drying gives a powder of higher anti-scorbutic value than the spray-process, and Jephcott and Bacharach [67] found that guinea-pigs could be protected from scurvy by winter or summer milk, specially dried by a roller-process. Special precautions taken in the spray-process also have been found to yield a product in which none of the original antiscorbutic potency of the milk was destroyed.[68]

Generally the vitamin-content of dried milk is less than that of an equivalent amount of raw milk. Separated skim or half-cream

milk will be lower and deficient in fat-soluble vitamins ; animal fats, such as lard, or vegetable oils, such as olive oil, cannot make good the deficiency. The antirachitic power of dried milk can be increased by ultra-violet irradiation.

(e) *Condensing of Milk. Effect on Vitamins.* In condensing milk, the liquid is first pasteurised or forewarmed to 80-90° C. for a short time and then condensed at 50° C. under reduced pressure for two to three hours. Such treatment does not seriously affect the vitamin-content of the condensed product. Hume [69] determined the antiscorbutic potency of sweetened condensed milk and found (on monkeys) that the protective dose was identical with the equivalent of fresh milk. Hess [70] also found that the loss of vitamin C caused by the heat of condensing was very small.

In the sterilisation of evaporated milk after canning at 107-116° C., a greater loss of vitamin C occurs ; Hart *et al.*[71] found it to be about 40 per cent.

Condensed milk is either whole milk or machine-skimmed. Any form of machine-skimmed milk, whether raw or condensed, is unsuitable for infant nutrition owing to its low fat-content— which is further diminished when the milk is diluted before use. In a similar manner, the dilution necessary to reduce the effect of the added sucrose (42 down to, say, 4 per cent.) is so great as to result in the production of a foodstuff very deficient in fats, vitamins and salts.

195. Vitamins of Milk Products

Since the fat-soluble vitamins appear completely in the butter churned out of cream or milk,[11] the best source of study of these factors is butter. In feeding-experiments with milk also the total effect of all the vitamins has to be taken into account. With butter a more concentrated source is available. The fat-soluble vitamins can be still further concentrated in the unsaponifiable fraction ; this fraction can be diluted at will in an inert medium for experimental work, *e.g.*, with coconut or olive oil. Such a procedure is common in diluting vitamin A standards or vitamin concentrates from cod-liver oil.

VITAMIN A OF BUTTER. It had long been suspected, but only recently shown with certainty, that the yellow pigment, carotene, could be transformed in the animal body into the colourless vitamin A. There is little, if any, vitamin A in the green food of a cow's diet, and Moore [6] has shown that she can transform the pigment into the vitamin. Morton and Heilbron [72] were the first to demonstrate the presence of these two substances in butter.

Biological assays can measure the sum of the vitamin A-activities of these two substances but, since such methods are time-consuming and fraught with difficulties especially in the case of vitamin A, it is gratifying that methods have been discovered whereby both carotene and the vitamin can be measured by physical methods.   Physical measurements, however, although giving accurate estimations of carotene and vitamin A, cannot yet be translated into biological units, owing to the uncertainty of the relative values of carotene and vitamin A.   The spectro-photometric measurement of the absorption at 328 mμ for vitamin A and at about 460 mμ for carotene yields true figures with regard to their concentration in butter.[73, 74]   A colorimetric estimation of the carotene in butter gives too high results,[75] but when the butter is dissolved in petroleum ether, satisfactory readings can be obtained.   The inactive xanthophyll should be allowed for or separated when carotene is measured by physical methods.   A simplified method has been suggested by Gillam [76] which is based on the constancy of the ratio of xanthophyll to carotene in butter, thus dispensing with the tedious separation.

Owing to the presence of an inhibitory substance which shows seasonal variation (greatest when cows are on pasture, least when fed indoors), the well-known Carr-Price antimony-trichloride reaction cannot be applied directly to butter, and figures so reported are valueless.   The unsaponifiable fraction has to be examined, and even then the measurement of the blue colour given by the Carr-Price [77] reagent yields lower vitamin A values than those measured by the absorption at 328 mμ.

Access to green pasture yields butter of high vitamin A-activity and indoor-feeding yields butter low in the vitamin.   Crawford et al.[78] showed that there was no difference in the vitamin A-activity of New Zealand butters (winter and summer) when the cows had been out on pasture throughout the whole experimental period.   Fraps and Treichler [79] found that cows on cotton-seed meal and hulls gave butter containing only a fifteenth of the amount of the vitamin in butter from cows similarly fed but allowed access to Sudan grass pasture.   The milk of the Wisconsin University Dairy throughout one year was examined by the spectro-photometric method for carotene and vitamin A by Baumann and Steenbock ; [74] they found a 400 per cent. variation in the carotene and a 100 per cent. variation in the vitamin A-content ; the lowest values were found in April and the highest in June and July.   Similar results were reported for British butter by Booth, Kon, et al.,[80] (Fig. 26), Watson et al.[81] and Gillam et al.[73] It is obvious, therefore, that the concentration of vitamin A

and carotene in butter is lowest in winter and early spring when they are mostly needed. Attempts have hence been made naturally to maintain the vitamin A-content of butter constant throughout the year. The feeding of lucerne hay and soya-bean hay were effective in maintaining a high vitamin A level or in restoring it to summer level after it had been depressed by indoor-feeding on dry food. The addition of grass, artificially dried by the methods used at the Imperial Chemical Industries Agricultural Research
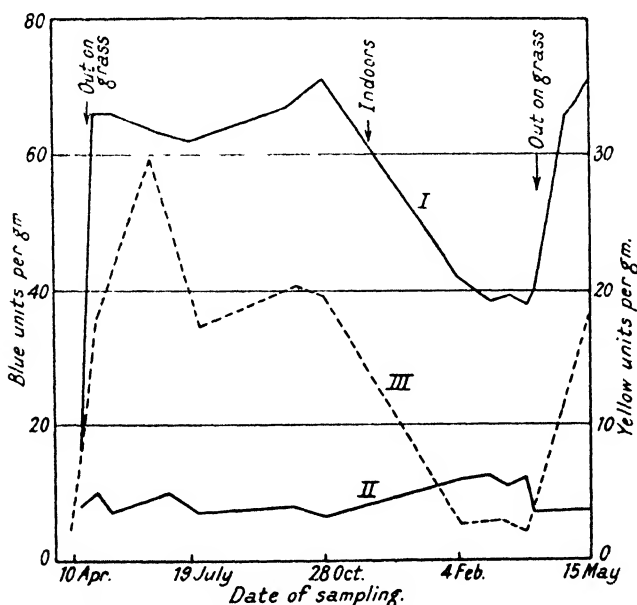
FIG. 26.—Season variations in the carotene and vitamin A content of butter-fat. (Kon and Booth.)

I. SbCl$_3$ blue values on unsaponifiable residue. II. SbCl$_3$ blue values on butter-fat. III. Carotene as yellow units in untreated fat. (Two yellow units of carotene are roughly equivalent biologically to 5.B.U. of vitamin A.)

Station, maintained the vitamin A- and carotene-contents of butter almost at summer level during the winter indoor-feeding period (Watson and collaborators [81]). Watson and Ferguson [82] found that the inclusion of 50 per cent. of the ration as dried grass raised the yellow colour of winter butter to summer level, and that the feeding of A.I.V. silage at the rate of 40 lb. per head per day was even better in this respect (Fig. 27).

Butter from the Channel Island breeds is more highly coloured than that of other breeds, and it has been claimed, owing to the

recognition of carotene as the precursor of vitamin A, that the vitamin A-content of the butter is higher. This is not borne out by experimental evidence; on the contrary, no difference has been found by Wilbur, Hilton and Hange [83] in the vitamin A-content of butter-fats from Guernsey and Ayrshire cows produced under identical conditions of feeding, although the butter-fat from the Guernseys contained twice as much carotene as that from Ayrshires. The samples of butter were tested biologically. Kon and Booth [84] made the same observations on Guernsey and Shorthorn butter-fat. They also measured the vitamin A-content by the antimony-
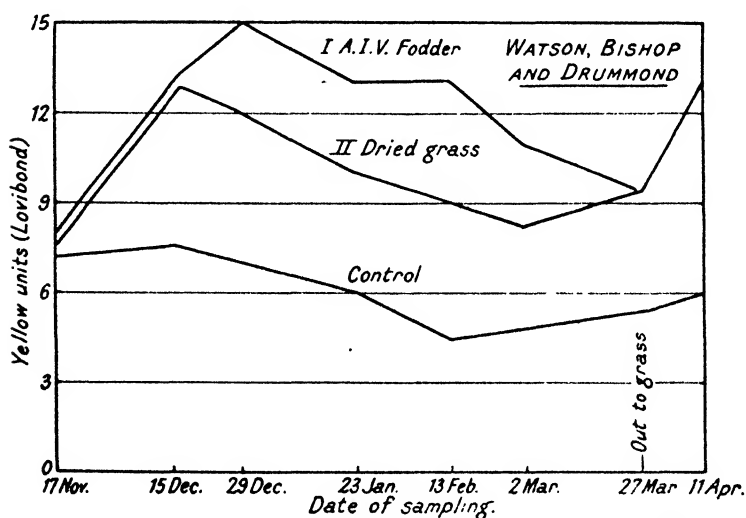
FIG. 27.—The carotene-content of butter-fat from the milk of cows fed on dried grass and A.I.V. fodder.

trichloride reaction on the unsaponifiable matter, and found that the Guernsey butter contained less vitamin A than the Shorthorn butter, although the yellow colour of the former was three times that of the latter. Davis and Hathaway [85] have compared the vitamin A-contents of Holstein (Friesian), Ayrshire, Jersey and Guernsey milk from cows kept under identical conditions of feeding, and have found no difference between these breeds. The Holstein milk contained less fat than the Jersey milk, there-fore, for similar results, the concentration of the vitamin in Hol-stein butter-fat must have been greater than that in Jersey butter-fat. They state in a later publication that if the butter-fat contents were equalised, Holstein milk would be more active than Jersey milk, the same applying to 20 per cent. cream from both breeds.[86]

VITAMIN D OF BUTTER. The vitamin D in butter varies seasonally in a manner analogous to vitamin A, the concentration being higher in summer than in winter. Crawford *et al.*[78] found the vitamin D-content of butter produced in winter in New Zealand was definitely lower than that produced there in the summer, although the cows were on pasture all the year round and the vitamin A was constant. Watson and his colleagues [81] found that the feeding indoors to cows of dried grass from plots heavily dressed with nitrogenous fertilisers did not increase the vitamin D-content of butter, although vitamin A was increased to summer level. These results emphasise the significance of the action of sunlight on the cow rather than that of the ration itself. " The direct insolation of the cow may play a dominant rôle in the formation of the antirachitic substance in milk under normal conditions " (Kon [22]). As an exception, Sabri and Fikry,[87] working in Egypt, were unable to show the presence of vitamin D in cows' butter or in the milk-fat of the buffalo even when these were incorporated to the extent of 12 per cent. in the Steenbock rachitogenic diet 2965. A very high vitamin D-content was observed by Bechtel and Hoppert [88] in summer butter off Sudan grass pasture.

In the assay of vitamin D, the rat is a convenient animal, but it is unable to differentiate between the vitamin D of cod-liver oil and irradiated ergosterol, as the infant can do ; the rat also cannot serve as a guide to the clinical value of various milks of which the antirachitic potency has been raised by one of the several methods now used for the purpose. Kon and Booth [89] found in experiments on rats that about 80 per cent. of the original antirachitic activity of autumn and winter butter was lost in the process of saponification, whilst the activity of cod-liver oil and of irradiated ergosterol remained constant under such treatment. Summer butter was more resistant, and highly potent butter derived from feeding irradiated yeast or irradiating the butter itself withstood saponification without loss. This suggested that the activity lost during saponification was that due to the fat of the butter, which affected the calcium and phosphorus metabolism of the rat when on the rachitogenic diet. Kon [22] states that " it is very doubtful whether the fat-moiety of butter will exert an antirachitic action on species in which absence of vitamin D alone leads to the appearance of rickets, independently of the mineral balance of the diet. The possibility is not excluded that the labile activity of butter is in part, at least, due to a genuine labile antirachitic factor. Whatever the ultimate finding may be, all the results indicate the necessity of extreme

caution in translating rat-values of vitamin D into clinical units."

VITAMINS OF CHEESE. The vitamin A-content of New Zealand cheese has been studied by Frengley and Herrick,[104] who state that cheese is more potent than butter on a fat basis. Coward and Morgan [105] report a value of 55 I.U. per gram for English Cheddar cheese.

A loss of vitamin B naturally occurs during cheese-making, owing to the separation in the whey. Vitamin B is lost from the curd, and cheese contains considerably less vitamin B than milk. The type of rennet used does not appear to affect the B content of curds.[106]

Determinations of vitamin C in cheese have given negative results.[106]

Calculated on a solids basis, the vitamin D-content of cheese, according to Mameli and Cultrera,[106] is higher than in milk ; this may be due partly to the different ratio of fat to solids-not-fat. The vitamin D-content of the fat of New Zealand cheese has shown seasonal variations.[104]

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CHAPTER XXII

THE NUTRITIVE VALUE OF MILK AS A WHOLE

196. Introductory

THE last two chapters have mainly dealt with the nutritive values of the major and minor constituents of milk. Regarded as a whole and as one element entering into human dietary, milk is the most satisfactory of foods and one for which there is no effective substitute. It must be remembered, however, that milk is not a perfect food, even for the infant organism, and that it is totally inadequate as the sole source of nutrition after the sixth month of life. Given as the sole source of food after this age, a considerable number of nutritional disorders ensue.

McCollum [1] subdivides the diets of the people of the world into two main classes, namely, those on a diet of cereals and leafy vegetables, *e.g.*, in Eastern and Southern Asia, and the diet of meat-milk products in arid areas. Both diets produce good physical development. It is to be feared that the diet of the Aryan races generally is too rich in phosphorus (from cereals), too low in calcium, and generally deficient in vitamins A, C and D. The deprivation of calcium from the body leads to serious damage to the skeletal structure and general tone of the tissues. The two main classes of foods rich in calcium are vegetables and milk.

197. Supplementing of Diet with Milk

Its richness in calcium and phosphorus makes milk an efficient food for supplementing or making up the deficiencies of cheap foods such as cereals, tubers and roots in these elements. There are many data to prove that an adequate milk ration added to a cereal diet causes satisfactory growth in young animals as well as better physical development, this being reflected in a larger average size, earlier maturity, a longer duration of reproductive life, and greater success in the rearing of young. Steenbock and Hart [2] have found also that no common mixture of proteins was as efficient as milk proteins to make up for the deficiencies in the biological value of cereal proteins. The best results in the feeding of growing pigs occurred when the ratio of liquid milk to maize meal was unity.

Prolonged experiments by Orr *et al.*[73] who fed to rats a diet

based on a dietary survey of an industrial population, have shown that such a dietary can be considerably improved by the addition of milk and vegetables.

Milk production in animals is favoured by using milk as a supplemental food ; it has been found that the energy of food, regardless of its source, is better used with ample milk in the diet than with little. Generally, cereals or vegetables are perhaps sufficient for maintenance, but for growth they have to be supplemented by milk ; even small quantities of milk, of negligible energy value, can cause better growth in pigs,[3] which means a better utilisation of the energy value of a feed and a better class of pork and bacon. The superior quality of dairy-fed pork is well known. McCollum [1] places milk as one of the most valuable protective foods.

198. Nutritional Value of Milk to Growing Children

Human beings have a longer growth-period than any of the other mammals, and this possibly has the advantage that early faults in malnutrition can be corrected at a later period. Hence the growth factors of nutrition have to be included in the diet for about twenty-one years of child and adolescent life, although perhaps not to the same intensity as in early years, or in the diet of quickly-maturing mammals. Various criteria of successful growth are : low infant mortality, perfect development of bone and teeth, ability to resist infection ; in mature life, additional criteria are high fertility and longevity before senile characteristics appear.

It is not surprising, therefore, that the various foods entering into a child's diet have been extensively studied with regard to supplying the growth factors in amount consistent with optimum nutrition and development. The correcting effect, in this direction, of vegetables and milk on the deficiencies of cheaper foods such as sugars, cereal products, legumes and tubers, has long been recognised, and many observations have been made on the effect of milk on the development and general health of children of school-age when fed with uniform amounts of milk daily. These experiments have attained classical distinction in the realm of nutritional chemistry.

McCollum, Parsons and Kalmbach (see McCollum,[1] Chap. 30) carried out an experiment on 84 children suffering from general severe malnutrition. These children had been fed on an institutional diet of satisfactory calorific value but containing no raw food. No change in the cooking of the diets was practicable, so that 42 of the children received one quart of whole milk daily (reconstituted

from milk powder) in addition to the institutional diet, which was given to the other 42 children to serve as a control. Possibly owing to the effect of external observation on the institution, both groups of children showed sharp increases in body-weight at the start, but this progress was not maintained in the control group ; but with the milk group, the weekly increase of from 2 to 5 lb. in body-weight was continued for the twenty-one-month period of observation. The response to the milk in the diet was phenomenal, and was reflected not only in gain in weight but in the vitality and vigour of the children. Further, when the control group was given one quart of milk per head per day, the capacity for growth was equal to that of the original milk-fed group.

A closer examination of the growth rates brought out the fact that the children on the milk diet were growing at a more rapid rate than expected from tables computed by other observers on the normal rate of child growth. These experiments, however, showed that a diet of cereals, tubers, roots and meat did not prove sufficient for the full development of the growing child, but that milk more than supplemented the deficiency.

It must be remembered that the children in the above experiments were in a severe state of malnutrition, and that their condition favoured maximum response to a better quality of diet. Data from surveys of the physical condition of children, generally, have shown that up to 70 per cent. suffer from some defect directly attributable to malnutrition, the most prominent being defective teeth as a serious interference to health. Observations on school-children to whose diet milk was added as a supplementary food have also been made. Morgan [4] and his collaborators found that the addition of small quantities of milk to the diet resulted in a better increase of weight and general health. He found that supplementing the diet by milk and biscuits gave a greater increase in weight than did oranges given as a supplement, although the latter gave a better gain in height.

Work in this country on the effect of milk on the well-being of school-children seems to narrow down the supplementary action to the effect of the minerals so added. It has been established by various workers that the calcium-intake is often below the calculated requirement. Thus Orr and Clark,[5] surveying the diets in certain Scottish towns, found that this occurred in 24·5 per cent. of the cases. Orr,[6] Leighton and Clark,[7] and Clark [8] found that the addition of whole milk and separated milk, respectively, to the diets of school-children attending day-schools in some Scottish towns promoted satisfactory growth, and separated milk proved as beneficial as whole milk ; the latter

effect may be referred to the mineral-content of the milk rather than to the vitamin D-content (which would be small, in any case). The extra supply of calcium salts would also enhance the value of any vitamin D present in the diet.

Mann [9] compared milk with several other supplements when added to the dietary of boys in an institution. The diet was considered adequate, although most of the boys were below normal height and weight. It was found that increase in growth, as measured by both height and weight, was promoted by the addition of one pint of whole milk per day. The milk used was pasteurised ; there was no effect on height by the addition of caseinogen in amount equivalent to that contained in the milk, or of margarine in amount equivalent to the extra calories. An improvement was noticeable with New Zealand butter. The missing growth-factors in the original diet were undoubtedly associated with the fat-soluble vitamins, the calcium salts, and the good quality of the protein of the milk added as supplement. In the twelve-month period of the experiment, 61 boys on the basal diet alone each gained an average of 3·85 lb. in weight and grew an average of 1·84 inches in height, whilst a group of 41 boys, receiving one pint of pasteurised milk daily in addition, gained 6·98 lb. in weight and grew an average of 2·63 inches.

Leighton and Clark,[7] in the studies cited above, found that the differences in height and weight between groups of children receiving whole and separated milk and those not receiving it were significant. There was a difference in favour of the whole-milk group of 45 per cent. in weight and 23·5 per cent. in height ; the children were also better in general appearance, were more alert, and did much better at school, this being more apparent in the second year. Clark [8] continued the observations for seven months in 100 rural English schools and found the benefit derived from milk to be well above the normal variation, whilst the condition and attendance of children regularly given a milk ration in school was superior. These experiments have proved conclusively that the addition of milk to the home ration of the child is of great value over the whole period of school-age.

Palmer [10] carried out an experiment extending over eleven weeks on two groups of Detroit school-children averaging 11 per cent. under normal weight. To one group milk and biscuits were given as an extra, and the other group received no supplement. At the end of the period it was found that 90 per cent. of the milk group had more than normal weight-gain, as against 42 per cent. in the control group. The scholarship of the milk group was also considerably superior. Chaney [11] confirmed the findings of

Palmer as regards milk generally, but was of the opinion that the deficiency of vitamin C in milk made it slightly inferior to orange juice for school-children, and that the drinking of large volumes of milk made the child lose its appetite for the other part of the dietary.

The optimum intake of fluid milk based on Atwater's [12] estimate of the food-intake of children from six to fifteen years of age (2,040 calories, 75 g. protein, 43 g. fat, 325 g. carbohydrate) appears to be one pint ; this amount is ample for supplying supplementary proteins. However, for an athletic boy consuming 4–5,000 calories daily, a quart of milk would be necessary. On the basis of efficient calcium-storage, which amounts, according to Sherman and Hawley,[13] to 0·01 g. daily in a healthy, growing child, a quart of milk daily means a calcium-intake of over one gram daily ; this will give an improved calcium-retention in the child and will therefore tend to the optimum development of bones and teeth. The calcium of milk is more efficiently utilised than that of vegetables ; milk also has a greater coefficient of digestibility when consumed as part of a mixed diet. Sherman strongly advocates the inclusion of milk in the diet of the child up to the age of fourteen years, irrespective of the quality of the other dietary constituents ; cereals should always be supplemented by milk. Although the above quantities represent the results of observations for optimum supplementing of protein and for calcium-retention, the value of much smaller quantities of milk must not be lost sight of. Crowther,[14] for instance, found that growing pigs showed an improvement in rate of growth of 13 per cent. when given one pint of milk daily and 22 per cent. on a quart per day. It was also observed that a fraction of a pint per day gave a significant improvement, thus confirming the findings of Golding,[3] who fed half a pint per head per day. Daniels and her co-workers [15] have lately found that children from three to five years old, on an ample diet and supply of vitamin D, retained as much calcium on one as on two pints of milk per day, and that the amount of phosphorus retained was greater on one than on two pints.

199. The Effect of Heat on the Nutritive Value of Milk

The heat-treatment of market milk is considered by health authorities and generally by the medical profession to be necessary for the destruction of pathogenic organisms and the safeguarding public health. It is not intended here to discuss this aspect of heat-treatment, but to consider the debatable point whether the loss, if any, of the nutritive value of milk by heat-treatment

is of economic significance in the light of our present knowledge of human dietary and nutrition.

The review of the position up to 1916 by Lane-Claypon [15] shows the chaotic state of the findings and the shortcomings of the methods of experimentation during the years before the significance of the vitamins was discovered. Most of the workers then concluded that there was no proof of inferiority of heated (boiled or pasteurised) as compared with raw milk and, indeed, some claimed that the raw milk was inferior to the boiled. Since that time, knowledge of the effect of heat on the vitamins and other milk constituents, and the variation in their amounts in milk, has advanced considerably, and the methods of experimentation have much improved.

The significance of any change in the nutritive value of milk by heating must be considered from several aspects. There is firstly, the species of animal for which the milk is intended. If some of the calcium is thrown down from a reactive colloidal form as a precipitate of calcium phosphate, it is evident that the human infant which naturally suckles milk of 0·040 per cent. CaO-content is not going to be seriously affected when cow's milk has its available CaO-content slightly lowered from a total CaO-content of 0·170 per cent. ; this is so even when the milk is diluted with an equal volume of water. Pasteurised cow's milk might easily show deficiencies, on the other hand, when fed to calves. Again, the susceptibility of any particular species to scorbutic tendencies will govern the degree to which the destruction of vitamin C by heat may be regarded as of significance. Generally, it is stated that milk is poor in vitamins and that these should be supplemented from an external source. The economic significance of vitamin C-destruction in milk thus hardly arises when its supply from an external source is advocated, and fortunately this vitamin can be obtained in abundance in fresh and cheap citrus and other fruits. The age of the subject fed is of significance in that the increasing acidity of gastric juice can re-dissolve precipitated calcium phosphate and thus overcome the effects of heat on milk in this direction ; the calcium is still entirely in the milk and only its availability is impaired. Possibly with a sufficiency of vitamin D, retention of calcium from feeding heated milk would not be greatly inferior to that from raw milk.[16]

200. Raw versus Pasteurised Milk

Much attention has been paid in the last decade to the possible differences in nutritive value of raw and pasteurised milk. It is to be feared that, considering the variation in milk composition

and its effect on the nutritive value and the already understood nutritional deficiencies of milk, too much stress has been laid on small differences which have been observed. A certain amount of bias towards the milk as it is given by the cow is natural, and perhaps investigators have proceeded to prove that pasteurisation does lower the nutritive value of milk. Conflicting evidence, therefore, exists both from superficial examination and statistical treatment of growth-data from such experiments. What is more to the point is to inquire whether the loss, if any, of the nutritive value during pasteurisation is of economic significance in relation to the benefit to the public health of safeguarding the milk supply from milk-borne diseases by the pasteurising process.

The present method of pasteurisation (143–145° F. for thirty minutes) was not extensively practised up to 1916 ; most experiments were done with milk which had been brought to boiling-point or, in some cases, with milk heated to 140° F. for twenty minutes.

Schroeder [17] carried out an extensive investigation with over 600 guinea-pigs which were fed on (a) their mother's milk and (b) raw, (c) pasteurised (140° F. for twenty minutes), and (d) boiled cow's milk (one minute at b.p.). Additional food, which was not specified, was also given. He measured the effect of feeding by the mortality rate of the animals and the average weights of the survivors at various periods. Those fed on their mother's milk were superior in weight and percentage of survival : the boiled-milk group came next, but were closely followed by the other two groups (raw and pasteurised) which showed almost identical results. This practically confirmed the findings of Keller [18] on mice, Lane-Claypon [15] on rats, Rodet,[19] Keller [18] and Bruning[20] on dogs, and the last-named worker also on pigs and goats.

Lane-Claypon (loc. cit.), summarising clinical data on the nutritive value of raw and boiled cow's milk as food for infants, states that natural feeding will produce the best results, but that if artificial feeding has to be employed there is no evidence that milk loses any of its nutritive value by boiling, and that the work of numerous observers indicates that rather more satisfactory progress is made with boiled than with raw milk.

Daniels and Stearns [21] observed the increase in weights of infants fed either on rapidly boiled or pasteurised (145° F. for thirty minutes) milk. They found that those on the pasteurised milk gave no weight-increase, but that on changing to the boiled milk a sharp increase was at once observed ; more calcium was found in the fæces after the pasteurised than after the boiled milk. Lewis,[22] comparing raw and pasteurised milk when fed to children,

found that certified milk (raw milk of very low bacterial count) fed to babies from birth to three months old gave an average gain of 29 oz., as against 8 oz. for pasteurised milk during a twelve-week period. With the three to six months old babies, the respective gains in a twelve-week period were 3lb. 15 oz. and 2 lb. 14 oz. and, with six to nine months babies, 5 lb. 8 oz. and 3 lb. 14 oz. The milk was heated before feeding. Ladd, Evarts and Franks [23] fed certified and pasteurised Grade A milk without heating, and claim a greater percentage of development for the former ; its feeding did not necessitate supplementing with cod-liver oil and orange juice.

Biological studies of the differences in nutritive value of raw and pasteurised milk have been made by various workers. Mattick and Golding [24] have brought evidence that rats can grow and breed in successive generations normally on a diet of raw milk supplemented by a white-flour biscuit ; similar animals on pasteurised milk behaved less satisfactorily in both these respects. Drummond,[25] however, in identical experiments found no evidence that pasteurisation adversely affected the nutritive value of milk. He found that raw milk supplemented by wheat-flour biscuit was insufficient to enable a young female rat to produce and rear a normal litter of young. His experiments suggested that additional vitamin B was required to adjust the balance for normal reproduction, and that additional amounts of copper and iron above those necessary to maintain the normal blood picture might influence reproduction beneficially. Other substances in the yeast extracts used might be responsible for the results observed.

Wilson and Cowell [26] carried out similar experiments with mice, and found that both raw and pasteurised milk were inadequate to promote normal growth ; the addition of copper and iron improved growth, but did not generally prove adequate to rear litters. The addition of Yeastrel (a concentrated food containing vitamin B) and mineral mixtures provided the necessary factors for satisfactory growth, reproduction and lactation. For mice, cow's milk is poor in iron, copper, and vitamin B. There was little difference between the value of raw and pasteurised milk in influencing general development, survival time, production of litters, and successful rearing of young in the case of half-grown mice, but with mice brought up from birth by does on a milk-and-biscuit diet, there was a considerable difference ; the weight of the mice at weaning-time was significantly heavier in the raw- than in the pasteurised-milk group.

The results of carefully controlled experiments with rats on the nutritive value of raw and pasteurised milk have recently been

published by the National Institute for Research in Dairying (Reading) and the Rowett Research Institute (Aberdeen).[71] By careful balance-experiments it was found that the retention of Ca and P and the digestibility and biological values of the milk proteins were identical for each type of milk. Pasteurisation had no effect on the vitamins A, B and D of milk but, on the average, there was 21 per cent. reduction in the vitamin C-content. The milks, supplemented with small amounts of Cu, Fe and Mn, showed no difference in nutritional value as judged by the criteria of weight-gains, body-length or composition of carcase of rats.

The outstanding investigation on the feeding of raw and pasteurised milk to school-children is the Lanarkshire experiment of Leighton and McKinlay.[27] For four months in certain schools in Lanarkshire, 5,000 children were given ¾ pint of raw milk daily and an equal number of children in these schools were used as controls. In a different group of schools, 5,000 children received ¾ pint of the same milk pasteurised, another 5,000 in the same group of schools acting as controls. The height and weight of the children were taken before and after the experiment. The authors claimed as a result of the experiment that milk had a beneficial effect when added to the diet of school-children, but that no difference was shown between raw and pasteurised milk. Elderton [28] statistically examined the result of this experiment and observed that : (a) extra milk (raw or pasteurised) generally increased the gain in height over the controls to an equal extent, except that on pasteurised milk older girls grew more than the younger ; (b) extra milk caused a gain in weight more often in girls than in boys, and in older than younger girls, and that the difference due to age was associated more with raw than with pasteurised milk ; (c) there was no evidence of the superiority of either raw or pasteurised milk in increasing the growth-rate, and that the value of pasteurisation turns practically on the elimination of infection.

" Student " [29] has noted two important defects in the experiment, in that the selection of controls was not at random, and that the weight, which included that of the clothing, was subject to variation, since the amount of clothing discarded between the beginning (February) and the end (June) of the experiment would vary according to the financial positions of the parents, the " better-off " children discarding more clothing in summer. To obtain an unequivocal result he suggested that the large-scale experiment should be repeated, or that a small experiment on twins of like sex be carried out.

Fisher and Bartlett [30] have also pointed out the unfortunate

planning of the experiment in that raw and pasteurised milk were not compared in the same schools, and that one conclusion should have been that the increase in height due to raw milk was significantly greater than that due to pasteurised milk.

Sprawson [31] has compared the two kinds of milk by examining the incidence of dental caries in children. He compared two small groups of children who received 12 oz. of pasteurised milk, with or without cod-liver oil, in one institution with children who received 20 oz. of raw milk in another institution. The differences were well marked, but did not lend themselves to statistical treatment, and the results may be accepted as an incentive to a more controlled investigation. Sprawson,[32] studying the feeding of raw milk and its effect on the incidence of caries in 750 boys in an orphan institution, found that the daily addition of raw milk to the diet caused a marked drop in the number of cases requiring treatment ; the effect was noticed two to three years after milk-feeding was begun.

Work on the relative values of raw and heated milk (pasteurised, boiled, evaporated, and milk powder) has been carried out by Frank *et al.*[33] on 3,000 children in 39 American cities by measuring increases in height and growth. The children were divided into two groups, one was given heated milk exclusively, and the other raw milk ; the latter had received raw milk only for more than the latter half of their lives. No difference was found in the average height and weight of these groups. It was unfortunate that this investigation took no account of the amount of milk-equivalent consumed, or of the composition of the remainder of the diet ; there was therefore no basis for the direct comparison of raw and heated milk.

The absorption of fat, protein and minerals by two healthy infants given raw and boiled milk, respectively, was identical in both cases.[34]

The gastric digestion of raw and boiled milk in seventeen infants (3–33 months) has been investigated.[70] The gastric contents were removed before, and at half-hourly intervals after test feeds with both milks. No significant differences were found.

Some authorities have observed that commercially pasteurised milk is superior to raw, due probably to the effect of the traces of Cu and Fe taken up from the metallic surfaces of processing plant in warding off anæmia. Lasby and Palmer [77] found this to be the case with the commercial product, but that laboratory pasteurised milk was inferior to raw. Hartel [78] observed the same results with flash-pasteurised milk (12 sec. at 76° C.).

Calf-feeding Experiments. McCandlish and Black [39] have

investigated the difference in nutritive value of raw and pasteurised milk for calves. Two groups of calves were selected, one being fed on raw milk and the other on pasteurised milk after five days from birth. After ninety days all the calves on raw milk were alive, whilst two out of eight on pasteurised milk had died. For sixty days there was no significant difference between the two groups but afterwards the raw-milk group went ahead. The pasteurised-milk group consumed more food per lb. live-weight gain than the raw-milk group. Orr and his co-workers [40] tested the relative value of raw milk, pasteurised milk, and pasteurised milk *plus* calcium lactate, for calves. The three groups of calves were fed on milk alone for fifty days and, for a further seventy-five-day period, an addition of concentrates was made. The average gain in weight of each group was 193, 176 and 197 lb. respectively. The calves on the pasteurised milk showed no signs of malnutrition but inferior " bloom." In a second experiment (8 calves), the same kinds of milk were given *ad lib.* for forty-five-day periods. Two calves receiving raw milk showed an average gain of 55 lb. in the first forty-five days. Three animals on pasteurised milk showed an average gain of 48·3 and 55·3 lb. in the first and second forty-five-day periods, whilst 3 calves on pasteurised milk *plus* calcium lactate showed an average gain of 55 and 68 lb. for the two periods, respectively ; in this case the calves on pasteurised milk showed effects of malnutrition and signs of rickets. A third experiment, extending over six months, on two groups of 3 bull calves showed an average weight-increase of the animals on pasteurised milk of 255 lb. and of those on pasteurised milk *plus* calcium lactate, 261 lb., the difference being insignificant.

The results of a calf-feeding experiment on raw and pasteurised milk (lasting over two years) have been reported by Wilson *et al.*[72] In a paired experiment each calf was given milk in strict propor-tion to its body weight, with hay *ad lib.* as supplement. The average increases in body-weight for an eight-week period were : raw group (25 animals), 53·72 lb. ; pasteurised group (23 animals, 53·86 lb. (61·2 and 62·9 per cent., respectively). The number of bull calves, which usually grow at a greater rate than heifer calves, was less on the " pasteurised " than on the " raw " group. It was not possible for any external observer to distinguish from condition, state of coat and other points, any difference between the two groups.

In the interpretation of these comparative experiments it has to be borne in mind that : (*a*) the results of experiments on one species cannot be indiscriminately applied to other species,

especially when feeding one species with the milk of another species ; (b) the raw and pasteurised milk for the comparative feeding experiment should be drawn from the same bulk, owing to the variation in composition of the major constituents and the wider variation of the minor constituents ; (c) when growth is the measure of response to the diet, normal seasonal variations in the growth-rate should be considered ; and (d) the scale of experimentation should be as large as possible ; the magnitude of the probable error has frequently overshadowed any small differences observed between raw and pasteurised milk in previous experiments, and it is only by careful planning of experimentation on a large number of subjects that the effect of other variables can be reduced to a minimum.

201. Infantile Scurvy from Feeding Heated Milk

It has been shown that if scurvy is to be prevented by feeding milk alone, a quantity must be consumed which practically amounts to a complete milk diet.[35] The paucity of vitamin C in milk and the ease with which it is partially destroyed by heat have caused many outbreaks of scurvy in children fed on heated milk. Neumann [36] (1902) attributed the many cases of infantile scurvy which he met with in his practice to the then recently-introduced practice of pasteurising milk ; the milk was heated to 90–95° C. before delivery, and again heated at near boiling-point for ten to fifteen minutes before consumption.

The American Medical Milk Commission in 1912 pronounced that for the purpose of infant-feeding, heated milk might be considered equal to raw milk. Hess and Fish [37] have described an outbreak of infantile scurvy among infants who had previously been fed for several months on milk pasteurised by the " holder " process (145° F. for thirty minutes) ; in this instance orange juice had been discontinued as a result of the above Commission's pronouncement, and the scorbutic symptoms rapidly cleared upon continuing with orange juice (see also Miller [38]).

202. The Use of Milk in Poultry Nutrition

Milk and milk by-products have been found to be valuable supplementary food for growth, egg-production and reproduction in poultry. Milk is not a complete food for growing chicks as, fed with an iron-free diet, it gives rise to anæmia in young chicks within twelve to fifteen days. The forms in which milk would be most commonly fed to poultry would be as skim or separated (sweet and sour) milk or butter-milk.

In regard to chickens, much work has been done to determine

the best form of milk to give to ensure rapid growth. Card [41] showed that equal growth was obtained when sour skim milk, or fresh butter-milk, was fed *ad lib.*, or when dried butter-milk formed 25 per cent. of the ration. Milk products showed no difference in value, and their use would depend upon the convenience in feeding and the availability and cost of the various forms of milk. Sweet and sour milk have been found of equal value in relation to chick-growth and mortality.[42] Elford [43] found that sweet skim milk gave the best growth when compared with sour skim milk, sweet whole milk, sour whole milk and water ; sweet whole milk gave the lowest mortality.

The addition of milk to a ration of mixed cereals has been found by Prentice *et al.*[44] to double the rate of growth of chicks, separated milk being as good as, if not better than, whole milk. Newbigin and Linton [45] have found that chickens, given separated milk *ad lib.*, made more rapid gains on both complex and simple grain mixtures than chicks allowed water only. On replacing the milk with water the chicks fell in weight rapidly. The value of milk was dependent in some measure on the biological value of the protein of the basal ration ; the maximum benefit was derived from milk during the first four to five weeks after hatching. It is advisable when milk or milk products are given as the sole source of protein that the liquid products should be given in unlimited quantities in the undiluted state.

Moore [46] has shown that sour milk fed to laying hens, especially in combination with vegetable protein (pea- and bean-meal) increases egg-production. Martin [47] has found it unnecessary to feed dry mash, if unlimited amounts of sweet or sour milk or butter-milk are fed ; he obtained maximum results with cereals, meat-meal and milk. Prentice [48] also found that the addition of milk to a cereal diet gave better egg-production than cereals and water, and equal to that from a diet of cereals plus fish-meal, minerals or soya-bean-meal. Atwood [48] has observed that hens reared, from hatching, on a liberal supply of separated milk, when compared with those getting a limited supply only, showed a lower mortality, a higher egg-production in the first and second year, and that their daughters produced more eggs. Parkhurst [49] observed that sour skim milk proved slightly superior to sweet skim milk for egg-production, the latter causing digestive troubles. Sour skim milk gave better production, profit, and size of eggs than any milk constituent used in comparison—curd, casein, casein *plus* albumin, lactose, lactose *plus* milk minerals, milk salts, lactic acid, or whey. Milk curd gave the second most favourable result. Milk whey contains constituents valuable for egg-production

other than albumin. The addition of protein-rich foods as supplements to unlimited sour skim milk has been found to be unnecessary from the standpoint of production, but in some cases gave larger eggs. Such foods are usually costly : fish- and meat-meal, meat- and bone-meal, leguminous-seed meals and lucerne-meal.

Milk products have consistently been found to give superior production when they have been compared with other protein-rich foods. Parkhurst (*loc. cit.*) has carried out experiments lasting over six years comparing sour skim milk, meat-meal (60 per cent. protein) and meat- and bone-meal (50 per cent. protein). The milk groups gave superior production both in number of eggs and egg-size to the meat-meal groups. In another similar experiment where pea-meal was also compared, the average annual yields for the various foods were : milk 146, meat-meal (tankage) 120, meat-meal 115, pea-meal 106.

The value of milk for poultry stock is thus proved. Milk products have been used to advantage for feeding chickens, turkeys, ducks, geese and table pigeons, and in rations of every kind, namely, for growth, maintenance, egg-production and fattening. Fertility and hatchability are improved by feeding milk products.

All types of milk should be given undiluted, but when whey is fed, the remainder of the ration should be supplemented by a protein-food.

203. The Consistency of Milk Curd in Infant Nutrition. Soft-curd Milk

In the nutrition of infants a most important factor is the physical consistency of the milk clot in the stomach. The formation of a soft curd is desirable, since digestion is facilitated by allowing better penetration of digestive enzymes into the clot both in the stomach and small intestines ; a hard curd is accompanied by a considerable amount of undigested curd in the stools and a condition of malnutrition of the infant.

It has been demonstrated by Lane-Claypon [15] that the clot which results from the coagulation of heated milk consists of much finer particles and is of a more open texture than the clot from raw milk. This is the basis of the practice of giving young children only heated milk. Cow's milk, however, is seldom given to infants except in the diluted form. It has also been established that the rate of passage of milk into the small intestine is affected by heat-treatment and by dilution.[50] Mortenson [53] has proved that the curd of boiled or autoclaved milk passes into the intestine very quickly, because more surface is offered to the gastric juice.

Most samples of cow's milk when clotted with rennin or pepsin

yield a tough rubbery curd, but some cows yield milk which on coagulation gives a soft " junkety " curd. The differences between these two types of curd can be roughly demonstrated by wringing some of the curd in a cheese-cloth of fine mesh. The soft curd will be squeezed out through the meshes, but the hard type will knit together into a rubbery mass. On stirring the hard curd during coagulation it will coalesce to a stringy mass, but the soft type will break up into fine particles. These are only rough tests on the curd character, and a more quantitative measurement of " curd-tension " has been devised by Hill ; [51] the curd is formed in a container by a standard procedure and a knife, formed from 10 radial blades soldered to an upright rod, is slowly pulled up through the curd, the amount of pull required being measured with a spring balance. The number of grams of pull is arbitrarily used as an index of the " curd-tension " of the particular milk. (See Section 139.)

High fat-content in milk has also been the cause of digestive troubles in infants. Milk with a high fat-content is more likely to be hard-curded, whilst milk with low curd-tension is generally of low fat-content, although individual exceptions have been observed.

There is no doubt that the character of the curd of milk from different cows varies and the criterion of the value of these milks for infant nutrition rests on the digestibility of the milk. Hill [51] cites a number of cases, observed by specialists in infant-nutrition, where babies who showed signs of doing badly on cow's milk or proprietary milk preparations immediately showed satisfactory response to selected soft-curd milk. All classes of infants, namely, healthy infants deprived of mother's milk, persistent vomiters of hard curds, colic and curd-stooled babies, babies persistently doing badly on all modifications of cow's milk, babies with chronic indigestion, and infantile eczema cases, improved in well-being and growth with the regular introduction of soft-curd milk-feeding.[52]

Soft-curd milk may be fed undiluted and thus does not require much supplementing. Such milk has also been used for adult-feeding, remarkable results being claimed for it in the treatment of adult indigestion and gastric ulcers.

This demand for soft-curd milk has been commercially exploited, and New York, and other American cities, at least, have been supplied with this milk on demand for a number of years.

Attempts have been made to change the nature of hard-curd milk to make it applicable for infant-nutrition as soft-curd milk. Momentary boiling reduces the curd-tension by 30 per cent., and

more, if boiling is prolonged. The addition of alkalis or salts of weak inorganic or organic acids such as lime water, sodium citrate or bicarbonate, have a similar effect. That the best result from heat-treatment is obtained by the sterilising process is demonstrated by the easy digestibility of diluted evaporated milk.[54] Milk in which lactic acid has been allowed to form (*acidophilus* and *bulgaricus* milk) also shows a low curd-tension. Lundstedt [55] has observed that homogenisation of milk (3,000 lb. pressure) greatly reduces the curd-tension. The lowering of ionic calcium concentration by 20 per cent. by a base-exchange process has been found by Lyman, Browne and Otting [56] to lower curd-tension almost to zero. The process consists of allowing milk of $0 \cdot 3$ per cent. acidity (artificially added lactic acid) to flow through a bed of zeolite, when the treated milk acquires an acidity of $0 \cdot 15$ per cent. (pH $6 \cdot 50$).[57] Such milk caused higher absolute and percentage retentions of Ca when compared with whole boiled milk.[74] Feeding infants on the same type of soft-curd milk has produced favourable results.[75]

Lundstedt also suggests a modified churning process whereby lecithin is transferred from the surface of the fat globules to the casein particles. The lecithin acts as a protective colloid on the casein network, and the churned milk, losing its ability to form a firm coagulum, is rendered soft-curded. The transfer of the lecithin from the fat globules to the casein is effected by churning at a low temperature under conditions of complete crystallisation of the milk-fat.[76]

Theophilus and his co-workers [58] have found that homogenisation at pressures of 500, 1,000 or 2,000 lb. per sq. in. reduces the curd-tension of milk approximately 25, 46 and 53 per cent. respectively, single-stage and two-stage homogenisers being equally effective ; milk of highest original curd-tension shows the greatest reduction on homogenising. Hansen and his associates [59] have found that mastitis from streptococcic infection of the udder invariably lowers the curd-tension, but that from staphylococcic infection does not. Foot-and-mouth disease causes also a lowered curd-tension of the milk. (See also Section 139.)

204. The Nutritive Value of other Forms of Processed Milk

The nutritive value of pasteurised milk has already been discussed (Section 200).

(*a*) SWEETENED CONDENSED MILK. The forewarming process and evaporation in copper vacuum-pans, probably tend to lower, but not completely destroy, the vitamin C of milk manufactured into sweetened condensed milk. A volume of work has been done

on the vitamin-content of this product, and most authorities state that the amounts of the important vitamins are the same as in ordinary raw whole milk. Hume [60] states that the vitamins A and C are practically completely retained. Hess [61] states that owing to the condensing being carried out with little access to air, little loss of vitamin C occurs. Vitamin C has been found present in the sweetened product after fifteen months' storage.[62] Hawk *et al.*[63] find that the vitamins A, B and D are not altered in the method of preparation ; Hess [63] states that condensed milk does not tend to produce rickets.

Sweetened condensed whole milk has been widely used for infant-feeding for many years with success ; its high sugar-content is regarded by pediatricians as the cause of over-fat infants which have a low resistance to disease, but that it generally gives good results with proper and intelligent feeding. It is frequently used for the new-born and as a supplementary food for older children. With proper supervision condensed milk can be successfully fed to average babies over long periods.

(*b*) EVAPORATED MILK. The main changes brought about in the nutritive properties of evaporated milk during manufacture are those due to the sterilising process. Vitamin C is completely destroyed ; some advantageous physical properties of the curd are developed (see *soft-curd milk* above). The calcium and phosphorus of evaporated milk are as well assimilated by the human digestive system as when taken in ordinary raw milk.[64]

Some diminution of vitamin B possibly occurs but other vitamins are not materially reduced. Dutcher and his associates [65] conclude that although aeration and oxidation may contribute to a slight loss of vitamin B, the vitamin is not readily destroyed by the usual evaporative processes.

Evaporated milk has the advantage of sterility and superior digestibility. The product is largely used for infant-feeding. Marriott and Schoenthal,[54] who have conducted clinical experiments with evaporated milk on 752 cases, find that it not only has no disadvantages but possesses distinct advantages for infant-feeding.

The above, of course, applies to evaporated whole milk. On the other hand, evaporated skim milk is almost totally devoid of the fat-soluble vitamins and very rightly tins of this product have to be labelled as unfit for babies.

(*c*) DRIED MILK. The results of many studies on the vitamin-content of dried milk go to show that neither the spray- nor the roller-process substantially lowers the contents of vitamins A, B and D, but that vitamin C is reduced by varying amounts.

Supplee and Dow [66] have stated that vitamin C is destroyed to a greater extent in the spray- than in the roller-process but Cavanaugh *et al.* [67] have reported the presence of considerable amounts of this vitamin in spray-dried milk. Jephcott and Bacharach [68] have compared summer milk, winter milk and neutralised milk, dried by both processes, and found both winter and summer milk dried by the roller-process to possess an antiscorbutic activity about equal to that of the original raw milk. The spray-dried powder was very deficient in vitamin C, and the neutralised milk product contained less of the vitamin than the raw milk.

Many investigators have come to the conclusion that dried milk powders and their products are safe for the feeding of infants. (See also the wide clinical experience of Tobey.[69])

In his classical experiment, McCollum [1] (see Section 198) fed milk which was reconstituted from milk powder manufactured by the Merrel-Soule process ; the milk was reconstituted by agitation in cold water.

The drying of milk has economic advantages. It is first and foremost a method of preserving an almost complete food for a reasonable length of time without deterioration. The bulk of the raw milk is greatly reduced without economic loss of its nutritive value ; milk in powder form is thus available under conditions which preclude a supply of liquid milk. It is made from the raw milk which is produced in spring and summer, and has the highest nutritive value. A milk rich in vitamins is thus available in winter when the ordinary liquid supply is low in the fat-soluble vitamins. This is of special importance in infant-feeding in winter time and in the communal feeding of groups, *e.g.*, institutions and military forces.

All the improvements which have been effected in the manufacture of dried milk tend towards the maintenance, or even the increase of the nutritive value of the raw milk. Improved storage properties, effected mainly to safeguard the marketable value, also promote preservation of the nutritive value. If the stored dried product becomes " tallowy," the deterioration is accompanied by a serious loss of vitamins A and C and some vitamin B, whilst ageing-faults mean a loss in solubility during reconstitution. The incidence of a tallowy flavour has been considerably reduced by a better understanding of the conditions under which milk-fat is oxidised, and the processing of sweet milk uncontaminated with heavy metals, such as iron and copper, has largely eliminated this fault. As a parallel occurrence to this, improvement has been observed in the preservation of the vitamins.

Further, since the product is a cheap and available source of

vitamin D, the amount of this vitamin in dried milk can be artificially increased, without endangering its storage properties, by irradiating it with ultra-violet light. This is best done by a short intense irradiation, so that autoxidative changes are not initiated in the milk-fat. It has been found, in investigations on this procedure, that the amount of pro-vitamin is practically the same in milk produced at all seasons of the year ; this practice therefore will tend to provide a supply of a comparatively rich source of the antirachitic factor of fairly constant potency.

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