HOTOSYNTHESIS

by

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PREFACE

THIS is the third book to appear under the title of Photosynthesis. It differs from its two admirable predecessors, written by Professor Stiles and by Dr. Spoehr, in that the synthesis in living plants of carbohydrates from carbon dioxide and water by the agency of sunlight is discussed as a problem in physical chemistry.

Ever since the days of van Helmont photosynthesis has been a problem of outstanding importance and interest. Its importance lies in the fact that it is the fundamental process of life and that in the leaves of plants is produced the whole supply of carbohydrates and proteins which are needed as food by animal and man. Its interest has lain in the difficulty of its explanation.

In the following pages, after some of the more important characteristics of photosynthesis have been described, the story is told of an investigation which led in the end to the photosynthesis in the laboratory of carbohydrates from carbon dioxide and water. In subsequent chapters the photosynthesis of proteins, the mechanism of photosynthesis and the kinetics of the process are discussed.

I am fully conscious that the whole outlook on the problem is a personal one, and for this I beg forgiveness. It is not easy to write impersonally of one's own experiences and at the same time accept full responsibility for their truth.

No one knows better than I the help and inspiration I received from my many colleagues who during dark days never lost hope of ultimate success. Their names have been recorded and to their unfailing enthusiasm I owe a debt that is inextinguishable.

Many friends too have rendered aid in other ways. I express my grateful thanks in particular to Dr. Winifred Brenchley, Professor Ruggles Gates and Professor J. M. Whittaker, whose contributions have been detailed in the following pages.

To Miss E. E. Kelly I am deeply indebted for her co-operation during the experimental work and for her valuable assistance in the writing of this book.

Above all I place on record my debt to my friend the late Sir Robert Mond, whose generous financial assistance rendered possible the experimental investigation described in Chapter V.

E. C. C. BALY

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PHOTOSYNTHESIS

CHAPTER I

PHOTOSYNTHESIS AND THE DIFFICULTIES OF ITS INTERPRETATION

THE name photosynthesis has now been generally adopted as defining the process whereby in the leaves of living plants complex organic products of the nature of carbohydrates are produced from carbon dioxide and water by the agency of sunlight. This process is one of outstanding scientific interest and one of fundamental importance to mankind. The truth of these two statements can be very simply demonstrated.

If any one of the carbohydrates which are known to be produced in plants is completely burned in oxygen, the sole products of the combustion are carbon dioxide and water, that is to say, the combustion process is the reversal of the process of photosynthesis. During the combustion a very large amount of energy is evolved and by the use of a calorimeter this amount of energy has been accurately measured. In the case of the well-known sugar, glucose, the combustion is expressed by the equation

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 673{,}000 \text{ calories}$$

It is evident from this equation that in the reverse process, the photosynthesis of 1 grm. molecular weight of glucose, a minimum amount of 673,000 calories is needed to promote it, that is to say, 112,167 calories for 1 grm. molecular weight of both carbon dioxide and water. As will be shown later, there are present in the light which reaches the earth from the sun no rays which can promote the simple reaction

$$CO_2 + H_2O + 112{,}167 \text{ calories} = \tfrac{1}{6}C_6H_{12}O_6$$

In spite of this perfectly sound argument it is known that glucose is actually produced in the leaves of many plants by the agency of sunlight, and the mechanism whereby the living plant achieves this end has given rise to much perplexity.

This is but one of many phenomena which have been observed and unexplained by physical chemists, botanists, and physiologists.

Then, again, photosynthesis is the fundamental basic reaction of all life. By its means the growing plant produces carbohydrates from carbon dioxide and water, and proteins from carbon dioxide, water, and nitrates. These products form the staple food of the graminiferous animals which in their turn form, together with certain plants, the food of man. If photosynthesis were to cease, all life on this earth would come to an end. Surely, then, it is right to regard the problem of photosynthesis as one of outstanding importance.

Yet another reason may be advanced in support of this statement. In all living animals and in man the vital processes may be divided into two classes : the synthetic or growth processes and the digestive or metabolic processes. Little or nothing is known of the mechanism of the former beyond the facts that they are highly endothermic and that special catalysts known as vitamins and hormones are necessary to promote them. In the living plant the synthetic processes are undoubtedly promoted by light, and it is well within the bounds of possibility that a knowledge of the mechanism of photosynthesis will enable us to gain some understanding of the mechanism of the vital reactions in animals and in man.

It is of great interest in considering the vital reactions of plants and those of man and animals to note that the two together form a completely closed cycle. The living plant during the day absorbs carbon dioxide from the air and water from the soil, and, by the agency of light, synthesizes carbohydrates. These carbohydrates, when the plants are eaten by men and animals, are staple food substances, and during the digestive processes are oxidized to carbon dioxide and water, the former being restored into the atmosphere by respiration. So far as carbohydrates are concerned, their digestion by man and animals is the reverse process of photosynthesis in plants, and the energy evolved during their digestion, which is equal to the energy absorbed in their photosynthesis, is of fundamental importance in the maintenance of vitality.

At the same time it must be remembered that a plant is not a mere machine the function of which is to photosynthesize carbohydrates and proteins. It is a living organism possessed of growth and digestive processes just like an animal. A plant has its respiratory system whereby it takes in oxygen from the atmosphere and respires carbon dioxide, this process being more evident when the plant is in the dark. The essential difference between the members of the two kingdoms is that in plants the synthesis of the compounds utilized in the growth processes is promoted by light, whilst in man and animals the necessary energy for the synthesis of the compounds utilized in the growth processes is supplied by a mechanism which, though probably one involving heterogeneous catalysis, has not yet been elucidated.

My object in this introductory chapter is to review some of the observations which have been made by scientists in their endeavours to solve the problem of the mechanism of photosynthesis. These observations have been both chemical and physical, because an explanation must entail both the formulation of the actual chemical reaction which takes place in the leaf and the correlation between this reaction and the light which promotes it.

In describing the experimental work which has a direct bearing on photosynthesis there are many observations which must be described in order that the difficulties which have been encountered may be fully appreciated. It is essential too that the importance of these observations be realized because any valid theory of the mechanism of photosynthesis must not only be in accord with but also give a completely satisfactory explanation of every observation that has been made.

The two primary facts of photosynthesis are (1) that carbohydrates are synthesized directly from carbon dioxide and water only, and (2) that this synthesis is promoted by the light which reaches the earth's surface from the sun. As regards the nature of the carbohydrates which are photosynthesized certain differences are known and, broadly speaking, it may be stated that in the leaves of the monocotyledonous plants hexoses, e.g. glucose, are formed and that in the leaves of the dicotyledonous plants the first visible product of photosynthesis is a starch. This starch soon undergoes hydrolysis, probably by enzyme action, into hexoses, and for this reason it is designated temporary starch. Incidentally, no explanation has as yet been brought forward of this difference in the products of photosynthesis in the two cases, but the difference is of relatively minor importance, since the major fact is the photosynthesis of a carbohydrate from carbon dioxide and water only.

We may initiate the discussion of the problem by considering the photosynthesis of a hexose from carbon dioxide and water, a process which can be expressed by the chemical equation

$$6CO_2 + 6H_2O = C_6H_{12}O_6 + 6O_2$$

and it has already been shown that the minimum amount of energy required to promote this reaction is 673,000 calories. Since it is known that the reaction is promoted by light, we may apply Einstein's law of photochemical equivalence which states that a minimum of one energy quantum of light is required to activate a single molecule of a substance and thereby cause it to react. The energy quantum is defined by $h\nu$, where h is the Planck constant and ν is the frequency of the light which promotes the reaction.

Now the life of an activated molecule is exceedingly short, being of the order of 10^{-7} second, and it is, therefore, impossible that the above equation is correct, because it entails the interaction of 12 molecules, all of which must remain activated for a sufficiently long period for this interaction to take place. It is obvious that the only possibility is the simple reaction

$$CO_2 + H_2O = \tfrac{1}{6}C_6H_{12}O_6 + O_2$$

and that the amount of light energy required is 673,000/6 or 112,167 calories for 1 grm. mole each of CO_2 and H_2O. It follows from Einstein's law that the product of the frequency of the light which promotes the reaction, Planck's constant and the Avogadro number, N, must equal 112,167 calories.

The relation between the wave-length of the activating light and the amount of energy absorbed in calories per gram-molecule of the reactant will frequently be needed in the following pages, and the method of calculation may be given. The absorption of a single quantum $h\nu$ by 1 molecule of a reactant means the absorption of $Nh\nu$ ergs or $Nh\nu/J$ calories by a gram molecule, where J is the mechanical equivalent of heat. Since the frequency ν is the velocity of light divided by the wave-length, this amount of energy is $Nhv/\lambda J$ calories, where v is the velocity of light in centimetres per second and λ is the wave-length of the light in centimetres. Adopting the most recent values of the constants, $N = 6{\cdot}064 \times 10^{23}$, $h = 6{\cdot}547 \times 10^{-27}$ erg sec., $v = 2{\cdot}99796 \times 10^{10}$ cm. sec. and $J = 4{\cdot}1852 \times 10^{7}$, the value of $Nhv/J = 2{\cdot}84388$, and this number divided by the wave-length of the light absorbed gives the amount of energy absorbed in calories per gram molecule. Conversely, the wave-length in centimetres of the light which promotes a reaction with a known energy intake in calories per gram molecule is given by 2·84388 divided by that energy intake.

In the above reaction which involves an energy intake of 112,167 calories, the wave-length of the light required to promote it is $2{\cdot}84388/112{,}167 = 2{\cdot}535 \times 10^{-5}$ cm. or 2535A, the Ångström being 10^{-8} cm. It must be remembered that this calculation is based on the assumption of maximum efficiency of the light, and hence $2{\cdot}535 \times 10^{-5}$ cm. is the maximum value of the wave-length of the light which can promote the reaction in a single operation. Now it is well known from spectroscopic observations that, owing to the absorptive power of the upper atmosphere, the light from the sun that reaches the surface of the earth contains no rays with a wave-length smaller than $2{\cdot}9 \times 10^{-5}$ cm. or 2900A. It is, therefore, impossible that the reaction

$$CO_2 + H_2O = \tfrac{1}{6}C_6H_{12}O_6 + O_2$$

can be promoted in a single operation by sunlight on the earth's surface. Moreover, it is well known that ultra-violet light with wave-length 2500A has a destructive action on organic tissue and it is certain that the living organisms now existent would be killed by sunlight if this light contained rays with wave-length 2535A. The foregoing calculation, vhich is based on the experience gained from the study of hotochemical reactions in general, gives rise to one of the ajor difficulties encountered in seeking the explanation of e mechanism of photosynthesis.

The photosynthesis of a hexose from carbon dioxide and water has been expressed by the equation

$$CO_2 + H_2O = \frac{1}{6}C_6H_{12}O_6 + O_2$$

because this is the form usually adopted. It is in the highest degree improbable that this equation is correct. The living plant photosynthesizes carbohydrates, not from a gaseous mixture of carbon dioxide and water vapour but from liquid water and atmospheric carbon dioxide. When carbon dioxide is in contact with liquid water a solution is formed which contains the equilibrium condition

$$CO_2H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^{\cdot} + HCO_3{}'$$

and in this equilibrium the component present in the largest amount is CO_2H_2O. It is probable, therefore, that the correct form of the equation representing photosynthesis is

$$CO_2H_2O + 112{,}167 \text{ calories} = \frac{1}{6}C_6H_{12}O_6 + O_2$$

This reaction may now be discussed from the chemical aspect, and two points of interest at once arise. In the first place it may be seen that in the equation the volume of the oxygen set free is equal to the volume of carbon dioxide which undergoes the reaction. The equation, therefore, cannot be accepted as correct unless it can be proved by experiments with living plants that during photosynthesis the volume of oxygen transpired into the surroundings is equal to the volume of the carbon dioxide assimilated. The volume ratio of the oxygen transpired to the carbon dioxide assimilated, or the photosynthetic quotient as it is called, has been the subject of careful study by several observers, notably by Maquenne and Demoussy,[1] who concluded from observations made with thirty-four different species of plants that the photosynthetic quotient is unity.

It must be borne in mind that these measurements are of necessity carried out under conditions of considerable difficulty. Mention has already been made of the respiration of plants in which oxygen is absorbed and carbon dioxide is respired. In the determination of the photosynthetic quotient the measurements must be corrected for the respiration which

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[1] *Echange Gazeux des Plantes vertes avec l'Atmosphère*, Paris, 1913; *Compt. rend.*, 1913, *156*, 506.

has taken place during the period of the irradiation. Then again, the relation between the rate of photosynthesis and the rate of respiration depends on the intensity of the irradiation and also on the concentration of the carbon dioxide in the surroundings.

Realizing these difficulties, Willstätter and Stoll,[2] in determining the photosynthetic quotient, used very large concentrations of carbon dioxide and great intensity of light. The rate of photosynthesis was thereby greatly increased, with the result that the possible errors due to the respiratory coefficient were reduced to a minimum. Under these conditions it was found that the photosynthetic quotient was exactly unity in all the species of plants examined. In the case of the succulents it was found that when the leaves were first irradiated the photosynthetic quotient was considerably greater than unity, but as the irradiation was continued it decreased to unity. The explanation of this phenomenon is the storage during the night of certain substances in the leaves of the succulents, and these undergo reactions which evolve oxygen and thus increase the volume of oxygen transpired during the first period of irradiation. As the result of these investigations there is no doubt that in the photosynthesis of carbohydrates the volume of oxygen set free is exactly equal to the volume of carbon dioxide assimilated. In this is to be found complete justification of the stoichiometric relation expressed in the equation

$$CO_2H_2O = \frac{1}{6}C_6H_{12}O_6 + O_2$$

The next question to be considered is the nature of the substance which is represented by $\frac{1}{6}C_6H_{12}O_6$, that is to say, the substance which is actually produced by the action of light on hydrated carbon dioxide and which undergoes polymerization into glucose. The most obvious substance to be formed from CO_2H_2O by the loss of a molecule of oxygen is formaldehyde, and this was first suggested by von Baeyer in 1870.[3] His explanation, however, of the formation of the formaldehyde from carbon dioxide and water was open to

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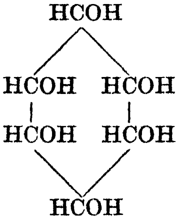
[2] *Untersuchungen über die Assimilation der Kohlensäure.* Berlin, 1918.

[3] *Ber. Deutsch. Chem. Ges.*, 1870, *3*, 63.

serious criticism. He was convinced that the formaldehyde is produced by the reduction of carbon dioxide to carbon monoxide, and he suggested that the carbon monoxide forms an addition compound with chlorophyll in the leaves similar to the addition compound of carbon monoxide and hemoglobin. von Baeyer also maintained that the reduction of carbon dioxide to carbon monoxide is promoted by hydrogen, a theory which involves the grave difficulty of specifying the source from which the necessary hydrogen is obtained.

It is unfortunate that von Baeyer, in advancing his theory that the production of formaldehyde is the intermediate stage in the photosynthesis of carbohydrates, gave an explanation of the mechanism of its formation which is untenable. The criticism of his explanation engendered doubt in the minds of many as to whether formaldehyde actually is the product of the action of light on carbon dioxide and water.

In his communication von Baeyer also discussed the polymerization of the formaldehyde into carbohydrates and advanced the very attractive view that it polymerizes into the cyclic compound



Five cogent arguments may be given in support of this view. In the first place, the six-membered ring is one of the most stable configurations of carbon atoms.[4] In the second place, the carbohydrates photosynthesized in living plants invariably contain six or a multiple of six carbon atoms in the molecule. In the third place inositol, which has the above formula, is found in the leaves of plants, and Crato [5] suggested that this substance is the first product of photosynthesis. In the fourth

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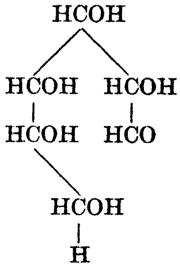
[4] von Baeyer, *Ber. Deutsch. Chem. Ges.*, 1885, *18*, 2278; 1890, *23*, 1275.

[5] *Ber. Bot. Ges.*, 1892, *10*, 250.

place the cyclic compound, by the wandering of one hydrogen atom, can form the open chain formula of glucose,



and glucose is the product of photosynthesis in the monocotyledons. Lastly, the cyclic molecules can readily undergo condensation into complex carbohydrates having the general formula $(C_6H_{10}O_5)_n$.

It will at once be realized that the formation of a molecule of formaldehyde and a molecule of oxygen from a molecule of hydrated carbon dioxide, which has been activated by the absorption of light energy, obviates the difficulty of a chemical explanation such as was advanced by von Baeyer. This photochemical process, combined with the cyclic polymerization of the formaldehyde, offers a very reasonable explanation of the mechanism of photosynthesis in the living plant.

Mention has already been made of the difficulty of explaining the energy relations of photosynthesis, namely, that there are present in the light which reaches the earth from the sun no rays with smaller wave-length than 2900A. The Einstein law of photochemical equivalence indicates that in order to promote photosynthesis, involving a minimum energy intake of 112,167 calories, a maximum wave-length of 2535A is needed. The acceptance of the above explanation of the mechanism of photosynthesis involves two further difficulties which may now be discussed.

When a chemical process takes place in two separate and distinct stages, in the first of which a well-known substance is produced, it is possible by analytical methods to detect the presence of that substance in the reacting system. No convincing evidence has been obtained of the presence of formaldehyde in the leaves of plants when they are irradiated.

Furthermore, formaldehyde is poisonous to plants and one has considerable reluctance in accepting the view that a living organism in its primary vital process produces a substance which is poisonous to itself.

Then again, it is well known that formaldehyde in aqueous solution is a stable substance. It is true that in the presence of alkali or of an alkaline surface formaldehyde undergoes polymerization, but the products of that polymerization are not the carbohydrates which are photosynthesized in the living plant. It has, however, been proved that when irradiated with ultra-violet light formaldehyde in aqueous solution undergoes polymerization into glucose, and the evidence of this will be given in Chapter III. The essential facts thereby established are that the polymerization is caused by ultraviolet light and that it is *activated* formaldehyde which polymerizes into the cyclic compound $C_6H_{12}O_6$. It is manifest from this evidence that the product of the action of light on hydrated carbon dioxide in the living plant cannot be ordinary formaldehyde, but must be activated formaldehyde, the energy content of which is considerably greater than that of ordinary formaldehyde. Whilst this conclusion explains the absence of formaldehyde in the leave of splants during their irradiation, it enhances the difficulty of explaining the energy relations of photosynthesis.

It is worthy of mention at this stage of the discussion that the formation of formaldehyde as the intermediate stage in photosynthesis is not the only possibility which conforms with a photosynthetic quotient of unity. It has been suggested that glycollic aldehyde may be produced in accordance with the equation

$$2CO_2H_2O = CH_2OH - CHO + 2O_2$$

and that three molecules of the aldehyde polymerize into one molecule of a hexose. It might equally well be suggested that glyceric aldehyde is formed in accordance with the equation

$$3CO_2H_2O = CH_2OH{-}CHOH{-}CHO + 3O_2$$

and that two molecules of the aldehyde combine to form one molecule of a hexose. These two suggestions may be critically examined in order to determine whether either would minimize the difficulties met with in the case of formaldehyde.

If glycollic aldehyde is the intermediate product, the first stage in the photosynthetic process must be the activation of two molecules of hydrated carbon dioxide as the result of the absorption by each of one quantum of light energy. These two activated molecules must then, before losing any energy by radiation, combine to form one molecule of glycollic aldehyde. This raises an important issue which has not hitherto been considered.

In the calculation of the wave-length of the light required to promote the photosynthesis of glucose from 1 grm. mole of hydrated carbon dioxide with an energy intake of 112,167 calories, it was tacitly assumed that the quantum efficiency of the process is unity. In other words, it was assumed that every single molecule of hydrated carbon dioxide which absorbs one quantum of light energy takes part in the photosynthetic process. This assumption is fully justified if formaldehyde is the intermediate compound, because if it be a characteristic property of an activated molecule of hydrated carbon dioxide to give a molecule of formaldehyde and a molecule of oxygen, every molecule so activated will give formaldehyde and oxygen.

Now the glycollic aldehyde theory, involving as it does the interaction of two activated molecules of hydrated carbon dioxide before either of them loses any part of its energy of activation, entails that very condition which is known to result in a quantum efficiency of less than unity. There is no doubt that if glyceric aldehyde be the intermediate compound produced, the quantum efficiency will be still smaller, since its formation entails the interaction of three activated molecules of hydrated carbon dioxide before any one of them loses any part of its energy of activation.

Let us now consider the result of the efficiency of photosynthesis being less than the maximum possible. It is obvious that when any photochemical process takes place with a quantum efficiency of less than unity, some of the light energy absorbed is lost by radiation, or, in other words, the amount of energy utilized is a fraction of the energy absorbed. It follows that the energy needed to promote the reaction must be greater than the amount calculated from the Einstein law. We have already seen that the maximum wave-length of the light needed on Einstein's law to promote photosynthesis with an energy intake of 112,167 calories is 2535A. The smaller efficiency in the cases of glycollic and glyceric aldehydes must result in more than 112,167 calories being needed to promote photosynthesis and in a proportional decrease in the wave-length required. So far from helping us to explain the energy difficulty met with in the production of formaldehyde by the agency of sunlight, the glycollic aldehyde and glyceric aldehyde alternatives very materially enhance that difficulty.

We have now discussed the only three compounds which can be produced from hydrated carbon dioxide with a photosynthetic quotient $O_2/CO_2 = 1$. As the result of this discussion there can be no doubt that photosynthesis is achieved by the photochemical production of formaldehyde which undergoes polymerization into a carbohydrate. Although a definite step forward has been gained, the major difficulty still remains to be solved, namely, the explanation of the conversion of hydrated carbon dioxide into activated formaldehyde by the agency of sunlight.

One of the most important contributions to our knowledge of the chemistry of photosynthesis was made by Willstätter and Stoll.[6] It is not necessary to give the details of their experimental methods, but they succeeded in extracting from leaves of plants the four plant pigments chlorophyll A, $C_{55}H_{72}O_5N_4Mg$, chlorophyll B, $C_{55}H_{70}O_6N_4MgH_2O$, carotin, $C_{40}H_{56}$, and xanthophyll, $C_{40}H_{56}O_2$. They also determined the amounts of the four pigments present in leaves and found that the total amount of chlorophyll A and B is not far from being constant, namely, 0·15 to 0·35 grm. per 100 grm. of fresh leaves, 0·6 to 1·4 grm. per 100 grm. of dried leaves, and 0·3 to 0·7 grm. per square metre of leaf surface. The molecular ratio of chlorophyll A to chlorophyll B was found to be about 2·9. The total weight of carotin and xanthophyll is from 0·07 to 0·2 grm. per 100 grm. of dried leaves, the ratio of carotin to xanthophyll being about 0·6.

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[6] *Untersuchungen über die Assimilation der Kohlensaüre.* Berlin, 1918.

As regards the function of these pigments in photosynthesis, it has been established by the work of several investigators that chlorophyll is essential to photosynthesis. The evidence for this is in the main the experimentally proved fact that photosynthesis does not take place in leaves which are deficient in chlorophyll. The possibility thus arises of chlorophyll promoting the conversion of hydrated carbon dioxide into formaldehyde under the influence of light, and Willstätter and Stoll investigated the action of carbon dioxide on solutions of chlorophyll.

In the course of their work on the plant pigments they had found that in alcohol chlorophyll passes into true solution and that in aqueous solution the chlorophyll is colloidal. In studying the effect of carbon dioxide, Willstätter and Stoll proved that carbon dioxide has no action on chlorophyll when in true solution in alcohol, the absorption coefficients of the gas in the case of alcohol and the alcoholic solution being identical. The colloidal solution of chlorophyll in water, on the other hand, was found readily to absorb carbon dioxide, and in the presence of excess of the gas the chlorophyll was decomposed to phaeophytin, the magnesium atom being split off as magnesium carbonate in accordance with the equation

$$C_{55}H_{72}O_5N_4Mg + CO_2H_2O = C_{55}H_{74}O_5N_4 + MgCO_3$$

Willstätter and Stoll also proved that when the concentration of carbon dioxide in the colloidal solution is small this reaction does not take place, but that an addition compound of chlorophyll and hydrated carbon dioxide is formed, the formation of this compound being reversible. In other words, they proved that when the concentration of carbon dioxide is small, an adsorption equilibrium is established, namely

$$C_{55}H_{72}O_5N_4Mg + CO_2H_2O \rightleftharpoons C_{55}H_{72}O_5N_4Mg.CO_2H_2O$$

Now it is to be noted from the composition of chlorophyll A, $C_{55}H_{72}O_5N_4Mg$, and chlorophyll B hydrate, $C_{55}H_{70}O_6N_4MgH_2O$, that the latter compound contains two atoms of oxygen more than the former. This fact, coupled with the fact that the conversion of a molecule of hydrated carbon dioxide into a molecule of formaldehyde entails the abstraction of two atoms of oxygen, led Willstätter and Stoll to advance an entirely new explanation of the mechanism of the primary photosynthetic process. Expressed in simpler terms than those used by them, their suggestion was that the adsorption complex of chlorophyll A and hydrated carbon dioxide is converted by light into chlorophyll B hydrate and formaldehyde in accordance with the equation

$$C_{55}H_{72}O_5N_4Mg.CO_2H_2O + \text{light energy} = C_{55}H_{70}O_6N_4MgH_2O + CH_2O$$

In short, Willstätter and Stoll were the first to suggest that photosynthesis is a photochemical oxidation-reduction reaction which takes place on a surface.

As they themselves pointed out, this theory assumes that the chlorophyll exists in the colloidal state in the leaves of plants, but they considered that this assumption was justified. They based their opinion on their measurements of the absorption spectra of chlorophyll in true and colloidal solution and of the chlorophyll *in situ* in the living leaf, and their finding that the absorption spectra of chlorophyll in the leaf and in colloidal solution are identical. A very exhaustive study of the absorption spectra of chlorophyll has recently been made by K. P. Meyer,[7] and the evidence obtained by him leaves no possible doubt that the chlorophyll in the leaves of living plants is colloidal.

The above equation expressing the photo-production of formaldehyde is, therefore, justified, but it only expresses one part of the process. It is necessary that there exists a second reaction in which the oxygen is set free from the chlorophyll B hydrate with the re-formation of chlorophyll A, namely

$$C_{55}H_{70}O_6N_4MgH_2O = C_{55}H_{72}O_5N_4Mg + O_2$$

Willstätter and Stoll believed that this reaction is promoted by an enzyme, with the result that the photosynthetic process becomes continuous so long as the external concentration of carbon dioxide remains constant.

In their endeavour to obtain evidence in support of this explanation of the mechanism of photosynthesis, Willstätter and Stoll determined the amounts of the four pigments present in the leaves of plants immediately before and after they were irradiated. They found that the ratio of chlorophyll A to

[7] *Helv. phys. Acta*, 1939, *12*, 349.

chlorophyll B remained practically constant, while the ratio of xanthophyll to carotin markedly increased. The constancy of the chlorophyll ratio is to be expected from the Willstätter and Stoll theory, provided that the velocities of the photochemical reaction and of the enzyme reaction are equal. They found, however, some difficulty in explaining the increase in the xanthophyll/carotin ratio, because they believed that these two pigments take no part in photosynthesis. This belief was founded on their measurements of the rate of photosynthesis in light which had been transmitted through a solution of carotin and xanthophyll. Willstätter and Stoll proved that the interposition of this absorbing screen only slightly reduced the rate of photosynthesis. It is, however, particularly to be noted that this observation only proves that carotin and xanthophyll do not take part in any photochemical reaction during photosynthesis. The possibility that these two pigments play a purely chemical role is by no means excluded.

Now Willstätter and Stoll, as was recorded above (p. 9), gave the final proof that the O_2/CO_2 ratio in the photosynthesis of carbohydrates is exactly unity. It is strange, therefore, that they do not appear to have noted that this is incompatible with an increase in the xanthophyll/carotin ratio while the chlorophyll A/B ratio remains constant. It is manifest that if the chlorophyll ratio remains constant a value of unity for the O_2/CO_2 ratio can only be obtained if the xanthophyll/carotin ratio is also constant. The observed increase in the latter ratio, therefore, must be due to a lag in the transpiration of the oxygen set free in the photosynthesis.

This phenomenon was first observed experimentally by Kostytschew [8] three years after Willstätter and Stoll published the results of their investigations. He found that when plants are first irradiated the volume of oxygen transpired is much less than the volume of carbon dioxide assimilated, the O_2/CO_2 ratio being about 0·33. After a short time the volume of oxygen transpired increases until the rate of transpiration exceeds that of carbon dioxide assimilation, the result being that in the end the volume of oxygen transpired becomes equal to that of the carbon dioxide assimilated. It is evident from Willstätter and Stoll's observations that this delay in transpira-

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[8] *Ber. Bot. Ges.*, **1921**, *39*, **319**.

tion is due to a temporary increase in the xanthophyll/carotin ratio. The phenomenon, therefore, is independent of the primary photosynthetic process.

Now that this difficulty has been surmounted, Willstätter and Stoll's theory may be discussed on its merits. It is the first scientific explanation advanced of the mechanism of photosynthesis and was based on the experimental proof that chlorophyll in the colloidal state, as it exists in the leaves of plants, adsorbs hydrated carbon dioxide. The fundamental basis of the theory is that the production of formaldehyde from the adsorbed layer of hydrated carbon dioxide is an oxidation-reduction reaction which is promoted on a surface by light. There are serious difficulties in the way of accepting the whole theory in the form enunciated by Willstätter and Stoll. Two of these difficulties have already been discussed, namely, the promotion of the oxidation-reduction reaction by sunlight, and the production of ordinary formaldehyde in place of activated formaldehyde. A third difficulty arises over the assumption that the second reaction, the reduction of chlorophyll B hydrate to chlorophyll A, is promoted by an enzyme. It is very difficult, if not impossible, to correlate an enzyme reaction with a photochemical reaction in a process which invariably has a photosynthetic quotient of unity. At the same time, it may be pointed out that these difficulties do not necessarily indicate that the theory is incorrect, as many authorities have believed. The fundamental principle of the photochemical production of formaldehyde is far too attractive to be lightly dismissed; rather let it be believed that the difficulties still outstanding indicate that the theory as enunciated by Willstätter and Stoll is incomplete and not incorrect.

✓ Attention may now be directed to the physical aspect of the problem of photosynthesis and the effect of the various controlling factors on the rate of photosynthesis may be discussed. There are four controlling factors and these are the temperature, the external concentration of carbon dioxide, the intensity of the light, and the nature of the light. In studying the effect of varying one of these factors it is manifest that care must be taken to guard against interference by any one of the three remaining factors. The realization of this necessity is due to

F. F. Blackman,[9] who in 1905 stated his principle of limiting factors in the following words : 'When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by that of the "slowest" factor.' Previous to the enunciation of this principle, many erroneous and contradictory results were observed. Any one of the controlling factors may be the limiting one, and it is obvious that in investigating the effect of varying one factor care must be taken to ensure the absence of restraint by any one of the other factors.

In view of its importance the effect of temperature on the rate of photosynthesis may be discussed first. It may be stated at the outset that when the carbon dioxide concentration and the light intensity are constant, the rate of photosynthesis increases as the temperature is increased. This fact is of great interest because this phenomenon is not characteristic of a purely photochemical reaction. It indicates that, associated with the primary photosynthetic process, there must exist a thermal or dark process, the velocity of which is a direct function of the temperature. The importance of this process was first recognized by Blackman and it is now universally known as the Blackman reaction. The mechanism of this process has not yet been fully explained, but in view of the fact that the photosynthetic quotient is unity, there can be little doubt that it is the restoration of the dark equilibrium which has been disturbed by the action of light.

In advancing their theory of the mechanism of the primary photosynthetic reaction, Willstätter and Stoll pointed out that in order that photosynthesis be continuous it is necessary that the chlorophyll produced in that reaction be reduced with the setting free of oxygen. It must be remembered that the oxygen is transpired into the surroundings through the stomata and hence, associated with the reduction process, there must exist a mechanism whereby the oxygen is conveyed from the chloroplasts to the stomata. The complete process whereby the oxygen is produced and transpired is the Blackman reaction.

The reality of the Blackman reaction was proved first by Warburg [10] and later by Emerson and Arnold [11] by the use of

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[9] *Ann. Bot.*, 1905, *19*, 281. [10] *Biochem. Zeitsch.*, 1919, *100*, 260.
[11] *J. Gen. Physiol.*, 1932, *15*, 391.

intermittent irradiation, that is to say, irradiation by a series of short flashes of light separated by short intervals of darkness. It was found that under these conditions the amount of carbohydrates photosynthesized by unit quantity of light was much greater than that photosynthesized by unit quantity of light when the irradiation was continuous. This increase is due to the fact that during the dark period the Blackman reaction continues, so that at the commencement of each light-flash the conditions are more favourable for photosynthesis than during continuous irradiation. Additional proof of this was obtained by Emerson and Arnold when they found that the amount of carbohydrate photosynthesized by unit quantity of light is a maximum when the light-flashes are exceedingly short and the

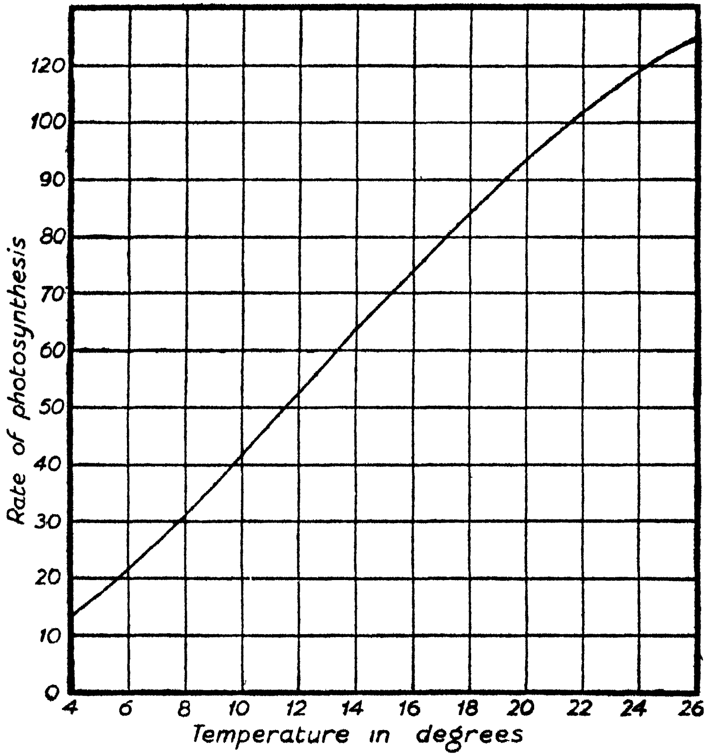

FIG. 1

dark intervals between them are sufficiently long for the Blackman reaction to complete itself.

Measurements of the rate of photosynthesis at various temperatures have been made by Warburg[12] and by Emerson[13] with suspensions of the unicellular alga *Chlorella*. The first series of Emerson's measurements is shown by the curve in Fig. 1, and, as can be seen, the relation between the rate of photosynthesis and the temperature is expressed by a sigmoid curve.

The object of these measurements was the determination of the temperature coefficient, that is to say, the ratio of the rate of photosynthesis at a given temperature to the rate at a temperature which is 10° lower. In Table I are given the temperature coefficients (Q_{10}) calculated from the curve in Fig. 1, together with those calculated from three additional series of measurements by Emerson and the values obtained by Warburg. It is to be noted that the values of Q_{10} for the temperature range of 5° to 10° are the squares of the values actually obtained.

TABLE I

TEMPERATURE COEFFICIENTS OF PHOTOSYNTHESIS (Q_{10})

Temperature range	Emerson				Warburg
	Series 1	Series 2	Series 3	Series 4	
5°–10°	4·47	6·32	5·86	4·90	5·0–4·3
10°–20°	2·42	3·32	3·81	2·72	2·1
20°–30°	1·27	1·62	1·80	1·37	1·6

Now, as has already been shown, the fact that the photosynthetic process has a temperature coefficient is due to the Blackman reaction, which is a thermal reaction. The relation between the energy of activation, E, of a thermal reaction and the temperature coefficient is given by

$$\log_e Q = \frac{E}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

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[12] *Biochem. Zeitsch.*, 1919, *100*, 268.

[13] *J. Gen. Physiol.*, 1928–9, *12*, 623.

where Q is the temperature coefficient, R is the gas constant, and T_1 and T_2 are the absolute temperatures at which the measurements are made. If the values of E are calculated from the above temperature coefficients by means of this formula, the values are by no means constant. For example, the values of the energy of activation of the Blackman reaction calculated from the three values of the temperature coefficient in Emerson's first series, 4·47, 2·42, and 1·27, are 23,422, 14,572, and 4,219 calories, respectively. This has given rise to much mystification, which is considerably enhanced by the fact that the temperature coefficient depends on the intensity of the light, Warburg having proved that the temperature coefficient decreases as the light intensity is reduced.

Turning once again to the effect of temperature, it will be seen from Fig. 1 that the rate of photosynthesis increases with the temperature up to 26°, the relation between the two being expressed by a sigmoid curve. It might be expected, therefore, that the rate would continue to increase progressively as the temperature is raised. This, however, is not the case, since Miss Matthaei, working in Professor Blackman's laboratory at Cambridge, discovered a very remarkable phenomenon.[14] She found that when the intensity of the irradiation was high the rate of photosynthesis increased with the temperature up to 37·5°. When the temperature exceeded this limit the rate of photosynthesis suddenly began to decrease rapidly until at 43° the rate was about one-half that observed at 37·5°. The curve in Fig. 2, which has been re-drawn from that published by Miss Matthaei, expresses her observations, and attention may be drawn to the remarkably sudden decrease in the rate of photosynthesis when the temperature exceeds 37·5°. Miss Matthaei also found that when the intensity of the irradiation was reduced the maximum rate of photosynthesis was observed at a lower temperature.

Miss Matthaei suggested that this strange phenomenon is due to the de-vitalization of the chloroplasts and considered that the combined effect of light and a temperature of 37·5° and over is lethal to the living plant. An analogy has been drawn with the well-known de-activation of enzymes when the temperature exceeds a definite limit. It may, however, be pointed out that

[14] *Phil. Trans.*, 1905, *197*B, 47.

a temperature of 37·5° is by no means uncommon in tropical climates, and if the de-vitalization of the chloroplasts is an irreversible process, as Miss Matthaei's own words appear to suggest, it is difficult to understand how any vegetation can flourish in a tropical summer. No convincing explanation of the phenomenon has been published.

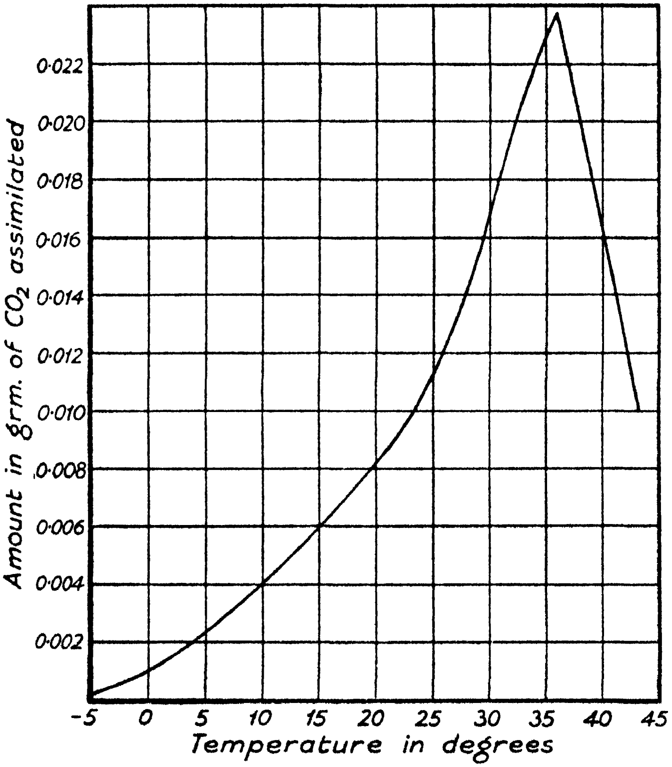

FIG. 2

We may next consider the relation between the rate of photosynthesis and the intensity of the light. This relation was determined by Warburg [15] and by Harder,[16] and Warburg's measurements are given in Table II.

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[15] *Biochem. Zeitsch.*, 1919, *100*, 255.
[16] *Jahr. wiss. Bot.*, 1921, *60*, 531.

TABLE II

Relative intensity of light	CO_2 assimilated	Relative intensity of light	CO_2 assimilated
1	45	7·1	172
2	82	16	220
4	135	c. 45	(240)

Omitting the last observation, the relation between the rate of photosynthesis and the light intensity is shown by the curve in Fig. 3, and this curve shows that when the light intensity is small the increase in the rate of photosynthesis is very nearly proportional to the increase in the light intensity. As the intensity of the light is increased the effect on the rate of photosynthesis becomes progressively smaller. This result was fully confirmed by Harder, who made four series of measurements with different external concentrations of carbon dioxide and found that as the concentration was increased the ratio of

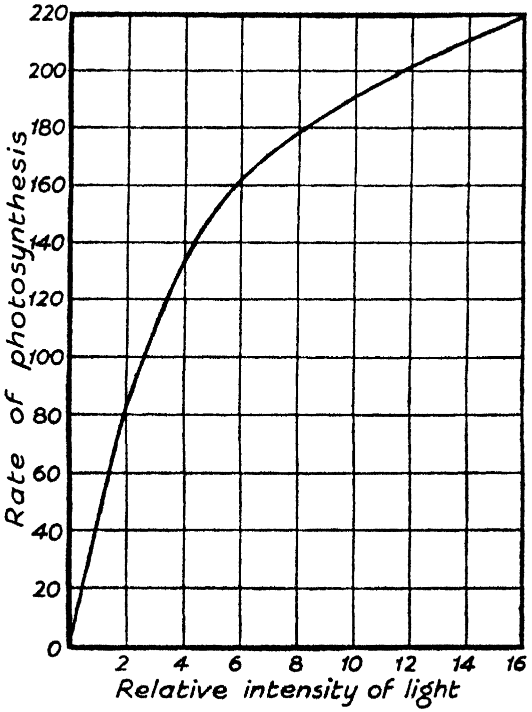

FIG. 3

the increase in the rate of photosynthesis to the increase of the light intensity became greater.

When the relation between the rate of photosynthesis and the light intensity is under discussion, the question of the efficiency of the photosynthetic mechanism is at once raised. This is one of the most important characteristics of the process and it is necessary to consider it in some detail. The term efficiency may first be defined. In an ideal photochemical reaction the absorption of one energy quantum $h\nu$ of the light which promotes the reaction will cause 1 molecule of the reactant to undergo the reaction, and hence the absorption of $Nh\nu$ calories will promote the reaction of 1 grm. mole of the reactant. In this case the quantum efficiency of the reaction is 1. If, on the other hand, as the result of the absorption of $Nh\nu$ calories, a fraction of 1 grm. mole undergoes a reaction, that fraction is a measure of the quantum efficiency. In order, therefore, that the quantum efficiency of photosynthesis in the living plant be determined, it will be necessary to determine the number of energy quanta absorbed at the light frequency selected and the number of molecules of hydrated carbon dioxide which have been caused to undergo assimilation. Since the number of molecules of oxygen transpired is equal to the number of molecules of hydrated carbon dioxide assimilated, the volume of oxygen transpired will give a measure of the photosynthesis promoted by the number of energy quanta absorbed. It follows, therefore, that the quantum efficiency of photosynthesis with light of frequency ν will be given by $e = V/22{,}400$, where V is the number of cubic centimetres of oxygen transpired as the result of the absorption of $Nh\nu$ calories.

The quantum efficiency of photosynthesis was measured by Warburg and Negelein.[17] They realized at the outset that the previous results obtained by Warburg and shown in Fig. 3 indicate that the efficiency of photosynthesis increases as the light intensity is decreased, and in their measurements of the quantum efficiency light of very low intensity was used. Very dense suspensions of *Chlorella* were irradiated in order to ensure that each plant irradiated received light of the same intensity.

A mercury-vapour lamp was used as the source of light and

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[17] *Zeitsch. phys. Chem.*, 1922, *102*, 235; 1923, *106*, 191.

by the interposition of absorbing screens three different spectral regions were used, namely, red light with wave-length 6900A to 6100A with maximum intensity at 6600A, yellow light with wave-length 5780A, and blue light with wave-length 4360A, the last two wave-lengths being characteristic of mercury vapour. It is to be noted that the red and blue rays selected by Warburg and Negelein lie well within the two characteristic absorption bands of chlorophyll, and hence it is certainly to be expected that one or other of the wave-lengths would promote photosynthesis. Warburg and Negelein's results for the three selected wave-lengths are given in Table III.

TABLE III

QUANTUM EFFICIENCY OF PHOTOSYNTHESIS

Wave-length	e	$\frac{1}{e}$
6600A	0·226	4·4
5780A	0·234	4·3
4360A	0·196	5·1

In the last column are given the number of energy quanta at each wave-length which would appear to be necessary to promote the assimilation of 1 molecule of hydrated carbon dioxide.

This, of course, is the original difficulty of the energy relation presenting itself in a different guise. We found previously that the minimum quantity of energy required for the conversion of 1 grm. mole of hydrated carbon dioxide into a carbohydrate, namely, 112,167 calories, can only be supplied by irradiation with light of wave-length 2535A, and this calculation was based on the assumption that the primary photosynthetic reaction has the maximum efficiency. Let us now consider the promotion of photosynthesis by $5Nh\nu$ at the wave-length 4360A. The value of $Nh\nu$ at 4360A is 65,227 calories and $5Nh\nu = 326,135$ calories, and hence Warburg and Negelein's result indicates that the absorption of 326,135 calories is necessary to promote a reaction the energy intake of which is 112,167 calories. Since $326,135 - 112,167 = 213,968$ calories, it follows that nearly two-thirds of the energy absorbed must be wasted, and hence the living plant would appear to be singularly inefficient in converting light energy into chemical energy.

An interesting question is raised by the assumption made by Warburg and Negelein that the whole of the light energy absorbed was utilized in promoting photosynthesis. It has been proved that the chlorophyll in living plants exists in the colloidal state, that is to say, it exists in the form of molecular aggregates which have a surface. The photosynthetic process, therefore, can only take place with molecules of hydrated carbon dioxide adsorbed on that surface. It thus becomes evident that there must be present a number of molecules of chlorophyll which take no part in photosynthesis. This conclusion is in accord with the result of an investigation by Emerson and Arnold,[18] who proved that in *Chlorella* the number of molecules of chlorophyll which actually take part in photosynthesis is only a small fraction of the total number present.

Let us consider the irradiation of a mass of chlorophyll the surface of which is coated with an adsorbed unimolecular layer of hydrated carbon dioxide, that is to say, a mass of chlorophyll, the surface layer of which consists of chlorophyll.CO_2H_2O complexes. The question at issue is whether this unimolecular layer quantitatively absorbs the blue and red light energy. If the whole of the light energy is not absorbed by the unimolecular layer, the remainder will be absorbed by the chlorophyll molecules underlying that layer, because the blue and red rays utilized by Warburg and Negelein lie within the absorption bands of chlorophyll. Indeed, the whole of the light energy would have been absorbed by the algae if no carbon dioxide had been present.

It is evident that, if only a fraction of the light energy is absorbed by the unimolecular layer of photosensitive adsorption complexes, the volume of oxygen transpired during the irradiation must be correlated with that fraction and not with the total amount absorbed. Warburg and Negelein's calculations of the quantum efficiency were based on the total amount of light energy absorbed and, in view of the uncertainty as to the absorptive power of a single layer of molecules, their values must be accepted with reserve.

It is to be noted that although chlorophyll absorbs light of wave-length 4360A as used by Warburg and Negelein, chlorophyll A, which on the Willstätter and Stoll theory promotes photosynthesis, exhibits a maximum absorptive power towards

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[18] *J. Gen. Physiol.*, 1932, *16*, 191.

light of wave-length 4175A. Any calculations of the energy relations of photosynthesis should, therefore, be based on this wave-length. The value of $Nh\nu$ at 4175A is 68,117 calories and there is no integral relationship between this amount of energy and the 112,167 calories known to be needed to promote photosynthesis. Since it has previously been shown that the photoconversion of hydrated carbon dioxide into formaldehyde should have a quantum efficiency of unity, the difficulty of explaining the production of formaldehyde in a single photochemical reaction appears to be insuperable.

More recently R. H. Dastur and his colleagues [19] have measured the rate of photosynthesis of carbohydrates in the leaves of plants when irradiated with lights of different spectral composition. They proved in the first place that in leaves irradiated with the light from an ordinary electric filament lamp the amount of carbohydrate photosynthesized was less than the amount photosynthesized in leaves irradiated with daylight of equal intensity. They proved in the second place that the low photosynthetic activity of artificial lights is due to the relatively low intensity of the blue-violet rays present in them.

By the use of daylight and of light from various lamps which differed in the amount of blue-violet rays they emitted, Dastur and his colleagues proved that, when the total intensity of the light falling on the leaves was maintained constant, the amount of carbohydrate photosynthesized increased as the ratio of the intensity of the blue-violet rays to the intensity of the red rays increased. The amounts of carbohydrates photosynthesized by the different lights were in the order: sunlight > arc lamp > 'daylight' lamp > ordinary electric filament lamp. They thus proved that the maximum photosynthetic efficiency is obtained with sunlight in which the intensities of the red and blue-violet rays are equal.

This discovery that both red and blue-violet rays are essential to photosynthesis is one of outstanding importance. It proves that the primary photosynthetic process, instead of being a single photochemical reaction as has hitherto been assumed, must consist of two photochemical reactions, one of which is promoted by blue-violet light and the other by red light. An

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[19] Dastur and Samant, *Ann. Bot.*, 1933, *47*, 295; Dastur and Mehta, *ibid.*, 1935, *49*, 809.

entirely new vision of the problem has thus come to us and, if these two photochemical reactions can be formulated and if the sum of one energy quantum of blue-violet light absorbed in one of these and one energy quantum of red light absorbed in the other equals the total amount of energy needed for the assimilation of 1 molecule of hydrated carbon dioxide, the major difficulty of photosynthesis will be solved.

Attention may next be directed to the influence of the external concentration of carbon dioxide on the rate of photosynthesis when the temperature and light intensity are maintained constant. This was also investigated by Warburg,[20] who irradiated suspensions of *Chlorella* in solutions of known amounts of sodium carbonate and sodium bicarbonate at 25°. The concentration of carbon dioxide in these solutions was calculated from the concentrations of the two salts. Warburg's measurements are given in Table IV.

TABLE IV

CO_2 concentration in grm. mole per litre	CO_2 assimilated	CO_2 concentration in grm. mole per litre	CO_2 assimilated
$0{\cdot}53 \times 10^{-6}$	29	$9{\cdot}8 \times 10^{-6}$	178
$1{\cdot}0 \times 10^{-6}$	47	23×10^{-6}	202
$2{\cdot}6 \times 10^{-6}$	120	43×10^{-6}	214
$5{\cdot}3 \times 10^{-6}$	144	91×10^{-6}	242

The relation between the rate of photosynthesis and the external carbon dioxide concentration is shown by the curve in Fig. 4. This curve indicates that when the external concentration of carbon dioxide is small the rate of photosynthesis is proportional to the concentration. When the concentration of carbon dioxide exceeds $2{\cdot}3 \times 10^{-5}$ grm. mole per litre, the relation again becomes linear.

Similar results were obtained by Harder,[21] who made four series of measurements with different intensities of light and found that as the intensity was increased the ratio of the increase in the rate of photosynthesis to the increase in carbon dioxide concentration became greater.

There now remains outstanding the one phenomenon characteristic of photosynthesis which transcends all others in the

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[20] *Biochem. Zeitsch.*, 1919, *100*, 230.

[21] *Jahr. wiss. Bot.*, 1921, *60*, 531.

difficulty of its explanation. The carbohydrates photosynthesized in living plants are invariably optically active, and indeed in every species it is invariably the same optical isomer which is photosynthesized. Thus in the monocotyledons the first product of photosynthesis is *d*-glucose, whilst in the dicotyledons the first product appears to be a starch which subsequently is hydrolysed by an enzyme into *d*-hexoses. No explanation of this phenomenon has been advanced and none of the observations which have been made with the living plant enable us to formulate a mechanism which must result in asymmetric photosynthesis. The phenomenon has a significance which extends far beyond photosynthesis since the

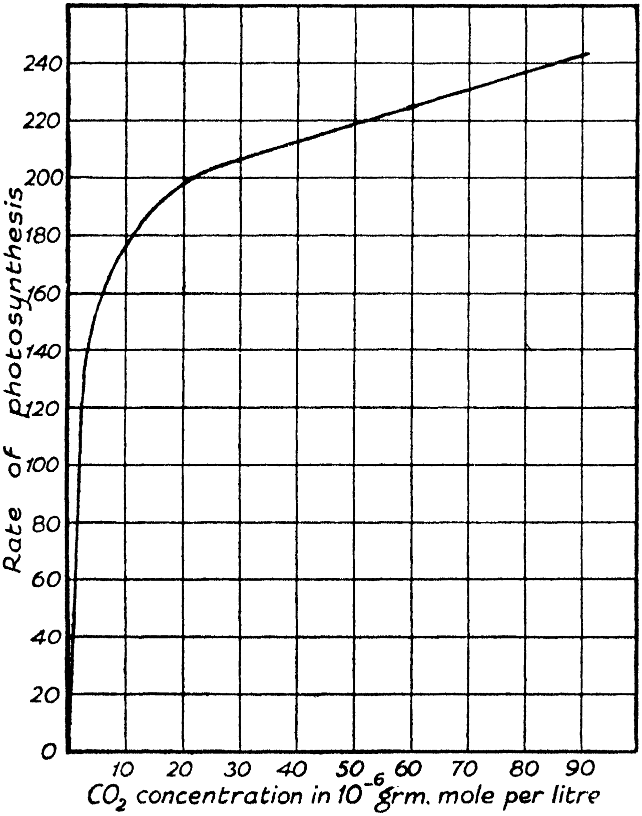

FIG. 4

optical bias first established in the leaves of plants persists in the whole gamut of vital chemistry in animal and man.

In this chapter a few selections have been made from the very many observations which have been recorded in the literature of photosynthesis. When considered singly, each of these presents a problem the explanation of which is fraught with difficulty. When considered as a whole, they engender wonder at the mystery which has for so many years lain beyond the power of man's understanding.

CHAPTER II

EARLY INVESTIGATIONS

1. THE ACTION OF ULTRA-VIOLET LIGHT ON AQUEOUS SOLUTIONS OF CARBON DIOXIDE

IN discussing the selected experimental observations of photosynthesis in the preceding chapter it was established beyond all reasonable doubt that the primary photochemical process is the synthesis of the carbohydrates from hydrated carbon dioxide through the intermediate formation of formaldehyde. It becomes evident from this, that the primary process of photosynthesis must consist of two separate and consecutive reactions, namely, the photochemical conversion of hydrated carbon dioxide into formaldehyde and oxygen, and the subsequent polymerization of the formaldehyde into a carbohydrate. Although the truth of this conclusion appears to be established, it cannot be accepted without experimental evidence in its support. Complete confidence in this explanation of the mechanism of photosynthesis can only be established by convincing proof that each of the two reactions can be achieved in the laboratory.

The story may first be told of the investigations which have been carried out on the action of light upon aqueous solutions of carbon dioxide. This story is not without interest, owing to the fact that in the early stages entirely contradictory results were obtained by various workers in this field. Usher and Priestley [22] were the first to obtain positive evidence of the formation of formaldehyde when pure aqueous solutions of carbon dioxide were irradiated with ultra-violet light from a quartz mercury lamp. The following paragraph may be quoted from their publication :

> Two transparent quartz tubes, of about 20 c.c. capacity, were filled

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[22] *Proc. Roy. Soc.*, 1911, *84*B, 101.

with the purest conductivity water obtainable, and the tubes were inverted in a trough of mercury and placed symmetrically near the mercury lamp. The water in one of the tubes was as nearly as possible gas-free, and a few cubic centimetres of carbon dioxide were introduced into the other. Both tubes were illuminated for about 12 hours, and the contents of each were then examined for the presence of formaldehyde and hydrogen peroxide. The solution of carbon dioxide was found to contain an easily recognisable quantity of formaldehyde, most of which was in a polymerised form, whereas none, either free or polymerised, could be detected in the water from the other tube. Traces of hydrogen peroxide were found in both. All the reagents used were carefully tested, and negative results were obtained with a solution of carbon dioxide which had not been exposed to ultra-violet light.

Usher and Priestley used Schryver's method of testing for formaldehyde, which is carried out as follows [23] :—2 ml. of a freshly prepared and filtered 2 per cent solution of phenylhydrazine hydrochloride are added to 10 ml. of the solution under test, after which 1 ml. of a freshly prepared 5 per cent solution of potassium ferricyanide is added. On the addition of 5 ml. of concentrated hydrochloric acid to the mixture a brilliant red colour is developed when the original liquid contained formaldehyde. This test is exceedingly sensitive and the presence of one part of formaldehyde in a million parts of water can easily be recognized.

Usher and Priestley's results carried conviction, but not long after their publication Spoehr,[24] on repeating their work, found that he was unable to obtain any evidence of the formation of formaldehyde. Various types of quartz mercury lamps were used and the pressure of the carbon dioxide, the temperature, the distance from the lamp and the period of irradiation were varied, but the results were completely negative. Similar negative results were obtained by other investigators, for example, by Coehn and Sieper,[25] Bauer and Rebmann,[26] and Bauer and Büchi.[27]

Some very interesting results were obtained by Moore and Webster,[28] who found that aqueous solutions of carbon dioxide,

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[23] *Proc. Roy. Soc.*, 1910, *82*B, 226. [24] *Biochem. Zeitsch.*, 1913, *57*, 110.

[25] *Zeitsch. phys. Chem.*, 1916, *91*, 347.

[26] *Helv. chim. Acta*, 1922, *5*, 828. [27] *Ibid.*, 1923, *6*, 959.

[28] *Proc. Roy. Soc.*, 1914, *87*B, 163 ; *Biochemistry*, pp. 38 to 52, Edward Arnold, London, 1921.

containing either colloidal uranium hydroxide or colloidal ferric hydroxide, after irradiation by direct sunlight for some days, contained appreciable quantities of formaldehyde. The colloidal uranium oxide was prepared by the following method. A saturated solution of ammonium carbonate was added to a 10 per cent solution of uranyl nitrate until the precipitate just ceased to re-dissolve, and then the solution was filtered and dialysed for several days. Moore laid stress upon the necessity of using the correct amount of ammonium carbonate and the advisability of continuing the dialysis until small traces of crystalloidal uranium still remained in solution. The stock solution of colloidal uranium hydroxide was diluted until the colloid content was only 0·035 to 0·024 per cent. Portions of this dilute solution were saturated with carbon dioxide and then sealed up in glass tubes and exposed to direct sunlight for two days. The tubes were then opened and about two-thirds of their contents were distilled off. The distillate gave in each case a marked positive reaction for formaldehyde. Moore and Webster proved that direct sunlight was necessary to promote the reaction, negative results being obtained when the tubes were irradiated by bright diffused daylight.

Identical results were obtained with colloidal ferric hydroxide which was prepared by the same method. Moore and Webster also found that the amount of formaldehyde obtained was greater when silica tubes were used, and this observation indicated that the active light rays were partly absorbed by glass.

Those of us who saw these experiments in Liverpool and discussed their significance with Moore were convinced by the evidence that formaldehyde was produced under the foregoing conditions, but Spoehr and Bauer and Rebmann, in the papers previously referred to, stated that they were unable on repeating Moore and Webster's work to obtain any evidence of the formation of formaldehyde.

My own interest in the whole problem was aroused by Professor I. M. Heilbron in 1920, who suggested that with the assistance of W. F. Barker we should investigate the action of short-wave ultra-violet light on carbon dioxide in aqueous solution. The results which were obtained are of some interest because they and the criticism made of them led in the end to the solution of the problem.

It was found at the outset that when conductivity water which had previously been saturated with carbon dioxide was irradiated in quartz tubes with the light from a quartz mercury lamp, no evidence of the formation of formaldehyde could be obtained. If, however, carbon dioxide were passed continuously through the water during the irradiation, definite evidence of the formation of formaldehyde was obtained, and this observation was many times confirmed.[29]

Now it is well known that carbon dioxide absorbs light of very short wave-length in the region of 2100A and that formaldehyde absorbs light of longer wave-length in the region of 2900A. In addition to their investigations previously described, Moore and Webster [30] also found that fairly concentrated solutions of formaldehyde, after irradiation with the light of a quartz mercury lamp, reduced Benedict's solution, and they concluded from this that reducing sugars were produced.

It is to be expected from this that when a solution of carbon dioxide is irradiated with light which contains rays of wave-lengths 2100A and 2900A, there will first be formed formaldehyde and then reducing sugars. Since it is known that even in the presence of some oxygen reducing sugars are broken down into formaldehyde by very short wave-length light, we concluded that when an aqueous solution of carbon dioxide is irradiated with the light from a quartz mercury lamp an equilibrium condition or photostationary state is established, which in all probability can be represented by

$$\begin{tikzcd}
\text{Sugar} \arrow[r] & \text{hydrated carbon dioxide} \arrow[d] \\
 & \text{formaldehyde} + \text{oxygen} \arrow[ul]
\end{tikzcd}$$

In our published paper the oxygen present in the photostationary state was omitted in error.

This photostationary state under normal conditions will lie very far over to the side of the hydrated carbon dioxide, with the result that the amounts of formaldehyde and reducing sugar present at any instant will be very small. We proved the presence of formaldehyde by distilling off about one-fifth

[29] *J. Chem. Soc.*, 1921, *119*, 1025.

[30] *Proc. Roy. Soc.*, 1918, *90*B, 168.

of the solution after irradiation and testing the distillate by Schryver's method, and its presence was invariably proved. The amount of the reducing sugars present in the photostationary state was insufficient to give any visible sign of reduction when the irradiated solution was boiled with Benedict's solution.

Now there are two methods whereby the concentration of formaldehyde in the photostationary state can be increased. The first method is to add to the solution before irradiation a substance which transmits the rays of wave-length 2100A and absorbs the rays with wave-length 2900A. Since the latter rays promote the polymerization of formaldehyde to reducing sugars, the amount of formaldehyde present in the irradiated solution should be increased. It is, of course, necessary that the substance used does not interfere with the photostationary state in any chemical sense. We found that paraldehyde fulfils these conditions. We first proved that paraldehyde in aqueous solution gives no formaldehyde when irradiated with the light from a quartz mercury lamp. We also proved that a solution of formaldehyde containing paraldehyde is not affected by that light, the formaldehyde being then completely protected. Finally, we proved that a solution of paraldehyde through which carbon dioxide was continuously passed gave after irradiation with the light from a quartz mercury lamp a marked positive reaction for formaldehyde.

The second method of increasing the concentration of formaldehyde is to increase the concentration of hydrated carbon dioxide in the irradiated solution, and this may be effected by the addition of potassium bicarbonate, which in solution is partly hydrolysed to potassium carbonate, carbon dioxide, and water. A similar increased concentration of hydrated carbon dioxide exists in saturated solutions of calcium and magnesium bicarbonates. We irradiated each of these three solutions with the light from a quartz mercury lamp and in each case obtained a strong reaction for formaldehyde, stronger, indeed, than was obtained with colloidal uranium hydroxide or colloidal ferric hydroxide as used by Moore and Webster.

In spite of the increased concentration of hydrated carbon dioxide used in these experiments, the amount of reducing sugar present in the photostationary state was insufficient to give a visible precipitate of cuprous oxide when the irradiated solution was boiled with Benedict's solution. In the course of these investigations we confirmed the discovery made by Moore and Webster that fairly concentrated solutions of formaldehyde, after irradiation with the light from a quartz mercury lamp, contained sufficient sugar to give a marked reduction when boiled with Benedict's solution. We also confirmed the fact that the concentration of formaldehyde in the irradiated solution must exceed a minimum value in order to obtain positive evidence of the formation of the reducing sugar. This observation suggested a method of proving whether a reducing sugar is a component of the photostationary state. It is evident that, if the concentration of formaldehyde normally present in the photostationary state be increased, the amount of reducing sugar in the photostationary state will be increased. Let the case be considered of the irradiation of a solution of potassium bicarbonate to which a small quantity of formaldehyde has been added, the resulting formaldehyde concentration being far less than the minimum value required to give a positive reaction for a reducing sugar when irradiated in the absence of carbon dioxide. If such a solution, after irradiation with the light from a quartz mercury lamp, gives a marked reduction when boiled with Benedict's solution, the presence of a reducing sugar in the photostationary state will be fully established. The evidence we obtained was convincing. Solutions of potassium bicarbonate containing small quantities of formaldehyde were irradiated for a few hours with the light from a quartz mercury lamp, and in every case sufficient reducing sugar was produced to give a strong reduction of Benedict's solution. As the result of this investigation Heilbron, Barker, and I were fully convinced that the evidence obtained proved that a reducing sugar can be photosynthesized from hydrated carbon dioxide through the formation of formaldehyde as an intermediate stage.

During the course of some further investigations involving the irradiation of solutions in large quartz tubes with ultraviolet light which were carried out after the above results were published, an interesting phenomenon was observed and it is relevant to mention this. My colleagues and I had met with great difficulty in understanding why we were unable to repeat certain observations which had certainly been made some months previously. One night it happened that when one of my colleagues was carrying a rack holding eight large quartz test-tubes down a dark stone staircase he slipped, and the result was that the tubes were broken into small fragments. He noted that as each tube fell on the stone steps its fracture was accompanied by the emission of a bright green phosphorescence. As the result of this fortunate accident we were able to prove that vitreosil tubes after long irradiation by the rays of the quartz mercury lamp become opaque to short wavelength light and at the same time develop a faint pink colour. Furthermore, we found that these tubes, when heated in a blast flame, emit a brilliant green phosphorescence and recover their original transparency. After this discovery had been made we met with no further difficulty in reproducing previous observations.

In the early part of 1924 I received a courteous letter from Professor C. W. Porter of the University of California, in which he told me that after taking stringent precautions against the presence of impurities he was quite unable to obtain any evidence of the formation of formaldehyde when carbon dioxide and water were irradiated with the light from a quartz mercury lamp. In reply I wrote that I could not understand his failure to reproduce our results in which I had complete confidence, and suggested as a possible explanation that his quartz vessels had become opaque to ultra-violet light as the result of prolonged irradiation.

Once again history had repeated itself. Usher and Priestley's pioneer results were combated by Spoehr and by others. On the present occasion our results were combated by Porter, and the complete discrepancy was considered to be of sufficient importance to justify a discussion of the impasse which had been reached.

It happened that in 1924 the British Association of Science had decided to hold their annual meeting in Canada, and Sir Robert Robertson, the President of Section B, arranged that at a meeting of that section at Saskatoon, Porter and I should each give an account of our work. The proceedings at the meeting were opened by a description of the Liverpool investigations, and I was followed by Porter, who gave an account of the experiments which had been carried out by himself in conjunction with H. C. Ramsperger.[31] In the preliminary experiments a mixture of carbon dioxide and water vapour was passed through a quartz tube which was irradiated with the light from a quartz mercury lamp. Rubber tubing and stopcocks were used, and in many cases measurable quantities of formaldehyde were obtained. In every case in which the irradiated gases came into contact with rubber tubing or the stopcock lubricant, small and variable quantities of formaldehyde were obtained. In later experiments the quartz tube used for the irradiation was sealed to pyrex glass by means of graded glass to quartz seals, the water vapour was obtained from conductivity water, and the carbon dioxide was prepared from pure sodium bicarbonate. After being irradiated, the mixed gases were passed through water, and this water, when tested with Schryver's reagent for formaldehyde, gave completely negative results. In a third series of experiments a mixture of carbon dioxide and water vapour was passed back and forth through an irradiated quartz tube. Two 300-ml. glass bulbs were joined at their lower ends by means of capillary tubing and at their upper ends they were connected through the quartz tube by means of graded glass to quartz seals. The two bulbs were filled with conductivity water and then half the water was displaced by pure carbon dioxide. The mixture of water vapour and carbon dioxide was caused to pass back and forth through the irradiated quartz tube by tilting the whole apparatus first in one direction and then in the other. The irradiation was continued for 45 hours, the apparatus being tilted ninety times during that period. The water was tested with Schryver's reagent after the irradiation and gave completely negative results.

Finally, a saturated aqueous solution of carbon dioxide which had been prepared with the same extreme care was irradiated in a sealed quartz tube, and once again it was proved that no formaldehyde was produced.

Now the negative results obtained by Porter and Ramsperger do not appear to contravene in any way the positive results obtained in Liverpool. In the first place the photostationary state, the existence of which in solution we claimed

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[31] *J. Amer. Chem. Soc.*, **1925**, *47*, **79.**

to have proved, cannot exist in a gaseous system, and consequently formaldehyde cannot be produced by the irradiation of a mixture of carbon dioxide and water vapour. In the second place, in Porter's experiments, in which the gaseous mixture was passed backwards and forwards through an irradiated quartz tube, the aqueous solution of carbon dioxide was contained in pyrex glass bulbs and thereby screened from the short-wave ultra-violet light. Formaldehyde, therefore, could not be produced by this method. In the third place, the fact that Porter found that no formaldehyde was produced when a saturated aqueous solution of carbon dioxide was irradiated in a sealed quartz tube confirms the result obtained in Liverpool, because we definitely stated in our communication that no formaldehyde was present in saturated aqueous solutions of carbon dioxide after irradiation, unless the gas had been passed continuously through the solution.

At the Saskatoon meeting Porter stressed the possible significance of his observation that in every case in which the irradiated mixture of carbon dioxide and water vapour had come into contact with the rubber tubing or stopcock lubricant, small and variable quantities of formaldehyde were obtained. There appeared to exist, however, three very good reasons why our Liverpool results could not have been due to contamination with extraneous organic matter. For example, if our solutions of carbon dioxide were contaminated, why was no formaldehyde formed when water through which carbon dioxide had been passed for several hours was subsequently irradiated ? Then again, how could contamination explain the greatly increased yield of formaldehyde we obtained when solutions of potassium bicarbonate were irradiated ? Finally, it is not possible to believe that the complete discrepancy between the positive results obtained by Usher and Priestley and the negative results obtained by Spoehr and by others can be explained on the supposition that Usher and Priestley were the only experimenters to use contaminated solutions. In spite of these arguments, there is no doubt that as the result of Porter's communications to the Saskatoon meeting, many held the opinion that the validity of the Liverpool results, and incidentally those of Usher and Priestley, had been called into question.

On my return to Liverpool the situation was reviewed and the definition of the photostationary state present in an irradiated aqueous solution of carbon dioxide was critically examined from the point of view of the energy relations involved. It was found that there were certain lacunae in the argument on which our formulation of the photostationary state had been based. It is known that carbon dioxide in aqueous solution, that is to say hydrated carbon dioxide, absorbs light of very short wave-length, namely 2100A, and hence the value of *Nhν* for a gram molecule of hydrated carbon dioxide is about 135,600 calories. It has already been shown that the conversion of 1 grm. mole of hydrated carbon dioxide into glucose involves an energy intake of 112,167 calories, and hence the polymerization into glucose of the formaldehyde produced by the action of ultra-violet light on hydrated carbon dioxide is exothermic to the extent of 135,600 − 112,167 = 23,433 calories. It may be concluded from this that activated and not ordinary formaldehyde is produced by the irradiation and that the recognizable product of the irradiation is glucose. The correct definition, therefore, of the photostationary state is

$$CO_2H_2O \rightleftharpoons \tfrac{1}{6}C_6H_{12}O_6 + O_2$$

Now it is well known that in the absence of oxygen, or when only a small quantity of oxygen is present, glucose is decomposed into formaldehyde by ultra-violet light. As will be shown in Chapter III, it has been proved that formaldehyde in aqueous solution is converted by ultra-violet light into glucose. It follows from this that if the oxygen concentration in the above photostationary state be reduced by any means, a secondary photostationary state between glucose and formaldehyde will be established. It must be emphasized that so long as the concentration of oxygen is that shown in the above expression of the photostationary state no ordinary formaldehyde will be formed. If the concentration of the oxygen be reduced below that concentration, a secondary photostationary state will be established, namely

$$\tfrac{1}{6}C_6H_{12}O_6 \rightleftharpoons CH_2O$$

It will be realized from this argument that the complete absence of formaldehyde in an irradiated solution of hydrated carbon dioxide in no way disproves the photosynthesis of glucose through the intermediate formation of activated formaldehyde.

This new definition of the equilibrium conditions established when an aqueous solution of carbon dioxide is irradiated with ultra-violet light gives a complete explanation of the Liverpool observations. In the first place, it was proved that no ordinary formaldehyde is formed when a saturated aqueous solution of carbon dioxide is irradiated with ultra-violet light in a closed quartz vessel. In this case no oxygen escapes from the system and hence the condition established is that of the primary photostationary state.

In the second place, it was proved that ordinary formaldehyde was formed when carbon dioxide was passed continuously through the solution during the irradiation. The oxygen content of the solution was thereby reduced and thus the secondary photostationary state between glucose and ordinary formaldehyde was established, with the result that formaldehyde was found in the solution after irradiation. It is particularly to be noted that the amount of ordinary formaldehyde present depends on the rate of removal of the oxygen. It is obvious that the amount of formaldehyde present will be greater, the greater is the rate at which carbon dioxide is passed through the irradiated solution. It is very possible that the negative results obtained by Spoehr in repeating our experiments were due to the fact that the rate at which the carbon dioxide was passed was not sufficient to produce a recognizable amount of formaldehyde.

In the third place, the irradiation of a solution of a soluble bicarbonate will cause an increase of the amount of glucose present in the photostationary state, with the result that a decrease in the oxygen concentration by means of a stream of bubbles of carbon dioxide will increase the amount of ordinary formaldehyde present in the secondary photostationary state. This, as already stated, was observed by Heilbron, Barker, and myself.

Now there is another factor the possible effect of which has not been discussed. It is well known that dust nuclei are invariably present in water unless special and elaborate methods have been adopted to remove them. If dust nuclei be present, they will adsorb hydrated carbon dioxide and the glucose will be photosynthesized on their surface. This condition will undoubtedly result in the formation of more ordinary formaldehyde than in a homogeneous system. It is possible that the discrepancies such as the positive results obtained by Usher and Priestley and the negative results obtained by other observers, are to be explained by the presence of greater and smaller numbers of dust nuclei.

The importance of the conclusions reached in this discussion of the photostationary state may be emphasized. The presence or absence of recognizable quantities of ordinary formaldehyde in solutions of hydrated carbon dioxide which have been irradiated with the light from a quartz mercury lamp is due to a photostationary state between glucose and formaldehyde, which is secondary to the photostationary state between hydrated carbon dioxide and glucose. The amount of ordinary formaldehyde present in the secondary photostationary state is inversely proportional to the oxygen concentration and thus depends on the rate at which carbon dioxide is passed through the irradiated solution. It is also probable that the amount of formaldehyde formed depends on the number of dust nuclei present. Finally, the absence of recognizable amounts of formaldehyde in an irradiated solution of carbon dioxide in no way disproves the photosynthesis of glucose from hydrated carbon dioxide through the intermediate formation of activated formaldehyde.

The conclusions thus reached indicated to us the necessity of obtaining experimental proof of the photostationary state

$$CO_2H_2O \rightleftharpoons \tfrac{1}{6}C_6H_{12}O_6 + O_2$$

It occurred to us [32] that the best method to adopt would be to irradiate an aqueous solution of carbon dioxide for a short time and then to add to it, while still being irradiated, a reagent for aldehydic compounds. This reagent must necessarily contain only inorganic compounds, and it was found that Feder's [33] solution was well suited for the purpose. This reagent, which contains mercuric chloride, sodium hydroxide,

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[32] Baly, Davies, Johnson and Shanassy, *Proc. Roy. Soc.*, 1927, *116*A, 197.

[33] *Arch. Pharm.*, 1907, *245*, 25.

and sodium thiosulphate, is reduced by all aldehydes and is very sensitive to formaldehyde. Two stock solutions were prepared, namely a 4 per cent solution of mercuric chloride and a solution containing 16 per cent sodium hydroxide and 20 per cent sodium thiosulphate. Feder's reagent is prepared by mixing equal volumes of these two solutions.

In each experiment 80 ml. of the mercuric chloride solution contained in a large transparent quartz test-tube were irradiated with the light from a quartz mercury lamp, pure carbon dioxide being continually passed through the solution. After the irradiation had been continued for 1 hour, 40 ml. of the solution were transferred in the light from the lamp to a second quartz test-tube, which was placed in the dark, and the irradiation of the remaining 40 ml. of the solution was continued. After an interval of 15 minutes 40 ml. of the alkaline thiosulphate solution were added to each of the quartz test-tubes, the one in the light and the one which had been kept in the dark. A very marked reduction took place in the irradiated solution, whilst in the tube which had been kept in the dark after irradiation the mixture remained perfectly transparent and colourless.

Blank experiments were then carried out in which the procedure was identical with that described, except that in each case the mercury chloride solution was completely deprived of carbon dioxide before it was irradiated. Considerable difficulty was at first experienced in preparing a solution of mercury chloride which contained no carbon dioxide, but the following method was finally found to be efficacious. A transparent quartz test-tube, 9 in. long and 1 in. wide, was obtained which was fitted with an accurately ground glass stopper with a tube containing soda-lime sealed to it. The quartz tube was almost completely filled with conductivity water and 1·6 grm. of mercury chloride was added. The solution was then heated to the boiling point and kept in gentle ebullition until the volume had been reduced to 40 ml., an operation which took about 1½ hours. The glass stopper and its soda-lime attachment were then placed in position, no lubricant being used, and the apparatus allowed to cool to room temperature. The cold CO_2-free solution of mercuric chloride was then irradiated with the light from a quartz mercury lamp for 1 hour, after which the glass stopper was removed from the quartz tube and 40 ml. of the alkaline thiosulphate solution were immediately added, the whole operation being carried out in the light of the quartz mercury lamp. No evidence whatever of reduction was obtained, the mixed solutions remaining perfectly transparent and colourless. These two experiments were repeated many times by independent observers, and on every occasion identical results were obtained.

These results undoubtedly proved that a photostationary state is established when an aqueous solution of carbon dioxide is irradiated by the rays from a quartz mercury lamp and that this photostationary state no longer exists after the irradiation has ceased. The reduction of the Feder's reagent further proved that one of the components of the photostationary state is an organic aldehyde.

Now when Feder's reagent is added to a very dilute solution of formaldehyde there is formed a finely divided grey precipitate of metallic mercury. When this reagent, however, was added to the irradiated aqueous solution of carbon dioxide there was formed a very flocculent brown precipitate which was entirely different from the finely divided grey precipitate given by formaldehyde. This undoubtedly proved that ordinary formaldehyde is not the organic component of the photostationary state and thus supports the conclusion reached from the consideration of the energy relations. Since this conclusion was that a hexose is the organic component of the photostationary state and since the experimental evidence indicated that the organic component is an aldehyde, we were led to examine the product of the reduction of glucose by Feder's reagent. An aqueous solution of glucose was prepared and allowed to remain for some hours until mutarotation was complete. On the addition of Feder's reagent the reduction product was a very flocculent brown precipitate identical in appearance and properties with that obtained when the reagent was added to the irradiated solution of carbon dioxide. The experimental evidence, therefore, supports the conclusion indicated by the energy relations, namely, that glucose is the component of the photostationary state.

The qualitative evidence thus obtained of the presence of glucose as a component of the photostationary state led us to visualize the possibility of photosynthesizing glucose by the irradiation of a saturated aqueous solution of carbon dioxide in the presence of a reducing agent which would remove the oxygen component of the photostationary state. Several experiments were carried out, but the results were disappointing owing to the reducing agents used being unsatisfactory. When we had almost lost hope of achieving success by this method, Professor C. O. Bannister told me that he had found that metallic iron in the presence of carbon dioxide and water rusted very rapidly when irradiated with ultra-violet light and asked me if I could explain this observation. A very probable explanation based on our results at once suggested itself, namely, that the action of ultra-violet light established the photostationary state and that ferrous bicarbonate was oxidized by the oxygen component of that state in accordance with the equation

$$4Fe(HCO_3)_2 + 2H_2O + O_2 = 4Fe(OH)_3 + 8CO_2$$

In fact, Bannister's discovery indicated that ferrous bicarbonate was the ideal reducing agent for us to use in the photosynthesis of glucose by the irradiation of aqueous solutions of carbon dioxide with the light from the quartz mercury lamp. It must be confessed that the use of ferrous bicarbonate as the reducing agent had not previously occurred to us and experiments were at once undertaken to determine whether the above conclusion, based on Bannister's discovery, was correct.

A rod of pure Swedish iron (99·8 per cent) was placed in each of eight 9 × 1 in. quartz test-tubes which were then filled with pure conductivity water. Carbon dioxide was then passed through the tubes until the water became saturated with respect to ferrous bicarbonate. This solution, when protected from the air, was colourless and quite stable in daylight.

The eight quartz test-tubes and their contents were then arranged round the mercury lamp and irradiated, and it was then observed that ferric hydroxide was slowly formed. After the irradiation had been continued for 2 hours, the liquid contents of the eight tubes were mixed and air was passed through until the whole of the excess of ferrous bicarbonate had been oxidized. The filtered solution, which was proved to contain no formaldehyde, was then concentrated to a small bulk under reduced pressure on a steam bath. The concentrated solution was found to contain a substance which reduced Benedict's solution and a small quantity of an acid which gave an unstable silver salt. The evidence thus obtained of the photosynthesis of a reducing sugar was very promising and the experiment was repeated many times, but in each case the amount of the reducing sugar produced was very small.

The complete absence of formaldehyde in the solutions after irradiation was interesting, since its formation by the photolysis of the photosynthesized reducing sugar might have been expected. The explanation was that the ferrous bicarbonate in solution absorbed the light rays which promote the photolysis and thus protected the reducing sugars which had been photosynthesized.

During these experiments a very interesting and important phenomenon was observed. The ferric hydroxide produced during irradiation was not formed in the body of the solution, this remaining transparent during the whole of the irradiation. The greater proportion of the ferric hydroxide was formed as a very coherent layer on the irradiated sides of the quartz test-tubes, a small amount being formed on the irradiated sides of the iron rods. Incidentally it was found that the ferric oxide layer formed on the irradiated walls of the quartz tubes was exceedingly difficult to remove and some of the tubes were thereby rendered useless. Since ferric hydroxide absorbs the ultra-violet rays from the quartz mercury lamp, the formation of a layer of this substance on the walls of the quartz test-tubes prevented any further photosynthesis taking place. This was proved by the irradiation of saturated solutions of carbon dioxide and ferrous bicarbonate in quartz test-tubes which had been coated with ferric oxide in previous irradiations, no evidence whatever of the photosynthesis of reducing sugars then being obtained. This is the explanation of the fact that the yields of photosynthesized reducing sugars in the previous experiments were small.

The formation of the strongly adsorbed layer of hydrated ferric oxide on the walls of the quartz tubes during the irradiation was of great physical significance, because it indicated to us that the photosynthesis of carbohydrates was taking place on a surface. The possibility of achieving photosynthesis by a photochemical surface reaction had not previously been envisaged and the first recognition of the reality of this marked a very definite stage in the progress of the Liverpool investigation.

During the course of our experiments with ferrous bicarbonate additional evidence of the heterogeneous nature of photosynthesis was independently obtained by R. A. Morton and R. W. Riding in my laboratory. A fine-grade Pasteur-Chamberland filter tube was filled with carbon dioxide under several atmospheres pressure from a cylinder of the liquid gas. The filter tube was then immersed in conductivity water contained in a quartz tube and irradiated with the light from a quartz mercury lamp for 24 hours. On examination the water was found to contain 0·01 per cent of formaldehyde, and on evaporation it yielded a minute but definite amount of organic matter. This experiment was several times repeated with Pasteur-Chamberland tubes of different degrees of fineness and the same results were obtained. In these experiments a layer of hydrated carbon dioxide on the surface of the filter tubes was irradiated, and it was evident that a carbohydrate was photosynthesized, this product being subsequently decomposed to formaldehyde by the rays of very short wave-length, as was explained in the discussion on the photostationary state.

The recognition of the fact that photosynthesis is a photochemical surface reaction initiated a new line of investigation, and an account of this will be given in Chapter IV.

CHAPTER III

EARLY INVESTIGATIONS

2. THE POLYMERIZATION OF FORMALDEHYDE INTO GLUCOSE

IN the preceding chapter the action of ultra-violet light on aqueous solutions of carbon dioxide was discussed and evidence was given that a photostationary state is established, namely

$$CO_2H_2O \rightleftharpoons \tfrac{1}{6}C_6H_{12}O_6 + O_2$$

and that glucose is the carbohydrate component. It was also shown that the glucose must be produced through the intermediate formation of activated formaldehyde, 6 molecules of which polymerize into 1 molecule of glucose. Before this argument can be accepted as proven, evidence must be obtained that activated formaldehyde does polymerize into glucose. It is well known that formaldehyde in aqueous solution absorbs light in the region of 2900A, and the question to be answered is whether formaldehyde when activated by the absorption of light of this wave-length polymerizes into glucose. If evidence can be obtained of the presence of glucose in solutions of formaldehyde which have been irradiated with ultra-violet light, the final proof of the reality of the above photostationary state will be secured.

The first evidence of the formation of substances which reduce Benedict's solution when aqueous solutions of formaldehyde are irradiated with ultra-violet light from a quartz mercury lamp was obtained by Moore and Webster.[34] They proved that the reduction was caused by the products of the irradiation, no reduction being obtained when a formaldehyde solution was boiled with Benedict's solution. They found that the minimum concentration of formaldehyde needed to

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[34] *Proc. Roy. Soc.*, 1918, *90*B, 168.

obtain evidence of the formation of the reducing compounds was about 2 per cent and that the maximum rate of formation was obtained when the concentration was 5 per cent and greater than 5 per cent. They noted that the rate of polymerization was retarded by the presence of acid and accelerated when the solutions were alkaline. Moore and Webster did not identify the products of the polymerization, but were convinced that they were, at any rate in part, reducing sugars, that is to say, hexoses.

This photo-polymerization was investigated by Heilbron, G. V. Francis and myself, and Moore and Webster's observations were fully confirmed. We found that when a 40 per cent solution of formaldehyde was irradiated in large quartz test-tubes by means of a quartz mercury lamp, the initial rate of the formation of a compound which reduced Benedict's solution was not maintained and decreased with time until it became very small. This was proved to be due to the fact that owing to secondary processes induced by the ultra-violet rays the irradiated solution became progressively more acid in reaction. This was overcome by the addition from time to time of small amounts of Merck's pure crystalline potassium bicarbonate, and it was then found that the rate of formation of the reducing compound became constant.

As the result of this information a series of eight 9 × 1 in. quartz test-tubes, filled with 40 per cent aqueous formaldehyde, were arranged round a U-shaped quartz mercury lamp. In each tube was placed a small cooling tube through which cold water was passed and small quantities of potassium bicarbonate crystals were added to each at 12-hour intervals. The reducing power of the irradiated solution was determined twice each day, and it was then found that the initial constant rate of formation of the reducing compounds was not long maintained. The rate soon began to decrease, and it became zero when the reducing power was equal to that of a 5 per cent solution of glucose. When this stage had been reached the irradiation was stopped and the combined contents of the quartz tubes were submitted to the following treatment.

The solution was neutralized with dilute sulphuric acid and then concentrated under reduced pressure on a steam bath. The potassium sulphate which separated was removed by filtration and the filtrate was diluted with water and again concentrated under reduced pressure on a steam bath, this process being repeated until the whole of the excess of formaldehyde had been removed. The final concentrated product was a viscous amber-coloured liquid which had a reducing power equivalent to that of a 25 per cent solution of glucose.

It happened that at this stage of our investigation Sir James Irvine visited Liverpool for the conferment on him of the honorary degree of Doctor of Science by the University, and he took the opportunity of discussing with us the analytical examination of our photosynthesized syrup. Sir James suggested that he should carry out this examination at St. Andrews with the assistance of Francis, a suggestion which gave us more than a little gratification.

During the preliminary examination of the syrup at St. Andrews the method of irradiation of the 40 per cent formaldehyde was critically examined because it appeared to be capable of improvement. In the first place the fact that the rate of photo-polymerization of formaldehyde is retarded by acid and accelerated in the presence of potassium bicarbonate indicated the existence of an optimum *p*H which should be kept constant. In the second place, the method of irradiation of the formaldehyde was very wasteful of light energy, and this suggested the irradiation of a large volume of the 40 per cent solution by means of quartz mercury lamps entirely surrounded by the solution.

Some preliminary experiments in which the formaldehyde solution was irradiated in quartz test tubes showed that the maximum rate of photo-polymerization was obtained in the presence of precipitated magnesium carbonate. When precipitated calcium carbonate was substituted for the magnesium salt, the rate of photo-polymerization was somewhat slower.

The final form of the apparatus used is shown in Fig. 5 in plan and elevation. It consisted of a glass vessel 15 in. square and 8 in. deep. In each of the four sides was drilled a circular hole 2¼ in. in diameter with centre 2½ in. above the bottom of the vessel. By means of a rubber ring a vitreosil tube 8 × 2 in. was fixed in each hole and into each of these quartz tubes a U-shaped quartz mercury lamp was inserted. In each of the four corners of the glass vessel was a cooling coil of glass tubing held by means of corks in holes drilled in a plate-glass cover resting on the walls of the glass vessel.

In the first experiment 20 litres of 40 per cent aqueous formaldehyde were irradiated in the presence of an excess of precipitated magnesium carbonate and a remarkable result was obtained in that 48 hours after the irradiation had been commenced the four quartz tubes surrounding the mercury lamps had become completely coated with a solid layer of magnesium carbonate which was impervious to light. No method of overcoming this was found, and in later irradiations an excess of pure precipitated calcium carbonate was used. It was not found necessary mechanically to stir the solution during the irradiation, the convection currents created by the heat of the lamps being found to be sufficient. It was observed

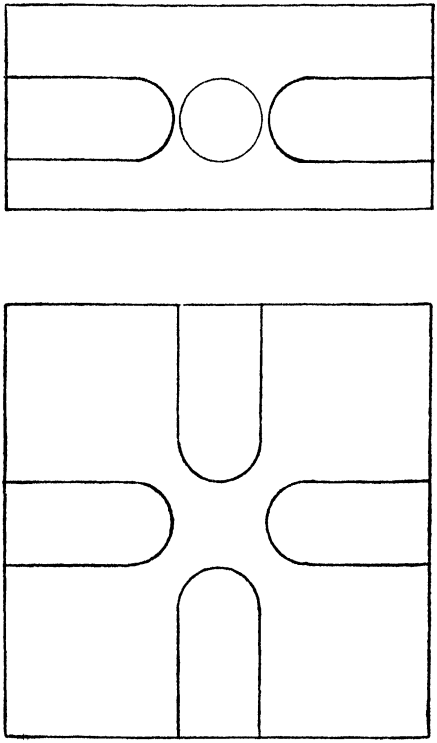

FIG. 5

that the optimum temperature was 35° and this was maintained by regulating the flow of water through the cooling coils.

An important factor in restricting the rate of photosynthesis was the formation of ozone by the action of the extreme short wave-lengths of light emitted by the quartz lamps on the air surrounding them in the quartz tubes. This formation of ozone was detrimental for two reasons. In the first place, ozone absorbs the same light rays as formaldehyde and hence its presence decreases the rate of the photo-polymerization. In the second place, the formation of ozone decreases the concentration of oxygen round the quartz lamps, with the result that some of the very short-wave light rays are transmitted, and these rays decompose the products of the photo-polymerization. The yield of these products was materially increased by a draught of air forced into the quartz tube, care being taken to guard against any decrease in efficiency of the quartz lamps being caused by cooling.

It was found that the products of the photo-polymerization are to a great extent decomposed by ultra-violet light, with the result that the maximum reducing power obtainable was equal to that of an 8 per cent solution of glucose. It was found that with 20 litres of 40 per cent formaldehyde solution this maximum reducing power could often be obtained after continuous irradiation for 14 days. The products of this photo-decomposition consisted partly of acids, one being glucuronic acid, and the presence of the calcium salts of these acids in the irradiated solutions had to be borne in mind in their treatment. This treatment proved to be a lengthy process and the most satisfactory method was devised by H. G. Littler.

The irradiated solution was concentrated under reduced pressure at 60° in a large steam-heated vacuum still. During this concentration it was necessary to add an excess of precipitated calcium carbonate in order to neutralize the formic acid produced by the formaldehyde undergoing the Cannizaro reaction.

$$2CH_2O + H_2O = HCOOH + CH_3OH$$

On the addition of absolute alcohol to the concentrated solution a great part of the calcium salts were precipitated and these were removed by filtration. The filtrate was then concentrated to as small a bulk as possible and then again mixed with absolute alcohol, this treatment being repeated several times.

Now the calcium salt of glucuronic acid is soluble in absolute alcohol and hence a special method was necessary for the removal of this salt. An equivalent quantity of zinc sulphate in aqueous solution was added, and after the precipitated calcium sulphate had been collected on a filter the filtrate was again concentrated under reduced pressure to a small volume. On the addition of several volumes of absolute alcohol zinc glucuronate separated and was removed by filtration. The filtrate was then once again concentrated, treated with several volumes of absolute alcohol and filtered, this process being repeated until all the zinc glucuronate had been removed.

The last stage of the process was the precipitation of the reducing compounds as a thick syrup by the addition of chloroform to their solution in absolute alcohol. After the removal of the chloroform from the precipitated syrup by warming under reduced pressure, the syrup was again dissolved in absolute alcohol and again precipitated by means of chloroform, this treatment being repeated until the syrup was completely free from formaldehyde.

The final product, which amounted to approximately 5,000 grm., was very thick and viscous. It was yellow to pale brown in colour, quite transparent and fluorescent. The reducing power varied with different preparations from 34 per cent to 25 per cent calculated as glucose. Most careful tests indicated the absence of pentoses and trioses. Every preparation had a marked sweet taste and a very pleasing flavour ; indeed, I found it necessary to protest against its continued use during afternoon tea in the laboratory. Quantities of this syrup were sent to St. Andrews and the following account of the result of its investigation is taken from that communicated by Irvine to the American Chemical Society during their annual meeting at Ithaca, N.Y., in 1924.[35] It is to be noted that the first quantity of syrup was that originally prepared by the irradiation in quartz test-tubes of 40 per cent formaldehyde containing potassium bicarbonate.

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[35] *Ind. Eng. Chem.*, **1924**, *16*, **1019.**

The material was a viscous amber-coloured liquid which readily reduced Fehling's solution. It contained a considerable amount of mineral salts and a variable quantity of water. In appearance and general properties the syrup resembled those obtained by condensing formaldehyde or trioxymethylene by means of dilute alkali, but the preliminary examination revealed the following irregular properties :

1. When heated, particularly with a trace of acid, rapid discoloration set in between 80° and 100°.
2. The reduction of Fehling's solution was equivalent to 35 per cent calculated as glucose, but this value diminished when the syrup had been heated with acid under conditions which would hydrolyse a glucoside or a polysaccharide. Part of the reducing power of the syrup was due to compounds other than sugars. A further deduction is that anhydro-sugars and polysaccharides of the type $C_6H_{10}O_5$ were absent.
3. No condensation with methanol took place in the presence of 0·5 per cent HCl at 15°, showing that ketoses were absent. In this respect the syrup differed sharply from α-acrose.
4. Alkyloxyl compounds were present and thus the original material gave an approximate methoxyl content varying from 9 per cent to 12 per cent. No doubt this was due to the fact that the formaldehyde had acted in part as a methylating agent. This view was confirmed by the observation that the alkyloxyl content was not diminished by alkali and that on heating the syrup under highly diminished pressure a small quantity of distillate was obtained which undoubtedly contained methoxyl compounds.

These observations introduced serious complications into the attempts to ascertain the analytical composition of this syrup.

The same irregular properties were discernible in further quantities of the syrup which had been subjected to repeated purification by precipitation from alcoholic solution by means of chloroform. The examination of this material, however, has provided evidence that compounds possessing the properties of hexoses are present to the extent of approximately 9·3 per cent.

EXAMINATION OF THE TOTAL CARBOHYDRATE CONTENT

It was desirable to ascertain not only the total material corresponding to the reducing hexoses, but also the amount, if any, of anhydro-hexoses ($C_6H_{10}O_5$) present either as such or in the form of polymerized aggregates.

4-grm. samples of the syrup were heated at 80° first under a pressure of 3 mm. and subsequently under a pressure of 0·02 mm. until the weight was constant. The apparent methoxyl content of the syrup had then been diminished to 6·3 per cent CH_3O and the reducing power had increased to 32 per cent calculated as glucose. The dried syrup was dissolved in methanol containing 0·25 per cent HCl and heated at 100° for 50 hours. The solution darkened at once, but this discoloration was not increased by continued heating. The colouring matter was removed by treatment with charcoal, and on working up the product in the usual manner a pale light-red syrup remained. After drying at 80° under a pressure of 10 mm., and thereafter at 80° under a pressure of 0·2 mm., it was ascertained that (*a*) the methoxyl content had increased from 6·8 per cent to 12 per cent, and (*b*) the reducing power had decreased from 32 per cent to 5·5 per cent.

The increase in methoxyl content due to glucoside formation is thus 5·2 per cent, whereas an increase of 16 per cent is required if the syrup examined is entirely $C_6H_{12}O_6$ or $C_6H_{10}O_5$. In other words, the amount of glucoside formed corresponds with the condensation of 32·5 per cent of glucose, whereas the titration results indicate that only 26·5 per cent of sugar took part in the condensation. According to the change in the reducing power the methoxyl content should have increased to the extent of 4·2 per cent, whereas an increase of 5·2 per cent was actually obtained.

The increase in the methoxyl content is thus approximately consistent with the diminution in the reducing power, and consequently two important conclusions are reached:

1. Polysaccharides or anhydro-sugars are absent.
2. The amount of aldose sugar present cannot exceed 26·5 per cent.

EVIDENCE OF METHYLATION

The purified dried syrup was subjected to methylation with methyl sulphate under conditions that would convert the reducing component into the corresponding methyl glucoside. The total syrup soluble in chloroform amounted to one-third of the syrup taken and this was subjected to the silver oxide reaction and the product soluble in ether was then distilled under reduced pressure.

100 grm. of the syrup gave 20 grm. of distillate with a methoxyl content of 49 per cent. After two further methylations by the silver oxide method and rectification of the product, distillates were obtained as follows:

	Temperature	Pressure	Weight
Fraction I	100°–110°	0·23 mm.	9·0 grm.
Fraction II	110°–130°	0·20 mm.	2·5 grm.
Fraction III	130° +	0·20 mm.	1·5 grm.

In addition, a small first fraction (2 grm.) was collected which consisted of methyl α-hydroxypropionate.

Examination of Fraction I. This fraction was a colourless mobile liquid which was freely soluble in organic solvents. Its solubility in water was not complete, a turbid solution being obtained, and this showed that compounds other than methylated glucosides were present. The behaviour towards Fehling's solution was correct for a glucoside, except that the cuprous oxide tended to remain colloidal, and these properties, together with the fact that the index of refraction for sodium light (μ_D) was 1·4561 instead of 1·4464, showed that further purification was necessary.

The main fraction was then distilled twice, the middle fraction being retained in each case,

the second fractionation gave 4·8 grm., b.p. 90° to 98° at 0·14 mm. $\mu_D = 1{\cdot}4539$

the third fractionation gave 1·3 grm. $\mu_D = 1{\cdot}4509$.

This fractionated material behaved as a glucoside and contained 54·5 per cent of methoxyl instead of the calculated value of 62 per cent for a fully methylated glucoside. This result may be explained by the presence of glucosides of aldoses lower than hexoses, or by contamination with alkyloxyesters. The molecular weight by the boiling-point method in benzene was found to be 232 instead of 250.

HYDROLYSIS OF THE METHYL GLUCOSIDE TO GIVE A METHYLATED HEXOSE

This was carried out as usual by means of 8 per cent HCl at the boiling-point. The solution turned yellow and then red, this being an indication that pentoses were present. After filtering from a little resin that had formed, the solution was neutralized and then extracted from chloroform. The chloroform extract yielded an amber-coloured syrup with b.p. 105° at 0·10 mm. As will be seen from Table V, the general properties of this syrup were those of an alkylated hexose.

TABLE V

	Methylated syrup	Tetramethylhexose
μ_D	1·4592	1·4580
CH_3O	51·44 per cent	52·50 per cent
C	51·08 ,, ,,	50·81 ,, ,,
H	8·73 ,, ,,	8·54 ,, ,,

In calculating yields the most favourable assumption is that the total methoxyl content of the distilled material can be attributed to the presence of tetramethylmethylhexoside and that all fractions have the same composition as the analytical sample. 100 grm. of the original syrup (CH_3O = 6·8 per cent) gave 13 grm. of distilled methylated glucoside with CH_3O = 54·5 per cent. The maximum amount of hexoside, therefore, was 10 per cent, and as the general experience is to obtain a yield of methylated sugar equal to that of the parent compound, this figure is equivalent to 9·3 per cent of sugar in the original syrup.

NATURE OF THE EXTRANEOUS PRODUCTS

The bulk of the photosynthesized syrup, at least to the extent of 80 per cent, consists of non-sugar compounds which contain hydroxyl groups. On methylation this material gives an amber syrup which is non-volatile and easily resinified on heating either alone or with acids, and this suggests polyhydroxyphenols. As already stated, hydroxyacids or their lactides also constitute a considerable proportion of the photo-polymerized product.

The foregoing results of the investigation of the syrup obtained by the photo-polymerization of the formaldehyde are of great importance in that they give a very definite indication that glucose is one of the products of the polymerization of activated formaldehyde, the data given in Table V for tetramethylhexose being in fact those recorded for tetramethylglucose.

The presence in large quantities of non-sugars in the syrup is not surprising. It is well known that glucose as well as other carbohydrates are decomposed by ultra-violet light, and the fact that in spite of the presence of a great excess of formaldehyde the reducing power of the irradiated solution never exceeded 8 per cent indicates that when this maximum had been reached the rate of decomposition of the glucose equalled the rate of its formation. The solution must, therefore, contain large amounts of these decomposition products owing to the fact that in order to secure the maximum reducing power the irradiation was continued for at least 14 days.

The presence of polyhydroxyphenols and their methyl derivatives is also of great interest in view of the von Baeyer theory that the first product of the polymerization of activated formaldehyde is the cyclic compound $C_6H_{12}O_6$, which, by wandering of the hydrogen atom, changes into glucose. The formation of this compound was mentioned in Chapter I and will be discussed in greater detail in Chapter VII. If this compound is methylated by the formaldehyde, a process suggested by Irvine and Francis, it will no longer change into glucose and will be characterized by the reactions of polyhydroxyphenols.

CHAPTER IV

EARLY INVESTIGATIONS

3. THE PHOTOSYNTHESIS OF CARBOHYDRATES BY THE ACTION OF LIGHT ON HYDRATED CARBON DIOXIDE ADSORBED ON A SURFACE

AT the end of Chapter II the experiments were described in which saturated aqueous solutions of ferrous bicarbonate in quartz tubes were irradiated with ultra-violet light, and it was shown that the irradiated solutions, when the excess of ferrous bicarbonate had been removed, yielded on concentration under reduced pressure small quantities of organic matter. This product contained a substance which reduced Benedict's solution and was probably a carbohydrate. The formation of a layer of hydrated ferric oxide adsorbed on the irradiated walls of the quartz tubes indicated that the photosynthetic reaction took place on a surface and suggested the investigation of the photosynthesis of carbohydrates by the action of ultra-violet light on hydrated carbon dioxide adsorbed on a suitable surface.

This suggestion at once offered an explanation of some very interesting experimental results which had been described by Zenghelis,[36] who irradiated by means of a quartz mercury lamp an ordinary paper fat-extraction thimble immersed in water in a uviol glass tube and filled with a mixture of carbon dioxide and hydrogen at a pressure equal to that of the water outside. The aqueous solutions after their irradiation were concentrated and yielded complex organic compounds which Zenghelis concluded were sugars because they were readily charred by strong sulphuric acid, and gave osazones when heated with phenylhydrazine. The amount of carbohydrate obtained from each thimble was about 0·02 grm. Zenghelis

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[36] *Compt. rend.*, 1920, *171*, 167.

found in the course of his experiments that the paper thimbles lost their efficiency after about 10 hours' exposure to the ultra-violet light.

My colleagues and I repeated Zenghelis's experiments in Liverpool, and we confirmed his observations in every particular. We also proved that the presence of hydrogen inside the thimbles is unnecessary and that the same quantities of carbohydrate were obtained when the thimbles were filled with pure carbon dioxide.[37]

There appeared to be little doubt that in these experiments the carbon dioxide inside the thimble entered into solution and formed an adsorbed layer of hydrated carbon dioxide on the outer side of the thimble, and that the ultra-violet light acting on this adsorbed layer promoted photosynthesis. These observations, therefore, gave support to the conclusion drawn from the irradiation of ferrous bicarbonate in quartz tubes.

Now the technique used by Zenghelis is very similar to that used by Morton and Riding when they irradiated by means of a quartz mercury lamp a fine-grade Pasteur-Chamberland filter tube filled with carbon dioxide under pressure and immersed in water in a quartz tube. As was stated on page 48, they found that after the irradiation the water contained 0·01 per cent of formaldehyde. Here, again, there is little doubt that the ultra-violet light was acting on a layer of hydrated carbon dioxide adsorbed on the outer surface of the filter tube.

The essential difference between Zenghelis's method and that used by Morton and Riding is that the former used uviol glass tubes whilst the latter used a quartz tube. As was proved on page 41, the formaldehyde found by Morton and Riding was produced by the photochemical decomposition of the carbohydrates by ultra-violet light of very short wavelength. In order to obtain confirmatory evidence of this we repeated Morton and Riding's experiments, using a uviol glass tube in place of the quartz tube. We then found that the amount of formaldehyde produced was very small and did not exceed one part in five million. The aqueous solutions after concentration under reduced pressure yielded small but

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[37] Baly, Davies, Johnson and Shanassy, *Proc. Roy. Soc.*, 1927, *116*A, 197.

definite amounts of photosynthesized organic matter and this was in harmony with Zenghelis's results.

The evidence thus obtained, that the photosynthesis of carbohydrates from hydrated carbon dioxide adsorbed on a surface can be promoted by ultra-violet light transmitted by uviol glass, was very remarkable in view of the proof which had previously been obtained that in a homogeneous solution of carbon dioxide the photostationary state is established by light of wave-length 2100A. Although accurate measurements of the absorptive power of uviol glass are not available, it is known that a layer of the glass 1 mm. thick absorbs 15 per cent of light of wave-length 3090A and 44 per cent of light of wave-length 2800A. Moreover, we found that the practical limit of transmission is 2700A, so far as the promotion of any chemical reaction is concerned, and hence it may be accepted that in Zenghelis's experiments the minimum wave-length of the light which promoted the photosynthesis was 2700A, and this indicates a maximum energy intake of 105,330 calories. When this maximum energy intake is compared with the minimum amount of energy required to convert a gram-molecule of hydrated carbon dioxide into a carbohydrate, namely, 112,167 calories, and with the energy needed to establish the photostationary state in an aqueous solution of carbon dioxide, namely, 135,400 calories, it is obvious that there must be a fundamental difference between photosynthesis on a surface and photosynthesis in homogeneous solution. Whatever may be the amount of energy needed to promote the photosynthesis of a carbohydrate from hydrated carbon dioxide adsorbed on a surface, it is necessary, in order to explain this result, that this substance must gain energy as the result of its being adsorbed. In a recent communication [38] it was shown that the phenomena of the adsorption of gases on solid surfaces can be explained by the formation of adsorption complexes in which energy has been donated by the adsorbing molecular units in the surface to the adsorbed molecules of the gas. Applying this to the present problem it will be seen that, if the amount of energy required to promote photosynthesis with an adsorbed layer of carbon dioxide is the same as that required with a homogeneous solution, 135,400

[38] Baly, *Proc. Roy. Soc.*, **1937**, *160*A, 465.

calories, the surface must donate 135,400 − 105,330 = 30,070 calories to each gram-molecule of carbon dioxide. This is an impossibly large amount of energy in the case of adsorption on filter paper. The only possible explanation is that when the hydrated carbon dioxide is adsorbed on a surface, the photosynthesis of carbohydrates is achieved by the supply of the energy which is actually needed, namely, 112,167 calories. This means that the filter paper in the Zenghelis experiments donated 112,167 − 105,300 = 6,867 calories to the adsorbed hydrated carbon dioxide, and this is a very reasonable amount. The conclusion thus reached that photosynthesis on a surface is promoted by the supply of the minimum amount of the energy needed is one of fundamental importance.

The first step in the investigation was to prepare aqueous suspensions of powders capable of adsorbing hydrated carbon dioxide and to irradiate them after saturation with carbon dioxide. In the first series of experiments the substance used was pure aluminium powder, a quantity of which was found in the University chemical museum in Liverpool. The powder was found to be free from organic matter and was washed first with alcohol and then with ether, after which it was kept under ether until required. It was hoped that the alumimium powder when in contact with water would become coated with hydrated oxide and that as a result of this it would adsorb hydrated carbon dioxide.

Eight 9 × 1 in. quartz tubes, each containing 90 ml. of conductivity water, were arranged round the quartz mercury lamp and a little of the washed and dried aluminium powder was added to each. A stream of carbon dioxide was passed through each suspension until the water was saturated with the gas and then the mercury lamp was lighted and the irradiation continued for 1 hour. The combined contents of the quartz tubes were then filtered and evaporated to dryness in a porcelain dish on a steam bath. Small quantities of aluminium hydroxide separated during the concentration and were removed by filtration. The dry residue was extracted with alcohol and the filtered alcoholic solution yielded on evaporation 0·02 grm. of a gummy residue, which did not reduce Benedict's solution, but was charred by warm concentrated sulphuric acid. No evidence of the formation of formaldehyde was obtained when the filtered solution after the irradiation was tested with Schryver's reagent.

These results, although they were fully confirmed by further experiments in which the time of irradiation varied from 1 to 8 hours, were disappointing, because we had hoped that the photosynthesized products would reduce Benedict's solution. The disappointment was increased when we found that the products of photosynthesis obtained when suspensions of pure barium sulphate in saturated aqueous solutions of carbon dioxide were irradiated also did not reduce Benedict's solution. It occurred to us, however, when we were considering the photosynthetic process in the living plant that the products of photosynthesis *in vitro* might be carbohydrates more complex than a reducing sugar. We were thus encouraged to observe the effect of acid hydrolysis on the gummy products of photosynthesis.

The experiment with aluminium powder was again repeated and the organic residue obtained by evaporation of the filtered suspension was dissolved in a little dilute hydrochloric acid and the resulting solution was heated on a steam bath for a few minutes. After neutralization, some Benedict's solution was added and the mixture was boiled. Considerable reduction then took place, the precipitated cuprous oxide being contaminated with a little aluminium hydroxide. An exactly similar result was obtained when barium sulphate was used in place of metallic aluminium.

These experiments with aluminium powder and barium sulphate were repeated many times, and in most cases, but not in all, reduction of Benedict's solution was observed. The reason for the occasional negative results was obscure, but as we soon realized that aluminium powder and barium sulphate are not the best suited for the adsorption of the hydrated carbon dioxide, the failure on occasion to obtain successful results did not appear to possess any serious significance.

It occurred to us that in view of the acidic nature of hydrated carbon dioxide the most suitable material to use as a surface would be a basic substance, since in that case the dissociation pressure of the adsorption complex would be less than it is in the case of a neutral salt such as barium sulphate. The first substance used in the second series of experiments was freshly precipitated aluminium hydroxide prepared by the addition of a solution of ammonia to a solution of aluminium nitrate. The precipitated hydroxide was thoroughly washed by centrifuging and was used immediately. A small quantity was suspended in conductivity water in each of eight 9 × 1 in. quartz test-tubes arranged around a quartz mercury lamp. After the suspensions had been saturated with carbon dioxide, they were irradiated for 2 hours. The united contents of the tubes, after filtration and evaporation, yielded 0·20 grm. of organic matter and this was dissolved in a little normal hydrochloric acid and heated at 100° for 10 minutes. After being made alkaline with potassium carbonate, the solution was filtered to remove the trace of alumina which had separated and then boiled with Benedict's solution. Marked reduction took place.

Similar results were obtained when suspensions of the aluminium hydroxide in solutions of potassium and sodium bicarbonates were irradiated. Evidence of the reduction of Benedict's solution was obtained in every case after the products of photosynthesis had been hydrolysed by means of acid.

One series of experiments carried out with aluminium hydroxide gave results of considerable importance. In all the previous experiments the aluminium hydroxide used had been freshly prepared. It was found that if the washed aluminium hydroxide were allowed to remain under water for 8 hours before use, the yield of photosynthesized products was very materially reduced. If the washed hydroxide were allowed to remain under water for 36 hours before use, no evidence whatever of photosynthesis was obtained. The explanation of these results is to be found in the fact that freshly prepared aluminium hydroxide is basic and when suspended in water is electropositively charged. When it is allowed to remain in contact with water for many hours it changes its character and becomes electronegatively charged and then no longer adsorbs hydrated carbon dioxide. The importance of these observations will be emphasized when the authenticity of the foregoing results is discussed.

The last series of experiments were carried out with the basic carbonates of aluminium, magnesium and zinc, each of which was prepared by precipitation methods and washed until it was free from all soluble salts. The methods of irradiation and subsequent treatment of the suspensions were identical with those described in the case of aluminium hydroxide, and in each experiment a good yield was obtained of the complex organic product which reduced Benedict's solution after acid hydrolysis.

When suspensions of basic aluminium carbonate were irradiated a new phenomenon was observed in that the suspensate became brown during the irradiation. The discoloured carbonate, after drying on a porous tile, gave considerable charring when treated with concentrated sulphuric acid. Evidently, therefore, some of the organic products of photosynthesis had remained adsorbed on the surface of the carbonate. This phenomenon was not observed with any of the other compounds which we used.

In view of the discrepancy between the results obtained by various authorities in this field, and more particularly in view of the doubt as to the correctness of the first Liverpool investigation engendered by Porter and Ramsperger's work, it is evident that confidence in the foregoing results can only be established by the most rigid proof of the complete absence of organic matter in the carbon dioxide and the other materials used.[39] Not only was this proof obligatory, but it was also necessary to carry out rigid control experiments which were identical with those described, except that surfaces incapable of absorbing hydrated carbon dioxide were irradiated.

Of all the materials used the carbon dioxide was the most probable source of impurity, since this gas is sometimes obtained from fermentation processes. The carbon dioxide used was supplied to us in cylinders by the Washington Chemical Company, Liverpool, under a written guarantee that it had been prepared entirely from limestone. The proof by the usual tests that the gas was pure was not sufficient in our opinion, and the following tests satisfied us that it was completely free from organic matter.

A slow stream of the gas was passed through 2 litres of distilled water for several hours in the dark, and the solution after evaporation to dryness under reduced pressure yielded no

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[39] **Baly, Heilbron and Barker, *J. Chem. Soc.*, 1921, *119*, 1025.**

trace of organic residue. Exactly the same negative result was obtained when the water was irradiated with the light from the quartz mercury lamp during the passage of the carbon dioxide. These tests were carried out both with and without the filtration of the gas through a layer 4 in. thick of finely powdered sodium bicarbonate between two packings of cotton wool. When the water was irradiated during the passage of the gas, the amount of formaldehyde formed did not exceed 1 part in 1,000,000. In order to guard against the presence of finely divided organic matter in the form of an aerosol, which might pass through the water unchanged, the carbon dioxide was passed through a quartz tube which was strongly irradiated by means of a quartz mercury lamp and then into conductivity water both with and without irradiation. In every experiment no trace of organic matter was obtained when the solution was evaporated to dryness. These observations proved that the carbon dioxide contained no organic impurity.

There still remained the possibility that organic matter was introduced from sources other than the carbon dioxide. In order to examine this possibility several series of control experiments were carried out and these gave conclusive evidence that the photosynthesis of organic matter was not due to the presence of impurity in any of the materials which had been used.

In the first place, during the confirmation of the results obtained by Zenghelis, paper thimbles in water which contained no carbon dioxide were irradiated in uviol glass tubes for 8 hours with the light from a quartz mercury lamp. No formaldehyde or other organic matter was produced and proof was thus obtained that the organic matter was photosynthesized solely from the hydrated carbon dioxide.

In the second place, a great number of experiments were carried out in which powders, such as silica, incapable of adsorbing hydrated carbon dioxide, were irradiated in water, whilst a stream of carbon dioxide was passed through the suspension. In no case was any organic residue obtained after filtration and evaporation of the solution. The most convincing evidence of all was based on the change in the properties of aluminium hydroxide. As has already been stated, this hydroxide when freshly prepared is basic and readily absorbs hydrated carbon dioxide, and after it has been allowed to remain in contact with water for 36 hours the hydroxide becomes acidic and is then incapable of adsorbing hydrated carbon dioxide. A quantity of the hydroxide was prepared, washed until it was quite free from all soluble impurity and then ground up with water to a thin paste. One-third of this paste was immediately diluted with conductivity water and the suspension, after saturation with carbon dioxide, was irradiated for 2 hours by a quartz mercury lamp whilst a stream of the gas was passed through it. After the remaining two-thirds of the hydroxide paste had been allowed to remain for 8 hours, half of it was irradiated in the same manner, the carbon dioxide used being obtained from the same cylinder of the liquefied gas. The last third of the hydroxide paste was allowed to remain for 36 hours and was then irradiated in the same manner, the carbon dioxide being again obtained from the same cylinder.

With the freshly prepared hydroxide a good yield of the organic products was obtained. With the hydroxide which had been allowed to remain for 8 hours, a very small yield of organic products was obtained. With the hydroxide which had been allowed to remain for 36 hours, no trace of organic matter was obtained and the irradiated hydroxide itself contained no trace of organic matter. These control experiments were carried out several times with identical results and, since the only variable factor was the physical property of the aluminium hydroxide, they gave conclusive proof of the photosynthesis of organic matter from hydrated carbon dioxide.

The position which had been reached at this stage of the investigation may now be summarized and discussed. It was first established from the energy relations that when hydrated carbon dioxide in homogeneous solution absorbs light of wavelength 2100A it is converted into activated formaldehyde which undergoes polymerization with the evolution of energy into a carbohydrate. This mechanism of photosynthesis is fully in accord with the observations made with living plants. Photosynthesis of a hexose, for example, can therefore be represented by

$$CO_2H_2O + Nh\nu = [CH_2O] + O_2 = \tfrac{1}{6}C_6H_{12}O_6 + O_2$$

where $[CH_2O]$ represents a gram-molecule of activated formaldehyde. Since it is known that a soluble carbohydrate, in the presence of sufficient oxygen, is decomposed by very short wave-length ultra-violet light into carbon dioxide and water, the irradiation of an aqueous solution of carbon dioxide must establish a photostationary state which may be represented by

$$CO_2H_2O \rightleftharpoons \tfrac{1}{6}C_6H_{12}O_6 + O_2$$

Proof of the existence of this photostationary state was obtained by the addition of Feder's solution to an aqueous solution of carbon dioxide whilst it was irradiated with the light from a quartz mercury lamp. Marked reduction took place and no reduction whatever was observed if the irradiation was stopped before the addition of the Feder's solution. Moreover, the product of the reduction was not metallic mercury, which is given by formaldehyde, but a flocculent material similar to that given by glucose.

When a surface is coated by an adsorbed layer of carbon dioxide the effective concentration of that reactant on the surface is much greater than it is in a saturated solution. When such a coated surface is irradiated by means of a quartz mercury lamp, the activated formaldehyde will be produced on the surface and hence the conditions for its polymerization into a carbohydrate will be the best possible. Lastly, the presence in suspension of a large number of coated solid particles which absorb the ultra-violet rays will screen the photosynthesized carbohydrate and thus prevent it being decomposed. It follows that under these conditions the photostationary state will not exist and that a carbohydrate should progressively be photosynthesized.

In the experiments described above in which powders coated with an adsorbed layer of hydrated carbon dioxide were irradiated with ultra-violet light there is no doubt whatever that organic matter was photosynthesized. The reduction of Benedict's solution indicates the presence of a hexose, but it is known that this reagent can be reduced by substances other than hexoses. The presence of these substances is most improbable and at the same time the evidence in favour of the photosynthesis of carbohydrates is very materially strengthened by the fact that the product of photosynthesis possessed no reducing power towards Benedict's solution until it had been hydrolysed by heating with dilute hydrochloric acid.

The next stage in the investigation of photosynthesis by the irradiation of hydrated carbon dioxide adsorbed on a surface was initiated by a suggestion made by one of my research students, W. E. Stephen. He drew attention to the fact that the photosynthesis of carbohydrates by the action of ultraviolet light on hydrated carbon dioxide adsorbed on a white surface was very different from the natural process in the living plant in which photosynthesis is promoted by daylight in the presence of chlorophyll. He proposed the irradiation of hydrated carbon dioxide, adsorbed on a green surface, by means of ordinary white light. Although somewhat sceptical of the possibilities of success, I suggested that Stephen should irradiate a suspension of nickel carbonate in water, saturated with carbon dioxide, using a tungsten filament lamp as the light source.[40] We were fortunate enough to find in the University chemical museum at Liverpool a quantity of nickel carbonate of unknown origin which was completely free from adsorbed alkali. A preliminary experiment was carried out in which 1 grm. of this material, suspended in water saturated with carbon dioxide, was irradiated in an ordinary German test-glass with the light from a tungsten filament lamp. After filtration and evaporation the solution yielded a gummy product which was undoubtedly organic in nature, since it readily charred when treated with concentrated sulphuric acid. This experiment was several times repeated with the same result, and we were thus convinced that it is possible to photosynthesize organic matter by the action of visible light on hydrated carbon dioxide adsorbed on a green surface.

In order to determine whether the photosynthesis was due to a unique property of a green surface, similar experiments were carried out with pure cobalt carbonate, a quantity of which was also found in the Liverpool chemical museum, and once again the photosynthesis of organic matter was proved. The photochemical reaction was then investigated in some detail and the results obtained were of some interest.

The experimental method was standardized in the following way: 11·5 grm. of one of the two carbonates was suspended

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[40] Baly, Stephen and Hood, *Proc. Roy. Soc.*, 1927, *116*A, 212.

in 1,500 ml. of distilled water and the suspension was irradiated in six rectangular glass cells, each holding 250 ml. The six cells were irradiated in two groups of three by 200-watt lamps, the total surface area irradiated being 294 sq. cm. A stream of carbon dioxide was passed through each cell during the irradiation and cooling coils were used to prevent the temperature rising. After the irradiation the suspensions were filtered and the filtrate was found to be almost saturated with the bicarbonate of cobalt or nickel and this separated as the insoluble carbonate when the solutions were concentrated under reduced pressure at 50°. The decomposition of the bicarbonates was accelerated by passing a very slow stream of air through the solution during the concentration and the precipitated carbonate was removed at intervals by filtration. This method of procedure was adopted because it was found that the two bicarbonates undergo a complex decomposition when their solutions are concentrated at 100° in air. The final residue obtained was a gummy material mixed with a minute quantity of the carbonate, and on treatment with methyl alcohol the former went into solution. After filtration the alcoholic solution was taken to dryness and the photosynthesized product was then obtained free from contamination by cobalt or nickel.

The complete absence of formaldehyde in the irradiated suspensions was proved by the following method. When a very dilute aqueous solution of formaldehyde is distilled under reduced pressure the concentration of formaldehyde in the first runnings of the distillate is about ten times that in the original solution. During the concentration under reduced pressure of the filtrates from the irradiated solutions the first runnings of the distillate were many times tested for the presence of formaldehyde with Schryver's reagent, and in every case a completely negative result was obtained. It may now be accepted, therefore, as having been conclusively proved that free formaldehyde is not produced during photosynthesis from hydrated carbon dioxide. This statement in no way contradicts the conclusion which was previously reached from the energy relations, namely, that the product of the action of light on hydrated carbon dioxide is activated formaldehyde which at once polymerizes to carbohydrates.

The evidence now adduced proves that under the experimental conditions none of the activated formaldehyde escapes from the reaction sphere and becomes ordinary formaldehyde by loss of its energy of activation. Direct evidence of the formation of activated formaldehyde as the intermediate stage in photosynthesis will be given when the photosynthesis of nitrogen compounds is discussed in Chapter VI.

The results which were obtained under the conditions detailed above may now be described. In the first irradiations the glass vessels containing the suspensions were placed close to the 100-watt lamp and it was disappointing to find that the yield of photosynthesized material was very small after 2 hours' irradiation. The time of irradiation was then increased to 4 hours, and it was then found that the yield of photosynthesized material was considerably smaller than that obtained by 2 hours' irradiation. It appeared, therefore, that the products formed in the first 2 hours were photochemically decomposed during the subsequent irradiation. A decrease in the intensity of the light was thus indicated and the vessels containing the suspensions were placed at a distance of 6 in. from the 100-watt lamps. Under these conditions a constant yield of about 0·035 grm. of photosynthesized product was obtained with the 294 sq. cm. of a suspension of cobalt carbonate, which compares very favourably with the 0·02 grm. previously obtained with 400 sq. cm. of suspensions of white powders irradiated with ultra-violet light. Further experiments proved that as the intensity of the light was decreased the quantity of the product photosynthesized by unit quantity of light progressively increased.

No attention has yet been paid to the question of the destination of the oxygen which is set free when the hydrated carbon dioxide is photochemically converted into activated formaldehyde. Professor E. K. Rideal suggested to us that, when a layer of hydrated carbon dioxide adsorbed on a surface is converted by the agency of light into activated formaldehyde, the oxygen set free remains adsorbed on the surface with the possible formation of a peroxide. This interpretation of the mechanism of the photosynthetic process is undoubtedly correct, because we observed that nickel carbonate when irradiated turned black on the surface, whilst cobalt carbonate turned brown, and that the darkened powders after being dried gave some chlorine when warmed with concentrated hydrochloric acid. This observation indicated that the darkening of the two compounds was due to the formation of Ni_2O_3 and CO_2O_3, respectively. Furthermore, we found that moist nickel carbonate, after it had become dark on the surface as the result of irradiation, regained its natural colour when allowed to remain in the presence of moist carbon dioxide.

These observations proved that, when irradiated in suspension in water saturated with carbon dioxide, the two carbonates become poisoned on the surface and that the poisoned surfaces tend to recover themselves, the rate of recovery being slower than that of the photosynthetic processes. The observations also offer an explanation of the fact that the yield of the photosynthesized products obtained with unit light intensity increases with decrease in the intensity of the light used. The slower is the actual rate of photosynthesis the greater will be the effect of the de-poisoning of the surface.

For the same reason it is to be expected that with constant intensity of radiation larger amounts of photosynthesized material will be obtained by increasing the amount of the carbonate in suspension. The more concentrated is the suspension the less frequently will a given particle of the carbonate be illuminated and hence the average time during which de-poisoning takes place will be increased. It has already been stated that with 11·5 grm. of cobalt carbonate, 0·035 grm. of photosynthesized product was obtained. When 46·2 grm. of the carbonate were used, all other conditions being the same, 0·075 grm. of product was obtained.

As regards the nature of the products of photosynthesis, the method of obtaining these free from metallic carbonate has already been described, and it may now be added that the gummy residue obtained with the evaporation of the methyl alcoholic extract was not completely soluble in water, in spite of the fact that the products were obtained in the first instance from an aqueous solution. The insoluble portion was probably colloidal and had been rendered insoluble by dehydration. The aqueous solution of the gummy residue gave reactions which convinced my colleagues and myself that it contained at least two carbohydrates. This conviction was based on the following five tests:

1. The solution on warming with Benedict's solution gave a copious precipitate of cuprous oxide.
2. The solution gave a well-marked Molisch reaction with α-naphthol and concentrated sulphuric acid. This test is accepted as being specific for carbohydrate.
3. The solution gave Rübner's test with basic lead acetate as modified by Rogerson.[41] This test is also specific for carbohydrates.
4. The solution, on warming for one hour with a little pure phenylhydrazine acetate, gave a solid osazone. This osazone was very soluble in cold alcohol and the solution after the addition of benzene and slow evaporation deposited the osazone again in the solid form.
5. The reducing power of the solution towards Benedict's reagent was materially increased on hydrolysis by hydrochloric acid.

The foregoing results were obtained with the specimens of nickel and cobalt carbonates from the Liverpool University chemical museum. These compounds were proved to be completely free from organic matter by carrying out the same series of operations with both substances, except that the passage of carbon dioxide through their suspensions was carried out in complete darkness. The residue obtained after filtration and evaporation of the solution was vanishingly small and contained no organic matter, since no darkening took place when it was warmed with concentrated sulphuric acid. The carbon dioxide used was proved to be free from organic impurity by the same rigid tests as were described above in connexion with the irradiation of hydrated carbon dioxide adsorbed on white powders with ultra-violet light.

Confirmation of the photosynthesis of carbohydrates was obtained with nickel and cobalt carbonates which were prepared by precipitation methods, but considerable difficulty was met with in obtaining these substances sufficiently free from alkali. It was found that evidence of photosynthesis could only be obtained when the amount of alkali present was exceedingly small. The difficulty of preparing the carbonates

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[41] *Biochem. Journ.*, **1915**, *9*, **245**.

in a sufficiently pure condition is due to their remarkable power of adsorbing hydroxyl ions. Owing to this adsorptive power the only satisfactory method of testing for the presence of alkali is to heat the carbonates until they are completely converted into the oxides, the product becoming black owing to the formation of Ni_2O_3 and Co_3O_4, respectively. On extraction with water the previously adsorbed alkali can be removed and the amount determined volumetrically. In order to illustrate the difficulty of preparing the two carbonates free from alkali an interesting story may be told. The experience gained from the cobalt carbonate from the Liverpool University chemical museum led me to believe that it should be possible for a manufacturing firm to prepare this substance in an equal state of purity. I approached a well-known firm and asked whether they would be willing to undertake the preparation of 1 kg. of cobalt carbonate which they could guarantee to be free from alkali. In due course, I received the material, together with a letter assuring me that it would be found free from alkali. We at once irradiated a suspension of the carbonate with water saturated with carbon dioxide and the filtrate was evaporated to dryness according to the method described above. A considerable crystalline residue was obtained and this consisted entirely of sodium carbonate. The firm were very much at a loss to explain this because, as they assured me, the material when extracted with water yielded no alkali. I then suggested that they make a complete quantitative analysis of the material, undertaking to pay their fee if no alkali were found to be present. Within a few days I received a very courteous letter from the firm in which they expressed their astonishment at having found that the cobalt carbonate contained over 1 per cent of sodium carbonate.

As the result of a protracted investigation we succeeded in preparing both carbonates sufficiently free from alkali to give evidence of photosynthesis, although their photosynthetic activity was very much smaller than that of the museum specimens. The method of preparation was the slow addition of a cold solution of nickel or cobalt nitrate to a cold solution of a slight excess of potassium carbonate. The precipitated carbonate was collected on a filter, washed with distilled water and then ground up with distilled water to a thin paste. This paste was then diluted to a large volume with distilled water and steam was passed in until the suspension boiled. The carbonate was then again filtered and the treatment repeated until the material was found by the following test to be free from alkali. The test adopted was to pass carbon dioxide for 2 hours through a suspension of the carbonate in distilled water and the material was judged to be alkali-free when the filtrate on evaporation yielded no residue of alkali. After the carbonates had passed this test, they were dried at 100°.

As will be described in Chapter V, very much better methods of preparing photosynthetically active nickel carbonate have more recently been found. The foregoing method of preparation has been given because it enabled us in 1927 to carry out very rigid control experiments which were of great value in establishing the authenticity of the photosynthesis of carbohydrates with the two museum specimens.

It is of considerable interest and importance to record the fact that the two carbonates prepared by the above method rarely gave any evidence of photosynthesis when suspensions of them in water were irradiated. In view of the fact that photosynthesis must be a surface reaction it was not surprising that we found variations in the activity of the surface such as are frequently experienced in heterogeneous catalysis. We envisaged the necessity of activating the two carbonates and two methods of activation were found. The first method was to heat them when completely dry in an oven at 120°–140° for 20 to 30 minutes, and the second method was to irradiate them with ultra-violet light for 30 minutes. The second method was the better of the two and the finely powdered carbonate was spread out in a large clock glass and irradiated by means of a quartz mercury lamp held about 10 in. above the powder which was frequently stirred so as to expose new surfaces.

The preparation of photosynthetically inactive nickel and cobalt carbonates and their subsequent activation by one or other of the two methods gave us the opportunity of carrying out rigid control experiments. A quantity of one of the freshly prepared carbonates was suspended in water and irradiated by means of a 100-watt lamp for 2 hours whilst a stream of carbon dioxide was passed continuously through the suspension. The suspension was then filtered and the carbonate collected on the filter was dried in an oven at 100°. The filtrate after evaporation to dryness by the method already described yielded no measurable quantity of organic matter. The irradiated carbonate which had been dried at 100° was then activated and again irradiated as before, the carbon dioxide being obtained from the same cylinder of the liquid gas. The filtrate from the second irradiation of the same carbonate after activation gave small, but definite, yields of carbohydrates. Over two hundred of these control tests were carried out during the investigation, both nickel and cobalt carbonates being used, and in every case no organic matter was obtained before activation, whilst after activation definite yields were obtained of organic products which gave the reactions for carbohydrates specified above. In view of all the evidence we felt justified in stating that carbohydrates had been photosynthesized in the laboratory from hydrated carbon dioxide by the agency of ordinary white light.

In a further communication [42] the experimental evidence obtained with nickel and cobalt carbonates was discussed in relation to photosynthesis in the living plant, this discussion being justified by the fact that the shift in the exciting wavelength from the ultra-violet region, characteristic of hydrated carbon dioxide in solution or adsorbed on a white surface, to the visible region, characteristic of natural photosynthesis, had been achieved in the laboratory.

As was explained in Chapter I, the elegant work of Willstätter and Stoll led them to the view that the primary photosynthetic process could be expressed by the equation

$$C_{55}H_{72}O_5N_4Mg,CO_2H_2O = C_{55}H_{70}O_6N_4MgH_2O + CH_2O$$

1 molecule of chlorophyll A being oxidized to 1 molecule of chlorophyll B hydrate in the formation of 1 molecule of formaldehyde. As was shown in Chapter I, one of the difficulties met with in accepting the above equation is the complete absence of formaldehyde in the living leaf during the photo-assimilation of carbon dioxide. As has already been

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[42] Baly and Davies, *Proc. Roy. Soc.*, 1927, *116*A, 219.

shown, the action of light on hydrated carbon dioxide must produce activated formaldehyde which at once undergoes polymerization into carbohydrates. Ordinary formaldehyde should not, therefore, be found at any stage of the process, and its complete absence has been proved during photosynthesis *in vitro.* So far as this fact is concerned there is agreement between the laboratory and vital processes.

The second point of interest is that the photosynthesis of carbohydrates in the laboratory has been achieved by the use of a surface, and the question is thereby raised as to whether the natural process is a photochemical surface reaction. It is generally agreed that in the living plant photosynthesis takes place in the chloroplasts which are heterogeneous systems consisting of water, proteins, lipoids, and the plant pigments. A considerable amount of evidence has been obtained that a limiting surface not only exists in the chloroplasts but is necessary for photosynthesis to take place. As the result of ultra-microscopic observations, Price [43] stated that the chloroplast appears as a slightly opaque and heterogeneous body with a motionless gel structure. The importance of the surface between the lipoid and aqueous phases of the chloroplast was emphasized by Stern,[44] since photosynthesis only takes place normally when this surface is intact. In his investigations of the effect of phenylurethane and methylurethane, Warburg [45] found that these substances retarded natural photosynthesis and concluded that their action depended on changes in a limiting surface. The relation between the rate of photosynthesis and the concentration of the narcotic was expressed by a curve very similar to the Freundlich adsorption isotherm. These observations very definitely indicate that natural photosynthesis is a heterogeneous reaction and it thus appeared that in this respect also there is a similarity between the process *in vivo* and *in vitro.*

In describing the photosynthesis of carbohydrates by the irradiation of suspensions of nickel and cobalt carbonates in water saturated with carbon dioxide, it was stated that the surfaces of these compounds tend to become poisoned by the

[43] *Ann. Bot.,* 1919, *28,* 601.

[44] *Zeitsch. fur Bot.,* 1921, *13,* 193.

[45] *Biochem. Zeitsch.,* 1919, *100,* 230; 1920, *103,* 188.

formation of a superficial layer of Ni_2O_3 or Co_2O_3. This layer, in the presence of hydrated carbon dioxide, reverts back in the dark to the carbonate. This thermal reaction controls the rate of photosynthesis, with the result, which was proved experimentally, that the amount of carbohydrate photosynthesized with unit light intensity increases as the light intensity is decreased. As was stated in Chapter I, it has long been known that a thermal reaction controls the rate of photosynthesis in the living plant as was first established by F. F. Blackman [46] in 1905. A third and indeed remarkable analogy is thus established between the natural and laboratory processes.

A fourth analogy between the two processes is that both are promoted by visible light. The explanation of this was unknown at that time and it is mentioned here only to emphasize the striking similarity between photosynthesis in the plant and that achieved in the laboratory.

As the result of quantitative determinations by Puriewitsch [47] of the amount of carbohydrates photosynthesized in the leaves of certain plants, it is possible to make a comparison between these amounts and that obtained under the best conditions in the laboratory. Puriewitsch determined the increase in the heat of combustion of excised leaves of four species of plants after irradiation by sunlight, the total intensity of the light being determined by means of a recording bolometer. He found that there is an inverse ratio between the amount of energy used by the leaves and the total amount of energy which fell on them. The following average values were obtained of the increase in the heat of combustion in calories per square centimetre per hour :

Acer plantanoides	0·34,	0·27,	1·09,	0·53		
Polygonum sacchalinense	4·1,	1·7,	1·4,	2·0,	0·3,	0·9
Helianthus annuus	1·3					
Saxifraga cordifolia	1·5					

As was recorded above, the maximum yield of carbohydrate photosynthesized with cobalt carbonate was 0·075 grm. in 2 hours. The heats of combustion of carbohydrates are, to a first approximation, proportional to their molecular weights,

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[46] *Ann. Bot.*, 1905, *19*, 281.

[47] *Jahrb. wiss. Bot.*, 1914, *53*, 229.

and it may therefore be assumed that the carbohydrate was glucose. On this assumption the gain in calorific value was 673,000 × 0·075/180 = 280·4 calories or 140·2 calories per hour. Since the irradiated area was 294 sq. cm., the gain in calorific value was 0·48 calorie per square centimetre per hour. The yield of carbohydrate obtained in the laboratory, therefore, was not seriously at variance with that observed in the vital process.

Now emphasis must be laid on the statement previously made that the preparations of nickel and cobalt carbonates made in the laboratory, even after activation, were not so photosynthetically active as those preparations which we found in the Liverpool University chemical museum. It was manifestly desirable, therefore, to find a method whereby one of these substances could be prepared free from adsorbed alkali and possessing a greater photosynthetic efficiency. During the investigations of various methods of preparation it occurred to us that it might be possible to prepare nickel carbonate by the electrolysis of an aqueous solution of carbon dioxide with pure nickel electrodes. On a trial of this method a very satisfactory product was obtained. In view of the subsequent events it is essential that the exact details of the method be given.[48]

A rectangular glass accumulator cell with internal dimensions of 8 × 6 in. and 8 in. deep was obtained from Messrs. Pilkington Bros., St. Helens. This cell was provided with a wooden cover to which were attached by means of binding screws three nickel plates 8 × 5 in. and $\frac{1}{8}$ in. thick. Two glass cooling coils were also attached to the wooden cover, one on each side of the central electrode, and cold water was passed through these during the electrolysis. The cell was filled with pure conductivity water to within 1 in. of the top and carbon dioxide was continuously passed into the water. The two outer electrodes were connected with the positive main of the 230-volt laboratory circuit and the central electrode was connected with the negative main, sufficient resistance being intercalated to maintain a current of from 2·2 to 2·0 amp. Since the area of each electrode immersed was 35 sq. in., the current density was about 1·0 amp. per square decimetre.

[48] Baly and Hood, *Proc. Roy. Soc.*, 1929, *122*A, 393.

About 30 grm. of the carbonate were obtained in 24 hours and the product tended to form a coherent layer on the cathode, but from time to time portions became detached and sank to the bottom of the cell.

The hydrated nickel carbonate was coarsely granular and became very friable on drying. It was almost free from adsorbed alkali and contained small quantities of black nickel oxide which, however, had no apparent effect on its photosynthetic efficiency. On analysis the substance was found to have a composition which lay between those corresponding to the formulae $10NiO,10H_2O,CO_2$, and $11NiO,11H_2O,CO_2$.

The hydrated carbonate after being dried at 100° was heated for 30 minutes in an oven at 140°. It was then powdered and passed through a 100-mesh sieve, an operation which was very easy to carry out owing to the friable nature of the material. 50 grm. of the powder were distributed by means of the 100-mesh sieve as evenly as possible over a sheet of plate glass having an area of about 5 sq. ft., thus giving about 11 mgm. per square centimetre. A light wooden frame 2 in. deep rested on the glass sheet and a second glass sheet was placed on this frame in order to protect the powder from contamination by dust. The layer of carbonate was then irradiated on both sides, three 100-watt lamps being placed above and below the apparatus. The carbonate required for its complete activation 6 hours' irradiation, no harm being done by irradiation for 18 hours. The actual period of irradiation was 15 to 16 hours and it was found necessary to use the powder immediately after activation. More than 2,000 grm. of the carbonate were prepared and the yield of organic matter obtained by the irradiation of the activated material in suspension in water saturated with carbon dioxide was remarkably constant when the variable factors, temperature, light intensity and concentration of the suspension were kept constant.

The filtrate from the irradiated suspension was concentrated under reduced pressure on a steam bath, the nickel carbonate which separated being removed by filtration at intervals. The gummy residue obtained was dissolved in methyl alcohol and the resulting solution after filtration was evaporated to dryness. The final product thus obtained gave the five reactions for carbohydrates detailed above on page 74, and these justify the statement that the product consisted of carbohydrates.

The remarkable constancy in the amount of carbohydrates photosynthesized under constant conditions enabled Hood and myself to determine the amount photosynthesized at different temperatures. In each of these determinations a suspension of 50 grm. of the activated carbonate suspended in 1,500 ml. of conductivity water were irradiated in a rectangular glass vessel, the irradiated area being 360 sq. cm. The temperature of the suspension was maintained at the desired value by means of two glass coils through which water at the necessary temperature was continuously passed. In each determination the 50 grm. of the carbonate were used immediately after activation and added to the 1,500 ml. of conductivity water, the temperature of which had previously been brought to the desired value.

The amounts of the carbohydrates photosynthesized in two hours at nine different temperatures are given in Table VI, and the relation between the yield and the temperature is expressed by the curve in Fig. 6. It is to be noted that in Table I of the original communication the yields stated for 36° and 41° were incorrect, as is shown by the curve given on the same page.

TABLE VI

Temperature	Yield of carbohydrates (grm.)	Temperature	Yield of carbohydrates (grm.)
5·0°	0·0101	31·0°	0·0783
11·5°	0·0281	36·0°	0·0460
17·0°	0·0412	41·0°	0·0238
21·0°	0 0512	46·0°	0·0061
26·0°	0·0648		

It may be seen that the amount of carbohydrates photosynthesized increased progressively with rising temperature from 5° to 31° and that when the temperature exceeded 31° the amount photosynthesized decreased very rapidly with rise in temperature.

It has long been known that in living plants there exists a temperature at which the rate of photosynthesis is a maximum, and that above this temperature the rate rapidly declines. This phenomenon has been investigated by plant physiologists and reference may be made in particular to an investigation carried out by Miss Matthaei.[49] The method adopted was the measurement of the maximum amount of carbon dioxide assimilated by excised leaves of plants when irradiated with light of constant intensity. Two series of Miss Matthaei's observations are expressed by the two portions of the full curve in Fig. 7, and it is to be noted that the two portions do not form a continuous curve. It is known that at temperatures above 25° the rate of photosynthesis decreases with the time of irradiation, and the dotted curve between 26·5° and 40° in Fig. 7 expresses the average rates of photosynthesis observed with longer periods of irradiation.

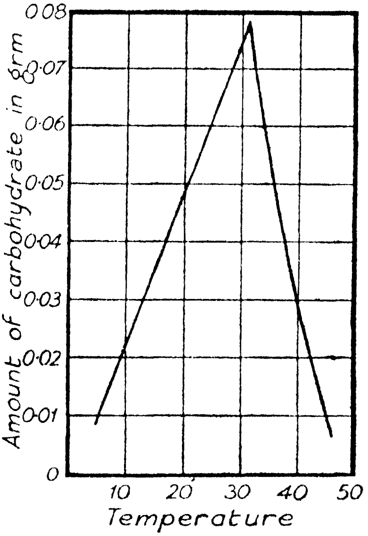

FIG. 6

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The curves which express the relation between the rate of photosynthesis and the temperature *in vivo* and *in vitro* are very similar in shape, and this is the most remarkable of all the analogies which have been established between the natural and laboratory processes. It is generally accepted that the sudden decrease in the rate of photosynthesis in the living leaf when the temperature exceeds a definite limit is due to the super-

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[49] *Phil. Trans.*, 1905, *197*B, 47.

position of a second process on the primary process, the second process having a large temperature coefficient. The same explanation is without doubt applicable to the laboratory process, but the evidence now obtained indicates that the superposed process cannot be one of purely biological origin.

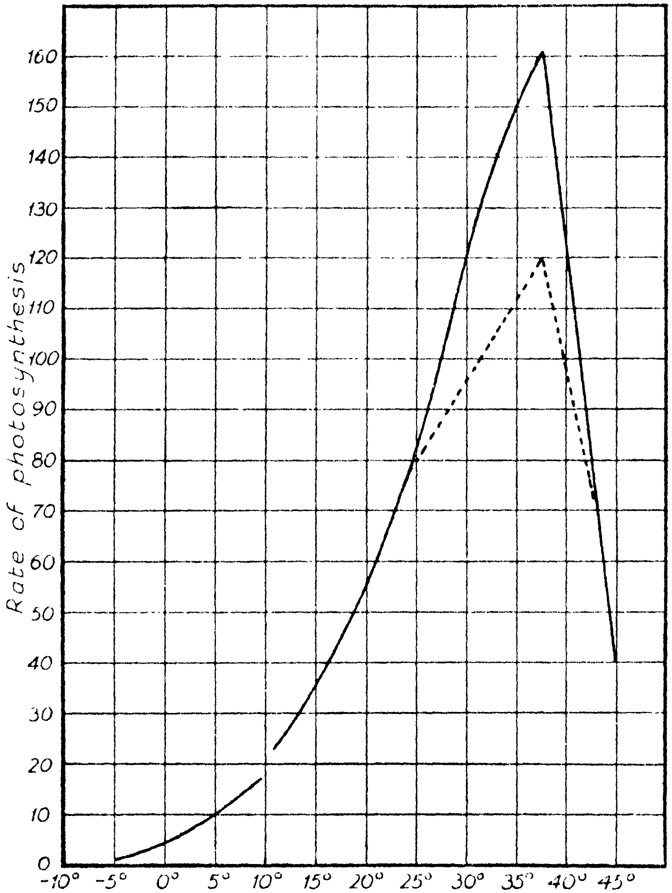

FIG. 7

(*After Miss Matthaei*)

The values of the temperature coefficient for 10° (Q_{10}) may be calculated from the amounts of carbohydrates obtained with the electrolytic nickel carbonate at different temperatures, and these are given in Table VII. The calculated values are given together with the values obtained by Warburg[50] for the same temperature intervals with the unicellular alga *Chlorella* under constant intensity of illumination.

TABLE VII

Temperature interval	5° to 10°	10° to 20°	20° to 30°
Q_{10} with nickel carbonate	4·50	2·11	1·54
Q_{10} with *Chlorella*	4·3	2·1	1·6

Once again a very striking similarity between the natural and laboratory processes is established by the values given in the table.

It must not be forgotten when the analogies between the two processes are being considered that in the living plant the products of photosynthesis are invariably optically active. Not only is this true, but in every known species of plant it is the same optical isomer, e.g. *d*-glucose, which is photosynthesized. It has been shown that in the laboratory process photosynthesis takes place on the surface, and many reasons have been given for the belief that the same is true in the case of the living plant. It may be suggested that the asymmetric photosynthesis which is characteristic of the vital process is due to the presence of a suitably oriented surface, and that when adsorbed on such a surface the molecules of hydrated carbon dioxide are themselves oriented all in the same way. Such an oriented layer might well give rise to asymmetric photosynthesis. It is at any rate easier to envisage asymmetric photosynthesis on a surface than in a homogeneous system.

The position that had been reached in the Liverpool investigation may now be summed up. Carbohydrates had been photosynthesized by the agency of white light from hydrated carbon dioxide in the presence of nickel carbonate, and the physical characteristics of the process had been found to be remarkably similar to those of the natural process. Over 2 kg. of the nickel carbonate had been prepared by the electrolytic method, and it had been proved by numerous repetitions of its irradiation that the photosynthetic activity of each separate preparation of 30 grm. was the same. We were convinced by this that any one who prepared the carbonate

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[50] *Biochem. Zeitsch.*, 1919, *100*, 258.

by the same method would find no difficulty in confirming our results.

It was, however, realized that the laboratory process had a great disadvantage in the dissolution of the nickel carbonate during its irradiation. This introduced a troublesome factor in the treatment of the filtrate from an irradiated suspension which was saturated with respect to nickel bicarbonate. Then again the necessity of activating the carbonate by pre-irradiation had not been explained. These two facts led to a further study of the methods of preparation of nickel carbonate suitable for photosynthesis. At that time Professor T. P. Hilditch drew my attention to the enhancement of the catalytic activity of nickel in the hydrogenation of oils, when the nickel is deposited on kieselguhr which has previously been coated with alumina.[51] The photosynthetic activity of nickel and cobalt carbonates deposited on the same support by precipitation methods was then investigated. It was found that during the irradiation of these preparations when suspended in water saturated in carbon dioxide, only a minute amount of nickel or cobalt went into solution. It was also found that their photosynthetic activity was much greater than that of the unsupported carbonate and that no activation by pre-irradiation was necessary.[52]

Now it is well known that the activity of certain heterogeneous catalysts is enhanced when they contain small quantities of thorium oxide. It appeared worth while, therefore, to determine whether the photosynthetic activity of nickel carbonate is enhanced when it contains a small quantity of thorium oxide. A preparation was made of nickel carbonate, containing 1·6 parts of ThO_2 to 100 parts of NiO, supported on aluminated kieselguhr. This preparation on irradiation with white light in suspension in water saturated with carbon dioxide yielded in two hours 0·0025 grm. of carbohydrates per gram of the powder. Evidence of photosynthesis was also obtained by the irradiation of manganous carbonate deposited on aluminated kieselguhr, but the amount of carbohydrates obtained was only 0·001 grm. per gram of the powder.

All these preparations had the disadvantage of being very

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[51] Armstrong and Hilditch, *Proc. Roy. Soc.*, 1923, *103*A, 586.

[52] Baly, *Trans. Faraday Soc.*, **1931**, *27*, 545.

difficult to wash free from alkali, and this led us to investigate the photosynthetic activity of supported ferric and chromic oxides and hydroxides. Two methods of preparing the supported substances were adopted, and in the first ammonia was added in slight excess to suspensions of the aluminated kieselguhr in solutions of ferric nitrate, chromic nitrate, or mixtures of the two. In the second method, suspensions of the aluminated kieselguhr in solutions of known amounts of ferric nitrate, ammonium bichromate or mixtures of the two were evaporated to dryness and then heated at 450° in order to obtain the oxides. Various amounts of thorium nitrate were added to the solution in each method.

With the supported hydroxides evidence of the photosynthesis of organic matter was obtained with every preparation, the amount being of the order of 0·002 grm. per gram of powder. With the supported oxides evidence of photosynthesis was only obtained with ferric oxide. With chromium oxide or mixtures of chromium and ferric oxides chromic acid was always produced on irradiation, the filtrate being coloured strongly yellow. With the pure supported ferric hydroxide and ferric oxide it was found that the amount of organic matter photosynthesized varied with the thorium oxide content.

The relation between the photosynthetic activity of supported ferric oxide and its thorium oxide content was then investigated and the method of preparation must be described in detail for a reason which will be explained later. This investigation was carried out in my laboratory by C. L. Moore and P. F. R. Venables during my visit to South Africa with the British Association of Science in 1929. My indebtedness to them is very great because the evidence they obtained led to the final solution of the problem in 1937.

The kieselguhr which was obtained from the laboratory store, was first heated with nitric acid in order to extract the small amount of clay it obtained and to oxidize any organic matter present, after which it was washed until free from acid and then dried at 125°. A suspension of 100 grm. of the purified kieselguhr in 1,000 ml. of water containing 150 grm. of crystalline aluminium chloride was boiled in an enamelled iron basin and there was then added in small portions at a time 200 grm. of anhydrous sodium carbonate, that is to say, twice the theoretical quantity. When half the quantity of alkali had been added the suspension became very thick, but on further addition of alkali it again became liquid. After the whole of the sodium carbonate had been added the mixture was boiled for a further 15 minutes and then transferred to 5 litres of distilled water in a 2-gallon enamelled iron bucket and disintegrated by means of an electrically driven stirrer. Steam at 15-lb. pressure was then slowly passed into the suspension until the bucket was full. The suspension was then allowed to settle and the supernatant liquid was syphoned off. The bucket was then again half filled with distilled water and the whole process was repeated until the supernatant liquid was neutral to B.D.H. indicator. This method of washing the coated kieselguhr free from alkali was a lengthy process, taking several days to complete. When the washing was nearly completed the suspension developed a very slow rate of sedimentation, but after further washing the rate of sedimentation once again became rapid. This more or less sudden decrease in the rate of sedimentation gave a valuable indication that the washing process was almost completed. Altogether 800 grm. of the aluminated kieselguhr were prepared by the above method and found to be quite uniform in properties.

In view of the fact that Armstrong and Hilditch's supported nickel catalyst contained 15 per cent of nickel, it was assumed that one-third of a molecule of ferric oxide was equivalent to one atom of nickel, and it was decided to prepare supported ferric oxide containing 16·67 per cent of ferric oxide. A standardized solution of pure ferric nitrate was prepared and 40 grm. of the aluminated kieselguhr was added to the volume of this solution which contained the equivalent of 8 grm. of ferric oxide. The mixture was cautiously evaporated to dryness with constant stirring on a sand bath. The dry product after being ground to a fine powder was heated at 460° in a silica tube in a current of dry CO_2-free and filtered air. The product was then heated under as low a pressure as possible at 400° in a silica tube closed at one end in order to remove the adsorbed nitric oxide. Preparations were also made in which different amounts of thorium nitrate were added to the ferric nitrate, the resulting ferric oxide layer containing from 1·0 to 2·4 per cent of thorium oxide. All the preparations had the colour of jewellers' rouge.

On irradiation with white light in suspension in water saturated with carbon dioxide each preparation gave evidence of photosynthesis of organic matter and the amount obtained varied periodically with the thorium oxide content. The maximum yields calculated on 1 grm. of the material were 0·004 grm. and 0·003 grm. when the ThO_2 content was 1·3 and 2·15 per cent respectively. Minimum yields of 0·0006 grm. and 0·0003 grm. were obtained when the ThO_2 content was 1·8 and 2·3 per cent respectively.

It was also found that the electropositive surface potentials of these powders, determined by the method of cataphoresis, varied with the thorium oxide content, maximum values being obtained with 1·3 and 2·15 per cent of ThO_2, and minimum values being obtained with 1·8 and 2·3 per cent of ThO_2.

It is to be noted that the products of photosynthesis were not carbohydrates. They were proved to be organic acids of sufficiently high molecular weight to undergo charring when warmed with concentrated sulphuric acid. The value of this investigation lay in the fact that proof was for the first time obtained of the enhancement of photosynthetic activity by means of thorium oxide and of this enhancement being a periodic function of the amount of thorium oxide present.

A systematic investigation of this method of photosynthesis was then undertaken, and owing to the fact that all the kieselguhr in the laboratory store had been used, a different variety was employed which had been presented to us by the Nobel section of Imperial Chemical Industries. Whilst the same method was adopted of coating the kieselguhr with alumina, the coated material was collected and well washed on a funnel before being treated with steam, as was described above. This change in procedure was adopted in order to shorten the washing process. No photosynthetic activity was observed with any of the preparations made by coating this aluminated kieselguhr with ferric oxide alone or mixed with thorium oxide. It was also noted that the supported ferric oxide, instead of having the colour of jewellers' rouge, was brown. This change in colour was found to be due to the dissolution of alumina from the aluminated kieselguhr during the evaporation to dryness of its suspensions in ferric nitrate solution. This was finally proved to be the result of the change which had been made in the method of washing the freshly prepared kieselguhr.

It was found that the aluminated kieselguhr which had been washed by the original method had a zero surface potential when suspended in water or dilute acid and yielded no alumina on boiling with nitric acid. The aluminated kieselguhr which had been washed on a funnel before being treated by the long process of steaming had an electropositive surface potential when suspended in water and yielded much alumina when boiled with nitric acid.

Now kieselguhr coated with an adsorbed unimolecular layer of alumina has an electropositive surface potential,[53] whilst the original preparations of aluminated kieselguhr had zero surface potential, the same being true of kaolin. There is little doubt, therefore, that during boiling with excess of sodium carbonate and alumina, kieselguhr becomes coated with an insoluble unimolecular layer of an alumino-silicic acid, and that the excess of alumina is leached out during the prolonged treatment with steam in the presence of gradually decreasing quantities of alkali. When the aluminated kieselguhr was collected and washed with water on a funnel, the excess of sodium carbonate was extracted, and hence the excess of alumina was not removed during the subsequent treatment with steam. This must be borne in mind if it be desired to investigate the photosynthetic activity of ferric oxide supported on aluminated kieselguhr.

This explanation was not found until after the discussion on photochemical processes held by the Faraday Society at Liverpool in April 1931, at which our successful results and subsequent failures were communicated.[54] At that meeting, my friend Dr. C. F. Goodeve told me that Dr. Bell at University College, London, had entirely failed to confirm our photosynthesis of carbohydrates with nickel carbonate prepared by the electrolytic method, and as the result of this, the work with

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[53] Baly and Pepper, *Nature*, 1935, *136*, 28; Baly, Pepper and Vernon, *Trans. Faraday Soc.*, 1939, *35*, 1165.

[54] Baly, *Trans. Faraday Soc.*, 1931, *27*, 545.

supported ferric oxide was abandoned in favour of a complete re-investigation of the nickel carbonate problem. The description of this investigation, which was carried out in the period 1931–7, will be given in the next chapter.

CHAPTER V

THE FINAL ACHIEVEMENT OF PHOTOSYNTHESIS OF CARBOHYDRATES

IN Chapter IV the story was told of the investigations of photosynthesis up to April 1931, when the discussion meeting on photochemical processes was held under the auspices of the Faraday Society. It was then that I learned that Bell had found that he could obtain no evidence whatever of photosynthesis by the irradiation of nickel carbonate which he had prepared by the electrolytic method described by Hood and myself in 1929.[55] In his communication published in December 1931, Bell [56] stated that he had also obtained negative results with ferric oxide supported on aluminated kieselguhr. Since he prepared the aluminated kieselguhr by the method which was stated in my communication [57] to lead to negative results, it is not necessary to discuss this part of his investigation.

Bell's complete failure to obtain any evidence of photosynthesis with nickel carbonate prepared by the electrolytic method was very surprising. We had prepared a photosynthetically active material by the activation of the carbonate which was obtained the very first time the electrolytic method was tried. As was stated in Chapter IV, more than 2,000 grm. of the nickel carbonate were prepared by this method and no variation in physical properties or in photosynthetic activity was observed with any one of the seventy preparations of 30 grm. each. Furthermore, photosynthesis with this material was demonstrated during a lecture before the Royal Institution and about 1 ml. of a concentrated solution of the carbohydrates photosynthesized in Liverpool was presented to the

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[55] *Proc. Roy. Soc.*, 1929, *122*A, 392.

[56] J. Bell, *Trans. Faraday Soc.*, 1931, *27*, 771.

[57] *Trans. Faraday Soc.*, 1931, *27*, 545.

Managers. We had felt confident that our results were reproducible and, indeed, it appeared to be a simple matter to prepare some more nickel carbonate by the electrolytic method and to confirm those results.

On commencing operations we found that the glass electrolytic cell used in 1928 had been broken, and a second cell of the same dimensions was obtained from Messrs. Pilkington Bros., St. Helens, who had supplied the first cell. Although in every other detail the apparatus was identical with that previously used, the nickel carbonate obtained was to my astonishment quite different from that originally prepared. The original material, which was dark emerald green in colour, tended to form a coherent layer on the cathode and was coarsely granular, becoming very friable on drying. The new material was pale green, very flocculent, and on drying, formed hard lumps which were very difficult to pulverize. It was also found to contain much adsorbed alkali and the original material was almost alkali-free. The new nickel carbonate gave no evidence of photosynthesis when irradiated after activation by pre-irradiation as described in Chapter IV.

A considerable quantity of this new material was prepared and 50 grm. at a time were pre-irradiated in the form of a dry powder, and then immediately irradiated in suspension in water saturated with carbon dioxide. Although the filtrates from these irradiated suspensions were treated in exactly the same way, as was previously adopted, no evidence of photosynthesis was obtained. In short, we fully confirmed Bell's negative results.

A quantity of 50 grm. of the original nickel carbonate prepared by Hood and myself was then pre-irradiated and immediately afterwards irradiated in suspension in water saturated with carbon dioxide. The filtrate after concentration yielded 0·04 grm. of carbohydrate, an amount which is in accord with that previously obtained, as may be seen from Table VI on page 82.

We were entirely at a loss to explain these observations. It seemed to be almost incredible that when using identically the same method of preparation in 1928 and 1931, two varieties of nickel carbonate could be obtained which were fundamentally different in their properties. It is true that the same glass cell had not been used in 1928 and 1931, but the possibility that this was the origin of the difference in properties did not occur to me until the significance of the fact that the original nickel carbonate was almost alkali-free, whilst the carbonate prepared in the new cell contained much alkali, was realized. This fact suggested the possibility that the two cells had been made of different kinds of glass. I then wrote to Messrs. Pilkington Bros., and asked if they could tell me whether there was any essential difference in the glass used in making the two cells. In their reply the firm wrote that after searching their sale records they had found that the first cell had been made of a type of glass with which they were experimenting at the time. This glass had an unusually small solubility in water. They went on to say that shortly after the first cell had been purchased, the manufacture of this glass had been given up, and that the second cell had been made of a glass having the usual solubility in water.

This information definitely indicated that the presence of adsorbed alkali in the nickel carbonate prepared in 1931 was due to the dissolution of alkali silicate from the glass cell, and that the very small amount of alkali in the original product was due to the small solubility of the glass cell used in 1928. The use of a cell and cooling coils made of pyrex, therefore, appeared to be desirable, and R. B. Twiss investigated this method, but the results were very disappointing. Considerable difficulties were met with owing to the passivation of the nickel electrodes, and although these were successfully overcome, the rate of formation of the nickel carbonate was very small, namely, from 2 to 4 grm. in 24 hours. Some evidence of photosynthesis was obtained with this product, but the yields were so small that further investigation appeared to be useless.

The electrolytic method of preparation of nickel and cobalt carbonates on a large scale with glass cells was then systematically investigated by Twiss, but the final conclusion was reached that it did not seem possible to reproduce the conditions which existed in the apparatus used in 1928.

The only avenue left open to us, therefore, was a complete re-investigation of the properties of nickel carbonate prepared by precipitation methods. The composition of the material varies considerably with the method of preparation, and the following compounds have been described in the literature, $NiO,CO_2,6H_2O,5NiO,2CO_2,8H_2O$, and $5NiO,2CO_2,5H_2O$. The last compound on heating at 150° loses carbon dioxide to give $3NiO,CO_2,3H_2O$. In view of the variation in composition of these substances we hoped to find a method whereby a photosynthetically active nickel carbonate could be prepared.

Three methods of preparation were used. In the first method equivalent amounts of either sodium or potassium carbonate in solution were added to cold and to boiling solutions of nickel nitrate. In the second method, potassium bicarbonate solutions were added to boiling solutions of nickel nitrate. The concentrations of the two reacting solutions and the rate of the addition of the alkali were varied within wide limits. The product in every case was collected on a filter and washed with distilled water. It was then disintegrated in 9 litres of distilled water by means of an electrically driven stirrer and again collected on a filter. This disintegration process was then repeated, after which the product was dried at 100°.

In the third method some of the preparations of nickel carbonate made by each of the above methods were disintegrated in 5 litres of distilled water and carbon dioxide was passed into the suspension until a saturated solution of nickel bicarbonate was obtained. This solution, after decantation, was heated under reduced pressure on a steam bath until almost the whole of the nickel was precipitated as the carbonate. The product was collected on a filter, washed and dried at 100°. We adopted this method in the hopes that the final product would be almost, if not entirely, free from adsorbed alkali.

A very large number of preparations were made by these three methods, but every preparation contained much adsorbed alkali and every preparation was photosynthetically inactive.

Now it was noted during this investigation that the nickel carbonates tended to become dark on the surface when exposed to light, the dark tinge disappearing when the irradiated powder was screened from light. It was also noted that the rate and extent of this darkening varied considerably with different preparations. For example, the pale green product obtained by the third method of preparation darkened very slowly in light, whilst the emerald green products obtained by one of the first two methods darkened much more quickly in light of the same intensity. This was the first time that a method was found of differentiating between different preparations of nickel carbonate. It was proved that the darkening was due to the formation of Ni_2O_3 on the surface, because the darkened powders evolved chlorine when treated with concentrated hydrochloric acid. In view of our observation in 1927 that both nickel and cobalt carbonate during photosynthesis turned dark on the surface owing to the formation of Ni_2O_3 and Co_2O_3, respectively, this observation appeared to have significance.

The phenomenon was further investigated by L. B. Morgan, who exposed to daylight 50 grm. of the emerald green nickel carbonate distributed in a thin layer on a large sheet of glass, a second glass sheet being suspended over it in order to protect the powder from dust. During the first day the powder developed a dark tinge on the irradiated surface, this dark tinge disappearing during the following night. On the second day the darkening became more pronounced and after four days' irradiation the carbonate had become permanently black on the surface. The irradiated carbonate was then extracted with warm water and the aqueous extract was found to contain minute traces of formaldehyde and the nickel salts of organic acids which gave unstable silver salts on the addition of silver nitrate. It thus appeared that some physical change in the nickel carbonate was caused by the prolonged action of daylight, because no organic matter was photosynthesized when the carbonate was irradiated for a short period only.

During the search through the literature in connexion with the composition of the various types of nickel carbonate two interesting statements were found. In the first place Proust stated that Ni_2O_3 can be prepared by gently heating nickel carbonate in the air.[58] In the second place, Brunck stated that anhydrous nickel nitrate on heating gives a black product which on being washed with hot water becomes progressively brighter in colour until finally a dark grey powder remains which consists mainly of NiO and only contains a small quantity

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[58] Gehlen's *Journal der Chemie*, 1805, *6*, 580.

of Ni_2O_3.[59] In view of the fact that Ni_2O_3 is endothermic with respect to NiO, these two statements appeared to be contradictory and Proust's method of preparing Ni_2O_3 was examined by W. P. Pepper in my laboratory. About 20 grm. of one of the nickel carbonate preparations were heated in a porcelain dish with constant stirring at 220° to 250° until the evolution of gaseous products had ceased.[60] The resulting black product was analysed and found to contain 96 per cent of NiO and 4 per cent of Ni_2O_3. As the result of further experiments it was found that the relative proportion of the two oxides in the heated product depended on the type of nickel carbonate. Thus the pale green carbonate prepared from a saturated solution of the bicarbonate gave on heating a product which contained 95·3 per cent NiO and 4·7 per cent Ni_2O_3, whilst the granular emerald green material prepared by the electrolytic method in 1928 gave a product which contained 97·6 per cent NiO and 2·4 per cent Ni_2O_3.

These observations with nickel carbonate clearly indicate that the formation of Ni_2O_3 must be due to the surface oxidation of crystal units of NiO. Since NiO crystallizes in the cubic (NaCl) system, the size of these crystal units can be calculated from the amount of Ni_2O_3 present in the product. If x be the number of Ni and O atoms in the edge of the cube, the number of atoms in the surface of the cube will be $x^3 - (x - 2)^3$, and, if the oxidation be restricted to the atoms in the surface, the product will contain $\frac{x^3 - (x - 2)^3}{4}$ molecules of Ni_2O_3 and $\frac{(x - 2)^3}{2}$ molecules of NiO. Since the distance between adjacent atoms of Ni and O in crystalline NiO is $2{\cdot}086 \times 10^{-8}$ cm., the length of the edge of the crystal unit will be $x \times 2{\cdot}086 \times 10^{-8}$ cm. It can be shown by this method that a product which contains 2·4 per cent Ni_2O_3 and 97·6 per cent NiO consists of cubes of NiO with an edge of about $5{\cdot}7 \times 10^{-6}$ cm. coated with a unimolecular layer of Ni_2O_3, and that a product containing 4·7 per cent Ni_2O_3 consists of cubes of NiO with an edge of about $2{\cdot}92 \times 10^{-6}$ cm. coated with a unimolecular layer of Ni_2O_3.

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[59] *Zeitsch. anorg. Chem.*, 1895, *10*, 240.

[60] Baly and Pepper, *Trans. Faraday Soc.*, 1936, *32*, 375.

It must be noted that the size of the crystal units of NiO as determined by this method may be larger than those existent in the nickel carbonate before it was heated. It was found that when the nickel carbonate was finely powdered before it was heated the Ni_2O_3 content of the product was a little smaller, and this indicates that the crystal units of NiO increase in size when the adsorbed water and carbon dioxide are evolved.

It is evident from these observations that nickel closely resembles iron, aluminium, tin, and indium, the hydrous oxides of which were investigated by Weiser and Milligan, who advanced convincing evidence that these oxides consist of minute crystal units of oxide and large amounts of water held by adsorption and capillary forces.[61] The evidence in the case of precipitated nickel carbonate indicates that this substance also consists of minute crystal units of the oxide together with water and carbon dioxide held by adsorption and capillary forces.

In the investigation described on page 89 of the photosynthetic activity of ferric oxide supported on aluminated kieselguhr it was found that the activity was materially enhanced when the ferric oxide contained 2·15 per cent and 1·3 per cent of thorium oxide, and also that the surface potential of these preparations had maximum values. This led us to investigate the effect of thorium oxide on nickel oxide and this was studied by J. Lord. A new method of preparation was adopted. A molar solution of pure nickel nitrate and a standardized solution of pure thorium nitrate were prepared. In each preparation a known volume of the thorium nitrate solution was added to 66·94 ml. of the nickel nitrate solution, which corresponds to 5 grm. of NiO, and the mixture was evaporated to dryness with continuous stirring on a sand bath. The product, after further heating, was finally ground and heated at 700° in a silica tube in a current of carbon dioxide until the black Ni_2O_3 had been completely decomposed to NiO, after which the product was allowed to cool in an atmosphere of carbon dioxide. The NiO thus obtained was stable in air owing to its being coated with an adsorbed layer of carbon dioxide. Altogether twenty-one preparations were made in which the

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[61] *Trans. Faraday Soc.*, 1936, *32*, 358.

thorium oxide content varied from 1·73 per cent to 6·00 per cent.

The surface potential of each of these preparations was determined by measuring the velocity of their movement in suspension when a known potential gradient was applied. The apparatus used is shown in Fig. 8 and is a modification of the well-known Burton tube.[62] This apparatus was found to be very convenient and to give strictly comparable results.

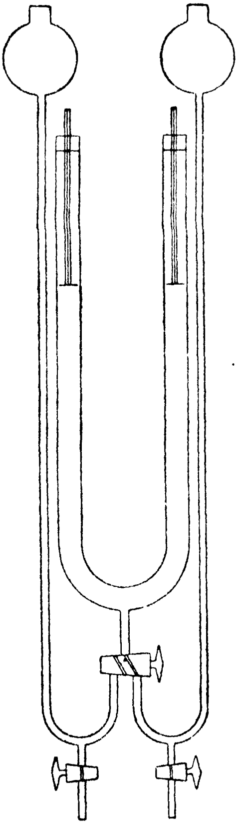

FIG. 8

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In the early measurements very finely ground suspensions of the substance in water saturated with carbon dioxide were used, but it was found that such suspensions were liable to undergo flocculation. As the result of this, suspensions in 0·0524N-acetic acid were used. It was proved that this acid, prepared by diluting 15 ml. of pure glacial acid to 5 litres, had very little action on the particles in suspension. The suspensions contained 0·5 grm. of the finely ground substance in 100 ml. of the 0·0524N-acetic acid.

The surface potential was calculated from the formula

$$\zeta = \frac{4\pi\eta v}{DV}$$

where η is the viscosity of the medium, v is the velocity of movement of the particles in centimetres per second, D is the dielectric constant, and V is the potential gradient in volts per centimetre. The viscosity of the 0·0524N-acetic acid was assumed to be the same as that of water and the dielectric constant was determined for us by H. Rogan and his value of 79·41 was used.

The surface potentials of the twenty-one preparations of

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[62] *Phil. Mag.*, 1906, *12*, 425, 472.

NiO containing ThO_2 were found to be a periodic function of the ThO_2 content, well-defined maximum values being observed with the preparations containing 4·9, 2·95, and 2·1 per cent of ThO_2. Expressing these percentages in molecular ratios of ThO_2 to NiO, they are $1ThO_2 : 72NiO$, $1ThO_2 : 120NiO$, and $1ThO_2 : 168NiO$, and it is of great interest to note that these ratios are $1ThO_2 : 24 \times 3, 5,$ and $7NiO$. The calculated and observed percentages of ThO_2 are given in Table VIII.

TABLE VIII

Molecular ratios	ThO_2 content obs. Per cent	ThO_2 content calc. Per cent
$1ThO_2 : 3 \times 24NiO$	4·9	4·9114
$1ThO_2 : 5 \times 24NiO$	2·95	2·9468
$1ThO_2 : 7 \times 24NiO$	2·1	2·1049

In order to determine whether the new method of preparation of these powders was suitable from the point of view of photosynthesis, preparations of NiO containing 4·9 per cent and 2·1 per cent ThO_2 were irradiated in suspension in water saturated with carbon dioxide. A new source of trouble was now met with in that, owing to the powders having the large surface potential of + 0·062 volt, they adhered to the glass walls of the irradiation vessel, which were electronegatively charged, and shielded the bulk of the suspension from the light. A further quantity of each material was then spread in a thin layer on a glass sheet and pre-irradiated from above and below for 15 hours in an atmosphere of carbon dioxide. Each preparation was then immediately irradiated in suspension in water saturated with carbon dioxide for 2 hours. It was then found that the powders did not adhere to the glass walls and a considerable amount of organic matter was obtained when each filtrate was evaporated to dryness. The photosynthesized material, however, did not give the Molisch reaction for carbohydrates.

These results, which were obtained in 1933, were the first evidence of photosynthesis obtained since Hood and I carried out the experiments with the nickel carbonate prepared by the electrolytic method. Encouraging as these results appeared to be, they were at the same time difficult to understand. They were encouraging because they indicated the correctness of the conclusion previously reached that photosynthetic activity depends on the presence of crystal units of NiO. They were difficult to understand because the products of photosynthesis previously obtained contained hexoses.

In the meantime a new method of preparing nickel carbonate had been devised by my son E. J. Baly and J. W. Ireland, and it was decided to investigate this method before proceeding further with Lord's preparations. The method consisted in the gradual addition of rather less than the equivalent quantity of potassium bicarbonate in M/2·5 solution to a cold M/5 solution of nickel nitrate. In view of the evidence that had been obtained of the effect of thorium oxide, preparations were made of nickel carbonate containing thorium oxide in the molecular ratio of $1ThO_2 : 24NiO$.[63]

When the potassium bicarbonate solution is added to the solution containing nickel and thorium nitrates, the first reaction is the neutralization of the free nitric acid produced by the hydrolysis of the thorium nitrate. Owing to the fact that the concentration of thorium is M/120, no hydrated thorium oxide separates when the solution has been completely neutralized. The further addition of potassium bicarbonate solution results in the following reaction

$$Ni(NO_3)_2 + 2KHCO_3 = Ni(HCO_3)_2 + 2KNO_3$$

and this proceeds until the solution becomes saturated with respect to nickel bicarbonate. The addition of more potassium bicarbonate solution then causes the separation of nickel carbonate containing thorium oxide with the evolution of carbon dioxide.

The method of preparation may be given in exact detail. 2 gram-molecular weights of pure nickel nitrate crystals were dissolved in 4 litres of distilled water and to the filtered solution was added a volume of a standardized solution of thorium nitrate containing one-twelfth of a gram-molecular weight or 22·010 grm. of ThO_2 and the mixture was diluted to 10 litres. 400 grm. of Merck's pure potassium bicarbonate crystals were dissolved in 2 litres of distilled water and, after filtration, the solution was diluted to 10 litres. The alkaline solution was added from burettes to the cold solution of the

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[63] Baly, *Proc. Roy. Soc.*, 1939, *172*A, 445.

two nitrates, which was vigorously stirred by means of two serrated discs electrically driven at 1,500 rev. per min.

The reason why 400 grm. of potassium bicarbonate were used in place of the theoretical quantity of 433·81 grm. was the belief that the danger of the presence of adsorbed alkali would be minimized by the presence of excess of the two nitrates. It was remembered that all the photosynthetically inactive preparations of nickel carbonate made in 1931 by the electrolytic method contained adsorbed alkali.

The precipitated material was collected on a large Buchner funnel, and after being washed with distilled water it was disintegrated by vigorous stirring in 9 litres of cold distilled water. After filtration the product was again disintegrated in cold distilled water and again collected on a Buchner funnel. The final treatment was to disintegrate the material in 6 litres of distilled water and to pass steam into the vigorously stirred suspension until it boiled. During this steam treatment some carbon dioxide was evolved and the colour of the material changed from pale green to a pure emerald green. The material was then collected and dried at 100°. Owing to the solubility of nickel bicarbonate and to some dissolution during the first washings of the precipitated material, the yield of the final product was only about 70 per cent of that calculated.

In order to forestall any possible criticism based on the use of boiler steam in the above process, it may be stated that the steam at 15 lb. pressure was that used for the laboratory supply of distilled water. The steam was supplied at 40 lb. pressure from the central installation at the University of Liverpool through over 100 yards of pipe and the pressure was adjusted to 15 lb. by means of reducing valves. Distilled water raised to 100° by passing in the steam and then cooled was proved to be free from organic matter.

It was found that the hydrated nickel carbonate containing thorium oxide prepared by this method gave some evidence of photosynthesis when, after pre-irradiation in the form of fine powder, it was irradiated in suspension in water saturated with carbon dioxide. In view of this observation and also of the evidence of photosynthesis obtained with nickel oxide containing thorium oxide in the molecular ratio of $1ThO_2 : 72NiO$, it was decided to prepare the mixed oxides by heating the hydrated nickel carbonate containing thorium oxide at as low a pressure as possible. It had previously been found that nickel oxide prepared in this way became superficially oxidized to Ni_2O_3 and, since it was believed that this poisoned the nickel oxide, it was necessary after the mixed oxides had cooled to room temperature to introduce into the exhausted vessel a saturated aqueous solution of carbon dioxide.

About 100 grm. of the hydrated nickel carbonate containing thorium oxide were heated in the apparatus shown in Fig. 9.

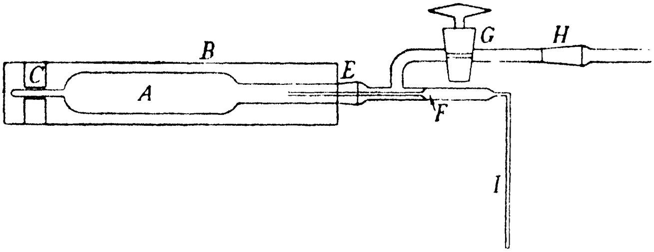

FIG. 9

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The vessel *A* was made of transparent fused quartz, the central portion being 15 in. long and 3 in. wide and the narrow portion was 1¼ in. wide. It was supported inside the vitreosil tube *B* of an electric resistance furnace at one end by the vitreosil reel *C* and at the other end by means of glass-wool packing. At the end of the narrow portion was an accurately ground quartz-to-glass joint *E*, and at *F* was internally sealed a long thin-walled capillary tube which projected well into the quartz vessel. The tube *F* was drawn out to a fine capillary tube *I*, which was bent at a right-angle and sealed at the end. The apparatus was connected with a Hyvac pump through the stopcock *G* and the ground-glass joint *H*.

Some preliminary experiments had shown that the adsorbed water is desorbed very rapidly when hydrated nickel carbonate is heated under reduced pressure, and it was necessary to devise a method to cope with this. A glass tube containing anhydrous calcium chloride was sealed at one end to the plug of the ground-glass joint *H* and at the other end to a glass tube containing phosphoric oxide which was connected through a second glass tube containing phosphoric oxide to the Hyvac pump. The quartz vessel and the connecting tubes were first evacuated to the lowest pressure obtainable and then the temperature of the quartz vessel was slowly raised until it finally reached 250°. Very soon some liquid water condensed in the tubes connecting the quartz vessel with the tube containing calcium chloride, and when this water had been completely evaporated the stopcock *G* was closed. The tube containing calcium chloride was then disconnected and replaced by a tube containing phosphoric oxide, and when the connecting tubes had been completely evacuated the stopcock *G* was again opened.

The temperature of the quartz vessel was then raised to 300°, at which it was maintained while the evacuation was continued until the pressure as measured by means of a McLeod gauge was reduced to below 0·001 mm., an operation which lasted 6 hours. When this low pressure had been attained the stopcock *G* was closed, the heating was discontinued and the whole apparatus allowed to remain overnight. On the following morning the ground-glass joint *H* was opened and the quartz vessel with its attachments removed from the resistance furnace. The tip of the capillary tube *I* was then broken off under 1,400 ml. of distilled water which had been charged with carbon dioxide in a sparklet apparatus. When this solution came into contact with the mixed oxides they became at once coated with adsorbed layers of hydrated carbon dioxide and thereby protected from atmospheric oxidation. The ground-glass plug of the joint *E* was then removed and after the lubricant had been carefully removed from the quartz socket the suspension of the mixed oxides was transferred to the irradiation vessel.

Now it had occurred to us during the previous investigations that the irradiation of a suspension of a powder had certain disadvantages. In the first place the area irradiated was small in relation to the surface area of the particles in suspension, and in the second place the period of irradiation of any one particle must be exceedingly short. For these two reasons we decided to irradiate a stationary layer of the nickel oxide containing thorium oxide whilst a slow stream of water, saturated with carbon dioxide, flowed over it. Before the mixed oxides were prepared as described above the apparatus shown in Fig. 10 was erected. The irradiation vessel *A*, which was a large photographic dish having a surface area of about

1,535 sq. cm., rested on a levelling table not shown in the diagram and placed near a large window. The dish was covered by a plate-glass lid in which had been drilled three holes in a straight line, one in the centre and one near each end. In each of these holes was fixed, by means of a rubber cork, a short length of glass tubing. The tube *B* was sealed at its lower end to the centre of a glass tube $\frac{3}{4}$ in. in diameter, closed at each end. This glass tube extended the whole width of the dish and was provided with a series of fine jets along its left-hand side in the diagram. The tube *D* was also sealed at its lower end to the centre of a glass tube $\frac{3}{4}$ in. in diameter, sealed at each end, which extended the whole width of the dish and was

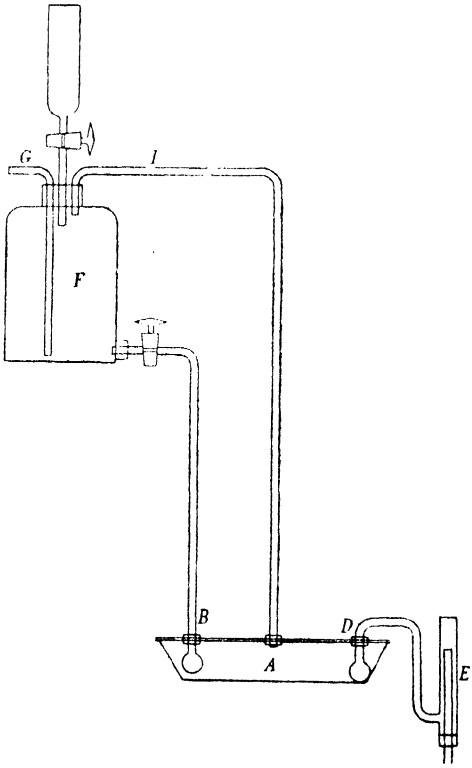

FIG. 10

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provided with a series of small holes along its left-hand side. The plate-glass lid with its attachments could, therefore, be removed and replaced whenever it was necessary. *E* was a constant-level apparatus which was later joined by means of rubber tubing to the tube *D*.

F was a 10-litre aspirator bottle which rested on a shelf 4 ft. above the porcelain dish. In the neck of the aspirator was fitted a rubber cork through which passed three glass tubes. The tube *G* was connected with a cylinder containing liquid carbon dioxide of guaranteed purity. The central tube with its funnel and stopcock enabled the aspirator to be filled with water, and the tube *I* conducted the carbon dioxide into the porcelain dish.

The aspirator bottle *F* was filled with distilled water, and when this had been saturated with carbon dioxide the suspension of the nickel oxide containing thorium oxide in 1,400 ml. of water saturated with carbon dioxide was poured into the dish. The plate-glass lid was then placed in position and the three connections as shown in Fig. 10 were made by short lengths of rubber tubing. The plate-glass lid was immediately covered with black velvet and the apparatus was allowed to remain for 3 hours in order that the mixed oxides might settle out and form a compact layer. It was found, however, that owing to the presence of very fine particles the supernatant liquid remained persistently cloudy. In order to remove this the stopcock of the aspirator was opened, with the result that water saturated with carbon dioxide was forced out through the jets attached to the tube *B*. The jets of solution impinged on the side wall of the dish, with the result that a slow and evenly distributed stream of water flowed over the mixed oxides, the rate of flow being 1 litre in about 13 minutes. During this operation it was necessary at intervals to refill the aspirator with water which had been saturated with carbon dioxide from a second cylinder of the liquid gas. The overflow from the constant level apparatus was allowed to run to waste.

When the overflow from the dish had become clear and free from suspended particles, the aspirator was filled with water saturated with carbon dioxide and the black velvet covering was removed from the plate-glass lid. The overflow from the irradiated vessel was now collected in a flask and returned to the aspirator through which a constant stream of carbon dioxide was maintained. The irradiation was continued for 3½ days, the dish being covered with black velvet during the night.

The solution, which had a volume of 12 litres and was almost saturated with respect to nickel bicarbonate, was concentrated under reduced pressure on steam baths and the hydrated nickel carbonate which separated was removed at intervals by filtration. Shortly after the nickel remaining in solution had been reduced in this way to a very small quantity there began to separate a white insoluble substance, and as the concentration was continued this white substance progressively increased in amount until quite suddenly it turned brown and acquired a gummy consistency which caused it to adhere to the walls of the containing flask. This gummy product had a characteristic organic odour and was readily charred by concentrated sulphuric acid. It was found that the solution had a strong alkaline reaction and there was little doubt that the gummy product had been produced by the action of the alkali on the white insoluble product of photosynthesis.

This result was most interesting because it indicated that by the irradiation of NiO containing ThO_2 in the molecular ratio of $1ThO_2 : 24NiO$ there had been photosynthesized a substance which was rendered insoluble when its solution was concentrated. The only possible explanation of this appeared to be that the product of photosynthesis was much more complex than a simple carbohydrate. The change from the photosynthesis of simple carbohydrates by the irradiation of nickel carbonate in 1927 and 1928 to the photosynthesis of much more complex compounds by the irradiation of NiO containing ThO_2 in the molecular ratio of $1ThO_2 : 24NiO$ indicated that the ThO_2 must play a definite role in establishing photosynthetic activity.

In order to prove this, several preparations of 75 grm. each of NiO containing no ThO_2 were made. The methods of precipitating the carbonate, decomposing the carbonate and the irradiation of the resulting NiO were identical with those described for the NiO containing ThO_2. Although several irradiations by the new and old methods were carried out, no evidence of photosynthesis was obtained. Conclusive proof was thus obtained that the presence of ThO_2 in the molecular ratios of $1ThO_2 : 24NiO$, $1ThO_2 : 72NiO$, or $1ThO_2 : 168NiO$ is essential in the preparation of a photosynthetically active product.

The fact that the new method of preparing NiO containing ThO_2 did not give a product free from alkali was disappointing. Although the presence of the alkali did not appear to inhibit photosynthesis, the alkalinity of the filtrate from each irradiation introduced a difficulty which it was necessary to overcome. At this stage of our investigation a new line of attack on the problem had been opened up by some observations made by W. P. Pepper in my laboratory, and in view of his results it was decided to postpone further investigation of the foregoing method until the new avenue had been thoroughly explored.

Pepper had examined the adsorption of the hydrous metallic oxides by kieselguhr and the results of his investigation led us to visualize the possibility of preparing a photosynthetically active material by coating kieselguhr with an anhydrous layer of NiO containing ThO_2 in the molecular ratio of $1ThO_2 : 24NiO$. After a very protracted investigation by Pepper, Vernon, and myself, we succeeded in so doing and in standardizing the method of preparation. Our hopes of obtaining in this way a photosynthetically active product were fully realized.

In order that the reasons for undertaking this investigation may be understood, a brief résumé of Pepper's observations may be given. When kieselguhr is suspended in a very dilute solution of aluminium, thorium, or ferric nitrate, each of which is almost completely hydrolysed, adsorption of the oxide takes place until an equilibrium state is reached. When a dilute solution of ammonia is slowly added to the suspension, the equilibrium is progressively shifted towards the adsorbed state until the whole of the oxide has been adsorbed.

The coated kieselguhr was then collected on a filter, washed with distilled water and dried at 100°. The surface of each preparation, suspended in 0·0524N-acetic acid, was determined by cataphoresis and it was proved that the adsorbed oxide was not acted on by this acid.

The relation between the surface potential and the amount of alumina adsorbed by 100 grm. of kieselguhr is shown by curve *A* in Fig 11. The first portion of this curve, which is linear, indicates that 100 grm. of the kieselguhr progressively adsorbs the same molecular units of alumina until it is completely coated by 0·00243 grm. mole. From observations to be described later, the effective surface area of 100 grm. of the kieselguhr was found to be 2·869 × 10^6 sq. cm. Assuming the symmetrical distribution of the molecular units on the surface, the diameter of each unit will be the square root of the area covered by two units. If these units are molecules of Al_2O_3,

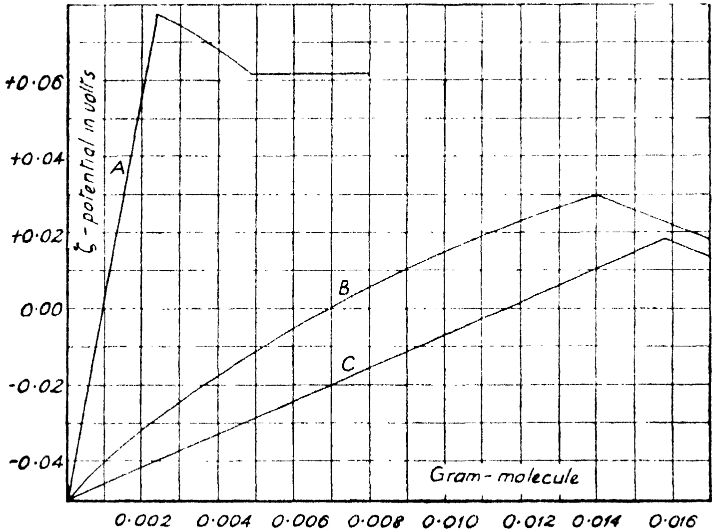

FIG. 11

A Al_2O_3
B NiO containing ThO_2 in the molecular ratio of $1ThO_2 : 24NiO$
C CoO

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the number of groups of two molecules adsorbed on 2·869 × 10^6 sq. cm. is 0·00243 × 3·03 × 10^{23} = 7·3629 × 10^{20}. The area covered by one of these groups is 2·869 × 10^6/7·3629 × 10^{20} = 3·8966 × 10^{-15}, and the square root of this is 6·242 × 10^{-8} cm. This is the same order of magnitude as that given by X-ray measurements of crystalline Al_2O_3. If the adsorbed units are molecules of $Al(OH)_3$, the number of groups of two molecules adsorbed on 2·869 × 10^6 sq. cm. is 0·00486 × 3·03 × 10^{23}, and from this the molecular diameter is found to be 4·414 × 10^{-8} cm. Since this is considerably smaller than the molecular diameter of crystalline $Al(OH)_3$, it may be concluded that the kieselguhr adsorbs a unimolecular layer of Al_2O_3.

A similar linear surface potential relation was obtained with thorium oxide and it was found that 100 grm. of the kieselguhr were completely coated by 0·002894 grm. mole of ThO_2. The molecular diameter of the ThO_2 molecule calculated from this is $5{\cdot}72 \times 10^{-8}$ cm., and this is only a little greater than $5{\cdot}59 \times 10^{-8}$ cm., which is the molecular diameter in the crystalline oxide.

A different method of procedure was necessary in measuring the adsorption of the oxides of cobalt and nickel, because the nitrates of these metals are not hydrolysed in solution and the use of ammonia as precipitant is precluded. Satisfactory evidence of the adsorption of cobalt oxide was obtained by the slow addition of the equivalent amount of sodium carbonate in 0·1M solution to a cold suspension of 10 grm. of kieselguhr in 100 ml. of a solution of cobalt nitrate, the maximum concentration of which was 0·018M. The relation between the surface potential and the amount of the oxide adsorbed as shown by curve *C* in Fig. 11 was linear and the maximum value was obtained when 0·0158 grm. mole had been adsorbed on 100 grm. of kieselguhr.

In the case of nickel 100 grm. of kieselguhr were coated by 0·01632 grm. mole of the oxide, but the relation between the surface potential and the amount adsorbed was expressed by a very pronounced curve.

The effect of thorium oxide when present in the molecular ratio of $1ThO_2 : 24CoO$ and $1ThO_2 : 24NiO$ was next investigated. It was found that 100 grm. of kieselguhr were coated by 0·01354 grm. mole of CoO + 0·01354/24 grm. mole of ThO_2, and by 0·0140 grm. mole of NiO + 0·0140/24 grm. mole of ThO_2, as compared with 0·0158 grm. mole of pure CoO and 0·01632 grm. mole of pure NiO. These observations were of great interest. Since in the case of CoO $0{\cdot}0158 \times 6/7 = 0{\cdot}01354$, it follows that each molecule of ThO_2 must replace one group of 4Co and 4O atoms out of every seven such groups. The same replacement must take place in the case of NiO, because $0{\cdot}01632 \times 6/7 = 0{\cdot}0140$.

Once again the relation between the surface potential and the amount of CoO containing ThO_2 was linear. In the case of

NiO containing ThO_2 the surface potential relation is shown by curve *B* in Fig. 11 and is almost linear, whilst in the absence of ThO_2 the relation was expressed by a very pronounced curve.

Now it was obviously necessary to obtain further information about these results. In the first place the nature of the adsorbate must be determined, and in the second place the explanation must be found of the difference between the surface potential relations in the two cases.

Valuable information was obtained [64] of the nature of the adsorbate when the maximum concentration of the nickel nitrate solution was reduced to 0·0018M and the equivalent volumes of 0·1M sodium carbonate solution were diluted to 1 litre before being added to the kieselguhr suspension. The relation then found between the surface potential and the amount of nickel oxide adsorbed is expressed by the curve in Fig. 12, which clearly indicates that the oxide was progressively adsorbed in three unimolecular layers. Owing to the fact that at the great dilution used a small amount of nickel remained in solution, this curve gives no accurate measure of the amount

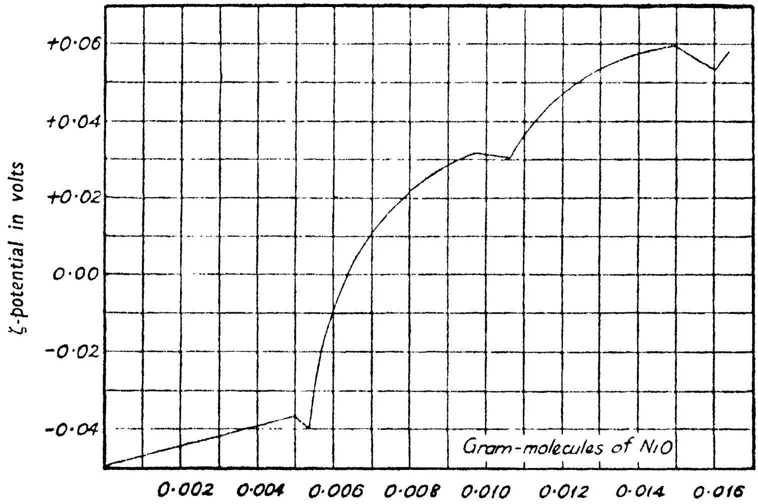

FIG. 12

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[64] Baly, Pepper and Vernon, *Trans. Faraday Soc.*, 1939, *35*, 1165.

of the oxide required completely to coat the kieselguhr. By the slow addition of a very dilute solution of the equivalent amounts of potassium bicarbonate to a boiling suspension of kieselguhr in nickel nitrate solution it was finally proved that 100 grm. of the kieselguhr were completely coated by 0·01632 grm. mole of the oxide.

In order to determine the amount of hydration of the adsorbed CoO, 37·250 grm. of the completely coated kieselguhr were first heated at 100° under reduced pressure in order to dry it, evidence having previously been obtained that neither the hydrated oxide nor the hydrated carbonate is decomposed to the oxide under these conditions. A weighed tube containing phosphoric oxide was then attached to the apparatus which was once again evacuated and heated at 300° for 3 hours. The amount of water collected was 0·0353 grm. or 0·001961 grm. mole.

Now 0·0158 grm. mole of CoO weighs $0{\cdot}0158 \times 74{\cdot}94 = 1{\cdot}1851$ grm. and hence 100 grm. of kieselguhr when completely coated weigh 101·1851 grm. The weight of CoO in 37·250 grm. of the coated kieselguhr is $1{\cdot}1851 \times 37{\cdot}250/101{\cdot}1851 = 0{\cdot}44644$ grm. or 0·00582 grm. mole. It follows that the number of water molecules associated with the adsorbed CoO molecules is in the ratio of $1H_2O : 3CoO$. In view of the fact that when 100 grm. of the kieselguhr is coated with 0·01632 grm. mole of NiO the coating consists of three unimolecular layers, there can be no doubt that the same is true of CoO. No other condition is possible when the amounts of the two oxides which completely coat 100 grm. of the kieselguhr are so closely similar. It thus becomes evident that in the case of cobalt the adsorbate consists of three unimolecular layers of anhydrous CoO and that on the outer surface, after drying at 100° under reduced pressure, there remains adsorbed a unimolecular layer of water.

It is now possible to calculate the surface area of the kieselguhr from the fact that 0·0158/3 grm. mole of CoO completely coat 100 grm. with a single unimolecular layer. The molecular diameter of crystalline CoO is $4{\cdot}24 \times 10^{-8}$ cm. and hence the area covered by 2 molecules is $(4{\cdot}24 \times 10^{-8})^2 = 1{\cdot}79776 \times 10^{-15}$ sq. cm. The number of groups of 2 molecules is $0{\cdot}0158/3 \times 3{\cdot}03 \times 10^{23} = 1{\cdot}5958 \times 10^{21}$ and hence the surface area of 100 grm. of the kieselguhr is $1{\cdot}79776 \times 10^{15} \times 1{\cdot}5058 \times 10^{21} = 2{\cdot}869 \times 10^{6}$ sq. cm. Exactly the same value of the surface area is obtained from the fact that 100 grm. of the kieselguhr are coated by 0·01632 grm. mole of NiO, and it would appear from this that the adsorbate consists of three unimolecular layers of anhydrous NiO. A determination, however, of the amount of water in the adsorbate showed that there was present an equal number of H_2O and NiO molecules. The only possible explanation of these two observations is that the adsorbate consisted of three unimolecular layers of anhydrous NiO separated by unimolecular layers of H_2O. Since it was also proved that, when the NiO contained ThO_2, unimolecular layers of H_2O were also present, the problem to be solved was the coating of kieselguhr with an anhydrous trimolecular layer of NiO containing ThO_2 in the molecular ratio of $1ThO_2 : 24NiO$. We succeeded in solving this problem, and for the benefit of anyone who wishes to prepare this product it is necessary to describe in exact detail the method of preparation.[65]

It must be noted at the outset that the kieselguhr used was the 'Superfloss' brand of the Johns-Manville Company and that the quantities of the various oxides which completely coat 100 grm. of kieselguhr refer to this product only. Since this product was found to contain some clay material and a little organic matter, it was necessary to purify it.

About 5 kg. of the kieselguhr were heated with 12 litres of 4N-HCl in a large enamelled iron basin for about 5 hours. The mixture was maintained in gentle ebullition and was frequently stirred. During this treatment small quantities of dark oily matter were set free and floated on the surface and were removed as far as possible by means of bibulous paper. It was necessary from time to time to add more 4N-acid in order to make good the loss by evaporation. When the oily matter had ceased to separate, the mixture was filtered, about one-third at a time, on a large Buchner funnel and the kieselguhr was washed on the funnel with distilled water until the filtrate was colourless.

It was found that the kieselguhr had a remarkable power

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[65] Baly, Pepper and Vernon, *Trans. Faraday Soc.*, 1939, *35*, 1165; Baly, *Proc. Roy. Soc.*, 1939, *172*A, 445.

of retaining water. Even when no further water could be extracted by suction from the bed of kieselguhr on the funnel, the material still contained enough water to cause it to flow when removed from the funnel. It was found necessary to disconnect the filter flask from the suction pump and gently to tap the bed of kieselguhr with a large flat glass stopper until the whole mass became semi-fluid. The separated water was then removed by suction and the treatment was repeated until no further water could be extracted.

The kieselguhr was then again boiled in quantities of 300 grm. at a time with 4N-HCl and this treatment was repeated until the acid extract gave no reaction for ferric iron with ammonium thiocyanate. In the great majority of cases it was found that two such extractions were sufficient.

The extracted kieselguhr was then washed until free from acid and the following treatment was found to be essential. The kieselguhr, after being washed on the funnel with 10 litres of distilled water, was divided into two approximately equal portions. One portion at a time was completely disintegrated by vigorous stirring in 7 litres of distilled water, and steam at 15 lb. pressure was passed in until the suspension boiled, after which it was filtered immediately. Owing to the fact that the kieselguhr consists of particles with widely different rates of sedimentation, it was necessary, in order to preserve its homogeneity, to stir the suspension continuously and to transfer it to the funnel by means of a ladle. This washing process was repeated until the filtrate was neutral to B.D.H. indicator, and when this end had been achieved the kieselguhr was dried overnight at 125°.

Although the greater proportion had been removed along with the clay, the kieselguhr still contained a very small amount of organic matter and this was oxidized by heating the finely disintegrated material in a current of air. The disintegration presented a problem of some difficulty, because when lumps of the kieselguhr were crushed by a spatula on glazed paper, small and very coherent laminae were formed which materially decreased the effective surface area. The only satisfactory method of disintegration was to shake small lumps of the kieselguhr in a tin-plate tube 30 in. long and 3 in. wide, which was provided with a tightly fitting lid at each end.

The finely disintegrated material was heated in a transparent vitreosil tube which was 4 ft. long and 3 in. wide, care being taken that not more than two-thirds of the diameter of the tube were filled. The tube and its contents were heated at 600° in an electric resistance furnace for 8 hours, a current of dry filtered air being passed continuously through it. At frequent intervals the tube was slowly rotated through several complete revolutions.

The purified kieselguhr was remarkably constant in its properties. A great number of batches were prepared during the investigation and these showed no variation in adsorptive power.

It had been found that in the case of pure NiO the most satisfactory results were obtained when a very dilute solution of potassium bicarbonate was added to a boiling suspension of kieselguhr in a very dilute solution of nickel nitrate. This method was not practicable with mixtures of nickel and thorium nitrates, because at the great dilution which was found to be necessary the latter salt would be almost completely hydrolysed, with the result that on adding the kieselguhr practically the whole of the ThO_2 would at once be adsorbed. It was necessary, therefore, to add a very dilute solution of the requisite amounts of potassium bicarbonate and thorium nitrate to a boiling suspension of the kieselguhr in a very dilute nickel nitrate solution. Considerable difficulty was at first experienced in obtaining a solution of potassium bicarbonate and thorium nitrate which did not deposit hydrated ThO_2. We fully expected that the stability of this solution would be increased by saturating it with carbon dioxide, but it was found that carbon dioxide at once precipitated the whole of the ThO_2. It was finally found that stable solutions of potassium bicarbonate and thorium nitrate could be obtained, provided that they were very dilute and freshly prepared from a concentrated solution of thorium nitrate which contained free nitric acid. It was also found necessary to clean the surface of the containing vessels with strong nitric acid and then to wash them free from acid with distilled water before use.

A number of lots of 10 grm. of the kieselguhr were then completely coated with 0·0014 grm. mole of NiO and 0·0014/24 grm. mole of ThO_2, and each of these was irradiated in suspension in 1,400 ml. of water saturated with carbon dioxide. Evidence of photosynthesis was obtained, but the results varied considerably with different preparations. In some cases a very intense Molisch reaction was obtained with the filtrate from the irradiated suspension, in others the Molisch reaction was faint, and in a few cases no Molisch reaction was given. Furthermore, a certain amount of nickel went into solution during the irradiation and this should not take place if the adsorbed layer consisted of anhydrous NiO and ThO_2.

It thus became evident that the method of coating the kieselguhr was faulty and it was necessary to find out where the fault lay.

In discussing the problem with my two colleagues two possible sources of trouble suggested themselves. One was the fact that we had used a stock solution of potassium bicarbonate, which had been kept in a glass flask and thus might contain some dissolved silica. This was found to be the case and that the silica was brought down with the NiO and interfered with the formation of the crystal lattice of NiO containing ThO_2. The second possibility was that a material improvement might be secured if the concentration of the nickel nitrate in the kieselguhr suspension were maintained constant during the addition of the correct amount of potassium bicarbonate and thorium nitrate. The final method of coating the kieselguhr was then devised, which gave products having a constant photosynthetic activity, and this method may be described in exact detail.

A concentrated solution of thorium nitrate was prepared by dissolving the commercially pure salt in water, adding 20 ml. of concentrated nitric acid and then water to make the volume up to 250 ml. This solution on analysis was found to be 0·40834M with respect to Th ions and 2·9091N with respect to H ions. This procedure was necessary owing to the uncertainty of the composition of the commercially pure salt. An exactly 0·1M solution of pure nickel nitrate and an exactly 0·2M solution of potassium bicarbonate were also prepared, the latter being stored in a quartz flask.

Two solutions were prepared, each by diluting 14·00 ml. of the 0·1M nickel nitrate solution to 2 litres. Exactly 10 grm. of the purified kieselguhr were de-flocculated in 100 ml. of one of these solutions by means of a serrated disc electrically driven at 1,500 rev. per min. The kieselguhr suspension was transferred to a 4-litre enamelled iron saucepan which rested on a powerful gas-ring burner and the remaining 1,900 ml. of the nickel nitrate solution were added. An electrically driven stirrer was then fixed in position so that its blades almost touched the bottom and one side of the saucepan, this position being adopted in order to guard against the formation of a central vortex in the suspension. The blades of the stirrer were 1¼ in. long and the speed was 240 rev. per min. and the direction of rotation was such as to drive the suspension downwards.

Two 250-ml. burettes of the design shown in Fig. 13 were placed in position so that the ends of their capillary delivery tubes were just above the blades of the stirrer, one on each side of its central axis. The stirrer was then started and the kieselguhr suspension was heated and boiled vigorously for 10 minutes in order completely to de-aerate it.

During these proceedings 0·07143 ml. of the thorium nitrate solution was measured out as accurately as possible by means of a high-grade micro-burette and diluted with water to 500 ml., 8·04 ml. of the 0·2M potassium bicarbonate solution were also diluted to 500 ml., and these two solutions were mixed. This mixed solution contained one-half of the total amounts of thorium nitrate and potassium bicarbonate required. One of the two burettes was filled with this alkaline thorium solution and the other was filled with the second dilute nickel nitrate solution. These two solutions were added to the vigorously boiling kieselguhr suspension at the same rate of four drops a second from the jets just below the stopcocks of the burettes.

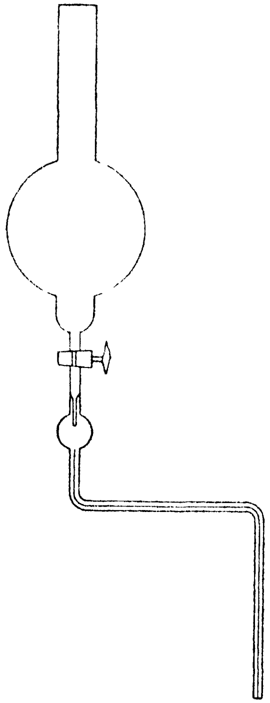

FIG. 13

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It was, of course, necessary from time to time to add boiling water in order to keep the volume of the suspension approximately constant. This water had previously been boiled for some time in pyrex flasks in order to de-aerate it. Shortly before the first half of the alkaline thorium solution had been added to the boiling suspension, the second half was made up as described above and added to that remaining in the burette.

When the whole of the alkaline thorium and nickel solutions had been added, an operation which took about 3 hours, the suspension was boiled for a further 15 minutes. The coated kieselguhr was then collected on a 4-in. Buchner funnel, washed with 1 litre of distilled water and then immediately transferred to an electrically heated oven and dried overnight at 60°. During the washing the coated kieselguhr turned grey-black on the surface and this phenomenon proved to be of great indicative value, since the darkening was very pronounced with the most photosynthetically active preparations. The grey-black colour disappeared during the drying in the oven.

The amount of adsorbed water in these preparations was determined by the same method as was described above in the case of adsorbed cobalt oxide and the amount found was equivalent to one-third of the nickel oxide. This indicated that the adsorbate consisted of anhydrous NiO and ThO_2 and was coated with a unimolecular layer of water.

It is worthy of record that during this investigation it was not found possible to coat kieselguhr with an anhydrous trimolecular layer of NiO in the absence of ThO_2. The evidence that was obtained definitely indicated that the presence of molecules of ThO_2 in the ratio of $1ThO_2 : 24NiO$ very materially aided the formation of the anhydrous adsorbate. It is well within the bounds of possibility that the ThO_2 molecules act as nuclei around which the NiO molecules arrange themselves in forming the crystal unit cells.

Several of these preparations were irradiated for 2 hours each in suspension in 1,400 ml. of water saturated with carbon dioxide. It was found that the amount of nickel present in the filtrate from each irradiation was less than the limit of the test with dimethylglyoxime. In this respect, therefore, these preparations were superior to those made by the previous method. In no case, however, was any evidence of photosynthesis obtained. This result was very disappointing and, indeed, extremely disconcerting until it occurred to us that, if we had succeeded by the new method in making preparations which were highly active photosynthetically, they might have become poisoned by the product of photosynthesis when brought out of the drying oven into daylight. Some evidence of this poisoning was obtained by extracting with HCl a number of preparations which had been brought out of the drying oven and exposed when dry to daylight. The filtrate from this extraction gave the Molisch reaction. Conclusive proof of this poisoning of the preparations by the product of photosynthesis was obtained at a subsequent stage of the investigation and will be given later.

In order to eliminate the danger of poisoning, the drying oven was removed to a dark room. About 1,400 ml. of a saturated solution of carbon dioxide in de-aerated distilled water were prepared in the glass irradiation vessel, the approximate dimensions of which were 8·5 × 5·5 × 2 in., and this was taken into the dark room. The coated kieselguhr was removed from the oven and added to the solution in complete darkness. The NiO and ThO_2 surface was thereby immediately coated with adsorbed hydrated carbon dioxide in the presence of excess of a saturated solution and hence the suspension could be brought into the light without danger of poisoning. The suspension after being dispersed by an electrically driven stirrer was irradiated by two 250-watt lamps, one being placed at a distance of 20 in. from each side of the vessel. By means of an electrically heated mat the temperature was maintained at 28°–30°, because it was proved in 1929 that the rate of photosynthesis increases with the temperature up to 31° and rapidly decreases when the temperature exceeds 31°.[66] During the irradiation the suspension was stirred at intervals, and after 2 hours it was filtered and the filtrate examined immediately.

First of all the presence of Ni ions was tested for with dimethylglyoxime, and, in general, the amount present was less than the limit of this test. Considerable importance attaches to this test, because it was found that when a visible

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[66] See page 83.

precipitate of nickeldimethylglyoxime was formed the coated kieselguhr was unsatisfactory. This had already been noted with the preparations made by the previous method.

It is of interest to record some observations which were made of the limit of sensitivity of the dimethylglyoxime test for nickel. In each test 20 ml. of the filtrate from an irradiation were taken, and after the addition of a little dimethylglyoxime solution and sufficient ammonia to make the solution alkaline the mixture was heated at 60° for 5 minutes. The limit of the test was taken to be the development of only a faint yellow colour when the solution was viewed through a depth of 12 cm. These faintly yellow solutions on being allowed to remain at room temperature for 16 hours deposited about 0·0005 grm. of nickeldimethylglyoxime. The total amount of nickel, therefore, in the 1,400 ml. of filtrate from an irradiation was about 0·009 grm. expressed as $Ni(OH)_2$.

When a small quantity of the filtrate was acidified with hydrochloric acid no evidence could be obtained of the hydrolysis of the product of photosynthesis. When, however, a small quantity of the filtrate was saturated with sulphur dioxide and allowed to remain for 10 minutes, a definite Molisch reaction was obtained indicating the formation of a carbohydrate. It will presently be shown that the concentration of the hydrolysable product of photosynthesis was very small, and it is probable that the failure to obtain any evidence of hydrolysis by hydrochloric acid was due to the oxidation of the product of the hydrolysis by dissolved oxygen. This oxidation would be eliminated when the filtrate was saturated with sulphur dioxide, since the dissolved oxygen reacts at once to give sulphuric acid. The presence of dissolved oxygen was proved by the immediate formation of sulphuric acid when the filtrate from an irradiated suspension was treated with sulphur dioxide.

When the filtrate from an irradiation had been allowed to remain for 2 hours at room temperature, or when it had been heated at 60°, it no longer gave the Molisch reaction after saturation with sulphur dioxide. This interesting fact was conclusively established by a great number of observations, and it definitely proves that the first product of photosynthesis is an unstable substance which changes slowly at room temperature and rapidly at 60° to a second substance which is not hydrolysed by sulphurous acid.

In the first series of irradiations of suspensions of 10 grm. of kieselguhr coated with NiO containing ThO_2 the filtrate was concentrated in a pyrex flask under reduced pressure on a steam bath. When the volume had been reduced to about one-third, there commenced to separate a small quantity of a finely divided white solid. This substance was similar in appearance to the white solid which separated during the concentration of the filtrate from the irradiation of a stationary layer of unsupported NiO containing ThO_2 and was described on page 107. After further concentration the white solid was collected on a Gooch crucible and dried at 60°. The dry substance had a greenish tinge, owing to the presence of a trace of nickel, and was proved to contain organic matter by the fact that it rapidly charred when warmed with concentrated sulphuric acid.

As the result of the irradiation of several lots of 10 grm. of the supported NiO containing ThO_2, about 0·035 grm. of this solid product was obtained. It was apparently unaffected by acid and a micro-combustion gave only minute quantities of water and carbon dioxide, in spite of the fact that it gave considerable charring with warm concentrated sulphuric acid. The possibility then occurred to me that we had realized the conditions which obtain in the leaves of living plants and that, since the first recognizable product of photosynthesis in the leaves of the dicotyledonous plants is a starch, our white product of photosynthesis was a type of starch.

In order to test this a portion of the greenish-white solid was suspended in very dilute acid, a little pure taka-diastase was added and the mixture allowed to remain for 2 hours at 37°. After filtration, the solution was boiled with Fehling's solution and reduction took place, a small quantity of cuprous oxide being obtained. A blank experiment was then carried out in which a solution of taka-diastase of the same concentration was boiled with Fehling's solution, and in this case no evidence of reduction was obtained. This hydrolysis of the second and stable product of photosynthesis to a reducing sugar by means of taka-diastase was confirmed several times.

Our cordial thanks are due to Messrs. Parke, Davis & Company for their gift of pure taka-diastase.

These results were extremely interesting and most encouraging. We were, however, quite unable to understand the failure to oxidize more than a very small fraction of the second and complex product of photosynthesis when the greenish-white solid was submitted to micro-combustion. In order further to investigate this, 20 grm. of the supported NiO containing ThO_2 were irradiated in suspension, and the filtrate was concentrated in a pyrex flask under reduced pressure on a steam bath until the greenish-white product commenced to separate. At this stage the liquid was tested with B.D.H. indicator and found to be alkaline. By titration with standard acid it was found to be about 0·0015M with respect to potassium carbonate. It was proved by experiment that this alkalinity was sufficient to dissolve a little silica from the walls of the pyrex flask and that this silica was brought down with the greenish-white solid and prevented it from being more than superficially oxidized during micro-combustion. The evidence for this statement was, firstly, that the greenish-white solid contained silica, and secondly, that when the filtrate from an irradiation had been exactly neutralized no solid separated during its concentration.

A second series of irradiations of 10 grm. of the supported NiO containing ThO_2 was then carried out, and in each case the filtrate, after the dimethylglyoxime test had proved the presence of only a minute quantity of nickel, was made neutral or only just acid by means of very dilute hydrochloric acid. Since the solution was itself acid owing to the dissolved carbon dioxide, this neutralization required great care. The dilute acid was added drop by drop until 2 ml. of the solution became on boiling neutral to B.D.H. indicator. The solution was then evaporated to dryness under reduced pressure in a pyrex flask on a steam bath. No solid matter separated during the concentration, and there was finally left on the bottom of the flask a thin film of a colloidal substance which was tinged yellow by a trace of anhydrous nickel chloride. This film was dissolved in a little water and transferred to a 50-ml. graduated flask. After the addition of 2 ml. of acetate buffer solution and 2 ml. of taka-diastase solution, water was added to make the total volume up to 50 ml. The flask was allowed to remain for 2 hours in an oven at 37° and then the reducing power of the solution was determined under the conditions laid down by Munson and Walker.[67] The amount of cuprous oxide obtained was 0·007 grm.

This result was confirmed many times, but the amount of cuprous oxide obtained did not appreciably vary. Since increasing the time of irradiation had no effect, it was evident that the amount of starch photosynthesized must be limited by some unknown factor coming into play. In order to investigate the origin of the restricted yield, a stationary layer of the coated kieselguhr was irradiated in the apparatus described on page 105. Immediately the irradiation was commenced evidence was obtained of the formation of the first product of photosynthesis, which gives the Molisch reaction after it has been hydrolysed by sulphurous acid. The concentration of this product in the solution steadily increased during the first few hours of the irradiation, and then the concentration began suddenly to decrease rapidly until no evidence of the presence of this first product or of the starch could be obtained.

This observation, which was recorded on two separate occasions with different preparations of the supported NiO containing ThO_2, is highly significant. The fact that the concentration of the first product in the solution progressively increases during the first period of the irradiation indicates that photosynthesis is taking place continuously during that period. The sudden and rapid decrease in the concentration of this product until no evidence of its presence in the solution is obtained can only be explained by a rapid decrease in the photosynthetic activity of the NiO and ThO_2 surface until it becomes quite inactive. The surface, therefore, must become poisoned during this second period of the irradiation. This poisoning cannot be caused by the first product of photosynthesis, because, if this product were a poison, little or no evidence of its formation would have been obtained. The only substance, therefore, which can poison the surface is the second product of photosynthesis, namely, the starch compound into which the first product slowly changes at room

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[67] *J. Amer. Chem. Soc.*, 1906, *28*, 663.

temperature. Confirmation of the poisoning of the NiO and ThO_2 surface was obtained by treating the irradiated powder with the minimum volume of 3N-HCl, when the filtrate gave a very definite Molisch reaction.

This poisoning of the surface undoubtedly explains the limitation of the amount of starch photosynthesized when the supported NiO containing ThO_2 was irradiated in suspension in water saturated in carbon dioxide. In order to test this explanation further quantities of 10 grm. were irradiated and the irradiated powders were treated with the minimum volume of 3N-HCl. Very definite Molisch reactions were obtained with the filtrates from these extractions. The poisoning is also quite in accord with the experience we met with during the investigation of the method of coating kieselguhr with NiO containing ThO_2. We found that the preparations became poisoned when they were brought out of the drying oven into the daylight. It is known that when in the oven the NiO and ThO_2 surface is coated with a layer of hydrated carbon dioxide which is only desorbed at 250°–300°. In the absence of liquid water this adsorbed layer appears to be changed at once by daylight into an adsorbed layer of the second product of photosynthesis.

The proof of the poisoning of the NiO and ThO_2 surface by the product of its irradiation indicates that, in order to photosynthesize the starch compound in quantity, it would be necessary to irradiate a stationary layer of kieselguhr coated with NiO containing ThO_2, with a continuous flow over it of a freshly prepared saturated solution of carbon dioxide. With the apparatus described on page 105 this would involve the use of 35–40 litres of this solution a day, and, since no apparatus was at that time available for the rapid concentration of large volumes of liquid, it was not found possible to carry out this method of irradiation before my retirement from Liverpool in 1937.

The experience we had gained as regards the treatment of the filtrates from the irradiation of suspensions of kieselguhr coated with NiO containing ThO_2 enabled us once again to investigate the irradiation of unsupported NiO containing ThO_2. We were encouraged to undertake this for two reasons. The first reason was the fact that the preparation of the unsupported material is much less difficult than the coating of kieselguhr with NiO containing ThO_2. The second reason will be appreciated from a brief résumé of the results obtained when a stationary layer of the mixed oxides were irradiated for 30 hours, as described on page 106. When the 12 litres of solution, which was almost saturated with respect to nickel bicarbonate, were concentrated under reduced pressure on steam baths and almost the whole of the nickel had been removed as the hydrated carbonate, there began to separate the greenish-white solid which was proved by later work to contain the starch compound. As was recorded, this substance was subsequently acted on by the alkali present in the solution and yielded a brown gummy material which adhered to the walls of the containing flask. Now the quantity obtained of the greenish-white solid was very considerable, so large, indeed, that we were led to believe that the unsupported NiO containing ThO_2 does not become poisoned in the same way as the supported material. This absence of poisoning appeared to be due to the fact that during the irradiation the nickel oxide passes into solution as the bicarbonate, the surface thereby being continuously renewed. Now the conversion of the greenish-white solid into the gummy material was caused by the alkali which had passed into solution as the result of the dissolution of the NiO containing ThO_2 during the irradiation. The amount of alkali which dissolves must be proportional to the amount of nickel bicarbonate formed and consequently must depend on the volume of water used. It was decided, therefore, not to repeat the original experiment, but to irradiate suspensions of 20 grm. of NiO containing ThO_2 in 1,400 ml. of water saturated with carbon dioxide. These suspensions were prepared by the method described with the apparatus shown in Fig. 9 (p. 103) and were irradiated in the rectangular glass cell for 2 hours. The oxides were maintained in suspension by an electrically driven stirrer and CO_2 was passed in during the whole period.

The treatment of the filtrates from these irradiations required very careful consideration. The method adopted with the filtrates from the irradiations of the supported NiO containing ThO_2 was to neutralize them at once by the careful addition of very dilute hydrochloric acid and then to evaporate them to dryness under reduced pressure, the residue being treated with water and hydrolysed by taka-diastase. These filtrates contained only minute quantities of nickel, whilst those from the irradiation of the unsupported oxides were saturated with respect to nickel bicarbonate.

The immediate neutralization of the filtrates in the latter case, with the attendant formation of $NiCl_2$, at once raised the question as to whether the activity of taka-diastase is affected by the presence of Ni ions. In order to investigate this question a carefully controlled series of experiments was carried out. Two solutions were prepared, one of boiled starch and the other of taka-diastase, and in each experiment 5 ml. of each of these solutions and 5 ml. of acetate buffer were used. In the first experiment this mixture was diluted to 50 ml. with distilled water and the whole allowed to remain at 37° for 2 hours. The reducing power was then determined by the method described above. The reducing power was next determined after 1, 2, 3, 4 and 5 ml. of $0{\cdot}1M\text{–}Ni(NO_3)_2$ had been added before the dilution to 50 ml. The amount of cuprous oxide obtained was the same in the first four experiments, but was appreciably less when 4 ml. of the nickel solution had been used and still smaller when 5 ml. had been used. These determinations, therefore, indicated that the activity of taka-diastase is appreciably decreased when the concentration of nickel ions exceeds 0·006M, that is to say, when 50 ml. of the starch, acetate buffer, and diastase solution contain more nickel than corresponds to 0·028 grm. of $Ni(OH)_2$. It followed from this that if the activity of the taka-diastase were not to be affected, it would be necessary to continue the concentration of the filtrate from an irradiated suspension of unsupported NiO containing ThO_2 until sufficient hydrated nickel carbonate had separated to reduce the total amount in solution to 0·028 grm. calculated as $Ni(OH)_2$.

Now evidence was given above on page 122 that the starch product of photosynthesis separated out in the form of insoluble complexes with hydrated nickel carbonate and some silica when the filtrate from an irradiation of supported NiO containing ThO_2 had been concentrated to about one-third of its volume. It is probable, therefore, that some of the starch will be brought down with the large amount of hydrated nickel carbonate which separates when the saturated solution of nickel bicarbonate obtained by the irradiation of the unsupported oxides is concentrated. This indicates that the amount of the starch product remaining in the concentrated solution will very probably be considerably smaller than that actually photosynthesized.

For these reasons we thought it desirable in treating the filtrates from the irradiations of the unsupported NiO containing ThO_2 to adopt a method which lay between the two extremes, namely, (1) immediate neutralization by means of acid, and (2) concentration until the total Ni content is reduced to 0·028 grm. expressed as $Ni(OH)_2$ and then neutralization by means of acid. The method adopted was to concentrate the filtrate under reduced pressure on a steam bath until the rate of separation of the hydrated carbonate began rapidly to decrease. The solution after filtration was then exactly neutralized with dilute hydrochloric acid and evaporated to dryness. The residue was dissolved in water, filtered, treated with taka-diastase and boiled with Fehling's solution in accordance with the method previously described.

Several irradiations of the unsupported NiO containing ThO_2 were carried out, and in each case evidence was obtained of the formation of a reducing sugar by the action of taka-diastase on the product of photosynthesis. The amount of cuprous oxide obtained on boiling with Fehling's solution varied from 0·008 to 0·005 grm. These results, therefore, confirmed those obtained by the irradiation of NiO containing ThO_2 in the molecular ratio of 1 : 24 adsorbed on kieselguhr.

Now it must be remembered that in the irradiation of a stationary layer of the unsupported NiO containing ThO_2 a considerable amount of alkali went into solution (p. 107). It was recorded that when the solution was concentrated, the white insoluble product of photosynthesis was decomposed to a brown gummy substance by this alkali. In these last irradiations of the unsupported oxides a large amount of alkali also went into solution, and as the result of this a considerable quantity of silica was dissolved from the walls of the pyrex glass during the concentration of the solution. When, after neutralization with hydrochloric acid, the concentrated solution was evaporated to dryness the silica in the dry residue was insoluble in water. On treatment with water the silica became gelatinous and was removed by filtration. In view of the insolubility of the greenish-white precipitate obtained in the first irradiations of the supported NiO containing ThO_2 and of the fact that this precipitate contained the starch product of photosynthesis and silica, it is certain that in the experiments described above with the unsupported oxides the whole of the starch product was not dissolved when the dry residue was extracted with water. The amounts of cuprous oxide recorded above, therefore, give no measure of the amounts of the starch product which had actually been photosynthesized.

In the account given in Chapter IV of the early experiments in 1927 with hydrated nickel carbonate, reference was made to the fact that when irradiated this substance turned dark on the surface owing to the formation of Ni_2O_3, and that this superficial layer of Ni_2O_3 reverts to NiO in the presence of hydrated carbon dioxide. Then again, it was found in 1936 and recorded on page 97 that the black product obtained by heating the hydrated carbonate at 250° until the evolution of gaseous products has ceased consists of minute cubic crystals of NiO coated with a unimolecular layer of Ni_2O_3. These observations suggested that it might be possible to prepare a photosynthetically active product by heating in air the hydrated nickel carbonate containing ThO_2, prepared by the method described on page 101. In order to put this suggestion to the test of experiment, 25 grm. of this material were heated in a porcelain basin at 250° with constant stirring until the evolution of gaseous products had ceased. The black residue was irradiated for 2 hours in suspension in water saturated with carbon dioxide. The filtrate was treated in exactly the same way as were the filtrates from the irradiation of suspensions of the mixed oxides prepared by heating under reduced pressure the precipitated hydrated nickel carbonate containing thorium oxide, and admitting water saturated with carbon dioxide into the evacuated apparatus. The same results were observed, about 0·006 grm. of cuprous oxide being obtained when the product of the hydrolysis of the photosynthesized starch by taka-diastase was boiled with Fehling's solution.

This result, which was obtained eight times, affords additional confirmation of the results obtained by the irradiation of NiO containing ThO_2 adsorbed on kieselguhr.

It must be emphasized that, although confirmation was thus obtained of the photosynthesis of the starch product by the irradiation of suspensions of unsupported NiO containing ThO_2 in water saturated with carbon dioxide, the difficulties associated with the use of this material are sufficiently great to render the method unsatisfactory. The material cannot be prepared free from alkali, and during its irradiation much NiO dissolves, with the result that the filtrate from the irradiated suspensions is saturated with nickel bicarbonate and is very alkaline.

The trimolecular layer of NiO containing ThO_2 adsorbed on kieselguhr is entirely free from these disadvantages. The amounts of nickel and alkali which pass into solution during its irradiation are exceedingly small, so that the filtrate from an irradiation may be immediately neutralized with dilute hydrochloric acid and evaporated to dryness under reduced pressure. Evidence of the photosynthesis of the first carbohydrate which is unstable can only be obtained by the irradiation of this material. The supported NiO containing ThO_2 is highly photosensitive, since it is almost instantaneously poisoned when it is brought into daylight after being dried in an oven at 60°. It is true that the supported oxides tend to become poisoned by the product of their irradiation, but this can be avoided by the irradiation of a stationary layer whilst a slow stream of a freshly prepared saturated solution of carbon dioxide flows over it.

The results of the investigations described in this chapter may now be summarized in the following statements :

1. It has been proved that a surface of NiO, which contains ThO_2 in the molecular ratio of $1ThO_2 : 24NiO$, is photosynthetically active, the presence of the ThO_2 being essential.
2. When kieselguhr, which is completely coated with a trimolecular layer of these oxides, is irradiated in presence of water, saturated with carbon dioxide, by daylight or the light of a tungsten filament lamp, there is photosynthesized a carbohydrate.
3. This carbohydrate, when its solution is saturated with

sulphur dioxide, is hydrolysed to a simpler carbohydrate which gives the Molisch reaction.

4. This first carbohydrate is unstable and changes slowly at room temperature and rapidly at 60° to a stable compound.

5. The stable compound, which is colourless, is hydrolysed by taka-diastase to a reducing sugar and is probably, therefore, a type of starch.

6. The first product of photosynthesis appears, therefore, to be the parent of the starch, the existence of which has not hitherto been recognized.

7. The photosynthesis of the starch compound was fully confirmed by the irradiation of unsupported NiO containing ThO_2.

8. When the unsupported oxides are irradiated the transitory existence of the first product of photosynthesis cannot be recognized owing to the fact that much nickel passes into solution as the bicarbonate.

9. The photosynthesis of carbohydrates on a surface of NiO is promoted by daylight and by light from an electric filament lamp. In this respect the process is exactly analogous to that in the living plant.

10. The final product of photosynthesis, which is formed by the condensation of the first product, is a type of starch and hence the process is similar to that in the dicotyledonous plants.

To these conclusions may be added the analogies between the laboratory and vital processes which were established in Chapter IV.

11. The rate of photosynthesis *in vitro* and *in vivo* increases with the temperature and the temperature coefficients, for the temperature ranges of 5° to 10°, 10° to 20°, and 20° to 30° are the same in the two processes.

12. In both processes the rate of photosynthesis with rise in temperature increases to a maximum value, and with further rise in temperature the rate of photosynthesis begins suddenly to decrease very rapidly.

13. In both processes the amount of carbohydrate photosynthesized by unit quantity of light energy decreases with increase in the intensity of the light.

CHAPTER VI

THE ASSIMILATION OF NITROGEN BY THE LIVING PLANT

A PROCESS which ranks in importance with the photosynthesis of carbohydrates is that by which proteins and other complex nitrogen compounds are produced in the living plant. The general opinion appears to be that nitrogen assimilation is not a photosynthetic process and the position is summed up by Stiles in the following words [68]:

> The opinion that an intimate relation exists between the production of carbohydrates in green plants and the production of proteins has frequently been expressed, but the evidence on the matter is contradictory. The view appeared to be general at one time that in higher plants the synthesis of proteins was confined, or almost confined, to the leaves [69] and might indeed depend on light, while to-day the general opinion appears to be that, although the leaves are the principal centres of protein formation, other organs of the plant possess the power to synthesize this material.[70] There appears to be no doubt that protein formation is possible in leaves in the dark.[71]

On the other hand, Spoehr wrote:

> The fact that the photosynthetic quotient, O_2/CO_2, is very close to unity has been very generally accepted as evidence that the product of reduction of carbon dioxide is a carbohydrate. There are, however, some experimental observations which, while not in direct contradiction to the fact just stated, are not in accord therewith and may nevertheless be of considerable importance in throwing some light on the chemical steps involved in the process.
>
> It has long been known that proteins are formed in the leaf when

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[68] Walter Stiles, *Photosynthesis*, p. 203. Longmans, Green & Co., London, 1925.

[69] W. Pfeffer, *The Physiology of Plants*, Vol. I. Oxford, 1900.

[70] F. Czapek, *Biochemie der Planzen*, Zweite Band. Jena, 1920.

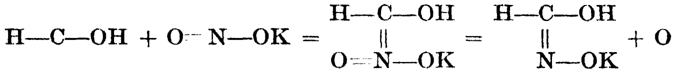
[71] U. Suzuki, *Bull. Coll. Agric. Imp. Univ. Tokio*, 1897, *2*, 409; *3*, 241; *Bot. Centralbl.*, 1898, *75*, 289.

there is an adequate supply of carbohydrates and there is some evidence that the rate of protein synthesis in leaves is greater in light than in the dark.[72]

Now there are on record a number of experimental observations which indicate that complex nitrogen compounds are photosynthesized by the action of activated formaldehyde on potassium nitrite and, since these compounds are similar to those produced in the living plant, these observations would appear to have an important significance in relation to the problem of nitrogen assimilation in the living plant.

There is no doubt that the parent substance from which living plants derive their nitrogen is potassium nitrate, some ammonium nitrate being also utilized. It is well known that potassium nitrate is reduced to nitrite in the roots and stems of plants, and Schimper[73] found that nitrites are always present in the living leaf in the dark and that they disappear in the light. This disappearance only takes place in the presence of chlorophyll, since when partly etiolated leaves are illuminated the nitrites remain unaltered in those parts which contain no chlorophyll.

Baudisch[74] was the first to suggest that the first step in nitrogen assimilation in the living plant is the production of formhydroxamic acid by the action of activated formaldehyde on potassium nitrite in accordance with the following scheme :



$$\mathrm{H{-}C{-}OH} + \mathrm{O{=}N{-}OK} = \begin{array}{c}\mathrm{H{-}C{-}OH}\\ \| \\ \mathrm{O{=}N{-}OK}\end{array} = \begin{array}{c}\mathrm{H{-}C{-}OH}\\ \| \\ \mathrm{N{-}OK}\end{array} + \mathrm{O}$$

the potassium salt being completely hydrolysed to the free acid. Baudisch also proved experimentally that formhydroxamic acid reacts rapidly with more activated formaldehyde to form complex nitrogen compounds, many of which are known to occur in the living plant.[75]

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[72] *Photosynthesis*, pp. 220–2. Chemical Catalogue Co., New York, 1926.

[73] *Bot. Zeit.*, 1888, *46*, 65.

[74] *Ber. Deutsch. Chem. Ges.*, 1911, *44*, 1009.

[75] *Zeitsch. angew. Chem.*, 1913, *26*, 612 ; *Ber. Deutsch. Chem. Ges.*, 1913, *46*, 115 ; 1916, *49*, 1159, 1167, 1176 ; 1917, *50*, 652 ; 1918, *51*, 793.

The photochemical production of formhydroxamic acid from potassium nitrite was confirmed in Liverpool[76] by the irradiation with ultra-violet light of aqueous solutions of this salt which were saturated with carbon dioxide and of solutions of the nitrite containing formaldehyde. In both cases the formhydroxamic acid was identified by the analysis of the insoluble cupric salt. In the latter irradiations no reducing sugars were formed even though the concentration of the formaldehyde was sufficient to give evidence of the reducing sugars in the absence of nitrite. It was also found that reducing sugars were formed along with formhydroxamic acid if the concentration of formaldehyde in the irradiated solution greatly exceeded that of the nitrite. Three important conclusions were thus established :

1. Activated formaldehyde such as is photochemically produced reacts with potassium nitrite to give formhydroxamic acid.
2. This reaction takes precedence of the polymerization of the activated formaldehyde to give a carbohydrate.
3. When the activated formaldehyde is produced at a greater rate than that at which it can react with potassium nitrite and with the formhydroxamic acid thereby formed, the excess polymerizes to reducing sugars. In this case the two reactions proceed simultaneously and independently.

These results certainly indicate that the first stage in the nitrogen assimilation process is the photosynthesis of formhydroxamic acid. This is established not only by the fact that this substance is actually produced by the interaction of activated formaldehyde and potassium nitrite, but also by the fact that formhydroxamic acid reacts with more activated formaldehyde to give the nitrogen compounds which are known to be produced in living plants. An aqueous solution of formhydroxamic acid is only very slowly acted on by ultra-violet light and, further, no interaction takes place when a solution of formhydroxamic acid and formaldehyde is allowed to remain in the dark. If the latter solution is irradiated with ultra-violet light the formhydroxamic acid and formaldehyde rapidly disappear, the test for reducing sugars being negative

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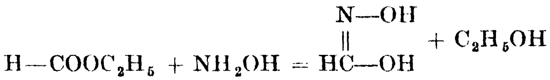
[76] **Baly, Heilbron and Hudson, *Trans. Chem. Soc.*, 1922, *121*, 1078.**

unless the formaldehyde is present in excess. It was also found that, by the action of ultra-violet light on aqueous solutions of formhydroxamic acid and formaldehyde, compounds more complex than formhydroxamic acid are produced.

Since the formation of formhydroxamic acid is the first stage in the photosynthesis, we used in the majority of our experiments formhydroxamic acid which had been prepared by the interaction of ethyl formate and hydroxylamine in solution in methyl alcohol in accordance with the equation



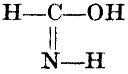
$$H{-}COOC_2H_5 + NH_2OH = \overset{\overset{\displaystyle N{-}OH}{\|}}{HC}{-}OH + C_2H_5OH$$

Exactly equivalent amounts of the ester and hydroxylamine hydrochloride were dissolved in absolute methyl alcohol and to the solution was added an exactly equivalent amount of sodium methoxide in methyl alcoholic solution. The mixture was allowed to remain at room temperature for 2 days. The precipitated sodium chloride was removed by filtration and the filtrate was concentrated under reduced pressure to a thick viscous syrup which slowly crystallized. The crystals were dried on porous tile in an evacuated desiccator over anhydrous calcium chloride.

Aqueous solutions of this formhydroxamic acid and formaldehyde were irradiated for various periods. The products varied considerably with the relative concentrations of the two components and the difficulties were much increased by the very remarkable velocity of the reactions which took place. Substances were produced during the first hour only to give place shortly afterwards to more complex compounds by further condensation with activated formaldehyde.

The variation in the results obtained established the fact that there are at least three different ways in which activated formaldehyde and formhydroxamic acid can condense together, a conclusion which also may be drawn from Baudisch's investigations.

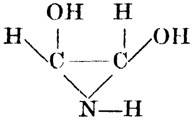
We observed an important fact, namely, that when activated formaldehyde and formhydroxamic acid condense together oxygen is set free, this being proved by the oxidation of much of the formaldehyde to formic acid. This leads to the view that formhydroxamic acid readily loses oxygen and, indeed, at the moment of its reaction it may be represented by the formula



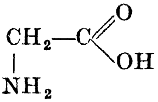
$$\begin{array}{l}\mathrm{H{-}C{-}OH}\\ \quad\ \| \\ \quad\mathrm{N{-}H}\end{array}$$

It may be noted in passing that this substance is a hydrate of HCN and attention may be drawn to this in connexion with the formation of the cyanogenetic glucosides in living plants. This compound can condense with activated formaldehyde in at least three different ways.

In the first place 1 molecule of each substance may react to give the labile ring compound

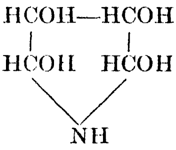


which by rearrangement would doubtless give glycine,

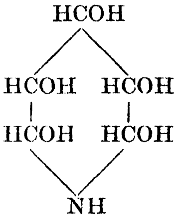


The median CH_2 group may react with more molecules of activated formaldehyde or with the condensation products of activated formaldehyde and formhydroxamic acid to give substituted glycines. This method of condensation necessitates that the NH_2 group must always be in the α-position with respect to the carboxyl group, and this is invariably the case in the products of nitrogen assimilation in the living plant. We proved the formation of α-aminoacids by the ninhydrin reaction, a pure blue colour being developed on heating with triketohydrindene hydrate. The aminoacids were isolated as their hydrochlorides, but a mixture was obtained in every case and it was not found possible to identify them. Baudisch also stated that he had obtained evidence of the formation of substances of the nature of α-aminoacids.

The second direction along which condensation may proceed is the union of 1 molecule of formhydroxamic acid with several molecules of activated formaldehyde. The two most stable condensation products are

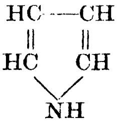


1



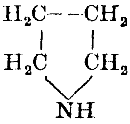
2

and by the loss of water and oxygen 1 will give



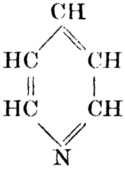
Pyrrole

or



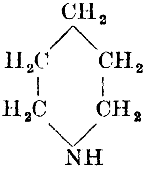
Pyrrolidine

and 2 will give



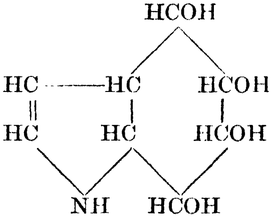
Pyridine

or

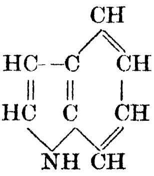


Piperidine

Then, again, pyrrole can condense with four more molecules of activated formaldehyde to give

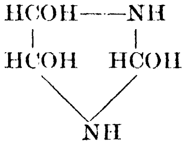


which by loss of water and oxygen will give

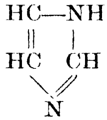


Indole

The third method is the condensation of 2 molecules of formhydroxamic acid with 1 molecule of activated formaldehyde to give the compound

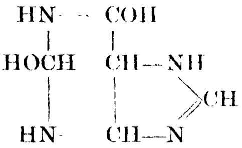


which by loss of water and oxygen will give

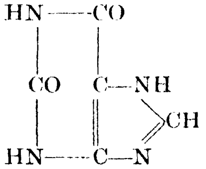


Imidazole or glyoxaline

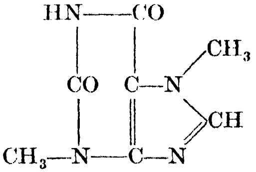
Furthermore, it is possible that 1 molecule of imidazole will condense with 2 more molecules of formhydroxamic acid to give



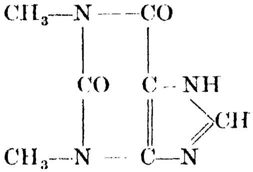
and it is very possible that this is the parent of xanthine, theobromine, theophylline, and caffeine :



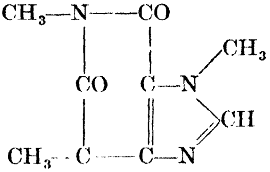
Xanthine



Theobromine

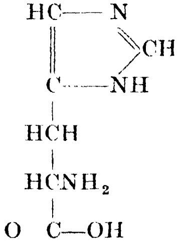


Theophylline



Caffeine

It was indicated above that by the action of activated formaldehyde or one of its condensation products with formhydroxamic acid on glycine substituted α-aminoacids may be formed. One of the many possible products of this substitution is histidine or α-amino-β-imidazolepropionic acid



The simplicity of these syntheses is peculiarly attractive, since they are the natural result of bringing together potassium nitrite and the activated formaldehyde which is produced from hydrated carbon dioxide by the action of light in the presence of chlorophyll. They are not too speculative, since Baudisch stated that he had proved the presence of both pyrrole and pyridine derivatives in solutions of potassium nitrite and methyl alcohol after long exposure to sunlight. It is to be noted that methyl alcohol is decomposed into formaldehyde slowly by sunlight and more rapidly by the light from a quartz mercury lamp. We found no evidence of the formation of pyrrole derivatives when aqueous solutions of formhydroxamic acid and formaldehyde were irradiated with the light from a quartz mercury lamp, but, as has already been explained, the photosynthesis is extremely rapid and soon proceeds far beyond the formation of simple compounds. We found that if an aqueous solution of formhydroxamic acid and formaldehyde were irradiated for 12 hours, evaporated to dryness with hydrochloric acid, and the dried residue, after being made alkaline with sodium hydroxide, were extracted with ether, the ethereal extract on evaporation always gave a nitrogen base which was of alkaloidal nature. We obtained at least two alkaloids this way, one being a volatile oil with an odour like tobacco, whilst the second was a solid with an odour like that of burnt feathers. Both these bases formed crystalline salts with acid and gave very strong positive reactions with all the usual reagents for alkaloids. Sufficient material was not obtained for the separation and identification of the two compounds. It is probable that the first alkaloid is identical with that obtained by Baudisch.

Further evidence was obtained that the photosynthesis of nitrogen compounds follows the courses indicated above. In one experiment an aqueous solution of formhydroxamic acid, containing a small quantity of formaldehyde, was irradiated with the light from a quartz mercury lamp for 2 hours. On evaporation, the solution yielded a small quantity of a crystalline nitrogen base. This compound melted at 85°–90° and was odourless when cold and developed on warming a fish-like odour. It gave a white silver salt and was oxidized at room temperature to formic acid by a neutral solution of potassium permanganate. This evidence convinced us that imidazole (m.p. 90°) had been photosynthesized. Evidence of the formation of imidazol was obtained in other experiments, but the product was a mixture. The odour of imidazole was very noticeable on warming and the reactions for silver nitrate and potassium permanganate were equally definite, but we were unable to isolate the imidazole in the crystalline form. This photosynthesis of imidazole removes the above explanation of the photosynthesis of xanthine and its derivatives from the realms of speculation and establishes confidence in its truth.

It was stated above that evidence had been obtained of the photosynthesis of α-aminoacids, and in further experiments we succeeded in proving the photosynthesis of at least one substituted α-aminoacid. The alkaline solution after its irradiation was extracted with ether in order to remove the alkaloidal substances and then acidified with hydrochloric acid and evaporated to dryness. The solid residue was extracted with boiling alcohol (90 per cent), and to the extract a mixture of absolute alcohol and ether was added in order to precipitate inorganic salts, and the filtrate was again evaporated to dryness on a steam bath. The residue consisted of the hydrochlorides of at least two substituted α-aminoacids and gave after neutralization with sodium carbonate solution the very characteristic Burgundy red colour on heating with triketohydrindene hydrate. The acids did not give the blue cupric salts which are characteristic of α-aminoacids.

The presence of histidine was tested for by means of Koessler and Hanke's method with *p*-diazobenzene sulphonic acid.[77] This test is a colour reaction given by histidine with an alkaline solution of the sulphonic acid and is specific under the given conditions. The colour reaction depends on the linkage between the diazo-compound and the imidazole ring. A yellow colour is first produced and this changes to orange and then shows a pink tinge in the meniscus. The colour reaches a maximum in 5 to 6 minutes and then fades to a yellow colour.

Two tests were carried out side by side, one with the photosynthesized material and one with a specimen of pure histidine, and, allowing for the slight trace of colour possessed by the photosynthetic material, the results were identical, both as regards the time of appearance of the colour and the tint produced.

The photosynthesis of nitrogen compounds from formhydroxamic acid and activated formaldehyde was further investigated in Liverpool by H. L. Saunders. The method of irradiation was improved by the maintenance of an approximately constant *p*H by the addition of an excess of pure calcium hydroxide and some new results were obtained which are of some importance. In one series of experiments the following solution was irradiated for 180 hours with the light from a quartz mercury lamp:

Formhydroxamic acid	2 grm.
Formaldehyde 40 per cent	5 ml.
Water	100 ml.

Excess of $Ca(OH)_2$ to give *p*H = 8 to 9

The irradiated solution was evaporated to dryness under reduced pressure, the distillate being collected in hydrochloric acid. The acid distillate was concentrated and made alkaline with sodium hydroxide. Piperidine was then set free and identified by preparation and analysis of its gold salt. In one experiment and one experiment only, pyridine was produced and not piperidine.

The residue in the distilling flask was dissolved in water, heated with hydrochloric acid on a steam bath and then made faintly alkaline with sodium carbonate. The precipitated material was dissolved in water to give a red solution which

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[77] *J. Biol. Chem.*, 1919, *39*, 497.

on evaporation yielded a red solid. This solid gave in aqueous solution on heating with triketohydrindene hydrate the Burgundy red colour characteristic of the substituted α-aminoacids. These acids were boiled with sodium hydroxide when nitrogen bases were evolved and the product then gave with triketohydrindene hydrate the blue colour characteristic of α-aminoacids. Two different cupric salts of these acids were obtained and analysed. Assuming that these were normal salts, the analytical results gave molecular weights of 179 and 127·5.

In another experiment the following solution was irradiated continuously for 11 days :

Formhydroxamic acid	28 grm.
Methyl alcohol	200 ml.
Formaldehyde 40 per cent	126 ml.
Water	76 ml.

$pH = 9{\cdot}5$

After the irradiation the solution contained crystals of calcium formate which were collected and the filtrate was extracted with ether in order to remove the alkaloidal substances. On the addition of five volumes of absolute alcohol more calcium formate separated and the filtrate was concentrated to a small bulk. The calcium remaining in solution was then precipitated by the addition of sodium carbonate and removed by filtration. The filtrate was neutralized with hydrochloric acid and was concentrated at 100° when most of the sodium chloride separated and was collected on a filter. On the addition of absolute alcohol to the filtrate there separated a finely divided yellowish precipitate which was exceedingly hygroscopic and was dried over phosphoric oxide in an evacuated desiccator.

This product gave all the characteristic reactions of a protein. It formed a gold salt insoluble in dilute acid, and analysis of this salt gave the molecular weight of the nitrogen compound to be 221·4. By combustion the nitrogen content of the gold salt was found to be 9·25 per cent, and hence the original material contained 17·5 per cent of nitrogen. It is to be noted that the natural proteins contain from 15·5 to 18·9 per cent of nitrogen.

It was found that the photosynthesized product was not affected on boiling with dilute acid, but on heating with concentrated hydrochloric acid for 2 hours at 100° it was hydrolysed into substituted α-aminoacids which, after neutralization, gave the characteristic Burgundy red colour on heating with triketohydrindene hydrate. The hydrochlorides of these acids were obtained in the crystalline form and when they were examined under the microscope three different crystalline compounds were recognized. The presence of histidine hydrochloride as one of these components was proved by the Koessel and Hanke colour test as detailed above.

The whole experiment was then repeated in the hope that it would be possible to separate the histidine. The irradiated solution was treated in the same way as was described above up to the stage when the calcium present as formate had been removed. Then after concentration the solution was heated with concentrated hydrochloric acid for 2 hours at 100°. The excess of hydrochloric acid was removed by distillation under reduced pressure on a steam bath, a process which also removed the excess of formaldehyde. This was followed by distillation under reduced pressure for 2 hours at 50° in the presence of magnesium hydroxide. The residual solution was divided into two equal portions, A and B.

Treatment of A. In half of this a Kjeldahl determination gave 0·65 grm. of total nitrogen or 2·6 grm. in the products of the irradiation.

In the second half, the amount of histidine present was determined colorimetrically and found to be 0·03 grm., and this corresponded to 0·032 grm. of nitrogen in the whole.

Treatment of B. To the whole of this solution an excess of phosphotungstic acid was added and the precipitate after washing and drying weighed 4·6 grm. The nitrogen content of this product was determined by combustion and found to be 1·956 per cent. This corresponded to 0·18 grm. of diamino nitrogen in the whole.

In the filtrate which contained monamino nitrogen only a series of Van Slyke determinations gave an average value of 0·1112 grm. of nitrogen on the whole.

The total amino-nitrogen, therefore, was 0·2912 grm., and as the total nitrogen in the histidine was only 0·032 grm., it is not surprising that all attempts to isolate crystalline histidine failed.

In this experiment a solution containing 28 grm. of formhydroxamic acid and 50 grm. of formaldehyde was irradiated, and thus the total nitrogen originally present was 6·43 grm. The total nitrogen present in the solution after irradiation was only 2·6 grm., and hence much photochemical decomposition of the products must have taken place with the loss of nitrogen. A similar photochemical decomposition of the products of photosynthesis was referred to in Chapter III, when the photosynthesis of glucose from formaldehyde in aqueous solution was described. This photochemical action of the very short-wave ultra-violet light cannot be avoided when a quartz mercury lamp is used as the light source. In the living plant the activated formaldehyde is produced by the agency of daylight and hence photochemical decomposition of the products of photosynthesis does not take place. In the laboratory experiments the products of photosynthesis found in the irradiated solution are the excess of those photosynthesized over the amount decomposed.

The experimental results obtained by Baudisch and in Liverpool indicate very strongly that by the action of activated formaldehyde on potassium nitrite compounds are photosynthesized which are the same as those found in the living plant. Amongst the products recognized were pyrrole, pyridine, piperidine, histidine, and a substance which by its properties justifies the statement that it was a protein.

It might be argued that the photosynthesis of naturally occurring compounds by the action of activated formaldehyde on ammonia has no direct bearing on the photosynthetic processes of the living plant, because ammonia in the free state does not occur in plants and indeed is a poison to them. On the other hand, it is impossible to deny that ammonium nitrate may be present and that this, as shown above, would react with activated formaldehyde to give formhydroxamic acid and free ammonia. It seemed advisable, therefore, to investigate the action of activated formaldehyde on ammonia in order to determine whether the products of such action bear any relation to substances known to be present in plants.[78]

In the first experiments aqueous solutions of ammonia, saturated with carbon dioxide, were irradiated in quartz tubes

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[78] Baly, Heilbron and Stern, *Trans. Chem. Soc.*, 1923, *123*, 185.

with the light from a quartz mercury lamp. The strength of the ammonia was in most cases 1·3M and the period of irradiation varied from 20 to 300 hours. The solutions after irradiation were evaporated under reduced pressure and the distillate was collected in aqueous hydrochloric acid. The acid solution was evaporated to dryness at 100° and the residue was found to be a mixture containing mainly ammonium chloride and methylamine hydrochloride.

During the course of these early experiments the mixed hydrochlorides were treated with sodium hydroxide, and it was then noted that a very strong odour of pyridine was developed. It was then realized that the photosynthetic process might possibly lead to the formation of more complex compounds than methylamine, that is to say, compounds of the type of pyrrole, pyrrolidine, pyridine, and piperidine. The production of one or more of these compounds might indeed be expected from the evidence obtained by Baudisch of the presence of pyrrole and pyridine in solutions of potassium nitrite and methyl alcohol after prolonged irradiation.[79]

The photosynthesis of the pyridine nucleus was then investigated in the following way. Batteries of eight 9 × 1 in. quartz tubes, each containing 100 ml. of 1·3M-ammonia saturated with carbon dioxide, were irradiated by the light from a quartz mercury lamp for different periods. The solutions were then concentrated under reduced pressure, the distillate being collected in hydrochloric acid. The acid distillate was evaporated to dryness and the dry residue was extracted with absolute alcohol and a considerable quantity of methylamine hydrochloride was obtained and identified. The alcoholic extract was also found to contain the hydrochloride of pyridine or sometimes of piperidine. The presence of one or other of these substances was proved by the borax test. A small quantity of the mixed hydrochlorides was rubbed in a mortar with a little borax when the odour of pyridine or piperidine at once developed very strongly, the odour of methylamine not being noticeable for at least 30 seconds. This test is commonly applied to ammonium salts suspected of containing pyridine and is capable of detecting 0·3 per cent of pyridine. Although in every experiment pyridine or piperidine was

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[79] *Ber. Deutsch. Chem. Ges.*, 1913, *46*, 113.

produced, the conditions for the photosynthesis of either one could not be found. In the majority of experiments pyridine was formed, but in a few cases piperidine was the product. These results proved that the pyridine nucleus is photosynthesized from activated formaldehyde and ammonia.

In order to confirm these results and also to determine whether the photosynthesis proceeds further than the pyridine nucleus, mixtures of ammonia and formaldehyde were irradiated with the light from a quartz mercury lamp. In the first experiments 1·3M-solutions of ammonia and formaldehyde were irradiated for 120 hours. The solutions were then evaporated several times with excess of hydrochloric acid in order to remove the excess of formaldehyde and finally taken to dryness. From the mixed hydrochlorides by extraction with absolute alcohol and fractional precipitation with ether nearly pure methylamine hydrochloride was obtained.

In a second series of experiments in which 1·3M-solutions of ammonia and formaldehyde were irradiated for longer periods the photosynthesis of pyridine was confirmed, but in no case was any evidence obtained of the formation of piperidine. The mixed hydrochlorides, prepared as described above, were extracted with warm absolute alcohol in which pyridine hydrochloride is much more soluble than methylamine hydrochloride. The addition of ether to the alcoholic extract precipitated the greater bulk of the methylamine hydrochloride. The filtrate was evaporated to dryness and the residue was extracted with a little chloroform in which pyridine hydrochloride is very much more soluble than methylamine hydrochloride. On evaporation of the chloroform solution a very small quantity of almost pure pyridine hydrochloride was obtained. The normal chloroplatinate was prepared and found to have a melting point which was about 15° too low owing to its contamination by an alkaloidal substance.

Although the presence of the alkaloidal substance was proved by its reactions with all the usual reagents, sufficient quantities for its investigation were not obtained. It was then decided to irradiate more concentrated solutions of ammonia and formaldehyde. Twelve quartz test-tubes, each containing 100 ml. of 2M-ammonia and formaldehyde, were then irradiated with ultra-violet light for 300 hours. During the irradiation the solutions turned brown and, after repeated evaporation to dryness with excess of hydrochloric acid and extraction of the dry residue finally obtained with absolute alcohol, a light brown solution was obtained which on evaporation deposited a brown deliquescent solid with an odour resembling that of liquorice. A minute portion of this solid when dissolved in water gave very strong reactions with all the reagents for alkaloids, the most noteworthy being a heavy purplish-brown precipitate with a solution of iodine and potassium iodide and a yellow precipitate and a characteristic odour on warming with Nessler solution. It was this odour, also developed on treating the hydrochloride with alkali, that led us to suspect the presence of an alkaloid of the coniine group.

In order to prepare as much as possible of the alkaloid, 2M-solutions of ammonia and formaldehyde were irradiated with ultra-violet light for periods of 6 to 10 days. The resulting solutions were worked up exactly as described above, and a chloroform solution of the pyridine and alkaloid hydrochlorides was obtained. On the addition of two volumes of ether to this solution most of the pyridine hydrochloride was rapidly precipitated. On the addition of a further small quantity of ether to the filtrate the remainder of the pyridine hydrochloride, together with a little alkaloid hydrochloride, separated. On evaporation of the second filtrate a brown solid was obtained, which on treatment with alkali gave no odour of pyridine, but only the characteristic odour of coniine.

In view of the difficulty in the absolute identification of coniine it was necessary to obtain convincing evidence that the photosynthesized alkaloid could not be any other substance than coniine. We were fortunate in obtaining the assistance of Professor W. J. Dilling,[80] who had made a special study of the identification of coniine and its differentiation from its allies.

The most characteristic test for coniine is the crystalline precipitate which it gives with potassium cadmium iodide. A neutral solution of coniine hydrochloride, cooled in a freezing mixture, on cautious addition of the iodide solution gives a white precipitate which, when seen under the microscope, consists of crystals shaped like an oak leaf. The synthetic

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[80] *Pharm. Journ. and Pharmacist*, 1909, *4*, 29.

alkaloid gave crystals which were identical in appearance with those obtained with natural coniine. The conhydrins only give amorphous precipitates with potassium cadmium iodide.

The synthetic alkaloid on oxidation with potassium bichromate in presence of sulphuric acid yielded butyric acid, a reaction also given by natural coniine.

Strong confirmatory evidence was obtained by Melzer's [81] test as modified by Dilling. 1 ml. of a solution of the hydrochloride is made alkaline with sodium carbonate, 0·5 ml. of alcohol and a few drops of carbon bisulphide are then added, and the mixture is heated to the boiling point. Distilled water is then added until the test-tube is nearly two-thirds full, and a few drops of a saturated solution of uranyl nitrate are added, when an orange-red colour is developed. The coloured compound is extracted by shaking with toluene or ether and the ethereal solution, on standing, deposits brown crystals. The solubility of the coloured compound in toluene sharply differentiates coniine from its allies and indeed is a specific test for coniine. When nickel chloride is substituted for uranyl nitrate a green colour is obtained, the green substance being soluble in ether and the ethereal solution, on standing, deposits green crystals. This at once excludes γ-coniceine, which gives a red colour with nickel chloride. With copper sulphate the colour is vandyke brown and the ethereal solution deposits brown crystals. Identical colours and crystals were obtained with the photosynthesized alkaloid and with natural coniine. These three tests are accepted as the best known for the identification of coniine, and in each case no difference whatever could be detected between the behaviour of the photosynthesized alkaloid and natural coniine.

The picrate of the photosynthesized alkaloid was prepared and three specimens obtained in three separate syntheses melted at 72°–74°, 73°–74·5°, and 74·5°, respectively, the melting point of natural coniine picrate being 75°.

Ladenburg found that the physiological action of his synthetic α-propylpiperidine is identical with that of the natural alkaloid.[82] Dilling determined the physiological action of our photosynthesized alkaloid and proved that this was

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[81] *Arch. pharm.*, 1898, *236*, 701.

[82] *Ber. Deutsch. Chem. Ges.*, 1886, *19*, 439, 2578.

identical with that of natural coniine. This physiological action is very characteristic and is given by alkaloids of the curare and coniine groups only. The former were definitely excluded by the complete failure of the tests characteristic of them. The conclusion, therefore, is reached that the physiological action must have been given by an alkaloid of the coniine group, a conclusion which is supported by the chemical tests which are very characteristic of this group.

Shortly after these results were published, Snow and Stone [83] stated that they had investigated the properties of the bases which are formed by the interaction of ammonium chloride and formaldehyde and had found that tetramethylmethylenediamine gave with the Melzer test and with potassium cadmium iodide, iodine in potassium iodide and Nessler reagent results which were similar to, but not absolutely identical with, those given by natural coniine. They also found that tetramethylmethylenediamine and methylmethyleneimine gave the same type of physiological action, the curare effect, as was advanced by us as evidence in favour of the identification of coniine. As the result of their findings Snow and Stone, whilst not denying the possibility of the photosynthesis of coniine, considered that the evidence adduced was not sufficient.

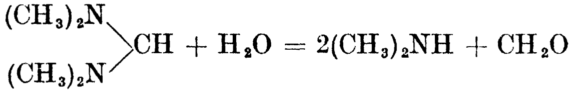
Now my colleagues and I were fully aware of the formation of condensation products of ammonia and formaldehyde, but we were convinced before publication that our results could not be due to either tetramethylmethylenediamine or methylmethyleneimine. In the first place it was highly improbable that ammonia and formaldehyde would react together at room temperature to form these compounds, since Werner [84] obtained them by heating 40 per cent formaldehyde and ammonium chloride at 104°. The normal reaction between ammonia and formaldehyde at room temperature is to form hexamethylene tetramine, $(CH_3)_6N_4$. In the second place we were satisfied that even if the Werner compounds had been produced in small amounts they would have been completely decomposed, like hexamethylenetetramine, during the concentration of our irradiated solutions with hydrochloric acid, a process which was several times repeated in order to remove all the residual formaldehyde.

[83] *Trans. Chem. Soc.*, 1923, *123*, 509. [84] *Ibid.*, 1917, *111*, 844.

In order to place the photosynthesis of coniine beyond all dispute the action of hydrochloric acid on tetramethylmethylenediamine and methylmethyleneimine was investigated in Liverpool by Miss Morrison (Mrs. J. W. Corran) soon after the publication of Snow and Stone's observations.

The two bases were prepared in the pure state by Henry's method.[85] Tetramethylmethylenediamine was prepared by the gradual addition of an aqueous solution of dimethylamine to 40 per cent formaldehyde in the proportion of 2 molecules of dimethylamine to 1 molecule of formaldehyde. Much heat was developed and, after cooling, the addition of solid potash caused the amine to separate as an upper layer, and after drying the amine boiled at 85°.

On treatment of the amine with aqueous hydrochloric acid formaldehyde was rapidly evolved and the solution on evaporation yielded only dimethylamine hydrochloride, which was identified by its melting point, 171°, and by analysis of the chloroplatinate which contained 38·95 per cent Pt (theory Pt = 39·04 per cent). It is evident, therefore, that the base is quantitatively decomposed by aqueous hydrochloric acid in accordance with the equation



$$\left.\begin{matrix}(CH_3)_2N\\ (CH_3)_2N\end{matrix}\right\rangle CH + H_2O = 2(CH_3)_2NH + CH_2O$$

Methylmethyleneimine was prepared by the gradual addition of aqueous methylamine to 40 per cent formaldehyde in equimolecular quantities in accordance with the equation

$$CH_3—NH_2 + CH_2O = CH_3—N = CH + H_2O$$

Much heat was evolved and, after cooling, the addition of solid potash caused the amine to separate as an upper layer. After drying, the amine boiled at 165°.

On treatment of the amine with aqueous hydrochloric acid, formaldehyde was rapidly evolved and the solution on evaporation yielded white crystalline hydrochlorides with melting point 159°–173°. This product was a mixture of two hydrochlorides which were separated by treatment with chloroform. The greater part, which was insoluble in chloroform, was identified as methylamine hydrochloride by its

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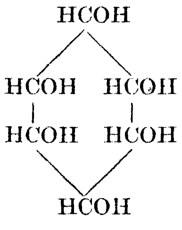
[85] *Bull. Acad. Roy. Belge,* **1893,** iii, *26,* 200.

melting point, 226°, and by the fact that the chloroplatinate contained 41·31 per cent Pt (theory Pt = 41·35 per cent). The small portion, which was soluble in chloroform, was identified as dimethylamine hydrochloride by its melting point, 171°, and analysis of the chloroplatinate (found Pt = 38·96 per cent, theory Pt = 39·04 per cent).

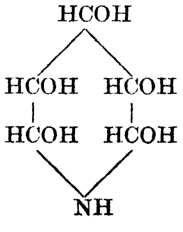
These results proved that my colleagues and I were perfectly correct in our opinion that even if the Werner bases had been produced by the interaction of ammonia and formaldehyde at room temperature, which is most improbable, they would certainly have been completely decomposed during the treatment of the irradiated solutions. Snow and Stone's criticism of the photosynthesis of coniine, therefore, was baseless.

The fundamental basis of the coniine photosynthesis is the formation of the pyridine ring by the action of activated formaldehyde on ammonia. As has already been stated, Baudisch obtained evidence of the photosynthesis of pyridine derivatives and my colleagues and I proved the formation of pyridine and also of piperidine. This photosynthesis was also confirmed by Miss Morrison, who separated and identified piperidine aurichloride.

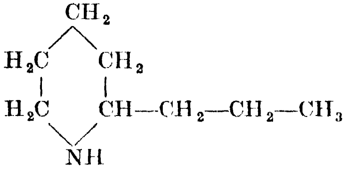
The photosynthesis of the pyridine ring is not surprising in view of the fact that the first stage in carbohydrate photosynthesis is the formation of the cyclic compound



In the presence of ammonia the cyclic compound

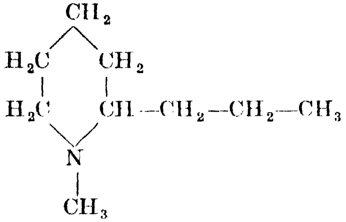


can equally well be formed, and this by loss of oxygen will give piperidine. Substitution of hydrogen atoms by alkyl groups will give the two naturally occurring alkaloids



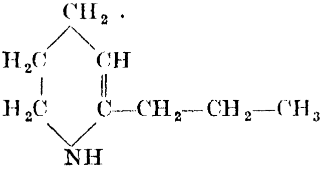
Coniine

and



n-methylconiine

and in the same way the formation of the third natural alkaloid of this group can be understood



γ-coniceine

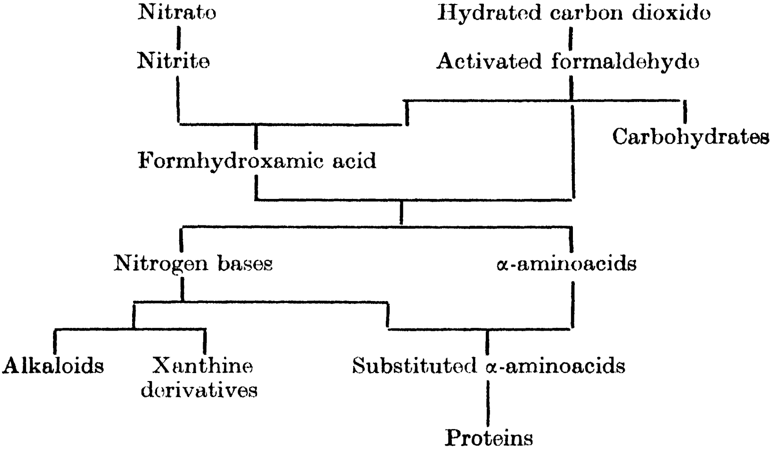
Evidence of the substitution by a methyl group of a hydrogen atom by means of activated formaldehyde was given in Chapter III, and there is little doubt that, adopting the Nef formula for activated formaldehyde, this substitution can be expressed by the equations

$$\begin{aligned} & R—CH_3 + CHOH = R—CH_2—CH_3 + O \\ & R—OH + CHOH = R—OCH_3 + O \\ & R—NH_2 + CHOH = R—NH—CH_3 + O \\ \text{and} \quad & R{=}NH + CHOH = R{=}N—CH_3 + O \end{aligned}$$

Reference has already been made to the fact that in all the foregoing investigations no evidence was obtained of the photosynthesis of carbohydrates unless the concentration of the formaldehyde was much in excess of that of the parent nitrogen compound. Miss Morrison carried out a series of irradiations of aqueous solutions of formaldehyde and ammonia in which the relative concentrations of the two reactants were varied within wide limits. She proved that the first evidence of the photosynthesis of carbohydrates along with nitrogen compounds was obtained when the molecular ratio of formaldehyde to ammonia was 3 : 1. When the molecular ratio was greater than this the photosynthesis of carbohydrates and nitrogen compounds took place simultaneously.

This clearly indicates that the action of the activated formaldehyde on the parent nitrogen compound is preferential to its polymerization. It follows, therefore, that if the concentration of the parent nitrogen compound, e.g. potassium nitrite, in the leaves of plants does not exceed a definite limit, photosynthesis of proteins will take place concurrently with the photosynthesis of carbohydrates, as is indeed the case. It is not possible to define that limit, because Miss Morrison's observations were made with a homogeneous solution, and in the living plant the activated formaldehyde is produced by a surface reaction.

The simultaneous photosynthesis of carbohydrates and nitrogen compounds may be indicated by the following scheme :



In view of the experimental results which have shown that by the progressive action of activated formaldehyde on nitrites the same types of products are photosynthesized as are known to be produced in living plants, it would appear to be highly probable that nitrogen assimilation in living plants is a photosynthetic process. It is necessary, therefore, to determine whether this conclusion is in any way at variance with the observations which have been made with living plants.

The quotation which was given above from Stiles's book indicates that the difficulties in the way of acceptance of the photosynthetic origin of the proteins arise from the observations that protein formation takes place in leaves in the dark, and that other organs appear to possess the power of synthesizing this material. Let us consider the various stages set forth in the scheme of photosynthesis given above. In the discussion of the action of activated formaldehyde on potassium nitrite and on the products of that action it was shown that labile substances are first produced which lose oxygen and give the reactants in the next stage of the process. These processes are thermal reactions and take place in the dark. Furthermore, the final stage in protein synthesis, namely, the combination of various substituted α-aminoacids, is also a process which takes place in the dark. Surely, therefore, if the process has been started by the irradiation of the leaves, increase in their protein content will be observed after the irradiation has ceased. The observation of this, therefore, is not necessarily antagonistic to the photosynthetic origin of the proteins or of any of the nitrogen compounds found in the plant.

Incidentally, in this connexion the presence in the leaves of plants of the enzyme catalase is noteworthy. The function of catalase is the acceleration of a process involving the evolution of oxygen. It may well be that the catalase promotes the change of the labile products into more stable forms with the evolution of oxygen.

Then, again, it is possible that some of the intermediate compounds, e.g. aminoacids, are translocated from the leaves to other organs of the plant, with the result that increase in the protein content of those organs will be observed. This observation, therefore, is not necessarily antagonistic to the photosynthesis of proteins.

The acceptance of the photosynthetic origin of the nitrogen compounds produced in living plants raises an issue which far outweighs in importance these two observations. This issue may be illustrated by the photosynthesis of histidine by the action of activated formaldehyde on potassium nitrite. It was shown above that the first reaction is the formation of the potassium salt of formhydroxamic acid and that this salt at once hydrolyses into the free acid and potassium hydroxide. We may, therefore, in expressing the whole process of forming histidine in a single equation, take as the starting substance nitrous acid instead of potassium nitrite. The formula of histidine is $C_6H_9O_2N_3$ and the equation must be

$$12CO_2,H_2O + 6HNO_2 = 2C_6H_9O_2N_3 + 6H_2O + 19O_2$$

that is to say, the volume ratio O_2/CO_2 is 19/12 or 1·583. Since in the photosynthesis of any substituted α-aminoacid the O_2/CO_2 ratio is greater than unity, it follows that the values of this ratio determined with living plants cannot be exactly unity if nitrogen assimilation is a photosynthetic process. It is essential, therefore, to clarify the position by calculating the O_2/CO_2 ratio which is to be expected from the nitrogen photosynthetic theory.

For this purpose a knowledge is necessary of the relative amounts of carbohydrates and proteins which are simultaneously produced in the leaves of plants. An estimate of this can be obtained from observations made by Krascheninnikoff which are quoted by Spoehr.[86] Krascheninnikoff determined the increase in the heat of combustion of leaves caused by the assimilation of a known amount of carbon dioxide. The heats of combustion of the leaves were determined after complete drying and, expressed in terms of 1 sq. m. of leaf surface, the following values were obtained :

Increase in heat of combustion of the dry photosynthesized matter	15,400 calories
Increase in heat of combustion if the increase in dry matter consisted entirely of carbohydrates	14,000 ,,

In order to express these values in terms of carbohydrate and protein, the heats of combustion of proteins are required. Owing to the fact that the molecular weights of proteins are not definitely known, no information as to their heats of combustion is available. Average values, however, may be obtained from the heats of combustion of their constituent aminoacids, because the heat of combustion of the product of combination of two compounds, with loss of water only, differs at most by very little from the sum of the heats of combustion of the two compounds. All the known heats of

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[86] *Photosynthesis*, p. 221. Chemical Catalogue Co., New York, 1926.

combustion in kilogram calories of the aminoacids synthesized in plants are given in Table IX, together with the values calculated for that weight which contains one gram atom of carbon.

TABLE IX

Aminoacid	Formula	Grm. mole	Grm. atom of C
Alanine	$C_3H_7O_2N$	387·7	129·23
Glycine	$C_2H_5O_2N$	234·5	117·25
Leucine	$C_6H_{13}O_2N$	855·6	142·60
Phenylalanine	$C_9H_{11}O_2N$	1,111·3	123·48
Tyrosine	$C_9H_{11}O_3N$	1,070·2	118·91

The mean of the values in the last column is 126,294 calories and the heat of combustion of glucose is 112,167 calories for 1 grm. atom of carbon. The heats of combustion determined by Krascheninnikoff indicate that the heat of combustion of the carbohydrates was 14,000 calories and that of the protein was 1,400 calories. The relative amounts of carbon in the carbohydrate and protein photosynthesized were 14,000/112,167 and 1400/126,240, and the ratio of these two amounts is 11·28. It may be concluded, therefore, that out of every 12 molecules of carbon dioxide assimilated, 11 form carbohydrate and 1 takes part in protein formation.

We are now in a position to calculate the O_2/CO_2 ratio which would be obtained from the simultaneous photosynthesis of glucose and each of the 15 aminoacids which are known to be produced in living plants. The method may be illustrated by the photosynthesis of glucose and of histidine which, if it were the sole product, would give $O_2/CO_2 = 1·583$. Since 11 molecules of CO_2H_2O form glucose with $O_2/CO_2 = 1$, while 1 molecule takes part in histidine photosynthesis, the observed value will be $(11 + 1·583)/12 = 1·049$. In Table X (p. 156) are given the values of the O_2/CO_2 ratio for each of the 15 aminoacids, together with the values which would be obtained if each acid were photosynthesized with glucose on the 11 : 1 basis. In the case of cystine it has been assumed that the sulphur atoms are obtained from sulphates.

In order to find the value of the O_2/CO_2 ratio to be expected when the leaves of plants are irradiated, it is necessary to know the relative proportions of the aminoacids which are photosynthesized in different plant species. Valuable information

TABLE X

Aminoacid	Formula	O_2/CO_2 ratio	
		Alone	11 : 1
Alanine	$C_3H_7O_2N$	1·500	1·042
Arginine	$C_6H_{14}O_2N_4$	1·917	1·076
Aspartic acid	$C_4H_7O_4N$	1·125	1·010
Cystine	$C_6H_{12}O_4N_2S_2$	1·917	1·076
Glutamic acid	$C_5H_9O_4N$	1·200	1·017
Glycine	$C_2H_5O_2N$	1·500	1·042
Histidine	$C_6H_9O_2N_3$	1·583	1·049
Leucine	$C_6H_{13}O_2N$	1·500	1·042
Lysine	$C_6H_{14}O_2N_2$	1·667	1·056
Phenylalanine	$C_9H_{11}O_2N$	1·278	1·023
Proline	$C_5H_9O_2N$	1·400	1·033
Serine	$C_3H_7O_3N$	1·333	1·028
Tryptophane	$C_{11}H_{12}O_2N_2$	1·318	1·027
Tyrosine	$C_9H_{11}O_3N$	1·222	1·019
Valine	$C_5H_{11}O_2N$	1·500	1·042

is afforded by the work of Osborne and Clapp, who determined the composition of various plant proteins by hydrolysing them and separating the constituent aminoacids.[87] The proteins were obtained from almonds, Brazil nuts, hemp, &c., and hence are those utilized in the next generation of the plant. The relative proportions of the aminoacids obtained by hydrolysis of these proteins may, therefore, be accepted as those photosynthesized in the leaves of the various plant species. Owing to the difficulty of the quantitative separation of the individual components of a mixture of aminoacids, Osborne and Clapp's results are not complete, but they indicate the variation in the relative proportions of the aminoacids photosynthesized in ten different species, and enable a calculation to be made of the O_2/CO_2 ratio which should be observed when the leaves of each species are irradiated. Osborne and Clapp's determinations are given in Table XI, together with the mean values of the O_2/CO_2 ratio calculated from the amounts of each aminoacid and the values of the ratio which should be found experimentally.

From the foregoing discussion of the photosynthesis of aminoacids along with carbohydrates, three important conclusions may be drawn, namely :

1. The observed values of the O_2/CO_2 ratio with living plants must be a little greater than unity.

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[87] *Amer. Journ. Physiol.*, 1907, *19*, 53, 475 ; *20*, 470 ; 1908, *22*, 362.

TABLE XI

	Amandin (almond)	Edestin (hemp)	Excelsin (Brazil nut)	Globulin (squash seeds)	Glyadin (rye)	Glyadin (wheat)	Hendein (barley)	Legumin (cow pea)	Legumin (vetch)	Zein (maize)
Alanine	1·40	3·60	2·33	1·92	1·33	2·00	0·43	2·08	1·15	9·79
Arginine	11·85	14·17	14·14	14·44	2·22	3·16	2·16	11·71	11·06	9·79
Aspartic acid	5·42	4·50	3·85	3·30	0·25	0·58	0·00	5·30	3·21	1·71
Cystine	?	1·00	?	0·23	?	0·45	?	*U**	*U*	?
Glutamic acid	23·14	18·84	12·94	12·35	37·80	42·98	43·19	16·97	18·30	26·17
Glycine	0·51	3·80	0·60	0·57	0·13	0·02	0·00	0·38	0·39	0·00
Histidine	1·58	2·19	1·47	2·63	0·39	0·61	1·28	1·69	2·94	0·43
Leucine	4·45	14·50	8·70	7·32	6·30	5·61	5·67	8·00	8·80	19·55
Lysine	0·70	1·65	1·64	1·99	0·00	0·00	0·00	4·98	3·99	0·00
Phenylalanine	2·53	3·09	3·55	3·32	2·70	2·35	5·03	3·75	2·87	6·55
Proline	2·44	4·10	3·65	2·82	9·82	7·06	13·73	3·22	4·04	9·04
Serine	?	0·33	0·00	?	0·06	0·13	?	0·53	?	1·02
Tryptophane	pres.	pres.	pres.	pres.	pres.	pres.	pres.	pres.	pres.	0·00
Tyrosine	1·12	2·13	3·03	3·07	1·19	1·20	1·67	1·55	2·42	3·55
Valine	0·16	6·20	1·51	0·26	?	0·21	0·13	?	1·36	1·88
O_2/CO_2 if alone	1·411	1·465	1·482	1·494	1·307	1·296	1·297	1·452	1·444	1·361
,, to be obs.	1·034	1·039	1·040	1·041	1·026	1·025	1·025	1·038	1·037	1·030

* *U* = undetermined

2. The observed values of the O_2/CO_2 ratio will be different with different species of plants.
3. The most probable extreme values of the O_2/CO_2 ratio are 1·020 and 1·050 with a mean value of 1·035.

The experimental determination of the photosynthetic ratio with living plants is fraught with difficulties, the chief of which is the fact that the plant absorbs oxygen and respires carbon dioxide at the same time as it absorbs carbon dioxide and transpires oxygen in the photosynthetic process. Then, again, there is some doubt as to whether the rate of respiration is the same in light and in the dark. If the volumes of CO_2 respired and O_2 absorbed in the dark are C_r and O_r, and if the volumes of CO_2 absorbed and O_2 transpired in the light are C_p and O_p, the true photosynthetic quotient will be $\frac{O_r + O_p}{C_r + C_p}$, provided that the rate of respiration is the same in darkness and in light. The two most trustworthy determinations of the photosynthetic quotient were made by Maquenne and Demoussy, and by Willstätter and Stoll. The former were carried out under the same conditions as those to which the plants were accustomed, whilst the latter were not. For this reason we may compare Maquenne and Demoussy's measurements [88] with those calculated for the ten mixtures of aminoacids and given in Table XI. Their measurements of the respiratory coefficients, *R*, and the photosynthetic coefficients, *P*, are given in Table XII.

Maquenne and Demoussy in discussing their measurements pointed out that in the great majority of cases the photosynthetic quotient lay between the respiratory quotient and unity, and, since the values of the latter did not differ much from unity, they concluded that the true values of the photosynthetic quotient must be nearer to unity than those actually determined. There does not appear to be any justification for this conclusion except the conviction that the photosynthesis of carbohydrates with $O_2/CO_2 = 1$ must result in an observed value of unity for the photosynthetic quotient.

As a matter of fact, the mean value of Maquenne and Demoussy's measurements of the photosynthetic quotient is

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[88] *Échanges gaseux des Plantes Vertes avec l'Atmosphère*, Paris, 1913; *Compt. rend.*, 1913, *156*, 506.

TABLE XII

Species	CO_2/O_2 R	O_2/CO_2 P	Species	CO_2/O_2 R	O_2/CO_2 P
Ailanthus	1·08	1·02	Kohlrabi	1·11	1·06
Ampelopsis	1·00	1·01	Lilac	1·07	1·03
Aspidistra	0·97	1·00	Lily	1·07	1·00
Aneuba (spring)	1·11	1·10	Mahonia (autumn)	0·95	0·99
Beetroot	1·03	1·06	Maize	1·07	1·05
Begonia	1·11	1·03	Pea	1·07	1·04
Cabbage	1·07	1·02	Pear	1·10	1·08
Cherry-laurel	1·03	0·97	Poppy	1·09	1·09
Chestnut (spring)	1·02	1·06	Privet	1·03	1·02
Chestnut (summer)	0·92	0·98	Rhubarb	1·02	1·00
Chrysanthemum	1·02	1·01	Ricinus	1·03	1·03
Dahlia	1·07	1·07	Rose	1·02	1·00
Geranium	1·02	1·05	Rose-laurel	1·05	1·01
Grape-Vine	1·01	0·99	Spindle-tree	1·08	1·02
Ivy	1·08	1·00	Sorrel	1·04	1·04
Kidney bean (young)	1·12	1·12	Tobacco	1·03	1·04
Kidney bean (average)	1·07	1·07	Wheat	1·03	1·02

1·035, and this was found above to be the most probable mean value. Moreover, their individual measurements are well distributed with respect to this mean value and, assuming that the limits of experimental error were the same in all the measurements, the agreement between the calculated and observed values of the photosynthetic quotient is very satisfactory.

Willstätter and Stoll's method of measurement of the photosynthetic quotient was entirely different from that used by all previous observers.[89] Realizing the difficulties caused by the respiratory process and its possible variation in light, they endeavoured to surmount them by very greatly increasing the rate of photosynthesis. They irradiated plants in a continuous flow of air which contained from 5 to 6·5 per cent by volume of CO_2 as compared with the normal amount of 0·03 per cent, and they used light of high intensity, namely, about 45,000 lux. By these means the rate of photosynthesis was increased to from twenty to thirty times the normal rate, with the result that the effect of any errors in the determination of the respiratory coefficient was reduced to a minimum. Under these conditions Willstätter and Stoll found that the photo-

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[89] *Untersuchungen über die Assimilation der Kohlensaüre.* Berlin, 1918.

synthetic quotient was exactly unity in all the plant species examined except the succulents. This result has generally been accepted as the final proof that under normal conditions the O_2/CO_2 ratio is unity in all plant species except the succulents.

Let us, however, consider the effect of increasing the rate of production of activated formaldehyde on the photosynthetic quotient when aminoacids are photosynthesized along with carbohydrates. When a plant is irradiated under normal conditions of light intensity and CO_2 concentration, the ratio of the rate of formation of activated formaldehyde to the rate of supply of nitrite to the leaves is approximately constant, and it has been shown that under these conditions 11 out of every 12 molecules of CO_2 assimilated form carbohydrates and 1 takes part in aminoacid formation. If the rate of formation of activated formaldehyde be increased while the rate of supply of nitrite remains constant, the relative number of CO_2 molecules which form carbohydrates will be increased in proportion. In Willstätter and Stoll's experiments when the rate of formation of activated formaldehyde was increased to from twenty to thirty times the normal rate, the relative number of CO_2 molecules taking part in carbohydrate and aminoacid formation was from 220 : 1 to 330 : 1. The values of the photosynthetic quotient to be expected under these conditions for the ten mixtures of aminoacids given in Table XI vary from 1·0008 to 1·002. Since the differences between these values and unity are too small to be detected experimentally, the photosynthetic quotient will be exactly unity as found by Willstätter and Stoll. In short, their observations only prove that the O_2/CO_2 ratio in carbohydrate photosynthesis is exactly unity. Their measurements by no means prove that under normal conditions this ratio is exactly unity or that aminoacid photosynthesis is not taking place.

We may now discuss the effect of increasing the quantity of nitrite while the rate of production of activated formaldehyde remains constant. Let it be assumed that in the leaves of certain plants there is stored during the night a quantity of nitrite which exceeds the normal and constant amount maintained during photosynthesis. Since it has experimentally been proved that the reaction of activated formaldehyde with nitrite is preferential to its polymerization to carbohydrates (p. 152), this condition will result in an increase in the relative amount of aminoacids photosynthesized. If the amount of nitrite stored during the night be sufficient to react with the whole of the activated formaldehyde, no carbohydrates will be formed. The assumption, therefore, leads to the expectation that when such plants are first irradiated large values of the O_2/CO_2 ratio will be observed, and that as the excess of nitrite is used up this ratio will progressively decrease to the normal value characteristic of the species. The maximum initial value of the O_2/CO_2 ratio will be that given by the photosynthesis of aminoacids only. The largest value of this ratio given in Table XI is 1·494, but an even higher value is possible with a different group of aminoacids. The final conclusion is thus reached, that when certain species of plants are first irradiated an initial value of at least 1·5 may be observed, and that as the irradiation is continued the O_2/CO_2 ratio will progressively decrease until the normal values for those species are reached.

It is of considerable interest that in the case of the succulents Willstätter and Stoll observed these very phenomena. With *Opuntia* the average value of the O_2/CO_2 ratio during the first half-hour of irradiation was 1·5, whilst the average value during 5½ hours' irradiation was 1·12. It is to be noted that the structure of the succulents is such as to favour the storage of nitrites during the night, since the volume of the leaves is large in relation to the surface and there are relatively few stomata. It would seem probable, therefore, that the observations made with these plants are to be explained by the accumulation of nitrites in their leaves during the night.

A different explanation of Willstätter and Stoll's results was advanced by Spoehr, who considered that the phenomena are caused by the fact that a considerable amount of organic acids is stored in the leaves of the succulents during the night.[90] These acids disappear when irradiation is commenced, partly owing to their oxidation to CO_2 by the O_2 produced in photosynthesis [91] and partly as the result of their photolysis by the direct action of light with the formation of CO_2.[92] The rate

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[90] *Photosynthesis*, p. 93. Chemical Catalogue Co., New York, 1926.

[91] Richards, Carnegie Inst., Pub. No. 209, 1915.

[92] Spoehr, *Biochem. Zeitsch.*, 1913, *57*, 95.

of photosynthesis is increased by the availability of the CO_2 produced in both these processes, and Spoehr considers that as the result of this the ratio of the O_2 evolved to the CO_2 absorbed when the irradiation is first commenced will be greater than unity.

It must be remembered, however, that the increase in the rate of photosynthesis caused by the internal supply of CO_2 will enhance the rate of oxidation of the organic acids and consequently no O_2 will be set free when the plants are first irradiated, except possibly under very intense illumination. This was experimentally observed by Richards. Then, again, the volume of CO_2 produced in the oxidation and photolysis of the organic acids will exceed the volume of O_2 used in their oxidation. For example, the oxidation of malic acid is expressed by the equation

$$C_4H_6O_5 + 3O_2 = 4CO_2 + 3H_2O$$

It is to be expected, therefore, that CO_2 will be evolved when the succulents are first irradiated, that is to say, the surroundings will be enriched with CO_2. Richards states that CO_2 is evolved when the succulents are first irradiated with diffuse light or direct sunlight. It is difficult to understand how under the above conditions any measurements could be made of the CO_2 absorbed and O_2 evolved in the photosynthetic process when the succulents are first irradiated.

The arguments in favour of nitrogen assimilation in living plants being a photosynthetic process may be summarized as follows :

1. It has experimentally been proved that by the action of activated formaldehyde on potassium nitrite formhydroxamic acid is produced, and that by the interaction between activated formaldehyde and formhydroxamic acid the same nitrogen compounds are photosynthesized as are produced in the living plant.

2. Amongst the nitrogen compounds photosynthesized *in vitro* from potassium nitrite were α-aminoacids, including histidine, alkaloids and a protein.

3. By the progressive action of activated formaldehyde on ammonia coniine was photosynthesized.

4. Each stage in the photosynthetic process is followed by

a dark reaction in which oxygen is evolved. The condensation of the aminoacids into a protein is also a dark reaction and hence the observation that some increase in the protein content of leaves takes place in the dark is in accord with the theory.

5. The translocation of the α-aminoacids photosynthesized in the leaves to other organs of the plant may result in protein formation in those organs. The observation that protein formation takes place in organs other than the leaves is, therefore, not at variance with the theory.

6. The O_2/CO_2 ratios for the photosynthesis of the fifteen aminoacids produced in living plants have the extreme values of 1·917 and 1·200.

7. It has experimentally been proved that the formation of aminoacids, &c., by the progressive action of activated formaldehyde on nitrite is preferential to its polymerization to form a carbohydrate, and that the latter is only produced when the concentration of nitrite is small.

8. Calculations based on the heats of combustion of leaves before and after irradiation indicate that out of every 12 molecules of CO_2 assimilated, 11 polymerize to carbohydrate and 1 takes part in aminoacid formation.

9. The O_2/CO_2 ratios to be expected on this 11 : 1 basis have been calculated for the different groups of aminoacids produced in ten plant species. The values differ from species to species and lie between 1·02 and 1·05, with a probable mean value of 1·035. The mean value observed with 34 species of plants was 1·035.

10. If the rate of formation of activated formaldehyde be increased twenty to thirty times by means of large external CO_2 concentrations and intense light, the O_2/CO_2 ratio will have the extreme values of 1·002 and 1·0008. Under these conditions the O_2/CO_2 ratio was experimentally found to be unity.

11. If during the night there is stored in the leaves of a plant an amount of nitrite which exceeds that normally present during the day, the O_2/CO_2 ratio will have a high initial value and will progressively decrease to the normal value characteristic of the species. If the amount of nitrite thus stored is sufficient to react with all the activated formaldehyde produced when irradiation is commenced, the initial value of the O_2/CO_2

ratio may be at least 1·5. In the case of the succulents an average value of 1·5 was observed during irradiation for the first half-hour and an average value of 1·12 during irradiation for 5½ hours.

In view of these arguments the photosynthetic explanation of nitrogen assimilation in green plants is advanced with some confidence in its truth.

CHAPTER VII

THE MECHANISM OF PHOTOSYNTHESIS

IN Chapters IV and V the story was told of the investigations in Liverpool which led in 1928 to the photosynthesis of hexoses by the irradiation of nickel carbonate prepared by the electrolytic process, and in 1937 to the photosynthesis of a type of starch by irradiation of nickel oxide containing thorium oxide, in the molecular ratio of $1ThO_2 : 24NiO$, adsorbed on specially purified kieselguhr. In this story all reference to the mechanism whereby hydrated carbon dioxide is converted by the action of white light into one or other type of carbohydrate was deliberately excluded in order not to confuse the issue. During the course of these investigations certain observations were made which indicated a very close analogy between the photosynthetic process with nickel oxide and photosynthesis in the living plant, and these were recorded as being of considerable interest. There appeared to be no doubt that, whatever the mechanism of photosynthesis may be, this mechanism must be the same in the two processes, that is to say, nickel oxide in the laboratory process must play the same role as chlorophyll in the vital process. It also appeared to be significant that these two substances, each of which is able to promote photosynthesis, is green in colour, this colour being due to the absorption of the red and blue rays present in daylight.

These similarities between the two processes become of great importance in any discussion of the mechanism of photosynthesis, not only because they indicate that the mechanism is the same in both, but because any theory of the mechanism can be put to the proof of experiment in the case of nickel oxide, such test not being possible with living plants. For this reason the mechanism of photosynthesis with nickel oxide may be discussed first.

THE MECHANISM OF PHOTOSYNTHESIS ON A SURFACE OF NICKEL OXIDE

The evidence that was given in Chapter II leaves no possible doubt that the conversion of hydrated carbon dioxide into any carbohydrate involves the intermediate formation of activated formaldehyde, and the problem to be solved is how this substance can be produced from hydrated carbon dioxide by the agency of white light and subsequently undergo quantitative polymerisation into a carbohydrate.

The starting point in discussing the process is the fact that the photosynthesis takes place on a surface of nickel oxide which is coated with an adsorbed layer of hydrated carbon dioxide. The evidence for this was given in Chapter V and was based on the fact that the preparations of NiO containing ThO_2 adsorbed on kieselguhr, became poisoned by the formation of a superficial layer of the starch product when, coated as they were with an adsorbed layer of hydrated carbon dioxide, they were brought out of the drying oven into daylight.

In the early experiments with nickel carbonate, which was later proved to consist of minute crystals of NiO together with carbon dioxide and water held by adsorption and capillary forces (p. 97), it was found that this substance when irradiated turned dark on the surface owing to the formation of a superficial layer of Ni_2O_3 (p. 73). It was proved at the same time that this superficial layer of Ni_2O_3 reverted in the dark to NiO. In view of Rideal's suggestion (p. 72) that the oxygen set free in the photochemical production of formaldehyde is retained on the surface, possibly in the form of a peroxide, it seemed possible that the action of light on a unimolecular layer of hydrated carbon dioxide adsorbed on a surface of nickel oxide might be expressed by the equation

$$4NiO.CO_2H_2O + \text{light energy} = 2Ni_2O_3 + CH_2O$$

This equation is, of course, identical with that proposed by Willstätter and Stoll (p. 16), except that the oxidation of 4NiO to $2Ni_2O_3$ by a molecule of oxygen is substituted for the oxidation of 1 molecule of chlorophyll A by a molecule of oxygen to a molecule of chlorophyll B hydrate. As in the case of Willstätter and Stoll's equation, there are two serious objections to the acceptance of the above equation as the

explanation of the pr
first place, the reaction i
daylight, and consequently it i
is absorbed by nickel oxide, be
hydrated carbon dioxide does not
rays present in daylight. The abso
nickel compounds have been measured and
lished that nickel oxide exhibits an absorpti
blue-violet region of the spectrum, the maximum
power being for rays of wave-length 4000A. Nickel ox
selectively absorbs red light of greater wave-length than 6
The characteristic green colour of inorganic compounds
nickel is due to this absorption of the blue-violet and red rays. The value of $Nh\nu$ corresponding to 4000A is 71,092 calories, and this is much smaller than the minimum amount of 112,167 calories needed to convert 1 grm. mole of hydrated carbon dioxide into glucose, and the discrepancy would be greater if the reaction were promoted by red light.

In the second place, the above equation involves the same difficulty which was stressed in discussing the Willstätter and Stoll equation, namely, that the primary photosynthetic process must produce activated formaldehyde in order that polymerization of the formaldehyde to glucose or starch may take place. If the equation in its present form were correct, it would be necessary to formulate a second reaction in which the formaldehyde produced is activated.

Now both these difficulties will be surmounted if the product of the action of blue-violet rays on the adsorption complex, $4NiO.CO_2H_2O$, be not 2 molecules of nickelic oxide and 1 free molecule of formaldehyde, but an adsorption complex consisting of 1 molecule of formaldehyde adsorbed on 2 molecules of nickelic oxide. It has been proved that photosynthesis with the aid of nickel oxide which contains thorium oxide is a surface reaction, and the suggestion now advanced is that the nickel oxide molecules in the surface layer form with hydrated carbon dioxide the adsorption complexes $4NiO.CO_2H_2O$, and that these complexes absorb blue-violet rays and are thereby converted into a unimolecular layer of the isomeric adsorption complexes of nickelic oxide and formaldehyde.

of alumina and other
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wn that this activation is due to
energy quanta by each adsorbing
ch molecule of the gas adsorbed.[94]
mplexes thus formed are thereby stabilized
dissociated into their components by the supply
of energy equal to that donated by the adsorbing
the adsorbed molecules. When that amount of energy
plied to the adsorption complexes they are resolved into
e original adsorbing units and the activated molecules of the
adsorbate.

Applying this to the adsorption complex of nickelic oxide and formaldehyde, the action of light on the adsorption complex $4NiO.CO_2H_2O$ can be expressed by the equation

$$4NiO.CO_2H_2O + h\nu_1 = 2Ni_2O_3.CHOH$$

where ν_1 is the frequency of the light which promotes the reaction and CHOH is a molecule of activated formaldehyde. The formula CHOH was originally suggested by Nef as explaining many of the reactions of formaldehyde.

In order to complete the process, it is necessary to dissociate the complexes, $2Ni_2O_3.CHOH$, by the supply to them of an amount of energy equal to that donated by the nickelic oxide to the formaldehyde. This energy can be supplied in the form of radiant energy and the dissociation can be represented by the equation

$$2Ni_2O_3.CHOH + h\nu_2 = 2Ni_2O_3 + CHOH$$

where ν_2 is the frequency of the radiation needed to promote the dissociation. The activated molecules of formaldehyde thus set free will at once polymerize on the surface into a carbohydrate. The evidence that activated formaldehyde polymerizes into glucose was given in Chapter III.

Now it was shown in Chapter V that photosynthesis on a surface of nickel oxide is a continuous process, and hence it

[93] Baly and Pepper, *Nature*, 1935, *136*, 28; Baly, Pepper and Vernon, *Trans. Faraday Soc.*, 1939, *35*, 1165.

[94] Baly, *Proc. Roy. Soc.*, 1937, *160*A, 465.

is necessary to add a third reaction to the above two photochemical reactions, namely

$$2Ni_2O_3 = 4NiO + O_2$$

As was stated above, this reaction was observed to take place in the dark, and hence it is a thermal process.

The theory now advanced of the mechanism of photosynthesis on a surface of nickel oxide is that the total amount of energy needed to convert hydrated carbon dioxide into a carbohydrate is supplied in two stages by means of two consecutive photochemical processes. This theory surmounts the difficulty which has been met with in all attempts to explain the process on the basis of a single light reaction. The theory states that the complete photosynthetic cycle consists of two photochemical processes and one thermal reaction, namely

1. $4NiO.CO_2H_2O + h\nu_1 = 2Ni_2O_3.CHOH$
2. $2Ni_2O_3.CHOH + h\nu_2 = 2Ni_2O_3 + CHOH$
3. $2Ni_2O_3 = 4NiO + O_2$

where ν_1 is the frequency of the blue-violet rays absorbed by nickel oxide and ν_2 is the frequency of the red rays absorbed by Ni_2O_3. It will be seen that as the result of these three reactions photosynthesis is a continuous process.

Before this theory can be accepted it is necessary to submit it to rigid critical examination, and the first step is to determine whether the minimum amount of energy to convert 1 grm. mole of hydrated carbon dioxide into a carbohydrate, namely, 112,167 calories, can be gained in the two photochemical processes. As has already been stated, nickel oxide absorbs the blue end of the spectrum in daylight and has the maximum absorptive power for light of wave-length 4000A, and the amount of energy corresponding to this is 71,092 calories per gram molecule. It must be remembered, however, that the formation of Ni_2O_3 from NiO is an endothermic process, the reaction being expressed by the equation

$$4NiO + O_2 = 2Ni_2O_3 - 2{,}600 \text{ calories}$$

and hence the net energy gain in the first photochemical reaction is 71,092 – 2,600 = 68,492 calories.

In order to complete the process, it is necessary to supply a further quantity of 112,167 – 68,492 = 43,675 calories, and the question is whether this amount of energy can be gained in the second photochemical reaction. The wave-length of light corresponding to this amount of energy is 6511A and, since all solid inorganic nickel compounds absorb all the red rays of the spectrum with wave-length greater than wave-length 6100A, it is manifest that the second photochemical reaction can be promoted by red light. It may be seen, therefore, that photosynthesis on a surface of nickel oxide can be promoted by the absorption first of blue light and second of red light, the frequencies of these lights, ν_1 and ν_2, being characteristic of nickel compounds. The two-stage mechanism thus gives a satisfactory explanation of the energetics of photosynthesis.

Although this evidence may be regarded as very satisfactory it is not sufficient completely to establish the validity of the theory. It is necessary also to prove (1) that Ni_2O_3 adsorbs formaldehyde, (2) that the adsorption complexes so formed consist of 2 molecules of nickelic oxide and 1 molecule of formaldehyde, and (3) that these adsorption complexes give evidence of photosynthesis when irradiated with red light. The investigation of these three phenomena was carried out in my laboratory by J. Lord and J. H. J. Bowen independently, and in view of their importance each method may be described in detail.

It was found at the outset that formaldehyde is not adsorbed on the surface layer of nickelic oxide of the black material obtained when hydrated nickel carbonate is heated at 250° in air until the evolution of gaseous products has ceased. This layer of nickelic oxide is quite stable and thus differs from the unimolecular layer of nickelic oxide formed when nickelous oxide is irradiated in presence of hydrated carbon dioxide. Preparations of hydrated nickelic oxide, therefore, were made by the addition of a cold alkaline solution of sodium hypobromite to a cold solution of nickel nitrate. It was, however, not found possible to wash the precipitated hydrated nickelic oxide free from alkali, because, when the alkali content was reduced below a certain limit, the hydrated oxide began to peptize, a dark brown solution passing through the filter. This difficulty was overcome by precipitating the hydrated oxide in the presence of kieselguhr, it having been found that the mixed product did not show any evidence of peptization during very prolonged washing. Many such preparations were made, containing different amounts of the hydrated oxide, and these were washed with distilled water until their alkali content was very small.

Proof of the adsorption of formaldehyde by the hydrated nickelic oxide was obtained by the measurement of the surface potential of several preparations when suspended in 0·0524N acetic acid and in 0·0524N acetic acid containing 0·3 per cent of formaldehyde. As was stated on page 99 this acid, which is prepared by diluting 3 ml. of glacial acetic acid to 1 litre, is well suited for cataphoresis measurements. The values of the surface potential are given in Table XIII, the first column of which gives the percentage of the oxide, calculated as Ni_2O_3, in the mixture with kieselguhr.

TABLE XIII

	Surface potential in volts	
Ni_2O_3 content	In 0·0524N acid	In 0·0524N acid and 0·3 per cent CH_2O
6·20	+ 0·0342	+ 0·0284
11·68	+ 0·0465	+ 0·0351
16·56	+ 0·0518	+ 0·0433
20·92	+ 0·0533	+ 0·0456

As will be seen from the table, the surface potentials of the hydrated nickel oxide are materially reduced in the presence of formaldehyde, and this definitely indicates that adsorption takes place.

The method of determining the composition of the adsorption complexes of nickelic oxide and formaldehyde was to suspend a known amount of the oxide in an aqueous solution of a known amount of formaldehyde. The nickelic oxide was then collected on a filter, washed with distilled water and analysed, and the amount of residual formaldehyde in the combined filtrate and washings was determined. From these analyses the ratio of the decrease in the amount of hydrated nickelic oxide, expressed as Ni_2O_3, to the decrease in the amount of formaldehyde was calculated. If the adsorption complexes have the formula $2Ni_2O_3.CHOH$, this ratio will be 11·018, and if the formula be $Ni_2O_3.CHOH$, the ratio will be 5·509.

It was found that the dry mixtures of hydrated nickelic oxide and kieselguhr were not absolutely stable, since analyses of them at intervals of 24 hours indicated a small decrease in their nickelic oxide content. It was necessary, therefore, to determine the nickelic oxide content of the mixture immediately before each experiment was carried out.

In the first experiments a suspension of a known amount of nickelic oxide in an aqueous solution of a known amount of formaldehyde was allowed to remain for some hours at room temperature before the residual amounts of the two were determined. The analytical results gave values of the Ni_2O_3/CH_2O ratio which were very discordant and unsatisfactory. The explanation of this was found to be the presence of alkali in the hydrated nickel oxide owing to insufficient washing, and this alkali caused some polymerization of the formaldehyde to formose or α-acrose.

Fresh preparations were then made of mixtures of hydrated nickelic oxide and kieselguhr and these were washed with distilled water until they were quite free from alkali, and this was found to be a very lengthy process. In each experiment, immediately after the dried nickelic oxide preparation had been analysed, a weighed quantity was added to an ice-cold aqueous solution of a known amount of formaldehyde. The mixture was gently stirred for 4 minutes and then the residual amounts of the oxide and formaldehyde were determined. The analytical results then obtained were very satisfactory, and in seven determinations the values of the Ni_2O_3/CH_2O ratio were found to be 10·81, 10·65, 10·71, 10·68, 11·40, 10·86, and 10·75 as compared with the calculated value of 11·018 for $2Ni_2O_3.CHOH$.

In appraising these values of the Ni_2O_3/CH_2O ratio, the limits of the analytical accuracy must be taken into consideration. In each experiment it was necessary to use an amount of the mixture of the hydrated nickelic oxide and kieselguhr, the oxide content of which could be determined in one single analytical operation. The use of larger quantities would have entailed drying the material after treatment with formaldehyde and analysing an aliquot part. This was precluded by the proof which had previously been obtained that during drying some oxygen was lost. Then again, it must be remembered that the adsorption of restricted to the surface of the particles of th and it was found that the amount of nickelic formed adsorption complexes was about 22 per c amount treated with formaldehyde. It will be realized fore, that the amounts of nickelic oxide and formald which in any one experiment formed adsorption comple were small.

This may be illustrated by the details of one experiment. 2·5084 grm. of a mixture of hydrated nickelic oxide and kieselguhr, which contained 8·559 per cent of Ni_2O_3, were added to 50 ml. of an ice-cold aqueous solution containing 0·6074 per cent of formaldehyde, and the mixture was gently stirred for 4 minutes. The initial quantities of nickelic oxide and formaldehyde, therefore, were 0·2147 grm. and 0·3037 grm., respectively. After collection on a filter and washing, the residual solid contained 0·1667 grm. of Ni_2O_3, and hence 0·2147 − 0·1667 = 0·0480 grm. of Ni_2O_3 had formed adsorption complexes. The combined filtrate and washings were found to contain 0·29928 grm. of formaldehyde, and hence 0·3037 − 0·29928 = 0·00442 grm. of formaldehyde had formed adsorption complexes. The ratio of the two reacting quantities, 0·0480/0·00442, is 10·86.

It will be realized from the foregoing that the amounts of formaldehyde which formed adsorption complexes were very small, and in fact they lay between 0·0045 grm. and 0·0040 grm. The margin of experimental error, therefore, was large, and hence the seven values of the Ni_2O_3/CH_2O ratio which were given above may be accepted as satisfactory evidence of the existence of the adsorption complexes $2Ni_2O_3 . CHOH$.

The second method of proving the existence of these adsorption complexes was based on the fact that they are isomeric with the adsorption complexes $4NiO . CO_2H_2O$. The two-stage theory advanced above indicates that the $2Ni_2O_3 . CHOH$ complexes are endothermic with respect to the $4NiO . CO_2H_2O$ complexes to the extent of 68,492 calories, and hence they should undergo change to $4NiO . CO_2H_2O$, provided that the energy of activation of this reverse reaction is supplied. In order to determine whether this reaction takes place, a small quantity of a mixture of hydrated nickelic oxide and kieseld to remain at 17° in contact with a dilute on of acetic acid and formaldehyde. The exceedingly slow and after 4 days the whole of c oxide had passed into solution as nickelous acetate, tion containing dissolved carbon dioxide. The velocity reaction was very greatly increased at higher tempera-es, and at 45° the nickelic oxide dissolved rapidly in a dilute aqueous solution of acetic acid and formaldehyde with the visible evolution of carbon dioxide. These observations, which were many times confirmed, prove that the adsorption complexes, $2Ni_2O_3.CHOH$, change slowly at 17° and rapidly at 45° into the adsorption complexes, $4NiO.CO_2H_3O$, which in the presence of dilute acetic acid pass into solution as nickelous acetate with the evolution of carbon dioxide.

The observations also indicate that the reaction

$$2Ni_2O_3.CHOH = 4NiO.CO_2H_2O$$

has an unusually large temperature coefficient, and hence the energy of activation must be very great. It is to be noted that the only other alternative to the formation of the adsorption complexes, $2Ni_2O_3.CHOH$, is that the complexes have the formula $Ni_2O_3.CHOH$. If the latter were formed when nickelic oxide is suspended in an aqueous solution of formaldehyde, the Ni_2O_3/CH_2O ratio would be one-half that experimentally found. Furthermore, the adsorption complexes $Ni_2O_3.CHOH$, would on treatment with acid give formic acid and not carbon dioxide and water. The experimental evidence, therefore, may be accepted as proving the existence of adsorption complexes having the formula $2Ni_2O_3.CHOH$, and that these are isomeric with and endothermic with respect to the adsorption complexes $4NiO.CO_2H_2O$.

The evidence that has been obtained of the formation of the $2Ni_2O_3.CHOH$ adsorption complexes when hydrated nickelic oxide is suspended in an aqueous solution of formaldehyde indicates a third method whereby the two-stage theory of photosynthesis can be put to the test of experiment. The theory states that by the action of blue light of wave-length 4000A the adsorption complexes $4NiO.CO_2H_2O$ are converted into the adsorption complexes $2Ni_2O_3.CHOH$, and that these adsorption complexes are subsequently dissociated by the action of red light of wave-length 6511A into nickelic oxide and activated formaldehyde which polymerizes on the surface to give a carbohydrate. Since it has been proved that the adsorption complexes $2Ni_2O_3.CHOH$ are produced directly from hydrated nickelic oxide and formaldehyde, the theory indicates that the irradiation of a suspension of hydrated nickelic oxide in an aqueous solution of formaldehyde should result in the formation of a carbohydrate.

Before any such irradiation was carried out it was necessary to determine whether any formose or other organic substance is produced when a suspension of hydrated nickelic oxide in an aqueous solution of formaldehyde is allowed to remain in the dark. In order to test this, 100 grm. of an alkali-free preparation of kieselguhr and hydrated nickelic oxide containing 6 per cent of Ni_2O_3 were suspended in a dilute aqueous solution of formaldehyde and allowed to remain at room temperature for some hours in the dark. The filtrate from the suspension was concentrated under reduced pressure, diluted with distilled water and again concentrated, this process being repeated until the whole of the formaldehyde had been removed. When the solution was free from formaldehyde it was concentrated under reduced pressure to dryness. The dry residue was proved to be completely free from organic matter.

A similar suspension of an alkali-free preparation of hydrated nickelic oxide and kieselguhr in a dilute aqueous solution of formaldehyde was then irradiated for some hours in the glass irradiation vessel. The filtrate from the suspension was freed from formaldehyde by the method described above and then evaporated to dryness under reduced pressure. The residue had a gummy consistency and was organic. It did not give the Molisch reaction, but on treatment with warm concentrated sulphuric acid much carbon was formed. Examination of the organic residues obtained by further irradiations of suspensions of hydrated nickelic oxide in aqueous solutions of formaldehyde indicated that these photosynthesized products were not carbohydrates, but acids of sufficiently large molecular weight to be charred by concentrated sulphuric acid.

When a surface of nickel oxide containing thorium oxide in the molecular ratio $1ThO_2 : 24NiO$ is irradiated in the presence of hydrated carbon dioxide, the photosynthesis is achieved by the NiO molecules in the surface layer, that is to say, the only molecules of Ni_2O_3 present are those actually produced during the photosynthetic cycle. When hydrated nickelic oxide is irradiated in the presence of formaldehyde there is a large excess of the nickelic oxide and it is more than probable that the oxidation to acids of the product of photosynthesis is caused by this excess. It thus would seem that the photosynthesis of complex organic acids by the irradiation of hydrated nickel oxide in the presence of formaldehyde is satisfactory evidence of the reality of the second photochemical reaction of the two-stage process.

The position now reached may be summarized. The two-stage hypothesis states that the complete photosynthetic cycle consists of two consecutive photochemical processes which are followed by a dark or thermal reaction. These reactions are

1. $4NiO.CO_2H_2O + h\nu_1 = 2Ni_2O_3.CHOH$
where $\nu_1 = 7{\cdot}4949 \times 10^{14}$ $(\lambda = 4000A)$

2. $2Ni_2O_3.CHOH + h\nu_2 = 2Ni_2O_3 + CHOH$
where $\nu_2 = 4{\cdot}6045 \times 10^{14}$ $(\lambda = 6511A)$

and CHOH represents an activated molecule of formaldehyde, n molecules of which polymerize on the surface to a carbohydrate $(CHOH)_n$.

3. $2Ni_2O_3 + O_2$.

In the first process the value of $Nh\nu_1$ is 71,092 calories and $4NiO + O_2 = 2Ni_2O_3 - 2{,}600$ calories, and hence the net energy gain is $71{,}092 - 2{,}600 = 68{,}492$ calories. In the second process the value of $Nh\nu_2$ is 43,675 calories and $68{,}492 + 43{,}675 = 112{,}167$ calories, which is the net amount of energy needed to convert 1 grm. mole of hydrated carbon dioxide into 1 grm. mole of glucose.[95]

The experimental evidence detailed above indicates that Ni_2O_3 and CH_2O form adsorption complexes consisting of 2 molecules of Ni_2O_3 and 1 molecule of CH_2O, and that these complexes change very slowly at 17° and very rapidly at 45° to the adsorption complexes $4NiO.CO_2H_2O$. Evidence has also been obtained that organic acids of sufficiently high molecular weight to carbonize on treatment with concentrated

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[95] Baly, *Roy. Photo. Journ.*, 1932, *72*, 474.

sulphuric acid are photosynthesized when hydrated nickelic oxide is irradiated in the presence of formaldehyde. It is probable that these acids are the result of the oxidation of carbohydrates by the excess of nickelic oxide present. The proof that the third reaction takes place in the presence of hydrated carbon dioxide has already been given on pages 73 and 128.

In view of this evidence the two-stage theory of photosynthesis may be advanced as a reasoned explanation of the mechanism of photosynthesis with the aid of nickelous oxide.

THE TWO-STAGE MECHANISM OF PHOTOSYNTHESIS IN THE LIVING PLANT

In Chapter I the work of Willstätter and Stoll on the four plant pigments, chlorophyll A, chlorophyll B, carotin, and xanthophyll, was described.[96] In the course of their investigation they proved that chlorophyll in colloidal solution in water at first adsorbs carbon dioxide, and that when the carbon dioxide is in excess the chlorophyll is decomposed into phaeophytin and magnesium carbonate. In view of the facts that in the living plant the chlorophyll exists in the colloidal state and that the carbon dioxide concentration is small, Willstätter and Stoll suggested that photosynthesis is achieved by the action of light on an adsorption complex of chlorophyll A and hydrated carbon dioxide. Expressed in simpler terms than those adopted by them, their suggestion was that this adsorption complex is converted by the agency of light into chlorophyll B hydrate and formaldehyde in accordance with the equation

$$C_{55}H_{72}O_5N_4Mg.CO_2H_2O + \text{light energy} = C_{55}H_{70}O_6N_4MgH_2O + CH_2O$$

In their quantitative determinations of the amounts present in plant leaves of the four pigments before and after irradiation, Willstätter and Stoll found that the ratio chlorophyll A to chlorophyll B remained constant during photosynthesis and that the ratio of xanthophyll to carotin increased. These

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[96] *Untersuchungen über die Assimilation der Kohlensaüre.* Berlin, 1918.

observations were difficult to reconcile with the above equation, because in the first place the equation certainly leads to the expectation that the ratio of chlorophyll B to chlorophyll A should increase, and in the second place Willstätter and Stoll proved that neither carotin nor xanthophyll takes part in any photochemical process during photosynthesis. As the result of these difficulties and also of the evidence that no free formaldehyde is formed during photosynthesis, the Willstätter and Stoll theory has not been generally accepted. In the discussion of this theory given in Chapter I emphasis was laid on the fact that the theory is not incorrect, but incomplete.

The striking analogies between photosynthesis in the living plant and that achieved in the laboratory with nickel oxide containing thorium oxide were emphasized in Chapter V. There is little doubt, therefore, that the mechanism of photosynthesis is the same in each. In applying the two-stage mechanism to the vital process we may commence with the fact established by Willstätter and Stoll that the photosensitive unit is an adsorption complex of chlorophyll A and hydrated carbon dioxide. By analogy with the mechanism in the case of nickel oxide and with the knowledge that the absorption spectra of the two chlorophylls are very similar to those of nickel compounds, we may conclude that the production of activated formaldehyde is achieved by two successive photochemical processes, namely

$$1.\quad C_{55}H_{72}O_5N_4Mg.CO_2H_2O + h\nu_1 = C_{55}H_{70}O_6N_4MgH_2O.CHOH$$

$$2.\quad C_{55}H_{70}O_6N_4MgH_2O.CHOH + h\nu_2 = C_{55}H_{70}O_6N_4MgH_2O + CHOH$$

where ν_1 is the frequency of the blue-violet rays absorbed by chlorophyll A and ν_2 is the frequency of the red rays absorbed by chlorophyll B.

Before the third reaction, in which the chlorophyll B hydrate is reduced to chlorophyll A, is formulated, we may inquire whether the necessary amount of energy can be supplied in these two photochemical processes. In order to decide this question a knowledge is necessary of the absorption spectra of the two chlorophylls. In their investigations Willstätter and Stoll measured the absorption spectra of solutions of pure chlorophyll A and B, and found that each is characterized by two absorption bands, one in the blue-violet region of the spectrum and one in the red. Chlorophyll A exhibits a maximum absorptive power towards light of the wave-lengths 4175A and 6575A, whilst chlorophyll B exerts a maximum absorptive power towards light of the wave-lengths 4600A and 6495A. A communication has been recently published by K. P. Meyer,[97] who gives a number of absorption curves characteristic of chlorophyll A and B, but it is not possible from such curves to determine the wave-lengths towards which each component separately possesses a maximum absorptive power. It is well known that in general the effect of increasing the concentration of an absorbing substance is to broaden the absorption band and not materially to change the wave-length at which the absorption is a maximum. Since Meyer's absorption curves of colloidal chlorophyll A and B indicate very strong absorption at 4200A, there is no reason whatever against the adoption of 4175A as the wave-length at which the chlorophyll A in the mixture exerts its maximum absorptive power. The same argument applies to the absorption band of chlorophyll B at 6495A, and hence the amounts of energy absorbed in the two photochemical processes given above may be calculated from these two wave-lengths.

In the first process the value of $Nh\nu_1$ at 4175A is 68,117 calories, and in the second process the value of $Nh\nu_2$ at 6495A is 43,785 calories. The sum of these two amounts of energy is 111,902 calories, and the difference between this and the amount actually required, 112,167 calories, is only 265 calories. In view of the fact that no information is available as to the energy change involved in the oxidation of chlorophyll A to chlorophyll B hydrate, this result may be accepted as being very satisfactory.

The third reaction, in which the chlorophyll B hydrate produced in the second light reaction is reduced to chlorophyll A with the evolution of oxygen, may now be considered. Willstätter and Stoll suggested that this reaction is promoted by an enzyme, but in the discussion of their theory in Chapter I the difficulty of accepting this view was emphasized. It is

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[97] *Helv. phys. Acta*, 1939, *12*, 349.

evident that this process must be more complex than the unimolecular reaction in the case of Ni_2O_3, because the oxygen is not set free *in situ* in the chloroplasts, but is transpired into the surroundings through the stomata.

In their quantitative determinations of the amounts of the four plant pigments present in plant leaves immediately before and after irradiation, Willstätter and Stoll found that the ratio of chlorophyll A to chlorophyll B remained constant, while the ratio of xanthophyll to carotin markedly increased. It is evident from this that the chlorophyll B hydrate produced in the second light reaction must be reduced to chlorophyll A as fast as it is formed. Since Willstätter and Stoll proved that the O_2/CO_2 ratio in the photosynthesis of a carbohydrate is exactly unity, and since the increase in the xanthophyll/carotin is caused by oxygen, it is manifest that these two observations are incompatible. The increase in the xanthophyll/carotin ratio, therefore, must be due to a temporary lag in the transpiration of the oxygen, a phenomenon which was discovered by Kostytschew [98] three years after Willstätter and Stoll published their observations. It thus becomes evident that the only possible mechanism of the third reaction of the photosynthetic cycle is the reduction of the chlorophyll B hydrate by carotin in accordance with the equation

$$C_{55}H_{70}O_6N_4MgH_2O + C_{40}H_{56} = C_{55}H_{72}O_5N_4Mg + C_{40}H_{56}O_2$$

Associated with this reaction there must exist a mechanism whereby the initial xanthophyll/carotin ratio is restored after it has been temporarily increased, and the oxygen is set free and transpired into the surroundings through the stomata. Little or nothing is known of this mechanism beyond the fact that the whole process is a thermal one, the velocity of which is increased by rise in temperature. There can be no doubt that the whole process is the one the existence of which was first recognized by F. F. Blackman [99] in 1905 and which is now universally known as the Blackman reaction.

It is a remarkable fact that in the living plant, in spite of the presence of a large excess of chlorophyll B, it is only the chlorophyll B produced in the two photochemical reactions

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[98] *Ber. Bot. Ges.*, 1921, *39*, 319.

[99] *Ann. Bot.*, 1905, *19*, 282.

which is reduced by carotin to chlorophyll A. It is equally remarkable that in spite of the great excess of xanthophyll present, it is only the xanthophyll produced in the third reaction of the photosynthetic cycle which is reduced to carotin in the Blackman reaction. The significance of these two facts does not appear to have been recognized. A possible explanation is that, owing to photosynthesis taking place on a surface, it is only those molecules of chlorophyll B hydrate and xanthophyll produced on that surface which are reduced to chlorophyll A and carotin, respectively. A second and more attractive explanation is that in the dark an equilibrium condition exists between the pressure of the oxygen produced in the Blackman reaction and the partial pressure of the oxygen in the surroundings. The existence of such an equilibrium would explain the initial lag in the transpiration of the oxygen when plants are first irradiated, because the restoration of an equilibrium is a slower process than an irreversible thermal reaction. It would be of great interest to determine whether the relative concentrations of chlorophyll A and B and of xanthophyll and carotin in the leaves of plants depend on the partial pressure of the oxygen in the surroundings.

The complete photosynthetic cycle in the living plant may now be stated. It consists of four separate processes, of which two are promoted by light and two are thermal processes. The first three processes are

1. $$C_{55}H_{72}O_5N_4Mg.CO_2H_2O + h\nu_1 = C_{55}H_{70}O_6N_4MgH_2O.CHOH,$$
where $\nu_1 = 7{\cdot}1799 \times 10^{14}$ ($\lambda = 4175A$)

2. $$C_{55}H_{70}O_6N_4MgH_2O.CHOH + h\nu_2 = C_{55}H_{70}O_6N_4MgH_2O + CHOH,$$
where $\nu_2 = 4{\cdot}1615 \times 10^{14}$ ($\lambda = 6495A$)

3. $$C_{55}H_{70}O_6N_4MgH_2O + C_{40}H_{56} = C_{55}H_{72}O_5N_4Mg + C_{40}H_{56}O_2$$

The fourth process, whereby the xanthophyll produced in the third reaction is reduced to carotin and the resulting oxygen is transpired through the stomata, cannot at present be formulated.

No experimental proof of this mechanism of photosynthesis in the living plant can be adduced, such as was detailed above in the case of nickel oxide. The two-stage mechanism of the production of activated formaldehyde, however, leads to a conclusion which was not referred to in the discussion of the process on a surface of nickel oxide, because it was not possible to submit it to the test of experiment before my retirement from Liverpool. The fundamental basis of the theory is that both blue and red light are needed to promote photosynthesis, but no attention has been paid to the relative intensities of the blue and red rays. It is obvious that in photosynthesis, which is achieved in two successive photochemical reactions, the rate of the process will be a maximum when the velocities of the two reactions are equal. In other words, the rate of photosynthesis will be a maximum when the intensities of the blue and red rays are equal. If the intensities of the blue and red rays are unequal, the rate of photosynthesis will be smaller than the maximum, even if the total amount of light energy is the same. It is to be expected, therefore, that reducing the intensity of the blue rays, while maintaining the total light intensity constant, will result in a decrease in the rate of photosynthesis. The mathematical proof of this will be given in Chapter VIII.

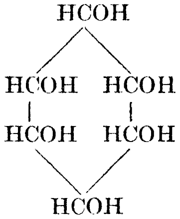
It is of great interest to record a series of observations by R. H. Dastur and his colleagues.[100] By the use of daylight and of the light from various lamps which differed in the amount of the blue rays they emitted, it was proved that when the total intensity of irradiation was maintained constant the amount of carbohydrates photosynthesized in unit time increased as the ratio of the intensity of the blue rays to the intensity of the red rays was increased. The amount of carbohydrates photosynthesized was in the order: daylight > arc lamp > ‘ daylight ’ lamp > ordinary electric filament lamp. It was thus proved that the maximum photosynthetic efficiency was obtained with daylight in which the intensities of the blue and red rays are equal.

These observations give the strongest possible evidence in favour of the two-stage mechanism of photosynthesis.

[100] Dastur and Samant, *Ann. Bot.*, 1933, *47*, 295 ; Dastur and Mehta, *ibid.*, 1935, *49*, 809.

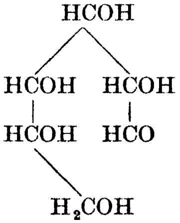
THE POLYMERIZATION OF ACTIVATED FORMALDEHYDE ON A SURFACE

The polymerization to a carbohydrate of the activated formaldehyde produced in the second photochemical process may now be discussed. In his communication dealing with the photosynthetic process, von Baeyer [101] suggested that the polymerization is cyclic, 6 molecules giving the cyclic compound



In the discussion of this suggestion in Chapter I (p. 10), five arguments were advanced in its favour. These were :

1. The six-membered ring is one of the most stable configurations of carbon atoms.
2. The carbohydrates photosynthesized in living plants invariably contain 6 or multiples of 6 carbon atoms in the molecule.
3. Inositol, which has the above formula, is a product of photosynthesis in plants.
4. The cyclic compound, by the wandering of 1 hydrogen atom to the γ-position can form the open chain structure of glucose



and glucose is the product of photosynthesis in the monocotyledons.

5. The cyclic molecules can readily undergo condensation

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[101] *Ber. Deutsch. Chem. Ges.*, 1870, *3*, 63.

> on the surface into complex carbohydrates having the general formula $(C_6H_{10}O_5)_n$, and starch is the product of photosynthesis in the dicotyledons.

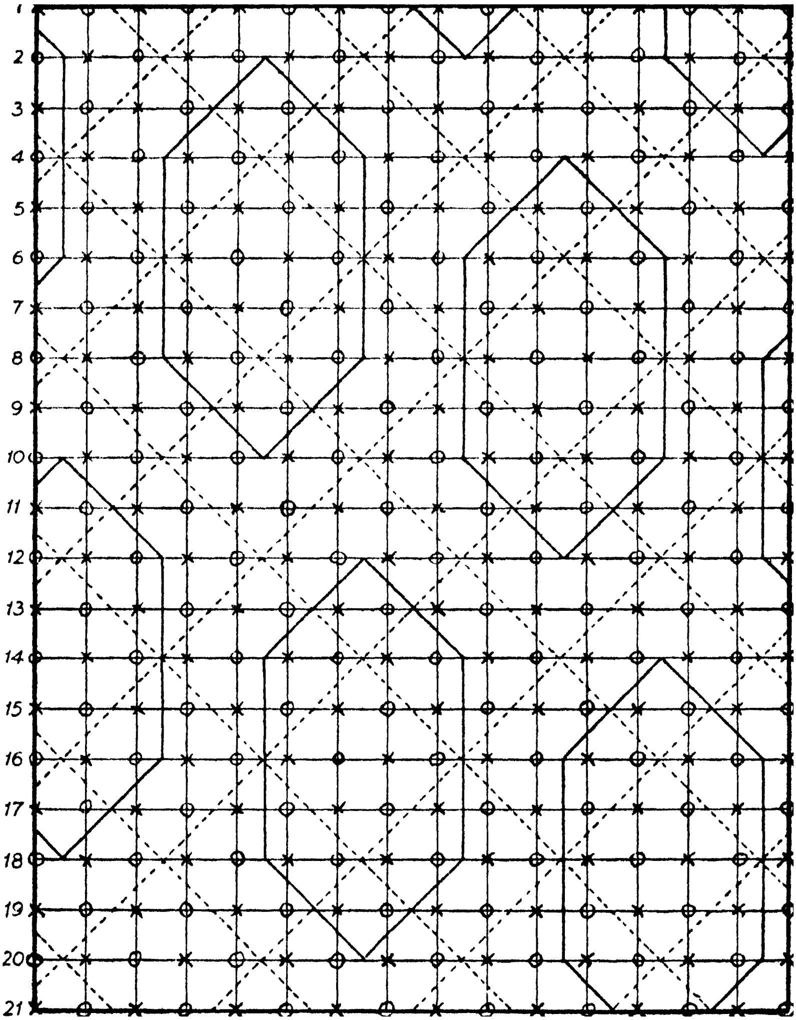
To these arguments may be added the evidence given in Chapter III of the formation of polyhydroxyphenols along with glucose when an aqueous solution of formaldehyde is irradiated with ultra-violet light.

In view of these arguments the von Baeyer theory may be accepted and applied in the first instance to photosynthesis on a surface of nickel oxide. For this purpose a knowledge is needed of the arrangement of the nickel and oxygen atoms in a surface of a crystal of nickel oxide. Nickel oxide crystallizes in the cubic (NaCl) system, and the arrangement of the nickel and oxygen atoms in the surface of a crystal is shown in Fig. 14, the distance between any pair of these atoms being $2{\cdot}086 \times 10^{-8}$ cm.

It has already been shown that 4 nickel and 4 oxygen atoms take part in the photochemical production of 1 molecule of activated formaldehyde from 1 molecule of hydrated carbon dioxide, and by means of the dotted diagonal lines the surface of nickel oxide has been divided into squares, each containing 4 nickel and 4 oxygen atoms. Each of these squares, therefore, is the area on which a single molecule of activated formaldehyde is produced.

In the polymerization to give 1 molecule of the cyclic compound $C_6H_{12}O_6$, 6 molecules of activated formaldehyde produced on six of the squares take part, and this is indicated by the hexagons in Fig. 14, each hexagon representing a single cyclic molecule of $C_6H_{12}O_6$. It is to be noted that the molecule of activated formaldehyde produced on the central square of each hexagon is not used in the cyclic polymerization, and that the number of molecules of activated formaldehyde which thereby become redundant is one out of every seven.

Now it can be seen that any one of the seven groups of 4 nickel and 4 oxygen atoms underlying 1 molecule of $C_6H_{12}O_6$ shown by a hexagon in Fig. 14 could be the redundant group, and hence there are seven different ways in which cyclic polymerization can take place on a surface of nickel oxide. In the absence of any directing influence there is no reason why any one of these seven possible cyclic polymerizations should take place. The conclusion is thus reached that in the absence of any directing influence a crystalline surface of nickel



X = Nickel atom O = Oxygen atom

FIG. 14

oxide is unsuitable for photosynthesis, because, if the cyclic polymerization does not take place, the activated molecules of formaldehyde will lose their energy of activation and re-form the adsorption complexes, $2Ni_2O_3.CHOH$, that is to say, the second photochemical process will become reversible. It is to be expected from this that a surface of pure nickel oxide will give no evidence of photosynthesis. This conclusion is of considerable interest in view of the evidence obtained in the Liverpool investigations that a surface of pure nickel oxide is photosynthetically inactive.

THE FUNCTION OF THORIUM OXIDE IN RENDERING A SURFACE OF NICKEL OXIDE PHOTOSYNTHETICALLY ACTIVE

In the investigation[102] of the absorption by kieselguhr of various metallic oxides it was proved that 100 grm. of the kieselguhr adsorbed exactly 0·01632 grm. mole of NiO, and that the adsorbate consisted of three unimolecular layers of anhydrous NiO separated by unimolecular layers of H_2O. It was also proved that 100 grm. of the kieselguhr adsorbed exactly 0·0140 grm. of NiO when the NiO contained ThO_2 in the molecular ratio of $1ThO_2 : 24NiO$, and that in this case the adsorbate was anhydrous. This evidence indicates that the molecules of ThO_2 promote the formation of crystalline NiO, probably because they act as nuclei around which the molecules of NiO arrange themselves in forming the crystal lattice.

Now it will be seen from the amounts of NiO which completely coat the same surface area in the presence and absence of ThO_2, namely, 0·0140 and 0·01632 grm. mole, respectively, that these amounts are in the ratio of 6 : 7. It is evident from this that the covering power of NiO is increased by one-sixth when it contains ThO_2 in the molecular ratio of $1ThO_2 : 24NiO$, and that each ThO_2 molecule replaces one group of 4Ni and 4O atoms out of every seven such groups. It may be noted incidentally that the replacement of a group of 4Ni and 4O atoms by 1 ThO_2 molecule is in accord with the surface area of one such group and the molecular diameter of ThO_2 in a unimolecular layer. In the same investigation it was proved that this molecular diameter is $5{\cdot}72 \times 10^{-8}$ cm.

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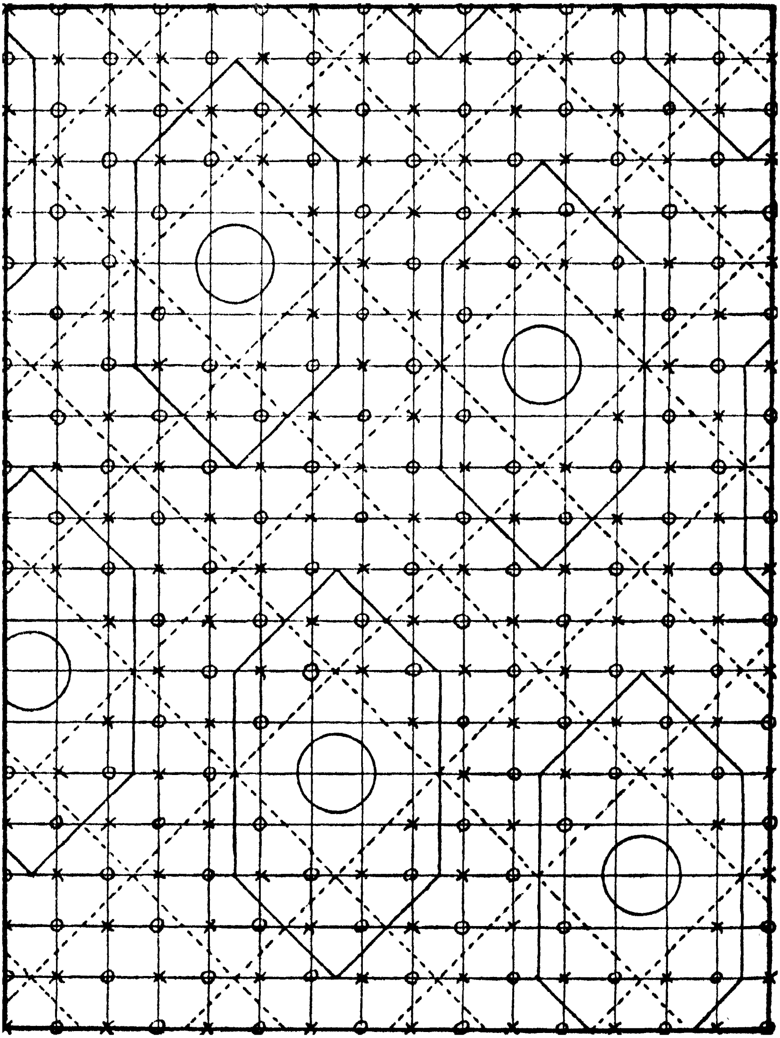
[102] **Baly, Pepper and Vernon, *Trans. Faraday Soc.*, 1939, *35*, 1165.**

and the side of each square in Fig. 14 containing 4Ni and 4O atoms is $5 \cdot 90 \times 10^{-8}$ cm.

In the discussion of the cyclic polymerization of activated formaldehyde to give the compound $C_6H_{12}O_6$ it was shown that this was highly improbable on a surface of pure nickel oxide owing to the fact that the molecule of activated formaldehyde produced from one group of 4Ni and 4O atoms out of every seven such groups is redundant, a conclusion which was fully borne out by the fact that no evidence of photosynthesis could be obtained with pure nickel oxide. As we have just seen, it was proved that, when the nickel oxide contains thorium oxide in the molecular ratio of $1ThO_2 : 24NiO$, each molecule of ThO_2 replaces one group of 4Ni and 4O atoms out of every seven such groups, that is to say, the same number of groups of 4Ni and 4O atoms as are redundant in photosynthesis on a surface of pure nickel oxide. This replacement, therefore, is exactly that needed to render a surface of nickel oxide ideal for photosynthesis. Experimentally it was proved that a surface of nickel oxide containing thorium oxide in the above molecular ratio possesses a remarkable photosynthetic activity, and hence the conclusion is reached that the replacement of groups of 4Ni and 4O atoms by thorium oxide molecules is exactly that needed to render a surface of nickel oxide ideal for photosynthesis.

In order to illustrate this a surface of nickel oxide containing thorium oxide in the molecular ratio of $1ThO_2 : 24NiO$ is shown in Fig. 15, in which the square areas occupied by the thorium oxide molecules are indicated by the large circles and the cyclic polymerizations of 6 molecules of activated formaldehyde to $C_6H_{12}O_6$ molecules are indicated by hexagons. It will be seen from the diagram that every single group of 4Ni and 4O atoms present in the surface takes part in the photosynthesis, and that as the result of this the conditions are the best possible for photosynthesis to take place. Furthermore, it is probable that the explanation of the effect of the thorium oxide molecules in promoting the crystallization of anhydrous nickel oxide when present in the molecular ratio of $1ThO_2 : 24NiO$ is to be found in the fact that each thorium oxide molecule in each layer is surrounded by six groups of 4Ni and 4O atoms.



X = Nickel atom O = Oxygen atom

FIG. 15

CYCLIC POLYMERIZATION OF ACTIVATED FORMALDEHYDE ON A SURFACE OF CHLOROPHYLL

In discussing the cyclic polymerization of the activated formaldehyde produced in the living plant it is necessary to consider the condition in which the chlorophyll exists in the leaves. As was stated on page 16, there is little doubt that the chlorophyll exists in the colloidal state and the absorption spectra measurements made by K. P. Meyer[103] undoubtedly prove that this is the case. Now it is manifest that the term colloidal as applied to the chlorophyll cannot have its usually accepted meaning, because it is beyond the bounds of possibility that the same carbohydrate could be photosynthesized in the same amounts in every plant of the same species on a surface containing a haphazard and random distribution and arrangement of the chlorophyll molecules. Such photosynthesis must be promoted on a surface in which all the chlorophyll molecules are similarly oriented.

It was pointed out above in the discussion of the Blackman reaction that in the third reaction of the photosynthetic cycle it is only the chlorophyll B produced in the second photochemical reaction which is reduced by carotin to chlorophyll A, and hence the carotin and xanthophyll must be present in close association with the chlorophyll. This association of the four plant pigments leads to the expectation of a non-crystalline condition. It would seem, therefore, that the term colloidal as applied to the chlorophyll in the leaves of plants means a non-crystalline state in which all the molecules are similarly oriented. Such similar orientation, indeed, is the most probable result of a series of similar growth processes.

We may now consider the cyclic polymerization of 6 molecules of activated formaldehyde, each of which is produced by 1 molecule of chlorophyll, on a surface in which the chlorophyll molecules are all similarly oriented. It will be realized that for the reason which was explained in detail for a surface of pure nickel oxide photosynthesis cannot take place on such a surface of chlorophyll. One out of every 7 molecules of activated formaldehyde produced will be redundant and there is no reason why any one of the seven possible cyclic polymerizations should take place on the surface. The same conclusion is thus reached as in the case of a surface of nickel oxide, namely, that it is essential in order that photosynthesis may take place that 1 out of every 7 molecules of chlorophyll be replaced by a molecule of an inactive substance.

[103] *Helv. phys. Acta,* 1939, *12,* 349.

The amount of the inactive substance required to render the chlorophyll photosynthetically active may readily be calculated. Willstätter and Stoll in their investigations of the plant pigments determined the total amount of chlorophyll present in the leaves of various species of plants, and found that in 1 square metre of leaf surface there is from 0·3 to 0·7 grm. The molecular weight of chlorophyll may be taken as 901 and, since 1 molecule of an inactive substance is required for 6 molecules of chlorophyll, the necessary amount of the inactive substance is from 0·3/5406 = 0·0000555 to 0·7/5406 = 0·0001295 grm. mole per square metre of leaf surface. These amounts are exceedingly small.

It is of outstanding interest in this connexion that investigations carried out at the Rothamsted Experimental Station have conclusively proved that very small quantities of a compound of one or other of certain inorganic elements are essential to the growth of plants, the complete absence of the element resulting in the death of the plant. This may be illustrated by the following quotation from a communication by Dr. Brenchley [104] :

> The available evidence makes it clear that boron and manganese are essential to the growth and health of many, if not all, species of plants. Copper and zinc have also been found to be necessary in many cases, although up to the present it is uncertain whether this need is universal. Apart from these four elements, isolated cases only of improvement due to traces of other minor elements have as yet been established. It may be, however, that specific elements are necessary for specific plants, and it is possible that the conclusive evidence already obtained with boron and manganese may further the opening of a wide field of investigation which may lead to results of far-reaching importance from scientific and economic standpoints.

In order that some idea may be gained of what is meant by traces of these elements, reference may be made to an investigation in which the essential need of boron was proved.[105] It was proved that broad bean and several other leguminous plants failed to complete their development in water or sand culture unless a trace of boron was present, 1 part of H_3BO_3 in 25,000 to 12,500,000 parts of nutrient solution being sufficient. It was found that 0·00005 grm. of boric acid per week

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[104] Winifred E. Brenchley, *Bot. Review*, 1936, *2*, 189.

[105] Katherine Warrington, *Ann. Bot.*, 1926, *40*, 27.

was sufficient to promote the growth of a single broad bean plant.[106]

In view of the necessity which was indicated above of the presence of an inactive substance in chlorophyll in the molecular ratio of 1 : 6, these observations are of great importance. The correlation between the necessary presence of an inactive substance in chlorophyll for photosynthesis and the necessity of boron and manganese for plant life would seem to be certain. In the calculation given above of the maximum amount of the inorganic substance needed, it was shown that this is from 0·0000555 to 0·0001295 grm. mole per square metre of leaf surface. The molecular weight of boric acid, H_3BO_3, is 61·84, and hence the maximum quantity of this substance needed is from 3·4 mgm. to 8·0 mgm. per square metre of leaf surface. This is by no means out of keeping with the observation that for a single broad bean plant 0·05 mgm. per week is sufficient.

The presence of boron in the leaves of plants has been proved and the fact that it is also present in other parts of the plant does not detract from the importance of its presence in the leaves. No explanation other than that now advanced has been found of the function of the boron. This function would undoubtedly appear to be identical with that of thorium oxide in the case of nickel oxide.

It is necessary to emphasize the fact that the amounts of boric acid calculated above are the maximum amounts needed, these amounts being those required to endow a surface of chlorophyll with its maximum efficiency. If greater quantities than the maximum of boric acid were present in the chlorophyll surface, the conditions would become progressively less favourable for photosynthesis. Excessive amounts of boric acid should, therefore, be definitely harmful, and this has been found to be the case.

The evidence given by the Rothamsted investigations and its concordance with the deduction drawn from the necessity of the presence of thorium oxide in a surface of nickel oxide leave little doubt that the function of boron, manganese, copper and zinc in promoting the growth of plants has found its explanation.

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[106] Brenchley and Warrington, *Ann. Bot.*, 1927, *41*, 179.

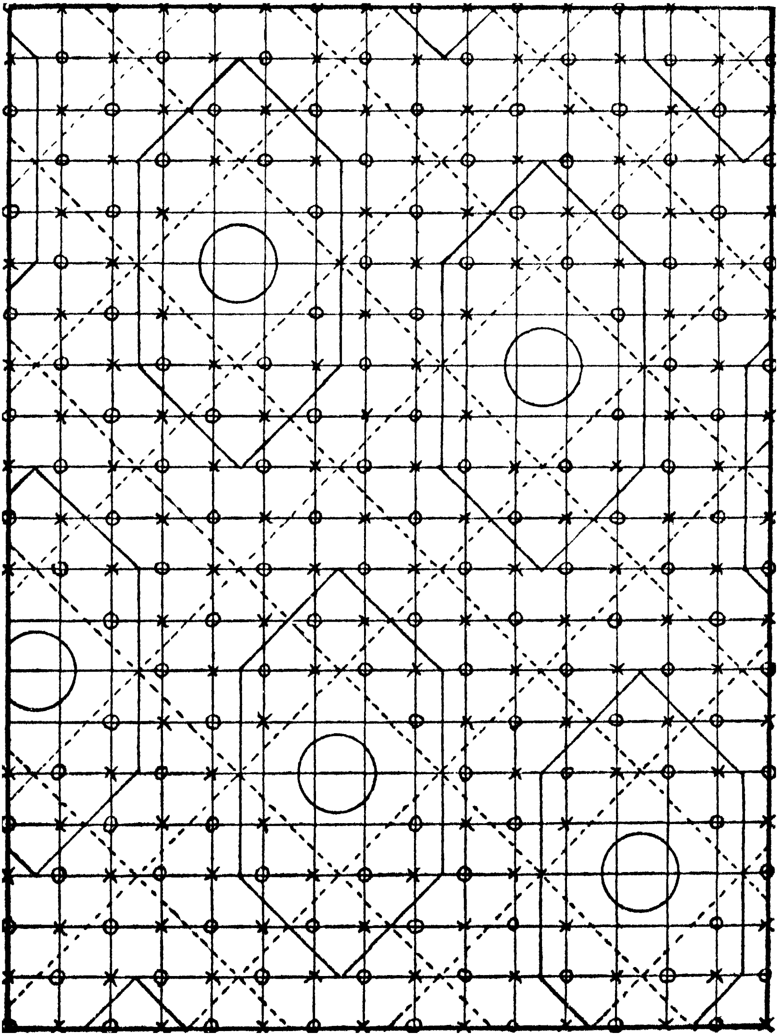
THE PHOTOSYNTHESIS OF OPTICALLY ACTIVE CARBOHYDRATES

One of the greatest mysteries of photosynthesis in nature has always been the fact that the carbohydrates produced in living plants are invariably optically active. This phenomenon is the more remarkable because in any one species the same optical isomer of the same carbohydrate is invariably photosynthesized. This invariability in the products of photosynthesis proves that there must exist a fundamental condition of the chlorophyll surface which can only promote asymmetric photosynthesis. Since in all photosynthesis the first product is the cyclic compound $C_6H_{12}O_6$, the fundamental condition of the chlorophyll must be such that one and one only of the optical isomers of this cyclic compound can be photosynthesized.

In the discussion of the mechanism of photosynthesis, a very close analogy has been established between the process on a surface of nickel oxide and that on a surface of chlorophyll. The energy relations are almost identical in the two cases, and in each the necessity of the presence in the surface of an indifferent substance has been proved. In both processes the same products are photosynthesized. Moreover, the rate of photosynthesis *in vitro* and *in vivo* increases with the temperature, and the temperature coefficients are the same in each. In both processes the rate of photosynthesis increases with rise in temperature to a maximum value, and with further rise in temperature the rate begins to decrease very rapidly. In both processes the amount of carbohydrate photosynthesized by unit quantity of light decreases with increase in the intensity of the light.

This remarkably close analogy justifies a further consideration of a surface of nickel oxide which contains thorium oxide in the molecular ratio of $1ThO_2 : 24NiO$, a surface which has been shown to be ideal for photosynthesis. There can be no doubt that a surface of chlorophyll which contains an indifferent substance in the molecular ratio of 1 : 6 is also ideal for photosynthesis and, since this surface invariably promotes the photosynthesis of optically active carbohydrates, we may inquire whether any condition exists in a surface of nickel oxide containing thorium oxide which can result in asymmetrical photosynthesis.

A surface of nickel oxide containing thorium oxide in the molecular ratio of $1ThO_2 : 24NiO$ is shown in Fig. 16. As in



X = Nickel atom O = Oxygen atom

FIG. 16

the case of Fig. 15 the surface is divided by dotted diagonal lines into square areas, each of which contains 4Ni and

4O atoms, with the exception of those occupied by ThO_2 molecules, which are indicated by circles. The cyclic polymerizations of 6 molecules of activated formaldehyde are indicated by the hexagons. Now the surface shown in Fig. 16 is characterized by the fact that in every square containing 4Ni and 4O atoms the Ni atoms are situated on the right-hand side of the O atoms. This arrangement has no physical significance in the two-dimensional diagram shown in Fig. 16, because the Ni atoms might equally well be situated on the left-hand side of the O atoms, as was the case in Fig. 15. The arrangement, however, becomes of real significance in the three-dimensional system created by the adsorption of a molecule of hydrated carbon dioxide on each of the groups of 4Ni and 4O atoms. In all the adsorption complexes, $4NiO.CO_2H_2O$, thereby formed, the adsorbed molecules of hydrated carbon dioxide must all be oriented in exactly the same way. As the result of this the adsorbed molecules of activated formaldehyde in the complexes, $2Ni_2O_3.CHOH$, which are produced in the first photochemical reaction, will all be oriented in exactly the same way. The final result will be that when the second photochemical process takes place the molecules of the cyclic $C_6H_{12}O_6$ compound will be formed, each by the polymerization on the surface of 6 molecules of activated formaldehyde, all of which are oriented in exactly the same way with respect to the surface. Since the criterion of optical activity is that in every molecule the arrangement of the atoms or groups of atoms attached to the asymmetric atom or atoms is identical, it is to be expected that the cyclic polymerization must result in the formation of optically active molecules of the $C_6H_{12}O_6$ compound. These optically active molecules will either form optically active glucose by the migration of a hydrogen atom or condense together with the loss of water to form the optically carbohydrate $(C_6H_{10}O_5)_n$.

It is unfortunate that for the reasons then given the amounts of carbohydrate photosynthesized in the investigations with NiO containing ThO_2 were too small to give any measurable rotation of polarized light. Some significance, however, may be found in the fact that the starch product was hydrolysed by diastase, it being well known that enzymes are selective in their action on one optical isomer only.

Turning now to the vital process, there is no doubt that the foregoing theory of asymmetric photosynthesis is applicable to a surface of chlorophyll which is oriented by the presence of an indifferent substance in the molecular ratio of 1 : 6. The problem of asymmetric photosynthesis in the living plant, however, is the reverse of that with nickel oxide. In the latter case the experimental evidence proves that the Ni and O atoms in the surface are all oriented in the same way by the presence of the ThO_2 molecules, and the theory indicates that this should result in the photosynthesis of optically active carbohydrates. In the case of the living plant it is known that optically active carbohydrates are invariably photosynthesized, and the theory indicates that this must be due to all the chlorophyll molecules in the irradiated surface being oriented in exactly the same way. The validity of the theory, therefore, will be established if it can be proved that the chlorophyll molecules in the irradiated surface are all oriented in exactly the same way. Although there is no direct proof of this similar orientation, convincing arguments can be adduced in favour of it. The chlorophyll molecules are synthesized and deposited in the leaves by a series of similar growth processes, and it is to be expected that, as the colloidal particles are built up, all the molecules in each layer will be similarly oriented. Then, again, it has been proved that the presence of an indifferent substance is essential for photosynthesis to take place on a surface of chlorophyll, and it has been demonstrated that the presence of the indifferent molecules results in the orientation of the chlorophyll molecules. Finally, the fact may be emphasized that the same carbohydrate is invariably photosynthesized in living plants, namely, glucose in the monocotyledons and starch in the dicotyledons. Such invariability can only be the result of an identical orientation of all the chlorophyll molecules in the leaves of the members of each class.

In view of these arguments, the theory of asymmetric photosynthesis is advanced with some confidence in its validity.

THE PRODUCTS OF PHOTOSYNTHESIS

Attention may now be directed to the nature of the carbohydrates which are the actual products of photosynthesis. The carbohydrates which are found in the greatest quantity in the leaves of plants are starch, sucrose, *d*-glucose, and *d*-fructose, and the important question is which carbohydrate is the actual product of photosynthesis. This question has been discussed at length by Stiles in his book,[107] to which I am indebted for the following brief epitome.

Sachs,[108] who was the first to demonstrate that carbohydrates are produced, held the view that starch is the first visible product of photosynthesis, but this view in the opinion of many was controverted by Kraus's [109] observation that the first appearance of starch was seen 5 minutes after photosynthesis had commenced.

In 1924 Weevers [110] made a series of determinations of the amounts of sucrose and hexoses in the green and yellow parts of the variegated leaves of many plant species, and found both hexose and sucrose in the green parts and sucrose in the yellow parts. Only two of the species examined differed, namely, *Cornus sanguinea* and *Aesculus hippocastanum*, in that in the leaves of the former the amount of hexose in the yellow parts was smaller than that in the green parts, and in the leaves of the latter the amount of hexose in the yellow parts was small. This evidence would appear to support the view that hexoses are the first products of photosynthesis and that sucrose is synthesized from them.

Since sucrose on hydrolysis by acid yields *d*-glucose and *d*-fructose, this view, as Stiles points out, would require the simultaneous photosynthesis of *d*-glucose and *d*-fructose. Some light is thrown on the problem by the observations of Nef,[111] who studied the reciprocal transformations of the hexoses promoted by very dilute alkali. Nef found that in

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[107] *Photosynthesis*, pp. 143–60. Longmans, Green & Co., London, 1925.

[108] *Bot. Zeitsch.*, 1862, *20*, 365.

[109] *Jahr. wiss. Bot.*, 1869, *7*, 511.

[110] *Proc. Kon. Akad. Amsterdam*, 1924, *27*, 1.

[111] *Ann. der Chem.*, 1913, *403*, 204.

an aqueous solution of *d*-glucose, *d*-mannose, or *d*-fructose containing 0·05 eq. $Ca(OH)_2$, an equilibrium condition was established at room temperature. This equilibrium contained *d*-glucose, *d*-mannose, *d*-fructose, *d*-pseudofructose, and α- and β-*d*-glutose. Starting with the hexoses of the galactose series, the equilibrium mixture contained *d*-galactose, *d*-talose, *d*-tagatose, *d*-sorbose, and α- and β-*d*-galtose. No conversion of a sugar from the former series to the latter series was observed.

The importance of these observations was stressed by Spoehr,[112] who considered that the similarity between Nef's equilibrium mixtures and the conditions existing in the living plant is highly suggestive. Stiles expressed the following opinion :

> While we have no grounds at present for suggesting that such sugar transformations are brought about in the cell by the existence of a definite degree of alkalinity, Nef's work shows that there is no reason to be surprised at the presence of a number of different sugars in the leaf cells, including those that enter into the constitution of the sucrose molecule. If one hexose, such as glucose, were the first sugar to be produced in the photosynthetic process, the action of, for example, an enzyme behaving similarly towards the sugars as a weak alkali, would lead to the presence of other hexose or hexoses.

This opinion that a hexose, probably *d*-glucose, is the actual product of photosynthesis in all plants, is to a certain extent at variance with the experimental results which were obtained by the irradiation of nickel oxide. Whilst a hexose or a mixture of hexoses was photosynthesized by the irradiation of certain preparations of nickel carbonate, the irradiation of both supported and unsupported nickel oxide containing thorium oxide invariably resulted in the photosynthesis of a complex carbohydrate which changed slowly at room temperature and rapidly at 60° into a starch. These observations throw a new light on the problem of photosynthesis in the living plant, because they indicate that starch, although not the actual product, can be the direct product of photosynthesis and that its formation from the actual product takes time, as was noted by Kraus in plant leaves. The difficulties experienced by plant physiologists in accepting the view that starch is

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[112] *Science Monthly,* July 1919, 32.

produced by photosynthesis appear to be surmounted, and it may be concluded that in the dicotyledons starch is the direct product of photosynthesis.

In view of this conclusion it is of some interest to consider the molecular weight of the first unstable carbohydrate which is actually photosynthesized and the mechanism whereby it forms starch. As was described in Chapter V, this product was obtained by the irradiation of unsupported nickel oxide containing thorium oxide in the molecular ratio of $1ThO_2 : 24NiO$. By the method described on page 97 it was found that this material consisted of cubic crystals, the edges of which had an average length of $5{\cdot}7 \times 10^{-6}$ cm. The average area, therefore, of the crystal faces irradiated was $3{\cdot}249 \times 10^{-11}$ sq. cm. It may be seen from Figs. 15 and 16 that the actual area of surface on which one cyclic $C_6H_{12}O_6$ molecule is photosynthesized is

$$10 \times 2{\cdot}086 \times 10^{-8} \times 7 \times 2{\cdot}086 \times 10^{-8} = 3{\cdot}046 \times 10^{-14} \text{ sq. cm.}$$

The number of cyclic $C_6H_{12}O_6$ molecules which can be simultaneously photosynthesized on an area of $3{\cdot}249 \times 10^{-11}$ sq. cm. is $3{\cdot}249 \times 10^{-11}/3{\cdot}046 \times 10^{-14} = 1{,}067$.

Now it is impossible that the cubic crystals of NiO containing thorium oxide were all of the same size. Furthermore, in the case of the supported NiO containing ThO_2 it was found from measurements of the rate of sedimentation that the extreme values of the surface areas of the particles were in the ratio of 100 : 1. In spite of this great variation in the surface area irradiated, one product only was photosynthesized, namely, the parent of the starch. It may be seen from Figs. 15 and 16 that there is no physical reason, except the surface area, why the number of cyclic $C_6H_{12}O_6$ molecules which condense together should be limited. The conclusion to be drawn from this appears to be that the molecular weight of the parent of the starch is indefinite and depends on the surface area irradiated.

During the irradiations of both supported and unsupported NiO containing ThO_2 the starch was invariably deposited on a surface, and, indeed, no evidence of its formation was obtained until after it had been so deposited. This indicates that the starch must be produced by the successive deposition of unimolecular layers of the first photosynthesized carbohydrate, or of some modification of it, on a surface. It thus appears that starch consists of a number of unimolecular layers of a carbohydrate $(C_6H_{10}O_5)_n$ and that it is stabilized by adsorption or chemical forces between each successive layer. Furthermore, it has already been pointed out that all the $C_6H_{10}O_5$ units in the condensation product $(C_6H_{10}O_5)_n$ are identical, with the result that all the unimolecular layers in the starch structure must be chemically and optically identical. These conclusions are in agreement with the evidence obtained from chemical investigations.

In this connexion some interesting observations may be referred to which were made during the investigation of photosynthesis on a surface of nickel oxide containing thorium oxide. When the filtrate from an irradiation of the supported oxides was exactly neutralized with very dilute acid and concentrated under reduced pressure on a steam bath, the starch compound remained in solution until it was finally deposited as a thin film on the bottom of the pyrex flask. This film was readily soluble in water and the resulting solution, after treatment with diastase, gave a small amount of cuprous oxide when boiled with Fehling solution. When the filtrate was not neutralized and contained a small amount of nickel bicarbonate, the starch was quantitatively deposited on the hydrated nickel oxide which separated during the concentration. Furthermore, it was proved (p. 123) that the surface of nickel oxide containing thorium oxide was completely poisoned by the deposition of the starch when it was allowed to remain in contact with the solution of the first product of photosynthesis.

Two facts are established by these observations. In the first place, the formation of the starch by the deposition of the first product of photosynthesis takes place with extraordinary rapidity on a surface of nickel oxide which is oriented by the presence of the thorium oxide. In the second place, the starch deposited on this oriented surface is insoluble in water, whereas when deposited as a film on a glass surface it remains soluble. The explanation of these facts must surely be found in the correlation between the molecular structure of the first product of photosynthesis and the oriented surface of nickel oxide, a correlation which, as previously stated, results in the optical activity of that product. It is evident from this that the soluble product of deposition is not true starch, but a substance consisting of a series of layers which are not fully co-ordinated, as is the case when the starch is deposited on an oriented surface.

Owing to the asymmetric bias normally existing in the living plant, the deposition of the first product of photosynthesis will result in the formation of true starch. It is not surprising, therefore, that the soluble material deposited on a glass surface, although it is hydrolysed to a hexose by diastase, does not give a blue colour with iodine. In discussing this with Professor Ruggles Gates he tells me that in some of the lower organisms, such as *Euglena*, a white substance is photosynthesized which is supposed to be related to starch. This substance, known as paramylum, is soluble in alcohol and gives no colour reaction with iodine. It is very probable that this paramylum is similar to the substance obtained when the first product of photosynthesis is deposited on a non-oriented surface.

We may now consider the photosynthesis of hexoses only such as only takes place in the monocotyledons, and was observed with the electrolytic nickel carbonate prepared by Hood and myself (p. 80). In the case of the monocotyledons it is evident from the exact similarity of the areas on which a single cyclic $C_6H_{12}O_6$ molecule is photosynthesized that only one hexose can be produced. The opinion, therefore, expressed by Stiles that *d*-glucose is the sole product of photosynthesis receives strong support, but only when the conditions are such as to prevent the condensation of the cyclic molecules to form the parent of starch.

The most obvious condition which would result in the photosynthesis of *d*-glucose only is the restriction of the irradiated area of chlorophyll or of nickel oxide to that on which a single cyclic $C_6H_{12}O_6$ molecule only can be formed. It is manifest that a minimum surface area is necessary for this to take place. Since nickel oxide crystallizes in regular cubes, this minimum area is that shown in Fig. 17, the position of the ThO_2 molecule being shown by the large circle. If a preparation of nickel oxide, containing thorium oxide in the molecular ratio of $1ThO_2 : 24NiO$, consists of cubes with sides having the area shown in Fig. 17, one hexose only will be photosynthesized. Similarly, if the chlorophyll surface irradiated be restricted to areas which contain 1 indifferent molecule surrounded by 6 chlorophyll molecules, *d*-glucose only will be photosynthesized. This does not necessarily entail that there is one such area in each chloroplast, since the same restriction to the photosynthesis of *d*-glucose only would result from a large area which by some means is subdivided into areas containing 1 indifferent and 6 chlorophyll molecules.

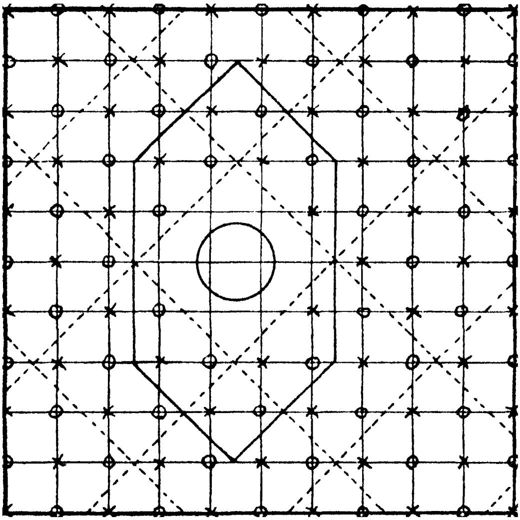

FIG. 17

There is an alternative possibility which arises from some observations made during the Liverpool investigations. It was found that the photosynthetic activity of nickel oxide containing thorium oxide is a periodic function of the thorium oxide content. Maximum activity was observed when the molecular ratio was $1ThO_2 : 24NiO$, minimum activity was observed when the molecular ratio was $1ThO_2 : 48NiO$, and a secondary maximum activity was observed when the molecular ratio was $1ThO_2 : 72NiO$. An exactly similar periodicity was observed with supported ferric oxide containing thorium oxide (p. 89).

The distribution of the ThO_2 molecules when present in the molecular ratio of 1 : 24 was shown in Figs. 15 and 16, this distribution being symmetrical and ideal for photosynthesis. Now a symmetrical distribution of the ThO_2 molecules does not appear to be possible when they are present in the molecu-

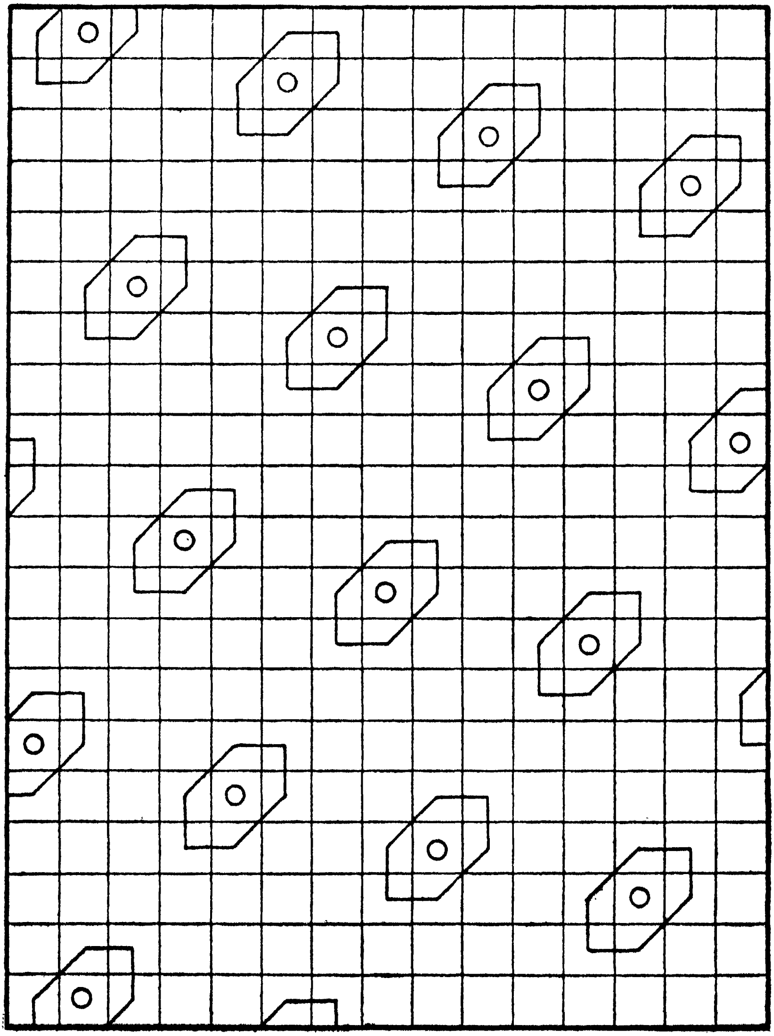

FIG. 18

lar ratio of $1ThO_2 : 48NiO$, and this is the most probable explanation of the minimum photosynthetic activity. When the molecular ratio is $1ThO_2 : 72NiO$ a symmetrical distribution is again possible, and this is the most probable explanation of the secondary maximum activity which was observed.

The distribution of the ThO_2 molecules, when present in the molecular ratio of $1ThO_2 : 72NiO$, is shown in Fig. 18, in which each square is the area containing 4Ni and 4O atoms. The directional influence of the ThO_2 molecules is now restricted to the six groups of 4Ni and 4O atoms which surround them, and hence the only cyclic $C_6H_{12}O_6$ molecules which can be photosynthesized are those indicated by the hexagons. These hexagons are not adjacent, as in Figs. 15 and 16, and hence it is to be expected that condensation of the cyclic molecules to a complex carbohydrate cannot take place. The irradiation of the surface, therefore, should result in the photosynthesis of a hexose only.

The same argument is applicable to a surface of chlorophyll, because the same orientation as that in Fig. 18 would exist if the indifferent molecules were present in the molecular ratio of 1 : 18 instead of 1 : 6 and would result in the photosynthesis of *d*-glucose only.

If this explanation of the photosynthesis of *d*-glucose only in the monocotyledons is correct, it is to be expected that this class of plants would require for their health and growth one-third of the amount of boron needed by the dicotyledons in which starch is photosynthesized. In reply to an inquiry whether any experimental evidence has been obtained at Rothamsted that the monocotyledons require less boron for their health and growth than the dicotyledons, Dr. Brenchley has written as follows:

> It is well recognized, both by ourselves and by other workers, that the monocotyledons as a class require less boron than dicotyledons. In the early days of our work, when the need for strictly pure reagents was not fully recognized, we were able to grow barley to complete maturity and ripe grain in solution in which broad beans died from boron deficiency at a very early stage. Under the present more rigorous conditions we have found
>
> 1. Absence of boron is fatal at a very early stage of growth to such dicotyledons as sugar beet, mangold, parsley, &c., in which the reserve in the seed is not very great; and similar deficiency shows as soon as seed reserves are exhausted in soybean, broad bean, peas, &c.

2. Without boron barley grows on quite satisfactorily until the time arrives when ears would normally be developed. No ears are formed, however, but numerous tillers are produced and a very bunchy vegetative plant results.

3. In our own experiments oats were able to produce flowering panicles, but no fertile seeds, in contrast to those receiving boron, which ripened their grain.

4. Maize varies in the amount of growth it can make without boron at different periods of the year. With us in all cases the primary male inflorescences perished, but later on axillary female inflorescences developed and some were still healthy when the experiment was discontinued in the autumn.

5. Onion grew for some time before any signs of boron deficiency were apparent.

Dr. Brenchley concludes by saying that these notes are as yet unpublished.

In view of this evidence there is little doubt that the second explanation of the photosynthesis of glucose only in the monocotyledons is in accord with experimental observations.

THE PHOTOSYNTHETIC ACTIVITY OF NICKEL CARBONATE

As was recorded in Chapter IV, a quantity of nickel carbonate was prepared by Hood and myself in 1928–9 by the electrolysis of an aqueous solution of carbon dioxide with nickel electrodes. This material when first prepared was photosynthetically inactive, but after it had been pre-irradiated for 18 hours in the form of a dry powder and subsequently irradiated in suspension in an aqueous solution of carbon dioxide, one or more hexoses were photosynthesized (p. 81). As was recorded in Chapter V, Bell was entirely unable to prepare a photosynthetically active material by this method, and further investigations in Liverpool proved that the original success was due to the fortuitous use of an electrolytic cell made of a glass which had an unusually small solubility in water. This cell was subsequently broken and could not be replaced, owing to the fact that the manufacture of that glass had been abandoned. In seeking an explanation of these observations there are two facts to be explained. The first is the activation of the nickel carbonate prepared in 1928 by pre-irradiation, and the second is the photosynthetic activity of the pre-irradiated material when it has been proved that pure nickel oxide does not and cannot promote photosynthesis.

It is known that nickel carbonate consists of minute crystals of nickel oxide, together with water and carbon dioxide held by adsorption and capillary forces. It was shown above that these cubic crystals must have a definite size for photosynthesis to take place, and the minimum area of the crystal face was shown in Fig. 17. If the irradiated area is smaller than this minimum, the photochemical production of activated formaldehyde becomes reversible, this reversibility being prevented solely by the cyclic polymerization of the activated formaldehyde when this is possible. There is no doubt that the photosynthetic inactivity of all freshly made preparations of nickel carbonate is due to the crystals of nickel oxide being too small.

Let us now consider the irradiation of a pure nickel carbonate in the form of a dry powder. When this powder is screened from light, the water and carbon dioxide it contains must have a definite dissociation pressure. When the powder is exposed to light, the reversible formation of activated formaldehyde will take place on all the irradiated crystal surfaces, a process which takes place with a single adsorbed layer of hydrated carbon dioxide. The result of this will be that the dissociation pressure will be increased, and the more so by the rise in temperature to about 50° caused by the six 250-watt lamps used. It is to be expected, therefore, that during prolonged irradiation the powder will slowly lose water and carbon dioxide and that the result will be the formation of crystal aggregates, the surface area of which is large enough to promote the photosynthesis of a hexose.

It is manifest that this crystal growth process cannot take place during pre-irradiation if, during its preparation, the nickel carbonate has adsorbed a non-volatile substance, such, for example, as alkali silicate dissolved from the glass of the electrolytic cell. It is very noteworthy that the electrolytic nickel carbonate, prepared in the cell having an unusually small solubility in water, contained only a minute quantity of alkali, and that all of the numerous preparations made in cells of ordinary soda glass contained much alkali. The conclusion is thus reached that in the first preparations of electrolytic nickel carbonate the amount of alkali silicate adsorbed was insufficient to prevent crystal growth during pre-irradiation, and that ordinary soda glass yielded sufficient alkali silicate to inhibit that growth.

The photosynthetic activity of the crystal aggregates formed by the pre-irradiation of the first electrolytic nickel carbonate is remarkable in view of the fact that it has been proved that the presence of an indifferent substance, such as ThO_2, in the molecular ratio of 1 : 24 or 1 : 72 is essential for photosynthesis to take place. In the electrolytic preparation conductivity water, carbon dioxide of proved purity and pure nickel electrodes were used, and thus the only possible impurity was the very small amount of alkali silicate dissolved from the glass electrolytic cell. Now it was recorded on page 116 that, during the investigation of the coating of kieselguhr with NiO containing ThO_2, it was found that the presence of a trace of alkali silicate in the reacting solutions destroyed the photosynthetic activity of the NiO and ThO_2 surface. It was found that this was due to the silica entering the crystal lattice along with the ThO_2, the effect being the same as that observed when the molecular ratio of the ThO_2 is greater than 1 : 24. This observation suggests that silica can function in the same way as thorium oxide in promoting crystal growth and in orienting the surfaces of the crystal faces.

It is, of course, necessary that the silica be present in the molecular ratio of either $1SiO_2 : 24NiO$ or $1SiO_2 : 72NiO$, the latter being the more probable because the material promoted the photosynthesis of hexoses only. The composition of the nickel carbonate lay between $11NiO,11H_2O,CO_2$ and $10NiO,10H_2O,CO_2$, and 30 grm. were prepared in each operation. The dimensions of the electrolytic cell were 8 × 6 in. and 8 in. deep, and since the cell was filled with conductivity water to within 1 in. of the top, the area of glass in contact with the electrolyte was 1,578 sq. cm. The amount of silica needed to give a molecular ratio of 1 : 72 with 30 grm. of the above compounds is 0·26 grm., and the dissolution of this amount in 18 hours from 1,578 sq. cm. of a glass with unusually small solubility is not unreasonable.

The amount of alkali which dissolved in all probability was equivalent to the silica, that is to say, the maximum amount dissolved and adsorbed was in the molecular ratio of 1 : 72NiO, and it is obvious that this would be insufficient to inhibit crystal growth during pre-irradiation.

In advancing this explanation of the photosynthetic activity of the electrolytic nickel carbonate prepared by Hood and myself, two facts may be emphasized. In the first place, this activity after pre-irradiation was remarkably constant, and this is in keeping with a constant rate of dissolution of alkali from the glass surface. In the second place, it is not essential that the amount of silica present in the product be exactly in the molecular ratio of 1 : 72, because, although the maximum activity would be secured by this ratio, activity would be observed if the molecular ratio were within 5 per cent of that ratio.

The arguments in favour of this explanation are greatly strengthened by the observations at Rothamsted, in which it was proved that in the absence of phosphates small amounts of silica materially promote the growth of barley and mustard.[113] Amongst the conclusions reached were the following :

1. Under controlled conditions in water cultures soluble silicate was found to have little effect upon the growth of barley if phosphorus were also present, but if the latter were absent a significant increase in dry weight was induced by the silicate.
2. The addition of silicate caused an appreciable increase in the height of the main shoot, which was most marked in phosphate-free solutions, becoming less evident as the quantity of phosphate present was increased.
3. Leaf development was retarded by phosphate deficiency and hastened by the addition of silicate.
4. A close association exists between the amount of phosphate present and the effect of silicate on the rate of tillering and the number of tillers developed.
5. Soil cultures with barley and mustard in pots with various forms of silicate showed that soluble silicates are more active than glass silicates, tending to cause increase in dry weight with deficient manuring, and in some cases also with complete manuring.

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[113] Brenchley, Maskell and Warrington, *Ann. Applied Biol.*, 1927, *14*, 45.

6. Further soil experiments reveal variations in the response of barley and mustard to silicate on different types of soil. A general improvement occurred with increasing doses of silicate together with various combinations of manures, notably when phosphorus or potash was absent.

This evidence that silica can function in living plants in the same way as boron, manganese, &c., justifies the suggestion made above that silica can function in the same way as thorium oxide in orienting a surface of nickel oxide, and that the photosynthetic activity of the electrolytic nickel carbonate prepared in 1928 was established by the presence of silica.

CHAPTER VIII

THE KINETICS OF PHOTOSYNTHESIS

IN Chapter VII the mechanism of photosynthesis was discussed and it was shown that the energy intake of 112,167 calories is accomplished in two successive photochemical processes, the first of which is promoted by blue light and the second by red light, and experimental evidence was adduced in support of this two-stage mechanism of activation. In the case of the surface of NiO the two photochemical processes are :

$$1.\ 4NiO.CO_2H_2O + h\nu_1 = 2Ni_2O_3.CHOH$$

$$2.\ 2Ni_2O_3.CHOH + h\nu_2 = 2Ni_2O_3 + CHOH$$

and in the living plant the two processes are

$$1.\ \underset{\text{Chlorophyll A}}{C_{55}H_{72}O_5N_4Mg}.CO_2H_2O + h\nu_1 = \underset{\text{Chlorophyll B}}{C_{55}H_{70}O_6N_4MgH_2O}.CHOH$$

$$2.\ C_{55}H_{70}O_6N_4MgH_2O.CHOH + h\nu_2 = C_{55}H_{70}O_6N_4MgH_2O + CHOH$$

where CHOH represents a molecule of activated formaldehyde, this substance undergoing polymerization to a carbohydrate.

In each case the two photochemical processes are followed by a dark or thermal reaction which in the case of nickel oxide is

$$2Ni_2O_3 = 4NiO + O_2$$

and in the vital process

$$C_{55}H_{70}O_6N_4MgH_2O + \underset{\text{Carotin}}{C_{40}H_{56}} = C_{55}H_{72}O_5N_4Mg + \underset{\text{Xanthophyll}}{C_{40}H_{56}O_2}$$

the latter being known as the Blackman reaction.

Now it is manifest from these equations that the rate of photosynthesis must depend upon three controlling factors, namely, the intensity of the light, the concentration of hydrated carbon dioxide and the temperature, the last governing the rate of the third reaction in each case.

During recent years accurate measurements have been made by Warburg, Emerson and others of the influence of each of these factors severally on the rate of photosynthesis in the living plant, but no explanation of the observed results was given by these experimenters. Now that the mechanism of photosynthesis has been found it is possible to derive equations which express the velocity of photosynthesis and the influence of each of the three factors on that velocity. The problem is one of physical chemistry and the influence of the temperature may be discussed first.

Let the case be considered of a living plant which has been kept in the dark under constant conditions of temperature and concentration of carbon dioxide. At the instant that the leaves are irradiated the rate of photosynthesis will have a certain initial value and then decrease until it becomes equal to that of the Blackman reaction. A photostationary state will thus be established in which the rate of photosynthesis will be constant, provided that the intensity of the light, the concentration of carbon dioxide and the temperature be kept constant. This decrease in the initial rate of photosynthesis was experimentally proved by Warburg and by Emerson. The method employed was to irradiate plants by a series of light flashes of very short duration, each flash being followed by a period of darkness. It was found that when flashing light was used, the amount of carbohydrate photosynthesized by unit quantity of light energy was greater than that photosynthesized by unit quantity of light during continuous irradiation.

The immediate problem is the formulation of the conditions which govern the photostationary state, and the effect of irradiation by means of flashing light may be considered later. In formulating these conditions it may first be assumed that the light employed is of constant quality, that is to say, that the ratio of the intensities of the blue and red rays is constant. This assumption is fully justified because in all the experimental observations in the laboratory light of constant quality has been used. This assumption enables us in the first instance to consider the photosynthetic process as consisting of a single reaction.[114]

As the result of this assumption there are three processes to be considered, namely, the light reaction in which the chlorophyll A.CO_2H_2O complex is converted into chlorophyll B and free activated formaldehyde, the Blackman reaction in which the chlorophyll B is reduced by carotin to chlorophyll A, and the conversion of chlorophyll A into its complex with CO_2H_2O. If a be the concentration of the chlorophyll A.CO_2H_2O complex in the surface when irradiation is commenced, the rate of photosynthesis when the photostationary state is established will be given by

$$r = k_1I(a - x - y) = k_2cxe^{-Q/RT} = k_3py$$

where I is the total light intensity, c is the concentration of the carotin which is assumed to be large and sensibly constant, Q is the energy of activation of the Blackman reaction, p is the pressure of the carbon dioxide, and k_1, k_2 and k_3 are constants.

This expression is based on the assumption that in the dark the concentration of the chlorophyll A.CO_2H_2O complex always returns to the same value a, whatever is the CO_2 concentration, and hence the initial rate of photosynthesis, k_1Ia, will be independent of the CO_2 concentration. Since it has been proved by irradiation with flashing light that the initial rate of photosynthesis is a direct function of the CO_2 concentration, it is evident that some modification in the assumption must be made.

It must be remembered that photosynthesis is a surface reaction, and hence it may be assumed that the rate of formation of the chlorophyll A.CO_2H_2O complexes from the chlorophyll A produced in the Blackman reaction is very great compared with the attainment of equilibrium between ordinary chlorophyll A and CO_2H_2O. On this assumption the above expression simplifies to

$$r = kI(a - x) = k_2cxe^{-Q/RT},$$

but a is now dependent on the pressure of the carbon dioxide.

If A be the total concentration of chlorophyll A in the

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[114] Baly, *Proc. Roy. Soc.*, 1935, *117*B, 218.

surface, both free and in the form of its complex with CO_2H_2O, the concentration a of the complex will be expressed by bA, where b is a function of the external pressure of carbon dioxide, the concentration of water being large and sensibly constant.

Inserting this value of a in the last equation we have

$$r = k_1I(bA - x) = k_2cxe^{-Q/RT} \quad . \quad . \quad . \quad (1)$$

whence by division by k_1Ix

$$\frac{r}{k_1Ix} = \left[\frac{bA - x}{x}\right] = \frac{k_2c}{k_1I}e^{-Q/RT}$$

and, since from (1) $k_1Ix = k_1IbA - r$,

$$\log \frac{r}{K - r} = \log \frac{k_2c}{k_1I} - Q'/T \quad . \quad . \quad . \quad (2)$$

where $K = k_1IbA$ and is the initial rate of photosynthesis, and $Q' = Q/2{\cdot}303R$.

Equations (1) and (2) are in accordance with two important observations, since they indicate that the initial rate of photosynthesis is a direct function of the external pressure of the carbon dioxide and that the temperature coefficient of photosynthesis is independent of the external concentration of the carbon dioxide. Since in any measurements of the rate of photosynthesis at different temperatures the intensity of the light is maintained constant, equation (2) indicates a linear relation between $\log \frac{r}{K - r}$ and $1/T$. It is, therefore, advisable before developing the complete formula, to determine whether this linear relation conforms with observation.

Four series of measurements were made by Emerson [115] of the rate of photosynthesis by the unicellular alga *Chlorella* at various temperatures and all four series are expressed by equation (2). His first series of measurements is expressed by

$$\log \frac{r}{130 - r} = 22{\cdot}88400 - 6{,}573{\cdot}8/T$$

and the values of r at temperatures from 0° to 30° given by this formula, together with Emerson's observed values, are given in Table XIV.

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[115] *J. Gen. Physiol.*, 1928–9, *12*, 623.

TABLE XIV

Temperature in degrees C.	Rate of photosynthesis	
	calc.	obs.
0	7·79	
1	9·40	
2	11·32	
3	13·24	
4	16·15	13·9
5	19·14	
6	22·55	22·7
7	26·39	
8	30·67	30·6
9	35·37	
10	40·46	42·8
11	45·89	46·8
12	51·58	52·3
13	57·45	
14	63·41	64·3
15	69·35	
16	75·19	75·3
17	80·82	78·5
18	86·17	
19	91·17	
20	95·83	
21	100·06	
22	103·90	
23	107·33	
24	110·37	
25	113·06	
26	115·40	115·5
27	117·46	
28	119·22	
29	120·76	
30	122·08	

The curve in Fig. 19 shows the relation between the calculated values of the rate of photosynthesis and the temperature,

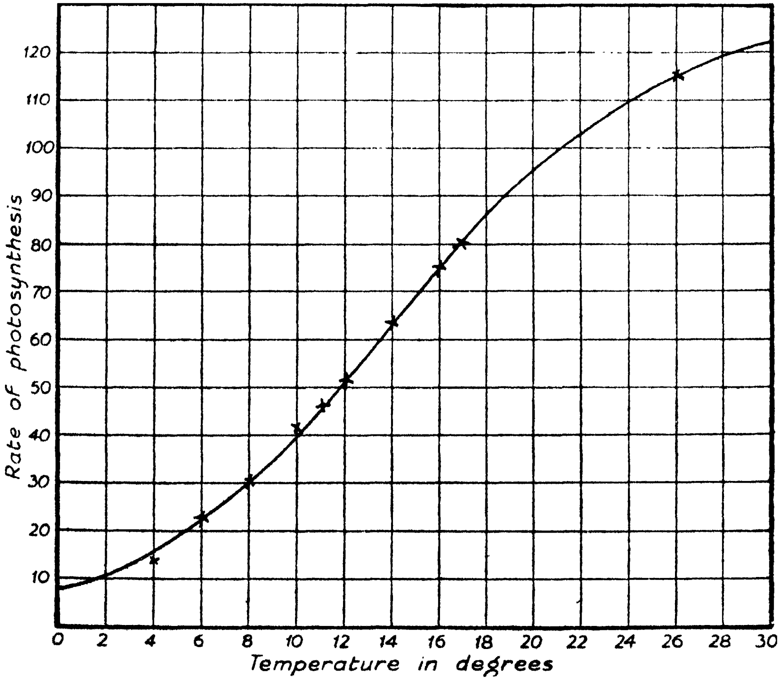

FIG. 19

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the crosses indicating Emerson's measurements. It is to be noted that the curve is sigmoid, a fact to which Emerson was the first to draw attention.

Emerson also made three series of measurements with *Chlorella* in order to determine the effect of varying the amount of chlorophyll in the alga. Three special cultures were prepared and the amount of chlorophyll present in each was determined. A known volume of the suspension of each culture was extracted and the absorptive powers of the solutions of chlorophyll thus obtained were measured. The extinction coefficients were $\varepsilon = 0{\cdot}101$, $0{\cdot}048$, and $0{\cdot}016$, respectively. Emerson's measurements with the three cultures are expressed by the equations

$$\varepsilon = 0{\cdot}101 \qquad \log \frac{r}{285 - r} = 21{\cdot}891 - 6{,}400/T$$

$$\varepsilon = 0{\cdot}048 \qquad \log \frac{r}{210 - r} = 22{\cdot}246 - 6{,}542/T$$

$$\varepsilon = 0{\cdot}016 \qquad \log \frac{r}{16{\cdot}3 - r} = 22{\cdot}584 - 6{,}530/T$$

In Table XV are given the calculated and observed rates of photosynthesis for the three cultures.

TABLE XV

Temperature	Rate of photosynthesis					
	ε = 0·101		ε = 0·048		ε = 0·016	
	calc.	obs.	calc.	obs.	calc.	obs.
0	7·60		3·95		0·72	
4	16·41	17·0	8·57	8·1	1·51	
5	19·63		10·30		1·80	1·1
9	38·73	40·5	21·04	21·9	3·44	
10	45·29		24·95		3·98	4·0
11	52·72	53·4	29·31	30·5	4·57	
12	61·10		34·36		5·18	5·0
15	90·57	85·5	53·21	53·2	7·23	
16	102·10		60·67		8·03	7·9
17	113·76		68·76	64·4	8·77	
18	125·90	123·9	77·09		9·48	10·7
20	150·32		95·06		10·84	
25	206·06	208·0	139·00	135·8	13·43	
27	223·36		153·82		14·13	14·1
30	243·79		171·80		14·92	

The values in Table XV are expressed by the curves in Fig. 20, the crosses indicating Emerson's measurements.

The values of Q, or the energy of activation of the Blackman reaction, may now be calculated from the four equations, it having been shown that $Q = Q' \times 2{\cdot}302585 \times 1{\cdot}9885$. The values are

Series 1	30,099·4	calories
$\varepsilon = 0{\cdot}101$	29,303·6	,,
$\varepsilon = 0{\cdot}048$	29,953·8	,,
$\varepsilon = 0{\cdot}016$	29,898·4	,,
Mean	29,813·8	,,

The fact that these values of Q do not diverge much from the mean value in spite of the great differences in the rate of photosynthesis, coupled with the good agreement between the calculated and the observed rates, affords strong evidence of the validity of the equation (2). In view of this satisfactory agreement we may now derive the complete formula for the photostationary state of which equation (2) is a simplified form and is valid only when the ratio of the intensities of the blue and red rays is constant.

As has been shown in Chapter VII, there are two successive photochemical processes which are promoted by blue and red rays, namely

$$1.\ C_{55}H_{72}O_5N_4Mg.CO_2H_2O + h\nu_1 = C_{55}H_{70}O_6N_4MgH_2O.CHOH$$

$$\text{and } 2.\ C_{55}H_{70}O_6N_4MgH_2O.CHOH + h\nu_2 = C_{55}H_{70}O_6N_4MgH_2O + CHOH$$

where ν_1 and ν_2 are the frequencies of the blue and the red rays, respectively. When the photostationary state has been established the velocities of these two reactions and of the Blackman reaction will be equal. It is evident from this that the photostationary state will be expressed by

$$r = k_1 I_1(bA - x - y) = k_2 I_2 x = k_3 c y e^{-Q/RT} \quad . \quad (3)$$

where I_1 is the intensity of the blue rays with frequency ν_1, I_2 is the intensity of the red rays with frequency ν_2, bA is the concentration of chlorophyll A.CO_2H_2O, x is the concentration of chlorophyll B.CHOH, y is the concentration of chlorophyll B, and c is the concentration of carotin.

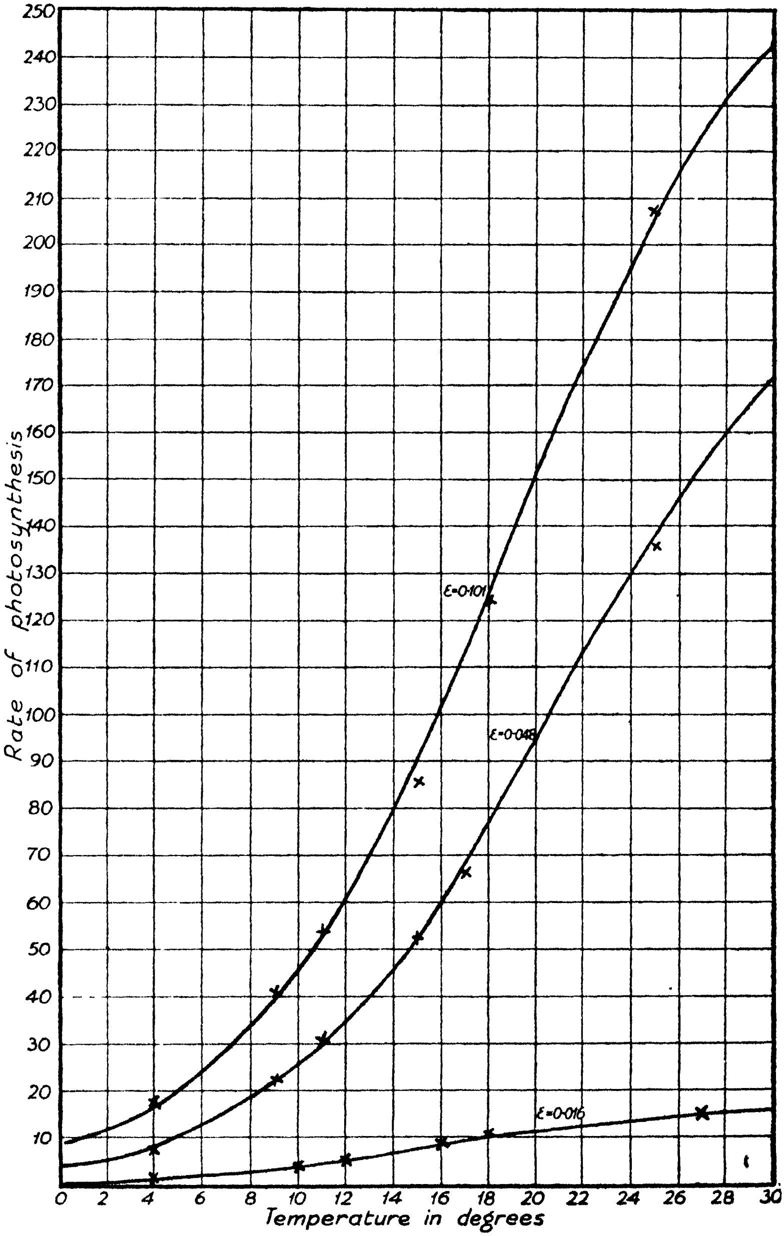

FIG. 20

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By the elimination of x and y we obtain

$$r = \frac{k_1 I_1 b A k_2 I_2 k_3 c e^{-Q/RT}}{k_1 I_1 k_2 I_2 + (k_1 I_1 + k_2 I_2) k_3 c e^{-Q/RT}} \qquad (4)$$

whence $$\frac{r}{K - r} = \frac{k_1 I_1 + k_2 I_2}{k_1 I_1 k_2 I_2} k_3 c e^{-Q/RT}$$

where $$K = \frac{k_1 I_1 k_2 I_2 b A}{k_1 I_1 + k_2 I_2}$$

and hence $$\log \frac{r}{K - r} = \log \frac{bAk_3c}{K} - Q'/T \quad . \quad . \quad . \quad . \quad . \qquad (5)$$

This equation (5) is identical in form with the simple equation (2), the only difference being that, whereas previously the light intensity was defined by I, in the complete equation (5) the light intensity is defined by $I_1 I_2/(I_1 + I_2)$, that is to say, the geometric mean of I_1 and I_2.

From equation (4) the following equations may be derived :

$$\frac{bA}{r} = \frac{1}{k_3 c e^{-Q/RT}} + \frac{k_1 I_1 + k_2 I_2}{k_1 I_1 k_2 I_2} \quad . \quad . \quad . \quad . \quad . \qquad (6)$$

and $$\frac{I_1 I_2}{r(I_1 + I_2)} = \frac{I_1 I_2}{(I_1 + I_2) b A k_3 c e^{-Q/RT}} + \frac{k_1 I_1 + k_2 I_2}{k_1 k_2 (I_1 + I_2) b A} \qquad (7)$$

Equation (6) indicates that the rate of photosynthesis is directly proportional to bA, that is the concentration of the chlorophyll $A.CO_2H_2O$ complex, when the light intensities and the temperature are constant. Equation (7), since k_1 and k_2 are the constants of two similar photochemical processes, indicates that the ratio of $\frac{I_1 I_2}{I_1 + I_2}$ to the rate of photosynthesis is a linear function of $\frac{I_1 I_2}{I_1 + I_2}$ when the temperature, carbon dioxide concentration and the ratio I_1/I_2 are constant. Equations (3) to (7) completely define the kinetics of photosynthesis in the living plant when the photostationary state has been established. These equations may now be discussed in relation to the experimental observations, and the effect of changing the light intensity may be considered first.

VARIATION OF LIGHT INTENSITY

The intensity of the light may be varied in two ways, since the total intensity may be varied whilst maintaining the ratio I_1/I_2 constant or the ratio I_1/I_2 may be varied whilst the total intensity, $I_1 + I_2$, is maintained constant. The former method was investigated by Warburg,[116] who varied the intensity by varying the distance from the light source and calculated the relative intensities by the inverse square law.

In order to determine the effect of reducing the light intensity, $\frac{I_1I_2}{I_1 + I_2}$, the equation given above for Emerson's first series of measurements,

$$\log \frac{r}{130 - r} = 22{\cdot}88400 - 6{,}573{\cdot}8/T,$$

may be taken. From equation (5) it is manifest that K is directly proportional to $I_1I_2/(I_1 + I_2)$ and that $\log bAk_3c/K$ is inversely proportional to $\log I_1I_2/(I_1 + I_2)$. It follows that in the above equation when the light intensity is reduced the value of 130 is reduced in direct proportion and that the term 22·88400 is increased in logarithmic proportion. In Table XVI are given the rates of photosynthesis at 5°, 10°, 20°, 25°, and 30° for various values of $I_1I_2/(I_1 + I_2)$, together with the temperature coefficients (Q_{10}). The first value of Q_{10} in each case is the square of the coefficient for the range of 5° to 10°.

TABLE XVI

Relative value of $\frac{I_1I_2}{I_1 + I_2}$	Rate of photosynthesis								
	K	5°	Q_{10}	10°	Q_{10}	20°	Q_{10}	25°	30°
1·00	130	19·14	4·47	40·46	2·37	95·83	1·27	113·06	122·08
0·75	97·5	18·25	4·04	36·66	2·09	76·75	1·21	87·65	92·98
0·50	65	16·69	3·42	30·86	1·79	55·16	1·12	60·47	62·92
0·25	32·5	13·31	2·47	20·92	1·43	29·84	1·05	31·33	31·98
0·10	13	8·23	1·67	10·64	1·18	12·55	1·03	12·81	12·92
0·05	6·5	5·04	1·35	5·85	1·10	6·39	1·01	6·45	6·48
0·025	3·25	2·86	1·18	3·08	1·05	3·22	1·007	3·24	3·24
0·01	1·3	1·23	1·07	1·27	1·02	1·30	1·003	1·30	1·30

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[116] *Biochem. Zeitsch.*, 1919, *100*, 255.

These results are of considerable interest because the effect of reducing the total light intensity, whilst maintaining the ratio I_1/I_2 constant, is progressively to decrease the temperature coefficient until it approximates to unity. In other words, the rate of photosynthesis is independent of the temperature when the light intensity is very small. This fact was experimentally proved by F. F. Blackman[117] and by Warburg.

As has already been noted, equation (7) indicates a linear relation between the ratio of $I_1I_2/(I_1 + I_2)$ to r and $I_1I_2/(I_1 + I_2)$ when the temperature, carbon dioxide concentration, and I_1/I_2 are constant. Warburg's measurements of the rate of photosynthesis with different light intensities, I_1/I_2, temperature and carbon dioxide concentration being constant, are expressed by the linear formula

$$\frac{I_1I_2}{r(I_1 + I_2)} = 0{\cdot}01697 + 0{\cdot}0034906 \times \frac{I_1I_2}{I_1 + I_2}$$

The curve in Fig. 21 shows the relation between $I_1I_2/(I_1 + I_2)$ and r calculated from this formula, the crosses indicating Warburg's measurements. As regards his last observation, which does not lie on the curve, Warburg expresses doubt of the value of the light intensity. If the true intensity were 25·1 instead of 45, his observation would lie on the curve.

It may be noted that equation (7) also gives a physical explanation of the well-established fact that the efficiency of photosynthesis increases with decreasing light intensity. This is illustrated by Table XVII, which gives the relative quantities of products photosynthesized by unit quantity of light at various relative intensities which have been calculated from the linear formula given above.

TABLE XVII

Relative intensity of light	Relative amount photosynthesized by unit quantity of light	Relative intensity of light	Relative amount photosynthesized by unit quantity of light
1	49·90	8	22·27
2	41·75	10	19·24
3	35·16	15	14·76
4	32·22	20	11·52
6	26·38	30	8·22

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[117] *Ann. Bot.*, 1905, *19*, 281.

The second method of varying the light intensities is to change the ratio I_1/I_2 while maintaining the total intensity of the blue and red rays, $I_1 + I_2$, constant. Now $I_1I_2/(I_1 + I_2)$ has a maximum value when $I_1 = I_2$, and consequently any change in I_1/I_2 will decrease the rate of photosynthesis, even though the total intensity of the blue and red rays remains constant. This is of considerable importance in view of the fact that various observers have used different light sources which differ in the relative intensities of the blue and red rays emitted by them. Since in all the usual light sources

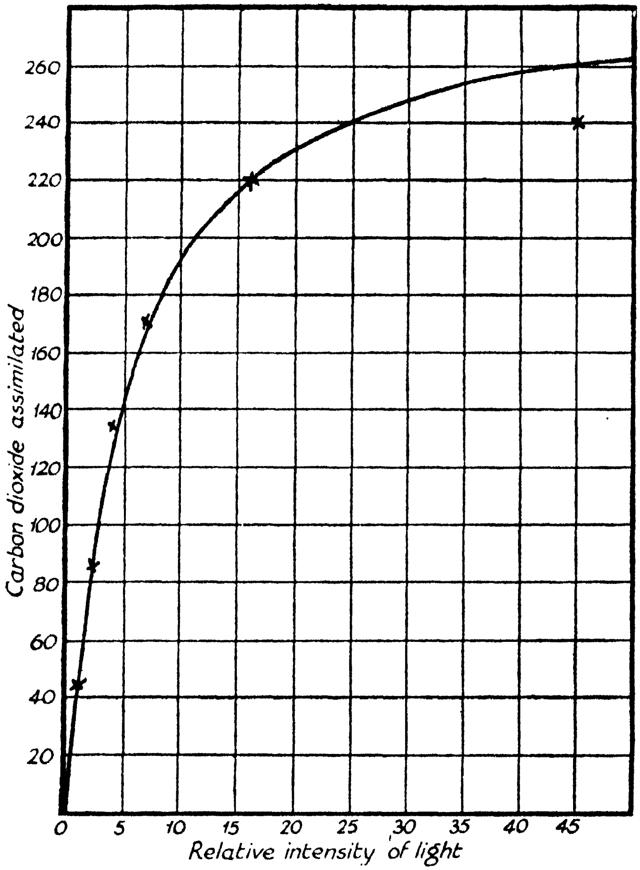

FIG. 21

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the ratio I_1/I_2 is equal to or less than unity, the effect on the rate of photosynthesis of reducing this ratio may be calculated, and for this purpose the equation expressing Emerson's first series may again be used.

In Table XVIII the first two columns contain the values of I_1 and I_2, the sum of these being 4 in each case. In the third column are given the values of $I_1I_2/(I_1 + I_2)$, and then follow the rates of photosynthesis at 5°, 10°, 20°, and 30°, together with the temperature coefficients (Q_{10}).

TABLE XVIII

I_1	I_2	$\frac{I_1I_2}{I_1+I_2}$	Rate of photosynthesis						
			5°	Q_{10}	10°	Q_{10}	20°	Q_{10}	30°
2·000	2·000	1·0000	19·14	4·47	40·46	2·37	95·83	1·27	122·08
1·778	2·222	0·9854	19·11	4·45	40·30	2·35	94·89	1·27	120·53
1·500	2·500	0·9375	18·96	4·37	39·64	2·30	91·34	1·26	115·16
1·333	2·667	0·8868	18·79	4·29	38·92	2·25	87·59	1·24	109·02
1·000	3·000	0·7500	18·25	4·04	36·66	2·10	76·92	1·21	92·98
0·800	3·200	0·6400	17·68	3·79	34·43	1·97	67·74	1·18	79·89
0·667	3·333	0·5556	17·13	3·58	32·39	1·86	60·28	1·16	69·71
0·500	3·500	0·4375	16·10	3·22	28·90	1·70	49·20	1·12	55·31
0·400	3·600	0·3600	14·99	3·02	26·05	1·59	41·47	1·08	44·71
0·250	3·750	0·2344	12·92	2·41	20·06	1·40	28·12	1·07	30·01
0·200	3·800	0·1900	11·76	2·19	17·39	1·33	23·13	1·05	24·40
0·100	3·900	0·0975	8·10	1·66	10·43	1·17	12·25	1·03	12·60
0·050	3·950	0·0494	4·99	1·34	5·77	1·09	6·31	1·01	6·40
0·020	3·980	0·0199	2·32	1·14	2·48	1·04	2·57	1·006	2·58

As may be seen from this table, a progressive reduction in the rate of photosynthesis and in the temperature coefficient is caused by a progressive decrease in the ratio I_1/I_2, even though the total intensity, $I_1 + I_2$, is maintained constant. In order to emphasize this, there are given in Table XIX (p. 222) the percentage reductions in the rate of photosynthesis when I_1/I_2 is reduced from unity to values which may be found in the light from various sources in common use.

It is obvious that the decreased photosynthetic efficiency for which I_1/I_2 is less than unity may be counterbalanced by increasing the total intensity of the light until $I_1I_2/(I_1 + I_2) = 1$. In Table XX are given the factors by which the total intensity must be multiplied in order to make $I_1I_2/(I_1 + I_2)$ equal to unity for each of the ratios of I_1 and I_2 given in

TABLE XIX

$I_1 : I_2$	Percentage reduction in rate of photosynthesis			
	5°	10°	20°	30°
1 : 3	4·6	9·4	19·7	23·8
1 : 4	7·6	14·9	29·3	34·6
1 : 5	10·5	20·0	37·1	42·9
1 : 7	15·9	28·6	48·7	55·5
1 : 9	21·7	35·6	56·7	63·4
1 : 15	32·5	50·4	70·7	75·4

Table XVIII. This is of importance in comparing the measurements of observers who have used light from different sources, and also in connexion with the use by Emerson and Arnold of neon light in which the ratio I_1/I_2 is small.

TABLE XX

$I_1 : I_2$	Factor	$I_1 : I_2$	Factor
1 : 1·25	1·0125	1 : 9	2·7778
1 : 1·667	1·0667	1 : 15	4·2667
1 : 2	1·2500	1 : 19	5·2632
1 : 3	1·3333	1 : 39	10·2564
1 : 4	1·5625	1 : 79	20·2532
1 : 5	1·8000	1 : 199	50·2513
1 : 7	2·2857		

It will be seen that as the ratio I_2/I_1 increases the factor approximates more nearly to one-quarter of that ratio.

A very striking confirmation of the effect of varying the relative intensities of the blue and red rays has been obtained by R. H. Dastur, who in collaboration with Samant[118] and with Mehta[119] found a considerable decrease in the amount of carbohydrates photosynthesized in the leaves of plants when daylight, in which the blue and red rays have equal intensity, was substituted by artificial light in which the intensity of the blue rays is materially less than that of the red rays. Dastur also proved by irradiating plants with daylight and with light of equal total intensity from various artificial sources that the smaller the ratio I_1/I_2, the greater was the reduction in the yield of carbohydrate.

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[118] Dastur and Samant, *Ann. Bot.*, 1933, *47*, 295.

[119] Dastur and Mehta, *ibid.*, 1935, *49*, 809.

There remain to be considered two observations, and the first of these is the reduction in the intensity of the blue rays by the use of an absorption screen. In an investigation of the induction period in photosynthesis G. E. Briggs[120] succeeded in proving the existence of the induction period even when the light had been passed through a strong solution of potassium dichromate. This observation might be accepted as evidence that photosynthesis can be promoted by red light only, but this is not the case. I am indebted to R. A. Morton for measuring the extinction coefficient of potassium dichromate, his value being 2·477 at 4705A, which lies well within the absorption band of chlorophyll A.CO_2H_2O complex. This value of the extinction coefficient indicates that the intensity of the blue rays is reduced to 1/300 after passing through 1 cm. of a molar solution or 5 cm. of a M/5 solution, the latter being a saturated solution in the case of potassium bichromate. Briggs does not mention the thickness of the solution used by him, but the rate of photosynthesis may be calculated on the assumption that the absorbing layer was 2·5 cm. thick. The ratio I_1/I_2 in this case is 1/150 and the value of $I_1I_2/(I_1 + I_2)$ is 0·013245. Applying this to the same equation as previously, the rates of photosynthesis are :

	10°	20°	25°	30°
No screen	40·46	95·83	113·06	122·08
With screen	1·67	1·71	1·72	1·72

It is evident, therefore, that the use of the absorbing screen does not prevent photosynthesis taking place.

These results are of considerable importance in connexion with the observations of Warburg and Negelein,[121] who determined the photosynthetic efficiency of various spectral regions isolated by means of absorbing screens from the radiation emitted by a quartz mercury-vapour lamp. No absorption screen is absolutely perfect in transmitting only those light rays for which it possesses a maximum transparency, and hence Warburg and Negelein were incorrect in assuming that the photosynthesis was promoted solely by the light rays in the isolated spectral regions. Their calculations

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[120] *Proc. Roy. Soc.*, 1933, *113*B, 5.

[121] *Zeitsch. phys. Chem.*, 1922, *102*, 235; 1923, *106*, 191. See page 25.

of the quantum efficiency at the three selected wave-lengths, therefore, were invalid.

THE TEMPERATURE COEFFICIENT

It is evident from equations (5), (6), and (7) that the temperature coefficient in photosynthesis is a function of $I_1I_2/(I_1 + I_2)$ and the carotin concentration only. The proof given by Warburg[122] that it is independent of the external concentration of carbon dioxide is the basis on which these equations rest. Nothing is known of the carotin concentration in the plants employed in the determination of the rates of photosynthesis, and hence it is not possible to consider this. It will be seen, however, from the three equations that the effect of decreasing the carotin concentration will be to decrease the velocity of the Blackman reaction and, as will be shown in the discussion of the action of poisons, this will increase the value of the temperature coefficient. The effect of decreasing $I_1I_2/(I_1 + I_2)$ has been shown in Tables XVI and XVIII and need not be discussed further, but the fact may again be emphasized that two observers using two light sources in which the ratio I_1/I_2 is different will obtain different values for the temperature coefficient for the same temperature range, even though the total light energy as measured in calories per second per square decimetre be the same in the two cases. In Table XXI are given the temperature coefficients calculated from the four equations expressing Emerson's measurements, together with those found by Warburg.

TABLE XXI

Temperature range	Temperature Coefficients (Q_{10})				
	Series 1	$\varepsilon = 0{\cdot}101$	$\varepsilon = 0{\cdot}048$	$\varepsilon = 0{\cdot}016$	Warburg
0°–10°	5·65	5·96	6·31	5·53	—
5°–10°	4·47	5·32	5·86	4·90	5 to 4·3
10°–20°	2·42	3·32	3·81	2·72	2·1
20°–30°	1·27	1·62	1·80	1·37	1·6

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[122] *Biochem. Zeitsch.*, 1919, *100*, 258.

VARIATION IN THE CARBON DIOXIDE CONCENTRATION

In discussing the effect of varying the external carbon dioxide concentration, we start from the fact that the rate is directly proportional to bA in equation (4), that is, to the fraction of the total number of chlorophyll A molecules in the surface which has adsorbed CO_2H_2O. The phenomenon of adsorption of gases has been discussed,[123] and it was shown that when a surface is in contact with a gas in a closed system the adsorption equilibrium is expressed by

$$k_1P(A - x) = k_2xe^{-Q/RT}$$

where P is the pressure of the gas, A is the total number of molecules or adsorbing units in the surface, x is the number of adsorption complexes and Q is the energy needed to dissociate the adsorption complexes. From this equation we have

$$x = A \times \frac{P\frac{k_1}{k_2}e^{Q/RT}}{1 + P\frac{k_1}{k_2}e^{Q/RT}}$$

and at constant temperature

$$x = A \times \frac{\text{const.}\,P}{1 + \text{const.}\,P}$$

Applying this formula to the adsorption of CO_2 on a surface of chlorophyll and assuming that the concentration of H_2O is large and sensibly constant, the fraction of A molecules of chlorophyll A coated by CO_2H_2O at constant temperature will be

$$A \times \frac{\text{const.}\,P}{1 + \text{const.}\,P}$$

where P is the external pressure of CO_2. Substituting this value for bA in equation (6) we have

$$\frac{P}{r} = B + CP$$

where B and C are constants when $I_1I_2/(I_1 + I_2)$ and the temperature are constant. This formula indicates that the ratio of the external CO_2 concentration to the rate of photo-

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[123] Baly, *Proc. Roy. Soc.*, 1937, *160*A, 465.

synthesis should be linearly proportional to the external CO_2 concentration.

The P,r curve in Fig. 22 is that given by the linear equation

$$\frac{P}{r} = 1{\cdot}38 \times 10^{-8} + 0{\cdot}0043580P$$

and Warburg's measurements[124] are indicated by the crosses. As will be seen, these lie sufficiently well with respect to the curve, with the exception of the last one, when $P = 91 \times 10^{-6}$ grm. mole of CO_2 per litre.

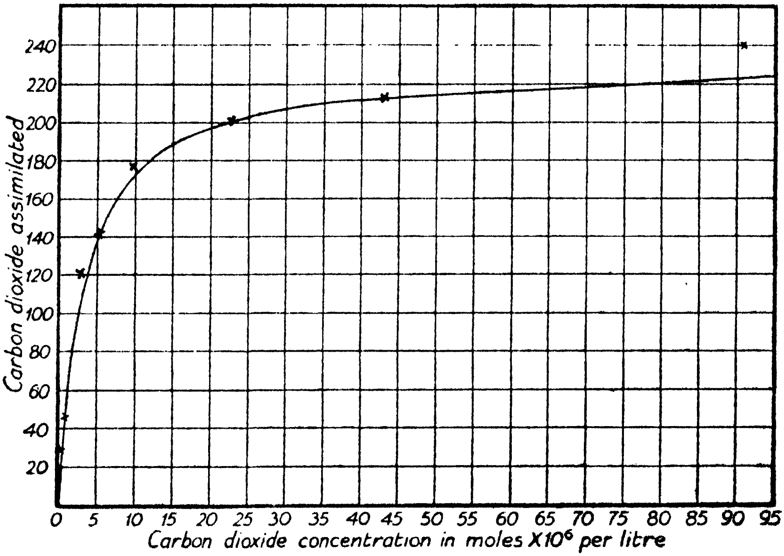

FIG. 22

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This deviation from the linear formula is of some interest, especially since Warburg pointed out that at the three highest concentrations of carbon dioxide the rate of photosynthesis is linear with the concentration. Whilst it is possible that the calculation of the CO_2 concentrations from the activity coefficients instead of the dissociation constants might give different values at the higher concentrations, it would seem more probable that the deviation is due to a more fundamental cause.

Now the external concentration of CO_2 under which photosynthesis normally takes place in plants is that present in

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[124] *Biochem. Zeitsch.*, 1919, *100*, 258.

the atmosphere, namely, from $1{\cdot}34 \times 10^{-5}$ to $1{\cdot}79 \times 10^{-5}$ grm. mole per litre. The highest concentration of CO_2 used by Warburg was $9{\cdot}1 \times 10^{-5}$ grm. mole per litre, which is from 5 to 6·8 times that to which plants are accustomed, and the question arises of the effect of this abnormal condition on the chloroplasts. It has been proved by Emerson and Arnold[125] that the number of molecules of chlorophyll which actually function in photosynthesis is only a small fraction of the total number present, that is to say, the number present in the irradiated surface is relatively very small. It is evident that as the external pressure of the CO_2 is increased, the fraction of the surface molecules converted into their complexes with CO_2H_2O will increase until the stage is reached when the whole surface has been coated with CO_2H_2O. It is possible that a further increase in the CO_2 concentration will cause the interpenetration of the chlorophyll structure by the CO_2H_2O and result in a greater number of chlorophyll $A.CO_2H_2O$ being formed than those in the surface layer. If this takes place, the rate of photosynthesis will be abnormally increased, and it is to be expected that the rate will then become directly proportional to the CO_2 concentration as Warburg found.

IRRADIATION WITH FLASHING LIGHT

It is well known that, all other factors being maintained constant, the photosynthetic efficiency of plants is materially increased when the irradiation is intermittent. It was shown by Warburg that the increase in the rate of photosynthesis is the greater the shorter is the period of each light flash, and Emerson and Arnold proved that the greatest increase is observed when the period of each flash is exceedingly short and the dark periods between successive flashes are sufficiently long for the Blackman reaction to complete itself.

In order to determine the effect of substituting intermittent for continuous irradiation, the equation expressing the rate of photosynthesis when the photostationary state has been established may be re-stated

$$r = k_1 I_1 (bA - x - y) = k_2 I_2 x = k_3 y c e^{-Q/RT}$$

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[125] *J. Gen. Physiol.*, 1932, *16*, 191.

It follows from this equation that when the irradiation is stopped and the Blackman reaction completes itself the concentration of the chlorophyll A.CO_2H_2O complex will increase from $bA - x - y$ to $bA - x$, whilst the concentration of the chlorophyll B.CHOH complex will remain unchanged. If after a period of darkness continuous irradiation with the same light be started, the initial velocity of the first photochemical reaction will be $k_1I_1(bA - x)$ and the initial velocity of the second photochemical reaction will be k_2I_2x. These two velocities, however, are not equal, and consequently there will be an interval of time before the photostationary state is re-established. It follows, therefore, that there must always be a period of induction before the rate of photosynthesis under constant conditions becomes constant.

On the other hand, if, after a period of darkness, irradiation be carried out for very short periods, each period being separated by a sufficiently long dark period for the Blackman reaction to complete itself, a new condition will be slowly established. The shorter the period of each light flash, the more nearly will this condition approximate to the equation

$$r = k_1I_1(bA - x') = k_2I_2x'$$

whence by elimination of x'

$$r = \frac{k_1I_1k_2I_2bA}{k_1I_1 + k_2I_2} \quad . \quad . \quad . \quad . \quad . \quad (8)$$

This expression gives the maximum rate of photosynthesis in flashing light when the period of each flash is reduced to a minimum. As will be seen from the equations for the photostationary state, this maximum rate of photosynthesis in flashing light is equal to K in equation (5), and hence the change in the rate of photosynthesis when flashing light is substituted for continuous irradiation will be given by the difference between the rate observed during continuous irradiation and the relevant value of K.

In Table XVI were given the rates of photosynthesis at five different temperatures, together with the relevant values of K, when the relative value of $I_1I_2/(I_1 + I_2)$ is reduced in the formula for Emerson's first series of measurements:

$$\log \frac{r}{130 - r} = 22{\cdot}88400 - 6{,}573{\cdot}8/T$$

It will be noted that the difference between the value of K and the rate in continuous light is smaller the smaller is the intensity of the light and the higher is the temperature. These two relations were established by Warburg[126] and by Emerson and Arnold.[127] The latter also found that the rate of photosynthesis in flashing light is linearly proportional to the total intensity, and this relation is to be expected because K is directly proportional to $I_1 + I_2$.

The increases indicated in Table XVI when flashing light is substituted for continuous irradiation are much smaller than those observed by Warburg and by Emerson and Arnold. This is due to the fact that, as stated by him, Emerson's measurements expressed by the formula given above were made with relatively low light intensity. This may be proved by increasing the value of $I_1I_2/(I_1 + I_2)$ in the formula, when very great increases in the rate of photosynthesis are obtained on changing from continuous to intermittent irradiation. In Table XXII is shown the effect of increasing $I_1I_2/(I_1 + I_2)$, together with the percentage increase in the rate, when by flashing light the rate is increased to the relevant value of K. The percentage increases are given because Warburg and Emerson and Arnold expressed their results in this way.

TABLE XXII

$\frac{I_1I_2}{I_1 + I_2}$	K	Rate of photosynthesis in continuous light				Percentage increase in rate in flashing light			
		10°	20°	25°	30°	10°	20°	25°	30°
1	130	40·46	95·85	113·06	122·08	221·3	35·6	15·0	6·5
5	650	53·87	233·57	371·56	490·88	1,106·5	178·3	74·9	32·4
10	1,300	56·20	284·71	520·26	788·74	2,213·0	355·3	149·9	64·8
50	6,500	58·22	345·19	765·30	1,523·70	11,065·0	1,783·0	749·3	324·1

THE EFFECT OF POISONS

A poison may affect the rate of photosynthesis in two ways. It may either decrease the velocity of the Blackman

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[126] *Biochem. Zeitsch.*, 1919, *100*, 258.
[127] *J. Gen. Physiol.*, 1932, *15*, 391.

reaction or it may decrease the velocity of the first photochemical reaction by being preferentially adsorbed on the chlorophyll A and thus reducing the concentration of the chlorophyll $A.CO_2H_2O$ complexes. Since the effect of the poison is different in the two cases, they may be separately discussed and, as previously, the formula

$$\log \frac{r}{130 - r} = 22{\cdot}88400 - 6{,}573{\cdot}8/T$$

may be used.

The effect of reducing the velocity of the Blackman reaction, that is to say the value of k_3c in equation (5), is shown for four temperatures in Table XXIII.

TABLE XXIII

Relative value of k_3c	Rate of photosynthesis						Percentage reduction in rate			
	10°	Q_{10}	20°	Q_{10}	25°	30°	10°	20°	25°	30°
1·00	40·46	2·42	95·83	1·27	113·06	122·08				
0·50	23·96	3·17	75·88	1·52	100·02	115·11	40·78	20·82	11·53	5·71
0·20	10·77	4·33	46·71	2·10	71·31	98·08	73·38	51·26	35·89	19·66
0·13	7·21	4·81	34·73	2·50	60·38	86·75	82·18	63·76	46·60	28·94
0·10	5·60	5·07	28·47	2·77	52·03	78·87	86·16	70·29	56·52	35·39
0·05	2·87	5·57	15·97	3·54	32·52	56·61	90·90	83·34	71·24	53·63
0·02	1·16	5·92	6·90	4·43	13·31	30·55	97·13	92·70	88·02	74·98
0·01	0·58	6·06	3·55	4·90	8·13	17·35	98·57	96·30	92·81	85·78
0·001	0·06	6·19	0·36	5·44	0·86	1·98	99·85	99·63	99·24	98·38

As may be seen from the table, the effect of progressively poisoning the Blackman reaction is very materially to reduce the rate of photosynthesis and also to increase the temperature coefficient.

The most interesting result is the effect of decreasing the light intensity with a constant amount of poisoning, and this is shown in Table XXIV for three amounts of poisoning and four intensities of light.

The figures given in Table XXIV show that the effect of a given amount of poisoning is progressively decreased when the intensity of the light is reduced, this effect being greatest at the higher temperatures. This effect was observed by Warburg when *Chlorella* was poisoned by HCN.[128] He found that with a concentration of $3{\cdot}8 \times 10^{-5}$ grm. mole of HCN per litre the effect of the poisoning was reduced from 48 per

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[128] *Biochem. Zeitsch.*, 1919, *100*, 258; 1920, *103*, 199.

TABLE XXIV

$\frac{I_1I_2}{I_1+I_2}$	k_3c	Rate of photosynthesis				Percentage reduction in rate			
		10°	20°	25°	30°	10°	20°	25°	30°
1·00	1·00	40·46	95·83	113·06	122·08				
	0·50	23·96	75·88	100·02	115·11	40·78	20·82	11·53	5·71
	0·13	7·21	34·73	60·38	86·75	82·18	63·76	46·60	28·90
	0·01	0·58	3·55	8·13	17·35	98·57	96·30	92·81	85·78
0·05	1·00	5·85	6·39	6·45	6·48				
	0·50	5·05	6·28	6·40	6·46	13·87	1·72	0·78	0·34
	0·13	3·51	5·72	6·15	6·34	40·00	10·49	4·65	2·16
	0·01	0·54	2·34	3·72	4·91	90·75	63·38	42·33	24·15
0·02	1·00	2·49	2·58	2·59	2·60				
	0·50	2·39	2·56	2·58	2·59	4·02	0·78	0·39	0·32
	0·13	1·94	2·15	2·54	2·57	22·09	16·67	1·93	1·15
	0·01	0·48	1·52	2·00	2·30	80·72	41·09	22·78	11·54
0·01	1·00	1·27	1·30	1·30	1·30				
	0·50	1·25	1·29	1·30	1·30	1·57	0·77	0	0
	0·13	1·11	1·27	1·28	1·30	14·56	2·31	1·54	0
	0·01	0·40	0·96	1·13	1·22	68·50	26·15	13·08	5·74

cent to 1 per cent when the light intensity was reduced to approximately 1/50. This reduction is very similar to that shown in Table XXIV, when the relative value of k_3c is reduced to 0·13; the reduction in the rate of photosynthesis at 25° when $I_1I_2/(I_1 + I_2) = 1$ is 46·6 per cent, and when $I_1I_2/(I_1 + I_2) =$ 0·02 the reduction is 1·93 per cent.

It is satisfactory to note that by reducing the velocity of the Blackman reaction alone the effects observed by Warburg can be quantitatively explained. The first photochemical reaction is not affected, therefore, as Warburg proved by his observations.

The second effect of a poison is to decrease the velocity of the first photochemical reaction owing to the chlorophyll A adsorbing the poison preferentially to hydrated carbon dioxide. Poisons which are so preferentially adsorbed are usually known as narcotics. If A molecules of chlorophyll A in a surface are exposed to a narcotic, it will be seen from the adsorption formula given on page 225 that the number which have adsorbed the narcotic will be

$$n = \frac{sN}{1 + sN} \times A$$

where s is a constant when the temperature is constant and N is the concentration of the narcotic. The number of unpoisoned molecules of chlorophyll A will be

$$A\left(1 - \frac{sN}{1 + sN}\right) = \frac{A}{1 + sN}$$

When this number of molecules is exposed to carbon dioxide and water the number of chlorophyll $A.CO_2H_2O$ complexes will be $\frac{bA}{1 + sN}$ where b is constant when the temperature and CO_2 concentration are constant. Since equation (4) shows that the rate of photosynthesis is directly proportional to bA, it follows that

$$\frac{1}{r} = C + DN,$$

where C and D are constants when the light intensity, temperature and CO_2 concentration are constant. The reciprocal of the rate of photosynthesis, therefore, should be linearly proportional to the external concentration of the narcotic.

Warburg's observations with phenylurethane are expressed with reasonable accuracy by the linear formula

$$\frac{1}{r} = 0{\cdot}01000 + 40{\cdot}264N$$

which gives $r = 100$ in the absence of the narcotic. The percentage reductions in the rate of photosynthesis given by this formula and those obtained by Warburg in the presence of six concentrations of phenylurethane are set forth in Table XXV.

TABLE XXV

N in 10^{-4} grm. mole per litre	1·2	2·4	4·8	9	18	36
$\frac{1}{r}$	0·014832	0·019663	0·029327	0·046238	0·082475	0·15495
r	67·42	50·77	34·10	21·63	12·12	6·45
Reduction in r	32·58	49·23	65·90	78·37	87·88	93·55
obs. (Warburg)	37	46	65	74	86	95

In summing up the foregoing discussion it may be claimed that the formulae derived for the rate of photosynthesis during the photostationary state give a satisfactory explanation of (1) the effect of changing the four variable factors, temperature, carbon dioxide concentration, total light intensity, and the relative intensities of the blue and red rays, (2) the effect of irradiation with flashing light, and (3) the effect of poisons in the case of the unicellular alga *Chlorella*.

THE MAXIMUM RATE OF PHOTOSYNTHESIS IN THE LIVING PLANT

When the effect of temperature on the rate of photosynthesis in the living plant was discussed, the range of temperature was restricted to 0° to 30°. The reason for this was that it is known that in the living plant the rate of photosynthesis increases with the temperature up to a maximum. When the temperature rises above a definite limit the rate of photosynthesis begins rapidly to decrease. This phenomenon was discussed in Chapters I and IV and the curves in Fig. 7 on page 84 represent Miss Matthaei's observations.[129] The usually accepted explanation of this phenomenon is that when the temperature exceeds the limiting value, de-activation of the chloroplasts begins, and the phenomenon has been compared with the de-activation by temperature of enzymes. It is well established that in fermentation and other enzyme reactions the velocity of the reaction first increases with rise in temperature and then suddenly begins rapidly to decrease owing to the de-activation of the enzymes.

There is, however, great difficulty in the way of accepting this as the correct explanation of the phenomenon in the living plant. In the first place Miss Matthaei found that the temperature at which the maximum rate of photosynthesis is observed decreases as the light intensity is reduced. In the second place, the same phenomenon was observed with the nickel carbonate prepared by the electrolytic method by Hood and myself and has already been described on page 82. In this case the phenomenon cannot be attributed to a biological process akin to the de-activation of enzymes and must be due to a physical process.

In Chapter VII, page 170, the evidence in favour of the two-stage mechanism of photosynthesis on a surface of nickel

[129] *Phil. Trans.*, 1905, *197*B, 47.

oxide was given and this included the experimental proof that the first photochemical process

$$4NiO.CO_2H_2O + 68{,}492 \text{ calories} = 2Ni_2O_3.CHOH$$

is reversible. In view of the fact that the first photochemical reaction in the living plant

$$C_{55}H_{72}O_5N_4Mg.CO_2H_2O + 68{,}117 \text{ calories} = C_{55}H_{70}O_6N_4MgH_2O.CHOH$$

is endothermic to almost exactly the same extent, it is in the highest degree probable that this reaction is also reversible. If the reverse reaction

$$C_{55}H_{70}O_6N_4MgH_2O.CHOH = C_{55}H_{72}O_5N_4Mg.CO_2H_2O + 68{,}117 \text{ calories}$$

takes place it is evident that this will decrease the rate of photosynthesis. Furthermore, the reverse reaction must have a temperature coefficient, and consequently its influence on the rate of photosynthesis will increase with the temperature.

The problem, therefore, is to evaluate the effect of this reverse reaction on the velocity of photosynthesis during the photostationary state, and I am indebted to my friend Professor J. M. Whittaker for the following solution.

In the complete photosynthetic cycle

1. $C_{55}H_{72}O_5N_4Mg.CO_2H_2O + h\nu_1 = C_{55}H_{70}O_6N_4MgH_2O.CHOH$
2. $C_{55}H_{70}O_6N_4MgH_2O.CHOH + h\nu_2 = C_{55}H_{70}O_6N_4MgH_2O + CHOH$
3. $C_{55}H_{70}O_6N_4MgH_2O + C_{40}H_{56} = C_{55}H_{72}O_5N_4Mg + C_{40}H_{56}O_2$

let x = the number of $C_{55}H_{70}O_6N_4MgH_2O.CHOH$ complexes
y = the number of $C_{55}H_{70}O_6N_4MgH_2O$ molecules
and z = the number of $C_{55}H_{72}O_5N_4Mg.CO_2H_2O$ molecules.

Then in time dt the loss in x, due to the fact that the first reaction is reversible, will be $x\phi(T)dt$, where $\phi(T)$ is the velocity of the reverse reaction. Hence, from equation (3) expressing the photostationary state

$$\begin{aligned}
r = k_1I_1(bA - x - y) &= k_2I_2x = k_3cye^{-Q_1/RT}\\
\text{loss of } z \text{ in } dt &= k_1I_1(bA - x - y)dt\\
\text{gain of } z \text{ in } dt &= k_3cye^{-Q_1/RT}dt + x\phi(T)dt\\
\text{loss of } y \text{ in } dt &= k_3cye^{-Q_1/RT}dt\\
\text{gain of } y \text{ in } dt &= k_2I_2xdt\\
\text{gain of carbohydrates} &= \text{gain of } y = rdt
\end{aligned}$$

where r is the rate of photosynthesis.

Then equating gains and losses

$$k_1I_1(bA - x - y) = k_3cye^{-Q_1/RT} + x\phi(T)$$

$$k_2I_2x = k_3cye^{-Q_1/RT} = r$$

These give $k_1I_1(bA - x - y) = k_2I_2x + x\phi(T)$

or $$k_1I_1\left(\frac{bA}{x} - 1 - \frac{y}{x}\right) = k_2I_2 + \phi(T)$$

or $$\frac{bA}{x} = \frac{k_2I_2 + \phi(T)}{k_1I_1} + 1 + \frac{k_2I_2}{k_3ce^{-Q_1/RT}}$$

so that $$r = k_2I_2x = \frac{k_2I_2bA}{\dfrac{k_2I_2 + \phi(T)}{k_1I_1} + 1 + \dfrac{k_2I_2}{k_3ce^{-Q_1/RT}}} \quad . \quad (9)$$

Now the reversal of the first photochemical reaction is a thermal process and hence $\phi(T)$ in Professor Whittaker's equation (9) may be substituted by $k_4e^{-Q_2/RT}$, where Q_2 is the energy of activation of the reverse reaction. As the result of this substitution we have

$$r = \frac{k_2I_2bA}{\dfrac{k_2I_2 + k_4e^{-Q_2/RT}}{k_1I_1} + 1 + \dfrac{k_2I_2}{k_3ce^{-Q_1/RT}}}$$

whence

$$r = \frac{k_1I_1k_2I_2bAk_3ce^{-Q_1/RT}}{k_1I_1k_2I_2 + (k_1I_1 + k_2I_2)k_3ce^{-Q_1/RT} + k_3k_4ce^{-(Q_1+Q_2)/RT}}$$

and $$\frac{1}{r} = \frac{1}{K} + \frac{1}{bAk_3c}e^{Q_1/RT} + \frac{k_4}{k_1I_1k_2I_2bA}e^{-Q_2/RT} \quad . \quad (10)$$

where $$K = \frac{k_1I_1k_2I_2bA}{k_1I_1 + k_2I_2}$$

In the discussion of the photostationary state it was shown that the rate of photosynthesis is given by equation (4), namely,

$$r = \frac{k_1 I_1 k_2 I_2 b A k_3 c e^{-Q_1/RT}}{k_1 I_1 k_2 I_2 + (k_1 I_1 + k_2 I_2) k_3 c e^{-Q_1/RT}}$$

and from this we have

$$\frac{1}{r} = \frac{1}{K} + \frac{1}{bAk_3c} e^{Q_1 RT} \quad . \quad . \quad . \quad . \quad . \quad (11)$$

It will be seen, therefore, that the effect of the reversal of the first photochemical reaction is expressed by the third term on the right-hand side of equation (10).

In order to determine the effect of the third term in equation (10) the first series of Emerson's measurements of the rate of photosynthesis at different temperatures may be taken, and it was shown above that these are expressed by

$$\log \frac{r}{130 - r} = 22{\cdot}88400 - 6{,}573{\cdot}8/T$$

This equation must first be expressed in the form of equation (11) and it may be seen from equation (5) that $K = 130$ and that $22{\cdot}88400 = \log \frac{bAk_3c}{K}$ and that $Q = 6{,}573{\cdot}8 \times 2{\cdot}302585 \times 1{\cdot}99885 = 30{,}099{\cdot}36$ calories. Emerson's measurements, therefore, may be expressed by the equation

$$\frac{1}{r} = 0{\cdot}007692307 + 1{\cdot}004745 \times 10^{-25} \times e^{30{,}099{\cdot}36/RT}$$

Attention was drawn on page 229 to the fact that Emerson's measurements were made with a small light intensity, and as the effect of reducing the light intensity will be discussed later, it is desirable to commence with an equation which expresses the rate of photosynthesis with an intensity of light which is ten times that used by Emerson. By increasing the value of K from 130 to 1,300 the above equation becomes

$$\frac{1}{r} = 0{\cdot}0007692307 + 1{\cdot}004745 \times 10^{-25} \times e^{30{,}099{\cdot}36/RT} \quad (12)$$

As regards the value of Q_2 in the last term of equation (10), nothing is known except the fact that it must be very large, because the influence of the last term in decreasing the rate of photosynthesis increases very rapidly with rise in temperature. In the experimental proof of the reaction

$$2Ni_2O_3.CHOH = 4NiO.CO_2H_2O$$

it was shown on page 174 that the rate increased very greatly with rise in temperature, and hence in this case Q_2 was very large. For reasons which will be given later, the value of $\frac{k_4}{k_1I_1k_2I_2bA}e^{-Q_2/RT}$ may be taken as $10^{66} \times e^{-100,000/RT}$ and the rates of photosynthesis calculated from the equation

$$\frac{1}{r} = 0{\cdot}0007692307 + 1{\cdot}004745 \times 10^{-25} \times e^{30,099{\cdot}36/RT} + 10^{66} \times e^{-100,000/RT} \quad . \quad (13)$$

The values of r calculated from this equation are given in Table XXVI.

TABLE XXVI

Temperature	Rate of photosynthesis	Temperature	Rate of photosynthesis
0°	8·23	35°	996·9
5°	22·07	37°	1,035·2
10°	56·21	37·5°	1,037·2
15°	133·8	38°	1,035·0
20°	284·7	40°	964·1
25°	520·3	45°	344·3
30°	788·2	47°	155·9
32°	884·2		

The relation between the rate of photosynthesis and the temperature is shown by the curve in Fig. 23, and this curve is very similar in shape to that obtained by Miss Matthaei and shown in Fig. 7 (p. 84).

It has not been found possible to express Miss Matthaei's measurements either by equation (10) or (11), equation (11) having been shown to express Emerson's four series of observations. The explanation of this is without doubt the fact that in each of his four series of measurements Emerson used the same suspension of *Chlorella*, whilst Miss Matthaei used an excised leaf for each measurement. Moreover, her curve in Fig. 7 was obtained by combining two separate series of measurements, one between −6° and 9·5°, and the other between 11° and 43°, and it can be seen that the two series do not give a truly continuous curve. In view of these two considerations, the fact that Miss Matthaei's observations cannot be expressed by either equation (10) or (11) does not appear to be significant.

The reasons for the adoption of $10^{66} \times e^{-100,000/RT}$ in equation (13) as the value of the term $\frac{k_4}{k_1 I_1 k_2 I_2 bA} e^{-Q_2/RT}$ in

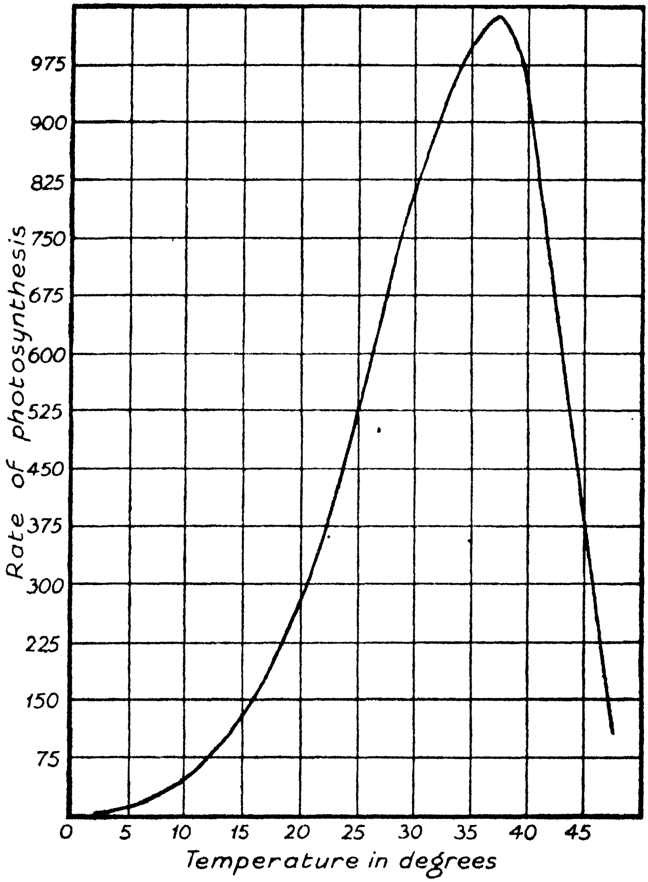

FIG. 23

equation (10) may now be explained. In the first instance, five series of calculations were made of the rate of photosynthesis from equation (13), together with the fourth term, const. $\times e^{-Q_2/RT}$, using for Q_2 the values 60,000, 70,000, 80,000, 90,000, and 100,000 calories, respectively. In each case the result of the calculations indicated the maximum rate of photosynthesis at a definite temperature and a decrease in the rate when the temperature exceeded that limit. Of these values of Q_2, 100,000 calories was the only one which gave a decrease in the rate of photosynthesis which was comparable to that observed by Miss Matthaei, namely, a decrease to half the maximum rate at 37·5° when the temperature was increased to 43°. For this reason the value of 100,000 calories for Q_2 was adopted.

The value of 10^{66} was adopted as the value of the term $\frac{k_4}{k_1 I_1 k_2 I_2 b A}$, because this value gives the maximum rate of photosynthesis at 37·5°, which agrees with Miss Matthaei's observation.

Now, in addition to her proof of the existence of a maximum rate of photosynthesis in the leaves of plants at a well-defined temperature, Miss Matthaei also proved that the temperature at which the rate of photosynthesis is a maximum is lowered when the intensity of the light is reduced. It is of great interest, therefore, to consider the effect of reducing the light intensity on the first and third terms on the right-hand side of equation (10). The discussion may be restricted to the case in which the relative intensity of the blue and red rays, I_1/I_2, is kept constant, and we may assume that the intensity of both I_1 and I_2 is reduced to one-tenth. The value of K in equation (10) will then be reduced to one-tenth and the value of $\frac{k_4}{k_1 I_1 k_2 I_2 b A}$ will be increased 100 times. If the light intensity in equation (13) is reduced to one-tenth, we have the relation expressed by

$$\frac{1}{r} = 0{\cdot}007692307 + 1{\cdot}0004745 \times 10^{-25} \times e^{30{,}099{\cdot}36/RT} + 10^{68} \times e^{-100{,}000/RT} \qquad (14)$$

and the values of r calculated from this equation are given in Table XXVII (p. 240).

As may be seen from the table, the maximum rate of photosynthesis is now given at 31°, as compared with 37·5° when the light intensity was ten times as great. The observations of Miss Matthaei, therefore, have been quantitatively explained.

TABLE XXVII

Temperature	Rate of photosynthesis	Temperature	Rate of photosynthesis
0°	7·79	30°	120·8
5°	19·14	31°	121·1
10°	40·46	32°	120·0
15°	69·36	35°	109·4
20°	95·83	37°	87·79
25°	113·0	40°	40·83

THE MAXIMUM RATE OF PHOTOSYNTHESIS ON A SURFACE OF NICKEL OXIDE

In the discussion of the mechanism of the process in Chapter VII it was shown that there is a close analogy between photosynthesis on a surface of chlorophyll A and photosynthesis on a surface of nickel oxide. In the former the three reactions are:

1. Chlorophyll A.$CO_2H_2O + h\nu_1 =$ Chlorophyll B.CHOH
2. Chlorophyll B.CHOH $+ h\nu_2 =$ Chlorophyll B + CHOH
3. Chlorophyll B + carotin = Chlorophyll A + xanthophyll

and in the latter the three processes are:

1. $4NiO.CO_2H_2O + h\nu_1 = 2Ni_2O_3.CHOH$
2. $2Ni_2O_3.CHOH + h\nu_2 = 2Ni_2O_3 + CHOH$
3. $2Ni_2O_3 = 4NiO + O_2$

It will be seen that the only essential difference between the two processes is that in the case of chlorophyll the third reaction is bimolecular, whilst in the case of nickel oxide the third reaction is unimolecular. In view of the very close analogy between the two processes the equation for the photostationary state when the surface consists of nickel oxide will be similar to equation (3), namely

$$r = k_1I_1(bA - x - y) = k_2I_2x = k_3ye^{-Q_1/RT} \quad . \quad (15)$$

where I_1 is the intensity of the blue rays with frequency ν_1, I_2 is the intensity of the red rays with frequency ν_2, bA is the concentration of the adsorption complex $4NiO.CO_2H_2O$, x is the concentration of the adsorption complex $2Ni_2O_3.CHOH$, and y is the concentration of Ni_2O_3. From equation (15), as in the case of chlorophyll, we obtain the following equations for the photostationary state:

$$\log \frac{r}{K - r} = \log \frac{bAk_3}{K} - Q_1'/T \qquad (16)$$

where $K = \dfrac{k_1I_1k_2I_2bA}{k_1I_1 + k_2I_2}$ and $Q_1' = Q_1/2{\cdot}303R$

$$\frac{1}{r} = \frac{1}{K} + \frac{1}{bAk_3}e^{Q_1/RT} \qquad (17)$$

$$\frac{bA}{r} = \frac{k_1I_1 + k_2I_2}{k_1I_1k_2I_2} + \frac{1}{k_3}e^{Q_1/RT} \qquad (18)$$

and $$\frac{I_1I_2}{r(I_1 + I_2)} = \frac{k_1I_1 + k_2I_2}{k_1k_2(I_1 + I_2)bA} + \frac{I_1I_2}{(I_1 + I_2)bAk_3}e^{Q_1/RT} \qquad (19)$$

Owing to the fact that the first photochemical reaction is reversible, the reverse reaction being a thermal process, a maximum rate of photosynthesis will be observed at a definite temperature as has been experimentally proved and shown by the curve in Fig. 6 on page 83. As the result of this, the above four equations only express the rate of photosynthesis up to a temperature which is about 7° below that at which the rate is a maximum.

The effect of the reversal of the first photochemical process can be found from Professor Whittaker's solution, since this is independent of the substances concerned. The solution in the case of chlorophyll was given above and the complete equation for NiO is identical in form with equation (10) and is

$$\frac{1}{r} = \frac{1}{K} + \frac{1}{bAk_3}e^{Q_1/RT} + \frac{k_4}{k_1I_1k_2I_2bA}e^{-Q_2/RT} \qquad (20)$$

where k_4 is a constant and Q_2 is the energy of activation of the reaction

$$2Ni_2O_3.CHOH = 4NiO.CO_2H_2O$$

The only quantitative measurements of the rate of photosynthesis which have been made with nickel oxide are those by Hood and myself in 1928.[130] These were described on page 82 and the relation between the rate of photosynthesis and the temperature was shown by the curve in Fig. 6. This

[130] *Proc. Roy. Soc.*, 1929, *122*A, 395.

curve shows a well defined maximum rate at 31° and this indicates that our observations should be expressed by equation (20). It is of interest, therefore, to record the fact that our observations are expressed with a reasonable degree of accuracy by the following equation:

$$\frac{1}{r} = 6{\cdot}5975 + 5{\cdot}128613 \times 10^{-10} \times e^{14,000/RT} + 5{\cdot}457579 \times 10^{71} \times e^{-100,000/RT}$$

The values of r calculated from this equation are given in Table XXVIII, together with the amount of carbohydrates photosynthesized.

TABLE XXVIII

	Amount photosynthesized in 2 hours			Amount photosynthesized in 2 hours	
Temperature	calc.	obs.	Temperature	calc.	obs.
5°	0·0173	0·0101	31°	0·0751	0·0783
11·5°	0·0283	0·0281	32°	0·0747	
17°	0·0408	0·0412	34°	0·0657	
21°	0·0513	0·0512	36°	0·0446	0·0460
26°	0·0656	0·0648	41°	0·0078	0·0238
28°	0·0708		42°	0·0038	
30°	0·0747		46°	0·0001	0·0061

The curve in Fig. 24 shows the relation between the temperature and the calculated amount of carbohydrate photosynthesized, the amounts obtained being indicated by the crosses. It will be seen that with the exception of the values at 41° and 46° the agreement is quite satisfactory.

SUMMARY

The factors which control the rate of photosynthesis in the living plant are the temperature, the total intensity of the light, the relative intensities of the blue and red rays absorbed by chlorophyll, and the external concentration of carbon dioxide. An equation has been formulated which expresses the rate of photosynthesis in terms of these four factors. It has been shown that this equation and others derived from it give a quantitative explanation of the experimental observations of the effect of varying each factor while the remaining three factors are maintained constant.

It having been established that the first photochemical reaction of the photosynthetic cycle is reversible, the effect of this on the rate of photosynthesis has been discussed. It has been shown that with progressive rise in the temperature the rate of photosynthesis increases up to a maximum, and then suddenly decreases rapidly. It has also been shown that the temperature at which the rate of photosynthesis is

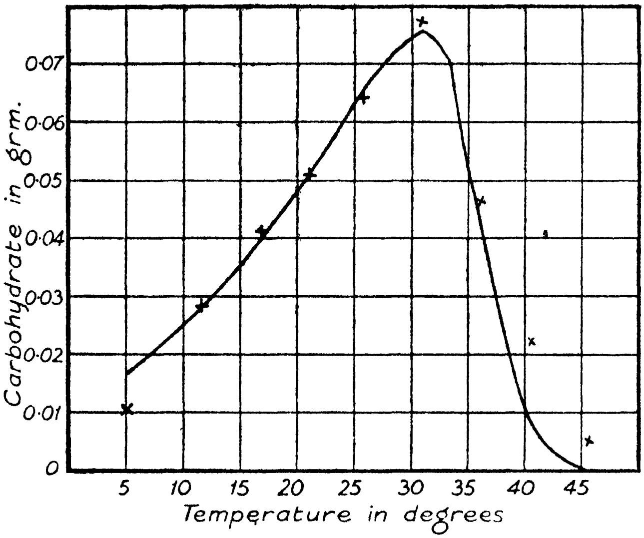

FIG. 24

a maximum is a direct function of the light intensity. The experimental observation of these phenomena has thus been quantitatively explained.

Lastly, quantitative explanations have been given of the effect of substituting intermittent for continuous irradiation and the effects of poisons and of narcotics on the rate of photosynthesis.

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