

### III

## THE PHOTOSYNTHESIS OF NATURALLY OCCURRING COMPOUNDS

**I**N my second lecture I laid some stress on the fact that every chemical reaction must consist of three separate and distinct phases. Since molecules normally exist in non-reactive phases, it is necessary before any reaction can take place to convert these phases into the reactive phases by the supply of energy. The first stage of every reaction consists in the supply of a definite increment of energy to each molecule, this increment being determined by the difference in energy content of the reactive and non-reactive phases. This first stage is followed by the second stage or reaction proper, in which the reactive phases of the resultants are produced. The third stage consists of the loss of energy by the reactive phases of the resultants, whereby their normal or non-reactive phases are formed.

It follows from this development of our knowledge of chemical change that reactions may be divided into two classes, namely, those in which the increment of energy necessary for the activation of the reactant molecules is small and those in which this increment is large. This subdivision is very important, because the ordinary methods used in the laboratory for the activation of molecules, namely, the action of heat, solvent, or material catalyst, are only capable of supplying small quantities of energy and hence of stimulating reactions the necessary energy increment of which is small. The experience of chemists who

use these methods is, therefore, entirely restricted to this class of reaction, and as long as we continue only to use these methods we cannot gain knowledge of those reactions the necessary increment of which is large.

This latter class of reaction is, to a great extent, a new field, a study of which promises well, since results may be expected which are entirely outside the experience gained by a study of the former. In my two previous lectures I have brought forward evidence in favour of a specific reactivity peculiar to each phase of a given molecule. The reactivities of the phases of a molecule with large energy content will be very different from those of the phases of the same molecule with small energy content; and I feel, therefore, that I am fully justified in saying that, if it be found possible to carry out reactions of the second class, the results obtained will be of more than ordinary interest.

A moment's consideration will show that any highly endothermic reaction must belong to the second class, since it is obvious that the larger is the excess of the activating increment of energy over and above the energy radiated in the third stage, the greater must be that activating increment. In all probability, therefore, a reaction of the second class must be sought amongst the highly endothermic reactions.

The question arises as to the possibility of supplying in such a reaction the very large energy increment necessary to activate the molecules, since, as has already been pointed out, the ordinary methods of laboratory practice are unable to supply to any molecule the large number of molecular quanta which together make this critical increment. It might be considered possible to supply the large critical increment by working at a very high temperature such as that of the electric furnace, but this is impossible owing

to the fact that the products of the reaction will be unstable at those high temperatures. On the other hand the molecular phase hypothesis at once suggests a method of supplying the necessary energy increment which is free from the difficulties caused by intensely high temperatures. If the energy is supplied to the reactant molecules at the frequency characteristic of their non-reactive phase, the energy so gained will be sufficient to activate them for any reaction, however endothermic this may be, since it may readily be proved that the energy quantum characteristic of a phase is sufficient just to resolve it into its component atoms. From the theoretical point of view, therefore, it should be possible to bring about any reaction, even a highly endothermic one, by activating the molecules by light of the same frequency as that characteristic of the phases of the reactants which are present.

A typical endothermic reaction is the conversion of carbonic acid into formaldehyde and oxygen, since the reverse reaction, the combination of oxygen with formaldehyde to form carbon dioxide and water, evolves about 127,000 calories per gram molecule. It is known that carbonic acid exhibits an absorption band with a central wave-length of about 2000 Angstroms and the energy quantum characteristic of that phase is  $9.84 \times 10^{-12}$  erg which corresponds to 144,000 calories per gram molecule. This is a measure of the energy absorbed by carbonic acid when exposed to light of the wave-length 2000 Angstroms and is much larger than the observed heat of the reaction. It follows that we have at hand in the absorption of light at the phase frequency a method of supplying the energy necessary to activate the molecules in a reaction of the second class, a method which is theoretically ideal in the sense that the activated molecules of the product are able at once to lose

their energy to the surroundings, which would not be the case if the reaction were carried out at very high temperatures. It will, however, be seen later that this ideal condition has not as yet been entirely realised in the laboratory.

The conversion of carbonic acid into formaldehyde has an added interest, since it has long been believed that this reaction forms the first stage in the photo-assimilation of carbon dioxide by the leaves of living plants whereby starches, sugars, etc., are synthesised. The observations made by Willstätter, that the molecular ratio of the oxygen transpired and of the carbon dioxide absorbed by leaves during photo-assimilation is unity, have been generally accepted as a proof of the formation of formaldehyde as the first product. For this reason the reaction in question is the fundamental reaction of all life, and its realisation in the laboratory becomes doubly important. It must, however, be remembered that the presence of formaldehyde has never been established in the living leaf; and there is no doubt that this fact introduces an element of considerable difficulty, since previous experience would lead us to believe, if a definite substance is formed in the first of a series of reactions, that the presence of that substance would be capable of detection, especially in the case of formaldehyde, the tests for which are peculiarly delicate.

Some years ago Moore and Webster investigated the action of ultra-violet light upon aqueous solutions of carbon dioxide and found that formaldehyde in small quantities was formed, but that this only occurred in presence of certain inorganic catalysts, such as colloidal ferric hydroxide, etc.<sup>1</sup> They argued from this that a catalyst is necessary for the photochemical reaction and suggested that

<sup>1</sup> Proc. Roy. Soc., 37, B, 163 (1914).

the importance of iron in the growth of a plant is to be found in this catalytic action. On the other hand, the necessity for a catalyst seems to be contrary to the theory, since the light energy alone should be able to promote the reaction without any further energy supplied by a catalyst. In spite of the negative results obtained by Moore and Webster the reaction was again investigated at Liverpool.<sup>1</sup> Carbon dioxide, prepared from pure calcium carbonate and synthetic hydrochloric acid, was passed through pure water contained in quartz test tubes, 8 x 1 inches, which were exposed to the light from a 220-volt quartz mercury lamp. Great care was taken to protect the carbonic acid solution from contamination by dust or any other impurities. It was then found that, if the distance between the lamp and test tubes was not less than 8 inches, small quantities of formaldehyde could always be detected in the solutions after twelve hours illumination. The presence of the formaldehyde was qualitatively proved by the Schryver test and the orcinol test, each of which is certainly capable of detecting the presence of one part in 1,000,000 parts of water. The amount of formaldehyde found was very small and was about 5 parts in 1,000,000, but there was no doubt whatever of its photochemical production from the carbonic acid.

In part at any rate the smallness of the yield of formaldehyde in these experiments is to be explained by the fact that formaldehyde is decomposed by very short wavelength radiations which are present in the light from the quartz mercury lamp. If dilute aqueous solutions of formaldehyde are exposed to this light, the formaldehyde is slowly decomposed, and after some hours no organic compound is to be found in the solution. It is evident, there-

<sup>1</sup> Baly, Heilbron, Barker, *Trans. Chem. Soc.*, 119, 1025 (1921).

fore, that when the rays from the quartz mercury lamp act on a solution of carbonic acid the formaldehyde produced undergoes decomposition, with the result that the amount present at any time can only be the excess of that formed over that decomposed, and this excess is very small. It is known that the destruction of the formaldehyde is caused by the very short wave-length light, since the interposition of a screen of calcite which absorbs these rays lessens the amount of decomposition. Unfortunately this screen also absorbs to a considerable extent the rays which activate carbonic acid, and, therefore, little benefit is gained by the use of such a screen between the lamp and the quartz tubes containing the carbonic acid.

The very short wave-length light is absorbed by air to a certain extent, so that its effect is diminished when the reaction vessels are placed at some distance from the lamp. This, no doubt, explains the negative results obtained by Moore and Webster with pure carbonic acid, since we found no evidence of formaldehyde when the distance between lamp and quartz test tubes was less than 8 inches.

It will be realised, therefore, that the conditions for the photochemical reaction are by no means ideal, since the source of energy emits rays which act on the products of the reaction. The ideal condition would of course be the entire exclusion of all rays except those which activate the carbonic acid. This failure to obtain the ideal conditions introduces serious difficulties into all work on photosynthesis, and these difficulties will become more apparent when the later work on this subject is described.

An obvious method of preventing the photochemical decomposition of the formaldehyde would be to introduce into the solution of carbonic acid a substance which absorbs the harmful rays. By direct observation it has been found

that the stability of formaldehyde, when illuminated by the unscreened rays of the quartz mercury lamp, is increased by the addition of certain inorganic compounds; and it may, therefore, be suggested that, in part at any rate, the rôle played by the colloidal ferric hydroxide, etc., in Moore and Webster's successful experiments on the photochemical production of formaldehyde from carbonic acid was protective rather than catalytic.

Many experiments have been carried out in order to determine the best conditions for demonstrating the photochemical production of formaldehyde from carbonic acid. The best method seems to be the formation of a fine spray of carbonic acid solution by means of an atomiser in the neighbourhood of the quartz mercury lamp. The spray falls into a large funnel and there is no doubt about the presence of formaldehyde in the liquid thus collected.

The photochemical reaction,  $\text{H}_2\text{CO}_3 = \text{CH}_2\text{O} + \text{O}_2$ , may now be further considered from the point of view of the three stages of the process. In the first stage we have the activation of the carbonic acid molecule by the absorption of the energy quantum,  $9.84 \times 10^{-12}$  erg, which takes place when the substance is exposed to light of the wavelength 2000 Angstroms. In the second stage we have the reaction proper with the formation of the activated molecules of formaldehyde and oxygen. In the third stage previous experience would lead us to believe that these activated resultant molecules lose energy and pass into their normal and non-reactive phases. On the other hand, as was first shown by Moore and Webster, a strong aqueous solution of formaldehyde, on exposure to the light from the quartz mercury lamp, develops the power of reducing Benedict's solution. By the action of the light the formaldehyde molecules are activated, and these activated mole-

cules undergo polymerisation to give compounds which are analogous to the hexose sugars in that they reduce both Benedict's and Fehling's solutions, the light rays which affect this reaction having the approximate wave-length of 2800 Angstroms.<sup>1</sup> Now, it is easy to prove that the reactive phase formed by the action of this light on formaldehyde has the same energy content, and hence the same reactivity, as the reactive phase formed by the action of light on carbonic acid. It is evident that we were incorrect in assuming that the third stage of the latter reaction consists in the loss of energy by the activated formaldehyde to give the normal and non-reactive form. I must confess that we were led astray by basing our expectations too much on previous experience, since the activated molecules produced from activated carbonic acid are characterised by their own specific reactivity, that is, the power of polymerisation to form reducing compounds analogous to the hexose sugars.

Recently the experiments on the action of ultra-violet light on aqueous solutions of carbonic acid have been repeated by Mr. M. R. Johnson in order to determine whether the reducing compounds are formed. The method was exactly the same as was previously described, and it may be stated that in every experiment the production of these compounds was proved. The amount formed is very small indeed and beyond the limit of the Benedict test as ordinarily carried out, but the delicacy of this test is very greatly enhanced if the reaction mixture after being heated for a few minutes is examined, not in ordinary light, but in the light from the quartz mercury lamp. Quantities of cuprous oxide quite invisible in daylight are readily seen in the light from the mercury lamp.

<sup>1</sup> Baly, Heilbron, and Barker, *loc. cit.*



It may be pointed out here that these reducing compounds must have been directly produced by the action of ultra-violet light on the carbonic acid, and not by the action of the light on the traces of formaldehyde simultaneously formed. Experiments carried out by Moore and Webster, and confirmed in Liverpool, have definitely proved that no reducing compounds are formed photochemically from formaldehyde at concentrations less than 0.5 per cent., and when light acts on carbonic acid the concentration of the formaldehyde produced does not exceed 0.001 per cent.

The smallness of the yield of the reducing compound is due, in part at any rate, to the absence of the ideal conditions previously defined. We have found that carbohydrates and analogous compounds are rapidly decomposed by the rays from a quartz mercury lamp, and consequently, once again, we never can find more than the excess of the amount produced over that decomposed. One of the products of photochemical decomposition of the carbohydrate, and analogous compounds, is ordinary formaldehyde; and thus the presence of ordinary formaldehyde in illuminated solutions of carbonic acid is undoubtedly a secondary product due to the photochemical decomposition of the reducing compounds, which are primary products of the action of light on carbonic acid. The formation of both products, reducing compounds and formaldehyde, afford strong evidence that the reaction,  $\text{H}_2\text{CO}_3 = \text{CH}_2\text{O} + \text{O}_2$ , does take place in ultra-violet light.

The foregoing evidence is by no means all that we have obtained in favour of the formation of activated formaldehyde by the action of light on carbonic acid. There is a considerable amount of evidence which is indirect in the sense that compounds formed from the activated formalde-

hyde can be obtained; and, for my own part, I believe this evidence to be of still greater value than that brought forward above.

If an aqueous solution of formaldehyde and potassium nitrite is exposed to the quartz mercury lamp, it becomes alkaline and there is synthesised the compound formhydroxamic acid,



This synthesis was first demonstrated by Baudisch who exposed an aqueous solution of potassium nitrite, containing methyl alcohol, to ultra-violet light.<sup>1</sup> No reaction takes place between these two substances in the dark at temperatures below 50°, but at higher temperatures the formaldehyde undergoes the Cannizaro reaction with the formation of methyl alcohol and formic acid. In the light from the quartz lamp formhydroxamic acid is synthesised at room temperatures, and it is thus evident that its formation is due to the combination of the activated formaldehyde with the potassium nitrite. As a photosynthetic product, formhydroxamic acid has the great advantage of giving an insoluble copper salt, the composition of which is perfectly definite. The formation of this compound when activated formaldehyde is produced in the presence of potassium nitrite has been established beyond any doubt by means of the copper salt. When solutions of carbonic acid containing potassium nitrite are exposed to the light from the mercury lamp, formhydroxamic acid again is produced, its presence being established by means of its copper salt.<sup>2</sup> This affords strong evidence in favour of the formation

<sup>1</sup> Ber., 44, 1009 (1911).

<sup>2</sup> Baly, Heilbron, and Hudson, Trans. Chem. Soc., 121, 1078 (1922).

of activated formaldehyde by the action of ultra-violet light on carbonic acid.

As will be seen presently from the detailed description of the experiments, mixtures of ammonia and formaldehyde give, under the influence of light, ring compounds containing nitrogen, such as piperidine and coniine. Since it is well known that these compounds are not formed by the action of ordinary formaldehyde on ammonia, their synthesis must be due to the activation of the ordinary formaldehyde by the light. When an aqueous solution of synthetic ammonia, saturated with carbon dioxide, is exposed to the light from the mercury lamp, compounds of the same type are produced, in one case piperidine hydrochloride, m.p. 233–235° being isolated in small quantities. Here, again, we have strong evidence of the formation of activated formaldehyde by the action of light on carbonic acid.

The third series of experiments are of a different type and deal with the action of the silent electric discharge on mixtures of hydrogen and carbon monoxide. As was first noted by W. Loeb, these gases condense to give compounds which reduce Benedict's solution, and recently this reaction has been investigated in Liverpool by Dr. J. H. Jones. We have found that a thick syrup is formed with a very strong reducing power towards Benedict's solution, and that some formaldehyde is simultaneously produced, but there is no evidence as to whether this is a direct or indirect product. Although these results are not exactly similar to those already described, it is of considerable interest that, by the supply of energy to mixtures of hydrogen and carbon monoxide, reducing compounds and formaldehyde are produced, especially since qualitative tests show that

the reducing compounds are analogous to those produced by the polymerisation of activated formaldehyde.

Returning once again to the fundamental reaction, the photochemical conversion of carbonic acid into formaldehyde and oxygen, I may refer to the difficulty caused by the total absence of formaldehyde in the living leaf. It would seem that this difficulty has now been disposed of, since the reaction does not give ordinary formaldehyde but activated formaldehyde which, in the absence of compounds with which it may combine, polymerises to form reducing compounds. The ordinary formaldehyde found in the laboratory experiments is a photochemical decomposition product of the reducing compounds and is not a component of the main reaction. Since the short wave ultra-violet light is not present in the sun's rays ordinary formaldehyde is not formed in the living leaf.

I will take this opportunity, before dealing with the criticism of this work, of describing the major difficulties which beset all these experiments on photosynthesis. I have already referred to one of these, caused by the impossibility of realising the ideal conditions, which results in the photochemical decomposition of the products of the photosynthesis. Not only does this reduce the yield to a very small amount, but it also introduces further complications caused by the presence of the decomposition products. In addition to this, trouble is also introduced by the well-known action of very short wave-length light on water to give hydrogen peroxide. Photochemical oxidation adds its quota to the total difficulties, but we have to a considerable extent succeeded in minimizing this by the use of an Al-Pt couple placed in the reaction mixture.

There is one phenomenon which caused a great deal of trouble in the early stages of the work and which was

entirely unexpected. It was found at one period of our investigations that the results obtained were alarmingly sporadic, being sometimes positive and sometimes negative, where previously they had been uniformly positive. As time went on the results became less and less satisfactory until a stage was reached when no positive result could be obtained, and no explanation of this could be found. One day, however, we found that one or two of the quartz tubes used in the work had developed an amethyst colour exactly similar to that of the natural variety, and obviously it had developed an absorptive power in the visible region. It was also found that the quartz had become opaque to ultra-violet light. On heating at about  $500^{\circ}$  these tubes developed a very fine green phosphorescence which soon vanished. On cooling, the tubes were found to be colourless and to have regained their transparency to ultra-violet light. On further investigation it was found that the opacity to ultra-violet light commences with the short waves and gradually extends towards the longer wave-lengths, until it finally reaches the visible region when the amethyst colour is developed. Long before the stage of visible colour is reached the quartz has become opaque to ultra-violet light and hence is useless for work on photosynthesis. When the significance of this phenomenon was realised every quartz tube used in the work, both coloured and colourless, was submitted to the heat treatment. Every tube phosphoresced, and when the treated tubes were employed in the photochemical experiments positive results were once again uniformly obtained.

The explanation of this phenomenon is not difficult to find on the molecular phase theory which was brought forward and discussed in my two previous lectures. By absorption of energy at the phase frequency in the extreme

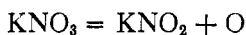
ultra-violet, the phase equilibrium normally present in quartz is displaced, and new, metastable phase equilibria of higher energy content are established, depending on the time of exposure to the light. Since the new phases have higher energy contents, their characteristic frequencies are smaller and are situated in the ultra-violet or visible region. As a result of this the quartz, when in a metastable condition, absorbs the ultra-violet rays and, in the extreme case, some of the visible rays. The time that a quartz tube takes to lose its transparency to the ultra-violet has not been determined, but a tube after it has once become opaque and undergone the heat treatment deteriorates more rapidly than an entirely new tube. If the tubes are in continuous use for photosynthetic work they should be subjected to the heat treatment once every fourteen days.

These observations have some significance in connection with the efficiency of the quartz mercury lamp. These lamps are usually made in one of three types which are designed to operate with continuous current at 100, 220, and 500 volts, respectively. In the case of the 100-volt lamps the temperature attained by the quartz during the operation of the lamp is not high, and as a result the phase change sets in and after a short time the lamp loses its efficiency as a source of ultra-violet light of short wavelength. The temperature attained by the 220-volt lamp is much higher and in this case the new phase equilibrium is not stable, so that this lamp loses little of its efficiency as a source of short wave ultra-violet light. We have had no experience of the 500-volt lamp, but in all probability the temperature reached during operation is higher still, so that the efficiency will not be impaired by continuous use.

At the meeting of the British Association for the Ad-

vancement of Science held at Saskatoon in August, Professor Porter communicated a very important paper on the photochemical conversion of carbonic acid into formaldehyde. In his early experiments he had no difficulty in proving the production of small quantities of formaldehyde when mixtures of carbon dioxide and water vapour were exposed to the light from a quartz mercury lamp. He found, however, that as certain sources of impurities were removed the yield of formaldehyde became progressively less, until in the complete absence of impurities no trace of either formaldehyde or reducing compounds could be detected. He found that the passage of the carbon dioxide through a lubricated stop-cock and through a short length of rubber tubing introduced sufficient impurities to "catalyse" the production of traces of formaldehyde.

These results are of singular importance and present a remarkable analogy with some observations recorded by W. T. Anderson.<sup>1</sup> The photochemical reaction



is a familiar one, and it is perfectly possible to demonstrate the presence of nitrite in a solution of potassium nitrate after it has been exposed to ultra-violet light.<sup>2</sup> On the other hand, Anderson has proved that in the complete absence of all impurities the reaction does not take place, but that a photochemical equilibrium is established



in which the amount of nitrite present is very small. When the exposed solution is removed from the radiation, the system reverts back to the nitrate and no nitrite can be detected. In the presence of oxidisable impurities one of

<sup>1</sup> J. Amer. Chem. Soc., 46, 797 (1924).

<sup>2</sup> Moore and Webster, Proc. Roy. Soc., 90, B, 158 (1919).

the components of the equilibrium is removed, and, therefore, the reaction will proceed from left to right when illuminated with light of the frequency characteristic of the nitrate.

The case of carbonic acid seems to me to be exactly analogous in that one molecule is photochemically converted into two molecules, one of these being oxygen. It may reasonably be suggested, therefore, that, in the complete absence of impurities, the illumination of a solution of carbonic acid will establish a photochemical equilibrium



in which the amount of activated formaldehyde is very small. If that is so, then, on removal of the illuminated solution from the radiation, the system will revert to carbonic acid and no formaldehyde or reducing compounds will be found.

It is a simple matter to put this suggestion to the test of experiment, since, if either of the components on the right-hand side be removed, the reaction will proceed from left to right. The removal of the oxygen can readily be effected by the addition of easily oxidisable impurities such as those very impurities Porter found were able to promote the reaction. It is also possible to remove the activated formaldehyde, since we know from independent observations that it combines with potassium nitrite to produce formhydroxamic acid. In the presence of potassium nitrite, therefore, the reaction should proceed from left to right with the formation of formhydroxamic acid. I have already described how formhydroxamic acid is actually produced when aqueous solutions of potassium nitrite, saturated with carbon dioxide, are exposed to short wave-length ultra-violet light.



The experimental evidence, therefore, supports the view that in the pure state a photochemical equilibrium is established when carbonic acid is illuminated and that the reaction will not proceed unless one or both of the components on the right-hand side are removed from the equilibrium. It may here be pointed out that in the case of the living plant the oxygen is removed, since it enters into combination with the pigments and remains in some form of combination until, finally, it is transpired into the atmosphere through the stomata.

The negative results obtained by Porter would seem to have a special value in that they have led to the realisation of the existence of the photochemical equilibrium,  $\text{H}_2\text{CO}_3 \rightleftharpoons \text{CH}_2\text{O} + \text{O}_2$ . They have led also to the correlation of various experimental results which afford a convincing proof of this equilibrium, and at the same time they give a second reason why the yield of the reducing compounds is so small when carbonic acid is illuminated, while the yields of the compounds formed by combination of the activated formaldehyde with other substances are relatively large. The existence of the photochemical equilibria with carbonic acid and with potassium nitrate is a matter of considerable theoretical interest, but this aspect of Anderson's and Porter's experiments cannot be discussed here.

Up to the present the photochemical production of activated formaldehyde only has been discussed from the point of view of the activation of the carbonic acid molecule by light of its phase frequency in the short wave ultra-violet region. When the conditions are considered under which this photochemical reaction is brought about in the living leaf, it is manifest that light of that frequency is not the activating agent, since it is well known that sunlight con-

tains no rays of such short wave-length. The problem of natural photosynthesis cannot be completely solved until an explanation is found of the mechanism utilised by the living leaf.

For our knowledge of the chemistry of the pigments which are present in the living chloroplast, and which undoubtedly form the basis of the photosynthetic mechanism, we are indebted to Willstätter. There are present four pigments, namely chlorophyll A,  $C_{55}H_{72}O_5N_4Mg$ ; chlorophyll B,  $C_{55}H_{70}O_6N_4Mg, H_2O$ ; carotin,  $C_{40}H_{56}$ ; and xanthophyll,  $C_{40}H_{56}O_2$ . The difference between chlorophyll A and chlorophyll B is just one molecule of oxygen and it is difficult to believe that this is purely fortuitous in view of Willstätter's statement that chlorophyll A forms an addition compound with one molecule of carbonic acid. Indeed Willstätter himself was the first to suggest the possibility that this addition compound, on exposure to light, is converted into formaldehyde and chlorophyll B in accordance with the equation



In the hopes of proving the truth of this suggestion Willstätter investigated the ratio between the amounts of the two chlorophylls during photo-assimilation of carbon dioxide, fully expecting to find that the ratio of chlorophyll B to chlorophyll A increased. He found, however, that the ratio remains constant and as a result of this he felt obliged to abandon his theory.

On the other hand, the relation between carotin and xanthophyll as regards oxygen is exactly the same as between the two chlorophylls, and consequently it is reasonable to suggest that carotin has the power of reducing chlorophyll B to chlorophyll A, itself being converted into xanthophyll.

The objection found by Willstätter in the constant ratio between the two chlorophylls now disappears, since the change in this ratio caused by the process represented in the equation above will be restored by the reduction of the newly formed chlorophyll B by the carotin. The place to look for change during photo-assimilation of carbon dioxide will be in the xanthophyll-carotin ratio which should tend to increase. This increase was actually found by Willstätter, but he did not link it up with his first suggestion regarding the function of the chlorophylls.

In view of this last mentioned observation by Willstätter I venture to suggest that the plant mechanism is a four component system involving three separate reactions as follows:

1.  $C_{55}H_{72}O_5N_4Mg, H_2CO_3 = C_{55}H_{70}O_6N_4Mg, H_2O + CH_2O.$
2.  $C_{55}H_{70}O_6N_4Mg, H_2O + C_{40}H_{56} = C_{55}H_{72}O_5N_4Mg + C_{40}H_{56}O_2.$
3.  $C_{40}H_{56}O_2 = C_{40}H_{58} + O_2.$

The final stage in which the xanthophyll is reduced to carotin must be effected with the help of an oxygen carrier, since the oxygen is not set free within the chloroplast but is translocated in the combined state through the leaf and transpired into the atmosphere through the stomata.

Two important points at once arise on this suggestion, first, the possibility of putting this scheme to the test of experiment and, second, the energy relations of the whole process. As regards the experimental proof, it may be said at the outset that one essential difficulty presents itself, namely, that of the solvent. Willstätter has shown that the properties of the two chlorophylls differ very materially in true and colloidal solution, and we have not yet succeeded in solving the problem. I may say, however, that

we have succeeded in establishing the reality of one step in the above scheme, namely, the oxidation of carotin into xanthophyll. Dr. Barker, Dr. Stead, and Dr. Robertson, working in Liverpool, have found that carotin in chloroform solution is oxidised by atmospheric oxygen, especially in light, to xanthophyll, the change having been proved by absorption spectra measurements. On the cautious addition of a solution of anhydrous ferric chloride in acetone to a solution of carotin in acetone, xanthophyll is also produced. In this case the xanthophyll was separated by the Tswett method of chromatographic analysis. This result is very encouraging, but as yet we have not succeeded in reducing xanthophyll to carotin. Many reducing compounds were tried, but it was found that either no action took place or the reduction was carried too far.

The energy relations are of peculiar importance, for we have already seen that in order to activate a molecule of carbonic acid, so that it may react to give activated formaldehyde and oxygen, a quantum of energy is required which is about  $9.84 \times 10^{-12}$  erg, and the question arises as to whether the pigments are able to supply this energy increment by absorbing their characteristic and smaller quanta of energy in the visible region. Of the three reactions specified above, the third may be ignored, since we know from Willstätter's observations that the ratio of xanthophyll to carotin tends to increase during photo-assimilation, and, therefore, the third reaction cannot play an integral part in the activation of the carbonic acid. Considering the first two reactions, we have three pigments on the left-hand side which must be activated, namely, chlorophyll A, chlorophyll B, and carotin. Now the absorption bands of these compounds differ to a certain extent in different solvents and we have no accurate measurement of the central

wave-lengths of the bands characteristic of the pigments in the chloroplast. In chloroform solution the central wave-lengths of the absorption bands of the two chlorophylls are 6670 and 6480 Angstroms, respectively, whilst the principal absorption band of carotin has a central wave-length of 4630 Angstroms. The characteristic quanta absorbed by the three compounds are, therefore,  $2.95 \times 10^{-12}$ ,  $3.04 \times 10^{-12}$ , and  $4.25 \times 10^{-12}$  erg, respectively. The sum of these three quanta is  $10.24 \times 10^{-12}$  erg which only differs from the quantum required to activate the carbonic acid molecule, namely,  $9.84 \times 10^{-12}$  erg, by 4 per cent. The three pigments, therefore, are able to absorb just the right amount of energy to activate the carbonic acid molecule, and, since we have an equilibrium condition between the three pigments with the carbonic acid in combination with one of them, we are safe in stating that the carbonic acid will be activated. The activated carbonic acid will at once react to give activated formaldehyde, since no question arises as to the photochemical equilibrium referred to on page 80 owing to the fact that the oxygen is not set free but remains in combination. On the other hand, further evidence of the reality of the photochemical equilibrium is to be found in the behaviour of the living plant, since photo-assimilation ceases if the plant is placed in oxygen under a pressure of two atmospheres.

The process detailed above, in which the light energy necessary for activating a substance is absorbed at frequencies other than those characteristic of the principal reactant, has been called photocatalysis, and a special case of this phenomenon has been studied by Daniels and Johnson.<sup>1</sup> I venture to believe that in the results detailed above, an explanation has been found of the mechanism

<sup>1</sup>J. Amer. Chem. Soc., 43, 72 (1921).

whereby photo-assimilation of carbon dioxide takes place in the living leaf. The results obtained in the laboratory with ultra-violet light afford convincing evidence that in the photochemical reaction formaldehyde is produced in an activated condition, and that, in this state, it possesses a reactivity which is entirely different from that observed in the laboratory under the ordinary methods of activation. The usually observed reactivities of this substance are those of phases of lower energy content than the phase which results from carbonic acid activated by its abnormally large energy increment. The highly activated formaldehyde obtained from carbonic acid does not lose energy to give ordinary formaldehyde (which therefore is not present in the living leaf) but undergoes certain definite reactions, two of which have been specified, namely, its ready polymerisation to give reducing compounds and its combination with potassium nitrite to give formhydroxamic acid.

An interesting feature of the photocatalytic production of activated formaldehyde in the living leaf and its subsequent reactions to give many of the products of plant life may be mentioned. I have already referred to the difficulties, associated with laboratory experiments on photosynthesis, which arise from the decomposition of the products by harmful rays emitted by the light force employed. This is not the case in the leaf where the photochemical conditions from this particular point of view are ideal.

I will now describe in detail the investigations we have made of the reactions characteristic of the activated formaldehyde, and will first deal with its polymerisation and the products formed in this reaction. In studying this process we have prepared the activated formaldehyde by the action of light on a concentrated aqueous solution of

ordinary formaldehyde. In the first place, Mr. H. G. Littler has determined the conditions for obtaining the best yield of the reducing compounds with a given intensity of the activating light, and the two most important controlling factors are the hydrogen ion concentration and the temperature. When the formaldehyde solution is exposed to the light the formation of the reducing compounds commences at once, but at the same time the solution develops an acidity due to secondary reactions. The yield of the reducing compounds is materially decreased in acid solution, and we further found that the yield is less in an alkaline solution than in a neutral solution. In order to neutralise the acid by-products it is necessary to have present an insoluble base which will react with them. Very many substances were tried and by far the best yield of reducing compounds is obtained if an excess of precipitated magnesium carbonate is present. When this reacts with the acids, carbon dioxide is set free, with the result that the final condition is obtained of a saturated solution of magnesium bicarbonate. The hydrogen ion concentration of this solution, judging from a considerable number of trials, is exactly the right condition for obtaining the best yield.

The use of precipitated magnesium carbonate, however, gives rise to an unexpected difficulty, since, when the concentration of the reducing compounds reaches a value of about 2 per cent., calculated as glucose, the carbonate dissolves in the solution and is photochemically deposited on the walls of the reaction vessel where the light enters the solution. This layer effectively protects the solution from the light and it becomes necessary to dismantle the apparatus and remove the layer by treatment with mineral acid, an operation which must be repeated at regular intervals. If calcium carbonate is substituted for the magnesium salt,

this action does not take place and, therefore, much time is saved. We have found that, in spite of the fact that the yield of reducing compounds obtained with the hydrogen ion concentration of a saturated solution of calcium bicarbonate is as much as 20 per cent. smaller, the time taken to obtain the same yield of reducing compounds is about the same in the two cases. In actual practice, therefore, we use precipitated calcium carbonate as the neutralising agent.

The remarkable influence of small differences in hydrogen ion concentration is a matter of considerable interest in view of the elaborate mechanism of buffer solutions in the living plant, obviously designed to maintain constant conditions of hydrogen ion concentration. It is probable, also, that this is a factor which controls all the reactions of activated formaldehyde, and not only its polymerisation. Again, it may be suggested that the special value of a saturated solution of magnesium bicarbonate is connected with the use of chlorophyll containing magnesium, particularly since Willstätter found that the chlorophyll A-carbonic acid complex in the free state gives magnesium carbonate on exposure to light. It may, very possibly, be the case that the decomposition is inhibited in the plant by the presence of magnesium bicarbonate in the chloroplast.

It is hardly necessary to point out that blank tests carried out with both magnesium carbonate and calcium carbonate and with formaldehyde in the absence of light have proved that no reducing compounds are formed. For example, a strong aqueous solution of formaldehyde, containing precipitated magnesium carbonate, gave no trace of reducing compounds after remaining in the dark for six weeks.

We have found it possible to carry out the photosyn-



thesis of the reducing compounds on a reasonably large scale by the use of a rectangular glass tank 15 inches square and 8 inches deep. Each of the four walls of the tank is drilled with a circular hole 2.25 inches in diameter, the centres of these holes being three inches from the bottom of the tank. In each hole a quartz tube, 6 x 2 inches, and closed at one end, is mounted as shown in Fig. II. About 20 litres of 40 per cent. formaldehyde solution is placed in the tank and a U-shape quartz mercury lamp is pushed into each of the quartz tubes. Cooling coils are mounted

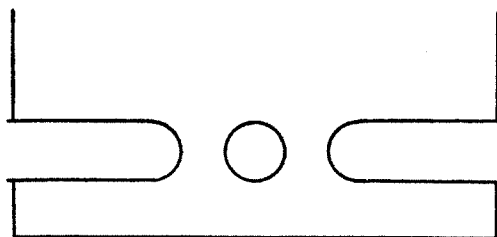


FIG. II.

in the tank and a constant stream of water is maintained through them. By controlling this stream of water any desired temperature can be obtained, and after many trials we found that the optimum temperature is about 37°. The tank is covered with a plate glass lid drilled with holes for the inlet and outlet water tubes, and a central hole for the rod of a stirring apparatus. During the exposure precipitated calcium carbonate is added from time to time so that it is always present in excess. Constant stirring is not maintained, as sufficient circulation is caused by the convection currents set up by the heat of the quartz lamps. An additional and necessary precaution may be mentioned, which must be taken in order to obtain the best yields. The very short wave ultra-violet rays from the lamps are

absorbed by atmospheric oxygen, which is thereby converted into ozone, and it happens that the absorption band of ozone is very near to that of formaldehyde. This reaction, which takes place within the two-inch quartz tubes, lessens the yield of the reducing compounds within the tank for two reasons. The formation of the ozone decreases the oxygen concentration, and thus allows more of the very short wave-length rays to pass into the solution and decompose the reducing compounds; and, further, the ozone absorbs some of the rays which activate the formaldehyde. A steady current of air is therefore passed into each of the two-inch tubes, and care must be taken that this current is not too rapid, since the efficiency of the lamps is materially reduced on cooling.

The amount of reducing compounds present is estimated every twelve hours and this reaches a maximum at about 6 per cent., calculated as glucose, since at this concentration the velocity of the photochemical decomposition of these compounds equals their velocity of formation. This maximum reducing power is reached with continuous illumination in about 14 days, there being present about 1200 grams of reducing compounds, in terms of glucose.

For the following method of separation of the reducing compounds from the unchanged formaldehyde, calcium salts, etc., we are indebted to Mr. Littler. The greater part of the formaldehyde is removed by evaporation under reduced pressure at 70°, a slow stream of air being continuously passed through the liquid, until the total volume is reduced to about 4 liters. During this operation a considerable amount of the formaldehyde undergoes the Cannizaro reaction to give formic acid and methyl alcohol; in order to neutralize the free acid, an excess of precipitated calcium carbonate is added before commencing the operation.

On addition of absolute alcohol to the concentrated liquid, the greater proportion of the calcium salts separates out. The filtrate is again concentrated under reduced pressure, in the presence of calcium carbonate, and again diluted with absolute alcohol, this being repeated until no further separation of calcium salts takes place. One of the photochemical decomposition products of the reducing compounds is glucuronic acid, and a special treatment is necessary to remove this compound since the calcium salt is soluble in alcohol. An exactly equivalent quantity of zinc sulphate in aqueous solution is added and the calcium sulphate removed by filtration. The filtrate is concentrated under reduced pressure in the presence of zinc carbonate, absolute alcohol is added, and the zinc glucuronate removed by filtration. This process is repeated until the zinc salt has been entirely removed.

The final filtrate is reduced in volume to a suitable concentration and chloroform is added, when the reducing compounds separate in the form of a thick syrup, which is collected, taken up in absolute alcohol, and again precipitated with chloroform. This process is repeated until the syrup is obtained free from formaldehyde. The small quantity of chloroform mixed with the syrup is removed by gentle heat under reduced pressure.

Thus prepared, the syrup is very viscous, transparent, and amber in colour. It exhibits a green fluorescence, is very sweet to the taste, and has a reducing power of 30–35 per cent., calculated as glucose. In a preliminary investigation of the syrup, attempts were made by Dr. Barker and Dr. Francis to prove the presence of hexose sugars by the process of exhaustive methylation. The results obtained were encouraging, since a small quantity of a methylated derivative was obtained, containing 60 per cent.

$\text{OCH}_3$ , which corresponds to that of a penta-methylated hexose. Formation of glucuronic acid is also of importance as evidence of hexose formation. Careful qualitative tests established the total absence of trioses and pentoses, non-formation of the latter being of considerable interest.

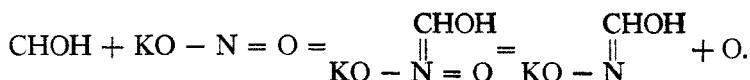
We are indebted to Principal Irvine, of St. Andrews, who, in conjunction with Dr. Francis, has carried out a systematic investigation of the photosynthesised syrup with the view of determining whether or no hexoses are present. Valuable evidence of their presence was first obtained in the formation of a methyl hexoside, and subsequently, by exhaustive methylation, they succeeded in preparing tetramethyl glucose. These results prove that there is present in the syrup about 10 per cent. of an aldose, probably glucose. This accounts for rather less than one-third of the total reducing power of the syrup, and the investigation of the remaining compounds is being actively pursued.

The proof that glucose is one of the products of the polymerisation of active formaldehyde is of considerable importance, since it establishes the fact that it is possible in the laboratory to reproduce the process taking place in the living plant to as far as the formation of one of the naturally occurring hexoses. The total absence of pentoses in our photosynthetic syrup indicates that these compounds in the living plant are not the direct products of photosynthesis, but that they are formed indirectly from hexoses or their condensation products, the starches and celluloses. The production of a hexose in preference to a pentose or triose, when activated formaldehyde polymerises, raises the very interesting question of the mechanism of this polymerisation, since it would be expected on the grounds of probability that the chances against hexose formation are the greatest. I venture to suggest the following mechanism as an explanation of the process.

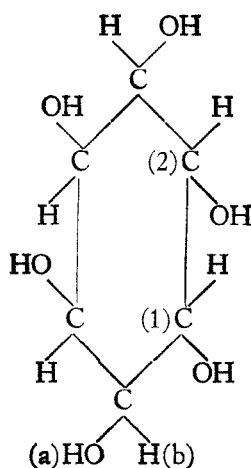
In view of the remarkable reactivity of activated formaldehyde it seems to me that it is necessary to picture a different formula from that given to ordinary formaldehyde. This abnormal reactivity is well expressed by the Nef

formula,  $C \begin{matrix} \diagup H \\ \diagdown OH \end{matrix}$ , with the carbon atom bivalent. The

formation of formhydroxamic acid, for example, by the union of activated formaldehyde with potassium nitrite can at once be understood



In the absence of compounds with which to combine, the substance CHOH will strive to attain two conditions, the most stable configuration possible and quadrivalence of the carbon atom. It may be accepted from the space lattice formulæ of diamond and graphite that the most stable configuration is the six-membered ring, and consequently by the polymerisation of six molecules of activated formaldehyde to form the ring compound



both aspirations will be satisfied. This molecule when freshly synthesised will be reactive, so we are justified in attributing to it properties such as are common in the experience of organic chemists. There are four changes which this molecule can undergo without introducing any novel properties:

1. The loss of water which, if the loss be symmetrical, will entail the loss of three molecules with the formation of phloroglucinol.
2. The migration of the hydrogen atom (a) to the carbon atom (1) and rupture of the ring to give the open chain formula of glucose.
3. The migration of the hydrogen atom (a) to the carbon atom (1) and of the hydrogen atom (b) to the carbon atom (2) with rupture of the ring to give the open chain formula of fructose.
4. The loss of energy by the molecule to give the non-reactive compound, inosite.

There are, therefore, four possible derivatives which in this way will be formed by the polymerisation of activated formaldehyde, namely, phloroglucinol, glucose, fructose, and inosite. All four substances are formed in the living leaf, and it would seem to be highly probable that they are all photosynthetically produced from carbonic acid through activated formaldehyde. It is to be expected, therefore, that all four substances should be present in the photosynthetic syrup prepared in the laboratory. The presence of one of these, glucose, has already been established, but Irvine has not been able to find any evidence of a ketose. Since fructose is less stable than glucose, the velocity of its photochemical decomposition will be greater than that of glucose, and consequently its absence from the syrup does not prove that it is not formed. Irvine is

of the opinion that a polyhydric phenol is present in the syrup, and we have obtained in Liverpool some evidence of the presence of a non-reducing hexose which gives the iodoform reaction. The further investigation of this syrup is being actively pursued and I have great hopes that still more evidence will be found in support of the above suggested mechanism of the polymerisation of activated formaldehyde.

We may now turn our attention to the problem of the photosynthesis of nitrogen compounds. It is known that the living plant obtains its supply of nitrogen from the nitrates of potassium and calcium, and also from ammonium salts. During their translocation from the roots to the leaves, the nitrates are reduced to nitrites, and, since it has been established that activated formaldehyde at once reacts with nitrites, it may be suggested that many of the nitrogen compounds found in plants are the direct products of photosynthesis. In order to test the truth of this suggestion a considerable amount of work has been carried out on the action of activated formaldehyde on nitrites and on ammonia with very promising results, since in both cases evidence has been obtained of the production of naturally occurring nitrogen compounds.

We may first deal with the action of activated formaldehyde on ammonia, a preliminary account of which has already been published.<sup>1</sup> Solutions of formaldehyde and specially purified ammonia were exposed to the light from the quartz mercury lamp for many hours, during which the solutions turned brown in colour. After a sufficient period of illumination had elapsed, the excess of formaldehyde was removed by repeated evaporation with fuming hydrochloric acid. Finally the dry hydrochlorides were

<sup>1</sup> Baly, Heilbron, and Stern, *Trans. Chem. Soc.*, 123, 185 (1923).

extracted with absolute alcohol, and the soluble hydrochlorides were again taken up in alcohol and fractionally precipitated with ether. In the end small quantities of a crystalline salt were obtained, which was the hydrochloride of a liquid base with the same odour as coniine. The hydrochloride gave all the well known alkaloidal reactions and the following tests specifically characteristic of coniine. It formed a double salt with potassium cadmium iodide, indistinguishable under the microscope from the same double salt prepared from the natural alkaloid. It gave with Dilling's modification of Melzer's test<sup>1</sup> the colour reactions which are specific for coniine. In addition to these, the photosynthesised alkaloid, gave the unmistakable odour of butyric acid on oxidation with potassium dichromate and sulphuric acid. Finally, its physiological action was the same as that of natural coniine.

This result was subsequently criticised by Snow and Stone,<sup>2</sup> who suggested that the substance was not coniine but tetramethylmethylenediamine, formed by the Werner reaction between ammonium chloride and formaldehyde. In support of this suggestion these authors showed that this base has the same physiological action as coniine and gives certain qualitative tests similarly to coniine, but not the two specific tests mentioned above. It is surprising that in putting forward this suggestion Snow and Stone ignored the fact that no Werner base could possibly have survived the repeated evaporation with concentrated hydrochloric acid, since these compounds are quantitatively hydrolysed by acid to formaldehyde and methylamine and dimethylamine. On this ground alone their criticism fails

<sup>1</sup> Arch. Pharm., 236, 701 (1898).

<sup>2</sup> Trans. Chem. Soc., 123, 1509 (1923).



and further work has given results which conclusively disprove their contention.

The investigation of the influence of light on mixtures of ammonia and formaldehyde has been repeated by Dr. Edith Morrison with an improved technique, including the use of the Al-Pt couple in order to eliminate photochemical oxidation as far as possible. In view of the peculiar difficulties of the identification of coniine in small quantities, it was decided to prove the intermediate formation of piperidine and subsequently to study the action of activated formaldehyde on this compound. Aqueous solutions of synthetic ammonia<sup>1</sup> and formaldehyde were exposed for several days to the rays from the quartz mercury lamp, an Al-Pt couple being placed in each reaction vessel. The resulting solution was repeatedly evaporated with concentrated hydrochloric acid until all the Werner bases had been completely hydrolysed. During this operation considerable quantities of ammonium chloride, methylamine and dimethylamine hydrochlorides separated out and were removed by filtration. The whole of the dry hydrochlorides were extracted with absolute alcohol, and the filtrate was evaporated to dryness and again extracted, until all the ammonium chloride was removed. A similar treatment was given with chloroform until all the methylamine hydrochloride was removed, this salt being insoluble in chloroform. The remaining mixture of hydrochlorides was again evaporated with fuming hydrochloric acid, once or twice, in order to decompose any possible residue of Werner base. The resulting mixed hydrochlorides, on the addition of alkali, now gave the unmistakable odour of

<sup>1</sup> The most rigid tests proved the total absence of all organic bases from this ammonia, for the gift of which we are indebted to Messrs. Brunner Mond, Ltd.

piperidine, and the following method was adopted of separating the piperidine from the dimethylamine and trimethylamine and also any coniine associated with it.

The mixed bases were set free by the addition of alkali, taken up in ether and the ethereal solution was gently warmed in order to drive off as much as possible of the two alkylamines. After cooling, dry hydrogen chloride was passed into the ether, when moist crystals separated out, and these were collected and evaporated to dryness with fuming hydrochloric acid. To an aqueous solution of the hydrochlorides gold chloride was added and, since dimethylamine and coniine do not form insoluble aurichlorides, the insoluble precipitate thereby obtained could only be the aurichlorides of piperidine and trimethylamine. The percentage of gold in this precipitate corresponded to a mixture of 92 per cent. of piperidine and 8 per cent. of trimethylamine. The following melting points were obtained, the aurichloride being used in each case

Piperidine .....	232.5-233.5°
Trimethylamine .....	243 -244°
50 per cent. Trimethylamine and 50 per cent. Piperidine	218 -229°
8 per cent. Trimethylamine and 92 per cent. Piperidine	230.5-231.5°
The last with more Piperidine.....	232.5-233.5°
Photosynthesised material .....	231.5-232.5°
Photosynthesised material and Piperidine.....	232.5-233.5°

This method of mixed melting points leaves no doubt of the reality of the photosynthesis of piperidine from ammonia and formaldehyde; and, therefore, additional evidence in favour of our original synthesis of coniine has been obtained. By the action of activated formaldehyde on piperidine more complex compounds are obtained, the nature of which is still under investigation.

I have already referred to the photosynthesis of form-hydroxamic acid by the action of activated formaldehyde on potassium nitrite, this having been first discovered by

Baudisch and fully confirmed in Liverpool. Mention may be made of the action of ultra-violet light on a solution of calcium nitrite in absolute methyl alcohol, which has recently been studied by Dr. Elwin Roberts. As is well known, short wave-length ultra-violet light decomposes methyl alcohol into formaldehyde and, probably, hydrogen, and consequently this method gives a convenient source of activated formaldehyde. The calcium nitrite solution in methyl alcohol, on exposure to the light from the quartz mercury lamp, deposits calcium formhydroxamate, together with some adsorbed nitrite, and the yield is almost quantitative. This result is important since it justifies our studying the further course of the reaction by acting on formhydroxamic acid, prepared from hydroxylamine and ethyl formate with activated formaldehyde. In a previous paper<sup>1</sup> it was stated that the photosynthetic process follows two courses, namely, the production of  $\alpha$ -amino acids and the production of nitrogen bases. Amongst the latter, glyoxalin and piperidine have been recognised. Evidence was obtained also of the condensation of individual compounds from each group to form substituted  $\alpha$ -amino acids, similar to those obtained by the hydrolysis of the proteins. The hope was expressed at that time that it should be possible to carry the photosynthesis further and obtain substances of a complex structure approaching that of the proteins themselves.

This work has been repeated and extended by Dr. H. L. Saunders, and many new and interesting results have been obtained, one of which may be described. Aqueous solutions of formhydroxamic acid and formaldehyde are exposed to the light from the quartz mercury lamp for eleven days, constant hydrogen ion concentration being

<sup>1</sup> Baly, Heilbron, and Hudson, *Trans. Chem. Soc.*, 121, 1078 (1922).

maintained by an excess of precipitated magnesium carbonate. After removal of excess of formaldehyde by distillation, about 0.5 gram of a substance is obtained, which is very soluble in water and insoluble in all the usual organic solvents. This compound does not give any characteristic qualitative tests for  $\alpha$ -amino acids, etc., but it contains nitrogen and forms a moderately stable hydrochloride. This salt gives an insoluble double salt with gold chloride, which has been analysed. By determination of the gold content the equivalent of the base was found to be 220, and further, 220 parts contain 35 parts of nitrogen. It follows that the minimum molecular weight of the compound is 440 and that in that minimum weight there are 5 atoms of nitrogen. This, in itself, is evidence of the complexity of the products which can be produced by photosynthesis from potassium or calcium nitrite and activated formaldehyde.

Since this compound evolves ammonia on boiling with sodium hydroxide, its hydrolysis by hydrochloric acid was studied. Dilute acid has no action, but on prolonged boiling with concentrated acid hydrolysis takes place, and amongst the products the presence of an  $\alpha$ -amino acid has been recognised. It is possible that this acid is histidine, since it gives the colour reactions with diazobenzenesulphonic acid identical with those given by natural histidine. Since the original compound, before hydrolysis, gives no reactions for  $\alpha$ -amino acids, the suggestion may be made that it is a condensation product of two or more  $\alpha$ -amino acids, and that it marks a further stage towards the photosynthesis of a protein. It is of some interest, therefore, that the compound gives insoluble products with all the usual reagents for proteins, including basic lead acetate,

potassium dichromate, potassium ferrocyanide, Millon's reagent, etc.

The above results afford strong support to the view that the activated formaldehyde, produced photochemically in the living leaf, not only undergoes polymerisation to give hexoses, but also reacts with nitrites and ammonium salts to give  $\alpha$ -amino acids and nitrogen bases, these in each case being the first products of the various reactions. As the result of the high energy content of these compounds when first synthesised, they are endowed with great reactivity and undergo condensation to give compounds which are actually found in nature. Direct evidence of such condensation has been found in the case of the  $\alpha$ -amino acids and nitrogen bases. Arising from this view some attractive possibilities suggest themselves, one of which may perhaps be mentioned. It is known that certain bases, such as piperidine, do not enter into the constitution of proteins; that is to say, they do not condense with  $\alpha$ -amino acids to form substituted  $\alpha$ -amino acids. On the other hand, we have found that piperidine is formed by the action of the activated formaldehyde on ammonia and also on formhydroxamic acid, and we may, therefore, conclude that it is formed in the living plant. The plant thus produces bases which it cannot use in making protein, and the suggestion may be made that it converts these unwanted children of photosynthesis into alkaloids by the further action of activated formaldehyde. More generally, it may be suggested that, even though the plant may have learnt how to make use of the alkaloids, these compounds represent the final non-toxic end-products obtained, under the particular conditions existing in the various species, by the action of activated formaldehyde on those bases which are toxic and cannot be utilised in the making of proteins. This sug-

gestion draws attention to the remarkable constancy of alkaloid production both as regards nature and amount in each species of plant. In view of the importance of hydrogen ion concentration in the polymerisation of activated formaldehyde, it is very possible that the nature of the alkaloid produced is also governed by the hydrogen ion concentration. Obviously, therefore, all experiments on photosynthesis with activated formaldehyde should be carried out under absolutely constant conditions of hydrogen ion concentration, as otherwise complex mixtures of different alkaloids will be obtained. This necessity was not realised in the earlier experiments, and a systematic investigation on these lines is now being carried out.

It is evident from what has already been said that when activated formaldehyde is produced in the presence of a nitrite the two substances react together and that this reaction takes precedence of the polymerisation to form hexoses. We have found, however, that if the concentration of the nitrite is small, compared with the amount of activated formaldehyde which is being produced, both reactions take place simultaneously; that is to say, reducing sugars and nitrogen compounds are synthesised at the same time. This is a condition which obtains in the living leaf, and there is no difficulty in understanding how the two processes take place concurrently. The photosynthetic production of nitrogen bases from activated formaldehyde and ammonia or nitrites must be accompanied by the evolution of oxygen over and above that associated with the formation of the activated formaldehyde from carbonic acid. If, therefore, the living plant produces nitrogen bases by photosynthesis, the amount of oxygen transpired into the atmosphere during the photo-assimilation of carbon dioxide must be greater than that expressed by the

molecular ratio  $O_2/CO_2 = 1$ . It is of considerable interest therefore that the actual value of this ratio as determined by Willstätter is 1.05.

At the commencement of this lecture I laid some stress on the possibility that the chemical processes associated with a reaction of the second class (a reaction with a very large activating increment of energy) might be very different from those with which we are familiar from our long experience of reactions of the first class. I think I was justified in making that statement, for there is no doubt that the photochemical conversion of carbonic acid to formaldehyde is a reaction of the second class and that the subsequent reactions, although few have as yet received the study they deserve, are of a somewhat novel type. The formation of glucose and the synthesis of piperidine may be classed as different from the reactions of every-day chemistry. Similarly, also, the condensation of  $\alpha$ -amino acids with certain nitrogen bases to form substituted  $\alpha$ -amino acids is outside the practical experience of organic chemists. It has long been realised that in the living plant hexoses condense together to give sucrose, starches, and celluloses, whilst bases and amino acids condense together to give first the substituted amino acids and then the proteins. Our ignorance of the forces at work has led at times to a belief in something mysterious, something perchance inexplicable, which is associated with life, for the whole is so strange to us in spite of the great advances made by the pioneers in organic research.

In a spirit of true reverence for Dame Nature and of high appreciation of her work, I have attempted to show you that the chemistry of the living plant may be but a chemistry of high energy. The great initial stimulus given to the carbonic acid by the energy which the sun pours

upon the earth inaugurates a series of chemical changes. Each and all of these form part of a sequence of events, every step of which marks an energy loss whereby the molecules come nearer and nearer to the states that are familiar to us. We have knowledge of the inorganic compounds from which the story starts, we have knowledge of the products where the story ends, but between the two there lies a tale of high energy, a tale of molecular derring do which the dull chemistry of the test-tube and beaker fails to envisage—and thus has been born the germ of mystery. I beg you not to hug this mystery as sacrosanct, but rather find delight in the romance of molecules, not so far removed from the romances of our youth which taught us all to emulate the noble deeds of men inspired by love and high ideal. I beg you find romance in the noble deeds of molecules inspired by light and high energy, for after all what nobler deed can there be than to promote and sustain life?

E. C. C. BALY.









