

*The Mechanism of Carbon Assimilation in Green Plants: the
Photolytic Decomposition of Carbon Dioxide in vitro.*

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In a previous paper* it was shown that carbon dioxide is decomposed in the green parts of plants independently of vital or enzymic activity, formaldehyde and hydrogen peroxide being produced. It follows from the analysis of the process of carbon assimilation there set out that this first step, the photolysis of carbon dioxide, should be capable of being artificially induced under laboratory conditions.

It is impossible here to give even a brief account of the work of previous investigators on these lines; it is sufficient to state that all experiments with chlorophyll solutions have given negative results, and as regards those with other forms of chlorophyll, such as dried powdered leaves or expressed juice, the balance of evidence favours the view that no decomposition takes place. Reference may be made to papers by Friedel† and Macchiati‡ on the positive side, and by Harroy,§ Herzog,|| and, quite recently, Bernard,¶ who obtained only negative results. Experiments with uranium compounds will be considered later.

I. Experiments on the Chlorophyll Catalysis of Carbon Dioxide.

In view of the very perfect chemical and physical arrangements which assist the decomposition of carbon dioxide in the plant, it is obvious that attention must be paid to these points in any experiment performed outside it. It appears likely that the failure of so many attempts on the part of previous observers to carry this out has been due to a neglect of such considerations—not so much with regard to the chemical as the physical conditions. The chemical conditions are those which are necessary when any other highly reversible action is being dealt with, viz., that one at least

* 'Roy. Soc. Proc.,' B, vol. 77, p. 369.

† 'Comptes Rendus,' 1901, vol. 132, p. 1138.

‡ 'Bull. Soc. Bot. Ital.,' 1903.

§ 'Comptes Rendus,' 1901, vol. 133, p. 890.

|| 'Zeit. physiol. Chem.,' 1902, vol. 35, p. 459.

¶ 'Comptes Rendus,' 1905, vol. 140, p. 509.

of the products be rapidly removed from the sphere of action. The physical conditions were first enunciated by Timiriazeff, in the Croonian Lecture for 1903, and are as follows:—(1) The optical sensitiser must be in the form of an extremely thin film; and (2) the concentration of the sensitiser in this film must be so great as to ensure a large transformation of the incident solar energy within a very small space. The fulfilment of both these conditions in the case of chlorophyll in the plant is a matter of common knowledge, and the experiments to be described were designed to reproduce as far as possible the essential mechanical, physical, and chemical arrangements which obtain in the green leaf.

It should be mentioned here, as the result of some observations by one of us in conjunction with Miss Irving* on the structure of the large chloroplasts of *Selaginella* and *Chlorophytum*, that the chlorophyll is restricted to the exterior of the granule, and that the thickness of the film is about 2.5×10^{-3} mm. Whether this applies generally or not is of no consequence in this connection, the essential point being that we have here an actual working arrangement, and one which can be imitated without difficulty.

The greatest possible concentration of chlorophyll is obtained when the solvent used for extracting it has been completely driven off, and if material is employed which contains very little else that is soluble in the liquid in which the final solution of the chlorophyll takes place; one may regard the concentration of the chlorophyll thus obtained as being approximately the same as in the plant. For these experiments the material used was the leaves of grass or wheat, chosen on account of their comparative freedom from fats and oils. Alcohol was used for the extraction, and petroleum ether for the final solution of the chlorophyll.

In the first series of experiments pieces of glass plate, 5×4 inches, were covered with an aqueous solution of gelatine so as to form a layer 1 to 2 mm. thick. When set, this was painted over with a solution of chlorophyll in petroleum ether or benzene. In this way a fairly uniform film of chlorophyll is obtained, and if, when the plate is placed in carbon dioxide, light is allowed to strike it in the direction \rightarrow carbon dioxide \rightarrow chlorophyll \rightarrow gelatine, we have a reproduction of the essential features of the arrangement in the living cell. Measurements of the thickness of the chlorophyll film gave an average value of about 6×10^{-3} mm.

On placing plates prepared in this manner in a bell jar containing moist carbon dioxide and exposing to light, the chlorophyll in the course of a few hours became completely bleached, and the gelatine developed a red

* At present unpublished.

colour when immersed in a solution of rosaniline decolourised with sulphurous acid. The experiment was repeated several times with the same result, and when performed on a larger scale the distillate from an aqueous solution of the gelatine was found to contain formaldehyde, the substance being identified by the characteristic methylenedianiline and tetrabromohexamethylenetetramine reactions. The melting point of the methylenedianiline was within 2° of that of the pure substance. It will be seen that the action which takes place under these conditions is the same as that which occurs in a green plant when the protoplasm has been killed and the enzymes destroyed; the accumulation of formaldehyde is possible so long as any chlorophyll remains to remove the hydrogen peroxide. The use of gelatine may be avoided by a modification of the arrangement just described. A layer of water in a shallow porcelain dish replaces the gelatine, and the chlorophyll film is obtained by dropping the petroleum ether solution over the surface from a pipette, at the same time directing a current of air against it so as to evaporate all the solvent before the chlorophyll can collect in patches. Experiments performed in this manner gave the same results as those with the gelatine films. The latter are more convenient to manipulate, but it is preferable to dispense with the gelatine if the object is to collect as much formaldehyde as possible, since this substance has a larger concentration in the residue than in the distillate.

A second series of experiments was undertaken with the object of ascertaining whether the process could be carried as far as the evolution of gaseous oxygen. It has already been pointed out in a previous paper that the decomposition of the hydrogen peroxide in the plant is brought about by an enzyme, probably a "catalase," and it was also shown that a certain amount of oxygen can be evolved from plants which have been killed, provided that the enzymes are not destroyed.

The catalase employed was obtained from sheep's liver by extracting the dried and powdered material with water containing a little thymol, the enzyme being precipitated by excess of absolute alcohol. The arrangement of the experiments was the same as in the first series, but the gelatine was made up with an aqueous solution of the catalase instead of with pure water, and the solution thus prepared was spread upon a strip of tinned iron 60 cm. long and 3 cm. wide. After painting it over with chlorophyll solution, the strip was placed inside a glass tube, sealed at one end, just large enough to contain it, and which was afterwards drawn out at the open end to a capillary, and sealed to a T-piece connecting it, through two stop-cocks, with a supply of carbon dioxide, and a pump. The carbon dioxide used was passed over red-hot copper in order to remove traces of oxygen, and the tube was

exhausted and refilled six times. A similar tube without any catalase was treated in the same way. When the tubes had been filled with oxygen-free carbon dioxide for the sixth time, they were sealed off at the capillary portion and exposed to sunlight. In the course of an hour the gelatine in the tube containing the enzyme was swollen with bubbles of gas, and the chlorophyll film became distorted and broken up. The only visible change in the other tube was the bleaching of the chlorophyll. When the tubes had been exposed to light for two days they were attached to a Töpler pump by a short piece of rubber pressure tubing. After completely exhausting the system on the pump side of the capillary, the latter was broken off inside the pressure tubing, and the gas pumped out through a tube packed tightly with soda-lime, which absorbed all but a trace of the carbon dioxide. The tube containing the catalase yielded 0.6 c.c. of oxygen, with a trace of nitrogen, while the other gave a minute bubble of nitrogen only, the latter being derived in each case from the air present in the carbon dioxide before its passage over the red-hot copper. Two more similar experiments gave 2 c.c. and 1.2 c.c. of oxygen respectively.

It was found that if the exposure to light was continued until the chlorophyll was bleached, the enzyme was poisoned by the accumulating formaldehyde. This is precisely what occurs in the plant under similar conditions; that is, when the protoplasm only has been killed.

According to the analysis of the photosynthetic process given in the paper already referred to, where it was shown that the condensation of formaldehyde to carbohydrates is dependent upon the healthy condition of the protoplasm, one would expect non-chlorophyllous living cells to be able to bring about this change. It appears from the feeding experiments of Bokorny, Bouilhac, and Tréboux that light is necessary in the case of green plants, and we have found that this is also the case with the white petals of *Saxifraga Wallacei*, which are capable of forming starch in the light from a 0.001-per-cent. solution of formaldehyde, though no condensation takes place in the dark. In a further series of experiments these petals, when quite free from starch, were painted with chlorophyll solution, and floated on water charged with carbon dioxide in a bottle placed in the light. In the course of a day they were found to contain starch. In this case we have what is essentially a green leaf arranged in a different way, though it is, of course, a very inefficient substitute for the natural organ.

As it was of interest to ascertain whether formic acid is actually produced as an intermediate product in the photolytic reduction of carbonic acid to formaldehyde, an experiment similar to those described in Series I above was performed, the gelatine being dissolved in a solution of sodium bicar-

bonate. In this case the whole of the bicarbonate was converted into formate. When neutral or slightly acid gelatine was used, no formic acid could be detected. With very large concentrations of carbon dioxide, formic acid appears to be the sole product, since when sealed tubes containing chlorophyll, water, and liquid carbon dioxide were exposed to light, no formaldehyde was found, though a considerable quantity of formic acid was produced.

If, however, formic acid is an intermediate product in the photolysis of carbon dioxide in the plant, the latter should be able to build up carbohydrates from this substance. This was found to be the case with *Elodea*, which, when exposed to light in a 0.02-per-cent. solution of formic acid, gave off oxygen and formed starch; no starch was formed, or oxygen evolved, from *Elodea* kept in the dark, carbon dioxide being carefully excluded in each case. When the plant was previously killed, the same changes took place as when carbon dioxide was used, that is to say, the chlorophyll became bleached, and the plant was subsequently found to contain formaldehyde.

II. *The Photolytic Decomposition of Carbon Dioxide in the Presence of Uranium Compounds.*

In a previous paper, to which reference has been made above, it was stated that Bach's observations had been repeated and confirmed in the case of uranium acetate, minute quantities of formaldehyde being found in the distillate. Since then our attention has been called to a recent paper by Euler,* who was unable to confirm Bach's observations. A more detailed investigation has therefore been made, and, since it cannot be proved that any particular organic substance which may be produced is formed from the carbon dioxide, and not from the electro-negative portion of the organic salt when the latter is employed, a purely inorganic sensitiser has been used in the present series of experiments.

In the first of these, which was a repetition on a larger scale of one which has already been described, a large Pettenkofer tube was filled with a litre of 2-per-cent. uranium sulphate solution, and a slow current of carbon dioxide was passed through it, the whole apparatus being placed on the roof in order to utilise all the available sunlight. Within an hour of passing the carbon dioxide the contents of the tube were considerably turbid, and at the end of the exposure, which lasted from January 27 till February 9, the precipitate amounted to several grammes, and was of a pale violet colour. The greater part was soluble in acetic acid, and was found to be a mixture of uranous

* 'Ber. deut. chem. Ges.,' 1904, vol. 37, p. 3415.

and ordinary uranic hydroxides. The insoluble residue* was a hydrate of uranium peroxide, being characterised by its insolubility in acetic acid, and also by the fact that it imparted a brownish-red colour to a solution of titanium oxide in sulphuric acid. The filtrate from the mixed hydroxides was distilled, and the distillate was examined for formaldehyde. None, however, was found, though the liquid energetically reduced Fehling's solution and silver nitrate. It was subsequently found to contain formic acid, the lead salt of which was prepared and identified. The undistilled residue was then evaporated down at 100°, and when nearly solid was repeatedly extracted with dry ether, in order to remove any formic acid which had not evaporated. The residual solid was extracted with absolute alcohol, and the solution on evaporation left a small quantity of a brown syrup, bitter to the taste, which reduced Fehling's solution. When treated with phenylhydrazine acetate no crystalline derivative could be separated, nor could the syrup itself be induced to crystallise. On account of its appearance, taste, and reducing action, it presents a striking resemblance to the substance obtained by Butlerow† from formaldehyde and milk of lime, and called by him "methylenitan." It has been shown that formaldehyde, when allowed to stand for some time in presence of uranic hydroxide, gives rise to a substance with similar properties. It does not appear possible to make any more explicit statement with regard to the identity of the substance produced from carbon dioxide in the manner described with methylenitan, since it is produced only in minute quantities, and does not admit of purification, or of the preparation of a derivative which can be purified; it is, however, worth while to notice that, as regards the two substances produced by the action of light on carbon dioxide in the presence of uranium sulphate on the one hand, and by the condensation of formaldehyde by uranic oxide on the other:

- (1) They are both organic substances which char on heating.
- (2) Both are insoluble in dry ether, and soluble in water and alcohol.
- (3) Both have a bitter taste, and reduce Fehling's solution.
- (4) Neither reacts with phenylhydrazine.

Since it was conclusively proved by this experiment and those which preceded it that formic acid is one of the products of the action of sunlight on carbon dioxide, and in view of the fact that the quantity of any product formed is materially influenced by the concentration of the substance giving rise to it—which is necessarily small when that substance is carbon dioxide—it was thought that some light would be thrown on the reaction if one started with

* UO_4 dissolves slowly in acetic acid, if uranous compounds are present.

† 'Comptes Rendus,' vol. 53, p. 145; 'Liebig's Annalen,' vol. 120, p. 296.

formic acid, instead of with carbon dioxide. For this purpose a solution of uranyl formate was employed, and the method has the advantage that any removal of formic acid from the system is indicated by a corresponding precipitation of uranic hydroxide. The uranyl formate for these experiments was prepared by heating uranyl nitrate (obtained from Kahlbaum) until oxides of nitrogen were no longer evolved, and dissolving the residual uranic oxide in the least possible quantity of 5-per-cent. formic acid. Preliminary experiments were carried out in order to ascertain whether any visible change takes place when the solution is exposed to sunlight. It was found that on placing a flask containing it in the light, the liquid became turbid after about 10 minutes' exposure, and in half an hour a considerable precipitate had accumulated. The precipitate at first produced is of a pure yellow colour, and consists of ordinary uranic hydroxide. After a short time it becomes violet-coloured, and, when a considerable quantity has collected, consists of a mixture of uranic and uranous hydroxides, together with small quantities of uranium peroxide. As in a large number of experiments no formaldehyde could be detected, several experiments were performed with a view to ascertain whether any gaseous products were formed. In one of these, a glass tube about 30 cm. long and 2 cm. bore was half filled with a solution of uranyl formate, drawn out to a capillary, exhausted, and sealed. After four days' exposure to sunlight the tube was connected with a Töpler pump and about 50 c.c. of gas were collected, which on analysis was found to consist of approximately equal volumes of carbon dioxide and hydrogen. This experiment was repeated several times, with the same result, though the carbon dioxide and hydrogen were not always produced in the same proportions. It should be stated that any uranium salt which remained in solution was invariably reduced to a green uranous compound, and this, no doubt, accounts for the variations observed in the relative volumes of the two gases.

The foregoing experiments show that carbon dioxide in the presence of uranium compounds gives rise, under the influence of light, to formic acid, and that formic acid under the same conditions gives rise to carbon dioxide and hydrogen. It is probable, for reasons which will appear later, that we are dealing with several consecutive reactions which, under the conditions of the experiments, have widely different velocities, and which are not strictly reversible. If this be so, it follows that it will be very difficult, if not impossible, to isolate certain intermediate products. For example, in the case of the first series of experiments with uranium sulphate, it may be questioned whether the formic acid found really represents the first stage in the photolytic decomposition of carbon dioxide; it may quite possibly be the final product of a reaction between formaldehyde and one of the hydroxides

present, following on the primary reaction which gave rise to the formaldehyde, and having a much higher velocity. In such a case, one could not expect to find formaldehyde in the final mixture. As the question at issue is whether or not formaldehyde is produced at any stage in the series of reactions which take place when sunlight acts on carbon dioxide under the conditions described, and as it was found that formaldehyde could not in any way be isolated from the mixture, it appeared that a careful study of the reactions of formic acid and aldehyde with such substances as were formed under the conditions of the experiments was the method most likely to settle the question. It may be worth while to point out here that a careful distinction must be maintained between those reactions which are endothermic, and derive the necessary energy from the sunlight, and those in which the action of light is purely catalytic.

Solutions of uranium salts—especially those like the formate—have always an acid reaction, and are probably hydrolysed to a considerable extent, so that any hydrogen peroxide that is formed is not completely removed from the system, but remains in equilibrium with the difficultly soluble uranium peroxide. We have, therefore, to consider reactions which may occur between

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|-------|-----------------|-----|-------------------|
| (i) | Formic aldehyde | and | uranium peroxide. |
| (ii) | „ acid | „ | „ „ |
| (iii) | „ „ | „ | hydrogen „ |
| (iv) | „ aldehyde | „ | „ „ |
| (v) | „ „ | „ | uranic oxide. |

It has been shown by Geisow* that when hydrogen peroxide reacts with solutions of formaldehyde in neutral or acid solution, hydrogen and carbon dioxide are the sole products, no formic acid being produced. On the other hand, the peroxides of lead and manganese give hydrogen and a metallic formate. It has been found that uranium peroxide reacts in the dark with formaldehyde, and that hydrogen and uranium formate are produced, but no carbon dioxide. Hence, in such a system as the one under consideration, where there is a constant but very small concentration of hydrogen peroxide, there is the possibility of explaining the production of both hydrogen and carbon dioxide, the reaction taking place between the formaldehyde and hydrogen peroxide, though, of course, at the expense of the uranium peroxide and this appears to be the probable explanation, when we consider that the velocity of reaction in the case of hydrogen peroxide must be very much greater than in the case of uranium peroxide. The fact that hydrogen and

* 'Ber. deut. chem. Ges.,' 1904, vol. 37, p. 515.

carbon dioxide are formed as the result of the interaction of hydrogen peroxide and formaldehyde is a piece of evidence which strongly supports the view that the latter substance is formed in the experiments we are considering, provided that formic acid does not give rise to these gases under similar conditions. As no record of experiments on these lines could be found the point was investigated. The mixture of formic acid and hydrogen or uranium peroxide was placed in a Zeisel flask with a condenser attached. This was first connected with some bulbs containing lime-water, and a current of CO_2 -free air was passed through the apparatus while the mixture was heated on a water-bath. Considerable quantities of carbon dioxide were evolved, as shown by the precipitation of calcium carbonate. A fresh quantity of the reaction-mixture was then taken, and the apparatus connected with a Schiff's nitrometer while a current of carbon dioxide was passed. Not a trace of hydrogen was evolved, although the mixture was boiled for three hours. There is apparently no difference between the reactions in the case of hydrogen peroxide and uranium peroxide, except that the latter proceeds more slowly. These experiments were carried out in the dark.

Experiments with ordinary uranic oxide showed that formaldehyde reduced it both in the dark and in the light, uranous oxide and a formate being produced, but only in the light was the violet colour observed. It appears that this coloration is merely a surface effect, since the precipitate, when dissolved in acid and reprecipitated, has the ordinary black colour of uranous oxide.

The following experiments bring out rather more clearly the distinction between formic acid and aldehyde mentioned above:—

To one of two tubes containing pure uranium peroxide, a dilute solution of formaldehyde was added; to the other, some dilute formic acid. After exposure to light from February 16 to February 19, no effect was visible in the tube which contained formic acid, whereas the other contained uranous formate and a violet-coloured precipitate. In order to examine the gases produced in the two cases, two more tubes were prepared, one of which (*a*) contained uranium peroxide, formic acid, and formaldehyde, while the other (*b*) contained uranium peroxide and formic acid only. Both were drawn out to a capillary, exhausted, and sealed. They were exposed to light from February 27 till March 6, when the gases were pumped out and examined: (*a*) contained 4.8 c.c. of carbon dioxide and 4.8 c.c. of hydrogen, while (*b*) contained 9.8 c.c. of carbon dioxide and no hydrogen.

There is thus no direct experimental evidence that formaldehyde is produced under these conditions, that is to say, it has not been isolated and

identified, but there is indirect evidence of its formation at some stage. Whilst hydrogen is always found among the products of the action of light on carbon dioxide in the presence of uranium compounds, under no conditions in any way approaching those of the experiments in question has hydrogen been obtained from formic acid. The reduction of uranic oxide, or of uranium peroxide, to a violet-coloured lower oxide, which was always observed in the experiments with carbon dioxide, can again only be brought about by formaldehyde; whilst the formation of a substance with the properties of methylenitan, which has only been prepared from formaldehyde, furnishes additional support to this view.

Summary.

I.—(1) Photolytic decomposition of aqueous carbon dioxide can take place in the presence of chlorophyll, independently of vital or enzymic activity, provided that the necessary physical and chemical conditions are strictly adhered to.

(2) The products of the decomposition are formaldehyde and hydrogen peroxide, formic acid being an intermediate product.

(3) It is possible to reconstruct the process of photosynthesis outside the green plant, (*a*) as far as the production of formaldehyde and oxygen, by introducing a suitable catalysing enzyme into the system, and (*b*) as far as the production of oxygen and starch, by introducing, in addition to the enzyme, certain kinds of non-chlorophyllous living protoplasm.

II.—(1) There is direct experimental proof that formic acid is a product of the photolytic decomposition of carbon dioxide in the presence of an inorganic uranium salt.

(2) Formaldehyde has not been isolated and identified in the case of an inorganic uranium salt, but a study of the reactions involved favours the view that it is formed as a transitory intermediate product.
