

A Study of the Mechanism of Carbon Assimilation in Green Plants.

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(Communicated by Professor M. W. Travers, F.R.S. Received December 16, 1905,—Read January 18, 1906.)

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Introduction.—The investigation to be described in this paper has had for its object the elucidation of certain problems concerning the nature of the first stages in the assimilation of carbon from carbon dioxide by the green parts of plants; and although far from complete, it has been thought advisable to publish the results already obtained, inasmuch as the weather is likely to hinder the experimental work for some time to come.

In 1870 Baeyer put forward the hypothesis that formic aldehyde is the first product of the decomposition of carbon dioxide in the plant. This suggestion received some support from Bokorny,* who proved in 1891 that starch was formed in the dark by the green filaments of *Spirogyra* when immersed in a solution of sodium oxymethyl-sulphonate of 0·1 to 1 per cent. strength.

Bokorny's experiments are possibly open to the objection that formaldehyde condenses very readily to non-poisonous carbohydrates in presence of sulphites or bisulphites, and it has been shown by Laurent and Acton† that starch is formed in the dark from most sugars.

Quite recently, Bouilhac and Tréboux‡ have succeeded in growing plants in a very dilute solution of pure formaldehyde. Tréboux has found that *Elodea* forms starch in the dark from a 0·001-per-cent. solution of formaldehyde, and Bouilhac has shown that this is also the case with *Sinapis alba* and some *Algæ*. Their experiments bring out in a striking manner the intensely poisonous nature of even very dilute solutions of formaldehyde.

Evidence of this kind, however, is quite indirect, and on this account greater importance attaches to the results obtained by Bach,§ who for the first time demonstrated the decomposition of carbon dioxide by light outside the plant. He showed that by passing pure carbon dioxide through a 1·5-per-cent.

* 'Berichte,' 1891, vol. 24, p. 103.

† 'Roy. Soc. Proc.,' 1890, vol. 47, p. 150.

‡ 'Flora,' 1903, p. 73.

§ 'Comptes Rendus,' 1893, vol. 116, p. 1145.

solution of uranium acetate exposed to sunlight in a glass apparatus, a precipitate consisting of a mixture of uranium peroxide with lower oxides was formed, and that the solution contained formaldehyde. Bach regarded the uranium acetate solution as playing the part of a chemical and an optical sensitiser, and considered the decomposition of the carbon dioxide to result primarily in the production of hydrogen peroxide and formaldehyde.

Decomposition of Carbon Dioxide Outside the Plant.—The experiments of Bach have been repeated and confirmed, both as to the production of peroxide and formaldehyde.

The amount of decomposition obtained in three weeks in bright weather was extremely small, and this appears to us to be explained by the fact that (1) as a chemical sensitiser uranium acetate is far inferior to that which exists in a green plant, inasmuch as the separated oxygen (in whatever form it may exist) is not entirely removed from the sphere of action, as in the case of the plant, but remains as a fairly insoluble peroxide which undergoes a reversible change with the other product, namely, formaldehyde; (2) as an optical sensitiser uranium acetate is inferior to chlorophyll to the extent that it possesses no absorption at all in the red, and only two faint bands between F and G.

In view of the extreme slowness of the reaction under these conditions, experiments were made with very large concentrations of carbon dioxide. Tubes of Jena glass, 40 cm. long and 8 to 10 mm. bore, were about three-quarters filled with 1·5 per cent. uranium acetate solution, and cooled in liquid air while some carbon dioxide was passed in. They were then sealed, and suspended outside a south window in bright sunshine. Within 15 minutes of warming up to the air temperature, a precipitate began to form, and in 24 hours the reaction was complete. The tubes when opened were found to contain uranium peroxide and formic acid, but no formaldehyde. The formic acid was obtained by distillation of the filtrate from the peroxide, and was characterised by (1) reduction of silver nitrate, (2) reduction of Fehling's solution, and (3) properties of lead salt.

Thus with very large concentrations of carbon dioxide, formic acid, and not formaldehyde, results.

These experiments are open to the objection that since uranium acetate is to a considerable extent hydrolysed in solution, the formaldehyde in one case and the formic acid in the other may possibly have been derived from the acetic acid present.

It has been found that uranium sulphate in a 2-per-cent. solution functions in the same way as the acetate. An experiment with the sulphate, conducted in the usual way, *i.e.*, bubbling carbon dioxide through the solution, which

lasted over three weeks in very dull weather, gave uranium peroxide and formic acid. The different results obtained here from those in the case of similar experiments with the acetate, may be due to the "reduction potential" falling below the limit required for the completion of the second stage of the decomposition.

In all the foregoing experiments, except in the case of the liquid carbon dioxide tubes, blank experiments were simultaneously performed, (1) with uranium solution and carbon dioxide in the dark, and (2) with carbon dioxide free solution in the light. In neither case was any precipitate formed.

Decomposition of Carbon Dioxide in the Plant.—If a similar reaction, resulting in the formation of formaldehyde and a peroxide, takes place in the first stage of the absorption of carbon dioxide by the plant, it is obvious that both the initial products of decomposition must undergo a rapid change.

On account of its intensely poisonous nature, formaldehyde must be very rapidly converted into some physiologically inert substance; and the peroxide must be decomposed with evolution of gaseous oxygen, a process which follows exposure to light by an interval of one or two seconds.

The problem, then, is to ascertain the process by which oxygen is disengaged; to show the actual presence of formaldehyde localised in the neighbourhood of the chloroplasts; and to trace the steps by which the formaldehyde is polymerised.

The Mechanism of the Evolution of Oxygen from the Green Plant.—In the experiments relating to the decomposition of carbon dioxide outside the plant, no evolution of oxygen gas is ever observed; it remains in the system as a peroxide.

There have been conflicting statements with regard to the presence of hydrogen peroxide in plants, but even if traces are to be found, there is no evidence that it is a product of decomposition of carbon dioxide. It has indeed been shown that several organic substances, notably the organic acids, *e.g.*, oxalic, give rise to hydrogen peroxide on exposure to light, and such substances as these are of common occurrence in the leaves of plants. If, however, hydrogen peroxide is one of the first products of the photolysis of carbon dioxide, we are more directly concerned with the elimination of oxygen in the gaseous form than with the detection of the peroxide.

Hitherto those writers who have recognised the difficulty at all have suggested some method of reduction, which, of course, leads back to the starting point. It appeared much more probable that this step in the process was brought about by a catalyst, probably an enzyme. To test this, some *Elodea* was immersed in a dilute solution of hydrogen peroxide. An

immediate and rapid decomposition set in, and a gas was freely evolved, which was found to be oxygen. The action proceeded as rapidly in the dark as in the light.

The following experiments were performed with the object of ascertaining the nature of the catalyst:—

(a) A plant was immersed in boiling water for 30 seconds and was subsequently found to be without action on hydrogen peroxide.

(b) After treatment with dilute solutions of iodine, mercuric chloride, and hydrogen sulphide, no action took place.

(c) Some *Elodea* was suspended in air charged with chloroform vapour for two hours in order to kill the protoplasm, and was then allowed to “recover” for a similar period. Rapid disengagement of oxygen took place.

(d) After immersion in very dilute formaldehyde solution, hydrogen peroxide was not decomposed.

These experiments seem to point to the existence of a catalysing enzyme. Several attempts to extract it by simple maceration with water or salt solution failed, and we were also unable to extract it after powdering leaves in liquid air. Following a suggestion of Dr. Horace Brown, we ultimately succeeded in obtaining it by previously drying a quantity of *Elodea*, and subsequently digesting with water at 30° for 48 hours. The enzyme was precipitated by an excess of absolute alcohol and dried.

By this process it is obtained as a light brown powder, containing diastase, whose aqueous solution energetically decomposes hydrogen peroxide, whereas ordinary malt diastase does not. Whether the enzyme is one already known, or whether it is secreted specially for the purpose of catalysing hydrogen peroxide, we cannot as yet say.

On mounting a leaf of *Elodea* in very dilute hydrogen peroxide, and examining microscopically under a high power, bubbles of gas were seen to emerge from the chloroplasts only, an observation which shows the strict localization of this enzyme to the seat of the photosynthetic process.

In regard to the distribution of this enzyme, we have examined the foliage leaves of plants belonging to 46 Natural Orders and representative of the Vascular Cryptogams and all the main groups of the Phanerogams, and have found the power of catalysing hydrogen peroxide in every case, though the energy of the decomposition varies considerably in different groups. It also occurs in etiolated leaves and in potato tubers, and, in fact, appears to be associated with amyloplasts, whether possessing chlorophyll or not.

The Production of Formaldehyde and the Manner of its Removal.—It has been found in the case of *Spirogyra* that starch appears in a previously

starchless filament within three minutes of exposure to light, and it is probable that some sort of carbohydrate is formed much sooner than this, for it has been shown by Brown and Morris* that starch is probably not elaborated within the cell until the supply of nutriment is in excess of the cell requirements.

It would therefore seem as though the arrangement which exists in the plant for the removal of formaldehyde is at least as efficient as any external arrangement we can make to remove it in a different way, without at the same time killing the plant, and thus eliminating one of the essential factors, namely, the vitality of the protoplasm.

For this reason it is useless to look for formaldehyde in healthy assimilating leaves. It is well known that certain chemical substances possess the property of condensing formaldehyde to various carbohydrates, chiefly formose, α -acrose, and methylenitan. It has been found by Loew† that such condensing agents are chiefly metallic oxides and acid sulphites, substances not likely to occur in plants.

Moreover, condensation by these bodies is a comparatively slow process, and quite inefficient when applied to the requirements of a plant. Nevertheless, if the condensation in the plant were due to some chemical agent stored in the neighbourhood of the chloroplast, it should still be capable of taking place when the protoplasm of the leaf is killed and its enzymes destroyed.

Some healthy green sprigs of *Elodea* were immersed in boiling water for 30 seconds, in order to kill the protoplasm and destroy the enzymes. They were then placed in water saturated with carbon dioxide and exposed to sunlight. In the course of a few hours the deep green colour of the leaves had been completely bleached, and on immersing the bleached sprigs in a solution of rosaniline decolourised with sulphurous acid, a red colour was developed.

The original green material when treated in this way exhibited no colouration. There was, therefore, some substance of an aldehydic nature present in the killed and bleached leaves which was absent in those which were alive. The sequence of events in this experiment may be described as follows:—Photolysis of carbon dioxide begins in the normal way, giving rise to hydrogen peroxide and formaldehyde. The enzymes having been destroyed, the hydrogen peroxide, instead of being catalysed in the usual manner, oxidises the chlorophyll to a colourless substance, at which point the reaction necessarily comes to an end. Meanwhile a quantity of

* 'J. C. S.,' 1893, 'Trans.,' p. 632.

† 'Berichte,' 1888, p. 271.

formaldehyde, equivalent to the hydrogen peroxide required to destroy the chlorophyll, accumulates, and thenceforward the reaction is strictly reversible.

The following experiments were performed to settle the points involved in this explanation:—It was in the first place necessary to show whether the colouration referred to above was due to formaldehyde. For this purpose some leaves, killed and bleached in carbon dioxide solution as described, were soaked for 12 hours in aniline water, and were then examined microscopically under a high power. Some leaves which had been killed and simply decolourised with hydrogen peroxide were treated in the same way.

In the first case the decolourised chloroplasts were observed to be the centres of clusters of well-defined crystals, identical in appearance with those of methylene aniline, artificially prepared from aniline water and formaldehyde. They were soluble in dilute mineral acids and also in warm alcohol, from which they crystallised in the cell on cooling. The leaves artificially decolourised with hydrogen peroxide showed no crystals.

An attempt was then made to obtain the formaldehyde outside the plant. For this purpose a large quantity of *Ulva* and *Enteromorpha* was killed and bleached in carbon dioxide solution, and subjected to steam distillation. The distillate was divided into two parts. To the larger of these was added some aniline water.

A white precipitate was formed after some time, which was collected, and heated side by side with a comparison tube containing methylene aniline. It melted, not quite sharply, three or four degrees below the pure artificially prepared substance. The other portion of the distillate was evaporated with ammonia on the water-bath, and the residue dissolved in water and treated with bromine water, gave the characteristic tetra-brom derivative of hexamethylene-tetramine.

Hence, leaves in which both protoplasm and enzymes have been killed, when placed under conditions favourable for assimilation, develop formaldehyde, until the photolytic process is brought to an end by the destruction of the chlorophyll.

It was next necessary to determine whether the condensation of the formaldehyde is due to an enzyme secreted by the chloroplast, or whether the protoplasm of the granule itself effected it. Some *Elodea* was suspended in air charged with chloroform vapour for two hours, by which means the protoplasm was killed without affecting the enzymes. It was then exposed to sunlight in saturated carbon dioxide solution. In a few hours the chlorophyll became bleached, and formaldehyde was subsequently found in the plant.

It follows from this that the protoplasm of the chloroplast is the condensing agent. The bleaching of the chlorophyll in this case is due to the fact that the enzyme, though unharmed at the commencement of the experiment, quickly becomes poisoned by the accumulating formaldehyde. In this last experiment the limit to the accumulation of formaldehyde in a plant is realised, since there is formed an amount equivalent to that amount of hydrogen peroxide which is catalysed before the enzyme ceases to act together with an amount equivalent to the hydrogen peroxide required to destroy the chlorophyll.

That a certain amount of hydrogen peroxide is catalysed when the protoplasm only is dead was shown in the following manner. Approximately equal quantities of *Elodea* were taken, one of which (A) was killed by immersion in boiling water, another (B) was suspended for two hours in air saturated with chloroform vapour to kill the protoplasm and not the enzymes, while the third (C) served as a control.

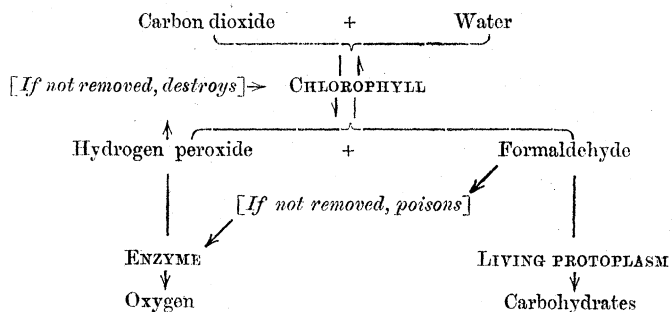
All three were placed in carbon dioxide solution under funnels with inverted test-tubes, and exposed to artificial light for 12 hours. From A there was no evolution of oxygen, from B 0.3 c.c. were given off, and from C 2.8 c.c. In the case of B, after exposure to light, no catalytic action on hydrogen peroxide could be observed, while previously vigorous decomposition had taken place.

Summary.

1. The photolysis of carbon dioxide may take place outside the plant in absence of chlorophyll, provided one of the products is removed.
2. The normal products of the photolysis are hydrogen peroxide and formaldehyde, though under certain conditions formic acid may be formed.
3. In the plant the decomposition of the hydrogen peroxide is provided for by a catalysing enzyme of general occurrence.
4. The condensation of the formaldehyde is dependent on the healthy condition of the protoplasm.

There are therefore three factors essential to photosynthesis from carbon dioxide and water in the plant, they are (i) vitality of the protoplasm, (ii) presence of a catalysing enzyme, and (iii) presence of chlorophyll. If any one of these factors be interfered with, the process of photosynthesis ultimately comes to an end, through the destruction of the optical sensitiser, chlorophyll.

The relations between the various factors in this process may be diagrammatically expressed thus :—



In conclusion, we wish to express our indebtedness to Dr. Travers, and to Dr. Horace Brown, for their valuable suggestions and help in the course of this research.
