

PROCEEDINGS OF
THE ROYAL SOCIETY.

SECTION B.—BIOLOGICAL SCIENCES.

On the Function of Chlorophyll.

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(Communicated by Prof. A. C. Seward, F.R.S. Received January 19, 1915.)

It has been generally assumed that the function of chlorophyll is to absorb radiant energy from light and to render this available for the production of formaldehyde from carbon dioxide and water, the formaldehyde being subsequently polymerised to carbohydrates, and oxygen being set free. This is supported by the fact that chlorophyll contains magnesium* and that in a cold watery solution of carbon dioxide this metal produces formaldehyde,† which readily polymerises spontaneously to paraformaldehyde, $C_2H_4O_2$, or metaformaldehyde, $C_3H_6O_3$, and into hexose sugars in the presence of lime-water or other weak alkali.

Usher and Priestly‡ stated that chlorophyll films when exposed to sunlight decomposed carbon dioxide, producing formaldehyde and hydrogen peroxide. The latter was responsible for the bleaching of chlorophyll in sunlight, and if a "katalase" enzyme was present the oxygen was set free and the chlorophyll remained green. "The bleaching of the chlorophyll in sunlight whether carbon dioxide is present or not is due to the formation of hydrogen peroxide." As a matter of fact chlorophyll films immersed in a solution of hydrogen peroxide remain green for a long time in darkness, and when

* A full account of the work on the chemistry of chlorophyll, by Willstätter and others, is given in the 'Chemistry of Plant Products,' by Haas and Hill, pp. 222-241 (1913).

† Fenton, 'Journ. Chem. Soc.,' vol. 91, p. 687 (1907).

‡ 'Roy. Soc. Proc.,' B, vol. 77, p. 369 (1906); vol. 78, p. 318 (1906); vol. 84, p. 101 (1911).

exposed to light do not bleach much more rapidly than similar films exposed to ordinary air.

In addition I was able to show* that formaldehyde is a direct product of the oxidation of chlorophyll when exposed to light, that none is produced in an atmosphere of pure carbon dioxide, and that when chlorophyll is exposed to light in the presence of oxygen and of carbon dioxide, oxygen is absorbed instead of being liberated. I pointed out that these observations supported the old view that chlorophyll itself is a stage in photosynthesis. Schryver† found that more formaldehyde was formed in the presence of carbon dioxide than in its absence, and suggested that any excess of formaldehyde might combine with the chlorophyll and be set free again on exposure to light. Direct tests by myself, using chlorophyll films and formaldehyde solutions kept in pure nitrogen in darkness, failed to give any support to this view. With various interruptions work in this direction has been in progress during the last five years. The appearance of an interesting paper by H. Wager, on the action of light on chlorophyll,‡ leads me to give an account of my own work up to the present time, leaving various points of detail for further papers.

Wager's conclusions are, in brief, that in sunlight, chlorophyll absorbs oxygen and decomposes into aldehydes, of which a small portion is formaldehyde, and into an oxidising agent capable of liberating iodine from potassium iodide, which is not hydrogen peroxide but may be an organic peroxide. This action takes place in the absence of carbon dioxide, and Wager inclines to the view that the production of sugar in the green leaf may be initiated by the photo-oxidation of the chlorophyll and the subsequent polymerisation of the aldehyde thus formed, rather than by the direct photosynthesis of carbon dioxide and water.

A possible objection that might be raised to these observations is that the methods of extraction given would not yield pure chlorophyll but a mixture of it with xanthophyll, carotin, lecithin, phytosterin and waxy substances. It is obviously of great importance to distinguish between the action of light upon these different substances, which can only be done by using them in pure form.

Separation and Purification of Chlorophyll, Carotin, and Xanthophyll.

The methods described by Willstätter for extracting and purifying chlorophyll were used with slight modifications. Grass leaves were immersed for

* 'Roy. Soc. Proc.,' B, vol. 80, p. 30 (1908).

† 'Roy. Soc. Proc.,' B, vol. 82, p. 226 (1910).

‡ 'Roy. Soc. Proc.,' B, vol. 87, p. 386 (1914).

1—3 minutes in boiling water. As much water as possible was then squeezed from them with a strong screw press. Sufficient absolute alcohol was added to moisten them, and within a few minutes all liquid was squeezed out in the press. The pale green liquid was thrown away. The residue was just covered with cold alcohol and digested in a closed glass cylinder in darkness in a cool chamber for at least one day. All the liquid was then poured off and squeezed out. One half of the volume of petrol ether was added, and then sufficient water to bring about separation. After standing in a large separating funnel the lower liquid was run off, then the scum between, and then the petrol ether. This was evaporated to dryness by a partial vacuum and gentle warming, redissolved in a minimum quantity of cold absolute alcohol, and again separated as before. The petrol-ether extract was allowed to stand in contact with water for some days. If any red carotin separated out it was again purified.*

This method is more satisfactory than when dried leaf meal is used, since the preliminary treatment removes a large amount of the waxy and other extractives that would otherwise be dissolved along with the chlorophyll. The separation is based upon the fact that carotin is only sparingly soluble in cold alcohol, that xanthophyll is soluble in water and in any mixture of alcohol and water, whereas carotin and chlorophyll are thrown out of an alcoholic solution by the addition of water and are readily soluble in petrol ether. Although xanthophyll is soluble in water, contact with the latter will not remove it from a petrol-ether solution of a waxy solid like chlorophyll unless sufficient alcohol is present, but pure solid xanthophyll dissolves slowly in water forming a solution which filters as a clear yellow liquid. If any carotin, wax, or chlorophyll is present, the xanthophyll dissolves in water with difficulty or not at all, owing to the lack of proper contact.

To obtain pure xanthophyll, the brownish-yellow alcoholic liquid was enriched with alcohol, shaken up again with petrol ether, and separation produced by the addition of water. After long standing in darkness the liquid beneath becomes perfectly clear and, after filtering, it may be evaporated to dryness by gentle warming and a partial vacuum in darkness. The residue was extracted with cold absolute alcohol, filtered again, evaporated to dryness, dissolved in petrol ether, and filtered. This gave a dark liquid, brownish yellow when shaken up, yellow when dilute, reddish brown by transmitted light when concentrated, but free from chlorophyll and with no red fluorescence.

Pure carotin can be obtained by treating the prepared grass material with

* A very strong solution of chlorophyll in petrol ether will always throw out some of its carotin in contact with water if any is present.

hot alcohol, separating with petrol ether, and allowing the strong chlorophyll solution to stand in contact with a little water for some days in the absence of light and oxygen. Small red particles of carotin separate out, which can be collected and washed with cold absolute alcohol.

To obtain larger quantities, the cortex of carrots can be grated, rubbed up with water, and then all the juice pressed out. On boiling the red liquid a scum of red carotin separates. This, after washing, can be dissolved in a minimum quantity of hot absolute alcohol, filtered hot, and allowed to stand. Most of the carotin separates out, and all of it if a little water is added. The residue is washed with water, then with a little cold absolute alcohol, dried, dissolved in petrol ether, and filtered.

All the processes must be carried out as far as possible out of contact with the air, and in darkness or very feeble light. When prepared, the solutions of all three pigments must be kept in small tightly stoppered and completely filled bottles in darkness until they are needed. Under these circumstances all three pigments keep for a long time without appreciable change.

The "Iodoxidase" Action of Chlorophyll in Light.

Wager concluded that one of the products of the decomposition of chlorophyll in light was "an active chemical agent capable of bringing about the liberation of iodine from potassium iodide," and that it was possibly an organic peroxide and was a gas (p. 394). The action is easily obtained by tinging potassium iodide and starch paper with a little chlorophyll, moistening and exposing to light in moist air. I am not, however, able to confirm Wager's statement that blue light will liberate iodine from potassium iodide. It must be remembered that the reaction is a very delicate one, which may be produced by a variety of causes. Touching the paper with the fingers or contact with the cork of a flask may cause a blue colour to be given on exposure to light, the presence of traces of acid, of certain organic or metallic oxidases will have the same effect, as well as traces of various gases often present in the air. Certain kinds of paper on moistening with potassium iodide and starch readily turn blue in sunlight, and may even develop a weak peroxidase reaction with guaiacum. Pure, well washed Swedish filter paper is free from this defect and may be used with safety. An important point is, also, that the solutions used must be perfectly neutral. Freshly prepared alcoholic chlorophyll is faintly acid, but the purified petrol-ether solution is neutral. Films of the former liberated iodine very readily in sunlight.

Wager found that paper tinged with eosin or methyl green would also give an "iodoxidase" reaction on exposure to light. Not only is this the case,

but carotin and xanthophyll show the same reaction when exposed to light. A long series of tests showed, however—(1) that the reaction was given only when actual contact was assured with the substance undergoing oxidation; (2) that it was not due to any volatile product of decomposition; (3) that none of the products of decomposition had any power of liberating iodine from potassium iodide; (4) that partially bleached paper treated with starch and potassium iodide and then kept in darkness gave no blue at whatever stage the bleaching was stopped; and (5) that no alkalinity was produced (red litmus paper + chlorophyll + potassium iodide). This last test shows that no hydrogen peroxide is formed.

A curious point worth noting is that moist blue litmus paper bleaches much more rapidly in sunlight than red litmus paper, and that the presence of a little chlorophyll, carotin, or xanthophyll on the paper hastens the bleaching, especially with the blue litmus.

The conclusion seems justified that chlorophyll, xanthophyll, carotin, etc., when exposed to sunlight, act not merely as oxidases to themselves but may also accelerate the oxidation of other substances, if in contact with them and if the supply of oxygen is abundant. This is borne out by the fact that if a watery solution of xanthophyll with a supernatant layer of guaiacum is exposed to sunlight, a green or blue layer forms between the two liquids which soon disappears again. All these actions are only shown when an abundance of oxygen is present, as when films are exposed to air. If the potassium iodide and starch are mixed with a watery solution of xanthophyll, or with a watery emulsion of carotin or chlorophyll, no perceptible liberation of iodine takes place on exposure to light. In the case of chlorophyll films or of carotin and xanthophyll films on filter paper, the possibility of traces of liberated potash combining with the chlorophyll or filter paper would naturally favour the liberation of appreciable amounts of iodine.

There is, therefore, no evidence that peroxidases, either organic or inorganic, are among the products of the decomposition of chlorophyll by oxygen and sunlight. The "iodoxidase" reaction in this case merely indicates that oxidation has been taking place. A slight liberation of iodine may even take place on a dry filter paper impregnated with chlorophyll and potassium iodide and then bleached, a blue colour showing on moistening.

The Oxidation of Chlorophyll.

Dry chlorophyll films will bleach fully when exposed to sunlight in dry air, but usually take five to ten times as long as similar films in moist air. If the chlorophyll film is at all thick, the bleaching is greatly retarded by the fact that the colourless products of oxidation form a waxy film on the

surface, which is almost impermeable to oxygen. Thus, in one case, 0.73 gm. of dry chlorophyll (petrol-ether extract), after one week's exposure in dry CO_2 -free air, was partly bleached and weighed 0.4 gm. For full bleaching four months' exposure was necessary, the weight then being 0.2 gm., and after extraction with water and drying being 0.13 gm. of white insoluble waxy solid.

Filter paper saturated with chlorophyll is unsuitable for exact experiments, and in addition moist filter paper after long exposure to sunlight may develop appreciable traces of reducing sugar, which can be extracted by water. Hence thin sheets of glass and mica were used on which solutions of chlorophyll in petrol ether were allowed to evaporate and form even films. For certain experiments thin-walled glass tubes 6 feet in length were employed.

To determine the influence of carbon dioxide on oxidation, dry films were exposed to sunlight in nearly dry air with and without carbon dioxide. In the presence of a little carbon dioxide the bleaching appeared to be accelerated and 0.44 and 0.453 gm. of chlorophyll left 0.198 and 0.22 gm. of white residue, whereas in the absence of carbon dioxide 0.462 and 0.43 gm. of chlorophyll left 0.145 and 0.123 gm. of dry white residue. This residue contains a little matter soluble in water and this is slightly more abundant in the films oxidised in the presence of carbon dioxide and the weight of the residue is greater. Dry films oxidised in dry air contain relatively little matter soluble in water.

Tubes were then lined with dry chlorophyll, filled with pure dry nitrogen and pure dry carbon dioxide and exposed to light for periods of 1 to 8 weeks. All remained green, but the films in CO_2 become a little more yellowish, and a little of the CO_2 disappeared. No oxygen or formaldehyde was formed. Similar tubes filled with dry air were exposed until bleaching ceased. The residual gas contained no oxygen but 2.2 per cent. of gas removable by potash. Further tests showed that this gas consisted wholly of formaldehyde gas.

Additional evidence that chlorophyll is able slowly to combine with CO_2 during its oxidation was obtained by exposing thick films to sunlight in air containing a little moisture and carbon dioxide until fully bleached, which took just over a month. In one case 0.8 gm. left a dry bleached residue of 0.785 gm. dry weight, whereas in pure air the bleaching was much slower and a similar quantity lost 68 per cent. by weight. A portion of this may be due to the fact that in pure dry air a slow loss of weight by photo-oxidation continues for some time in the bleached residues, whereas if the film is thin and the oxidation rapid in moist air, there may be little or no loss of weight by the time the film is bleached, the oxygen absorbed weighing nearly as much as the gas lost. Thus 0.49 gm. of chlorophyll spread out in a thin film

on mica sheets and oxidised rapidly in moist air and sunlight weighed when dried 0.478 gm. Possibly some water may also enter into combination.

The Gaseous Products of the Photo-oxidation of Chlorophyll, Carotin and Xanthophyll.

Thin-walled glass tubes 6 feet in length were warmed, and a strong solution of chlorophyll in petrol ether poured in. The heavy vapour falls out of the tube, and with a little practice a film of chlorophyll of even thickness can be left lining the tube. These were exposed to sunlight until bleached (usually 3 to 5 days) in a stream of moist CO₂-free air, which was led into water through a fine capillary exit tube. In one case where four lengths of six foot tubes were used 1.14 gm. of chlorophyll left 1.19 gm. of residue, while the water contained no CO₂, but smelt strongly of formaldehyde and, by Schiff's test, comparing with standard strengths, contained 0.32 gm. of formaldehyde. On evaporating to dryness, it left a white residue of paraformaldehyde equivalent to 0.14 gm. from the total solution. No other aldehyde was present in the solution or in the residue in the tube.

Similar experiments carried out with tubes lined with pure carotin and xanthophyll films showed that during the photo-oxidation of these substances, no carbon dioxide, but formaldehyde gas is produced, and in the case of xanthophyll possibly a little water vapour is also formed.

The question at once arises as to whether chlorophyll, like lime-water and feeble alkalis, has the power of polymerising formaldehyde to reducing sugars. If equal quantities of (a) water and (b) a watery solution of formaldehyde were added to an equal volume of an alcoholic solution of chlorophyll and the mixture exposed to sunlight in the presence of oxygen, (b) turned brown while (a) was still green, but on long exposure (a) became quite pale while (b) was still a darker brown. The liquids were then filtered and evaporated to dryness and gently heated, until in the case of (b) no smell of formaldehyde was perceptible and the loss of weight ceased. The residues and the dry residues from filtering were weighed.

	Insoluble residue.	Soluble solids.
A	0.31	0.81
B	0.425	0.925

In both cases the soluble solids gave a strong reduction with Fehling's solution. Alcoholic chlorophyll, however, sometimes contains traces of reducing sugar, and in this experiment carbon dioxide was not excluded.

Hence, in a second experiment, carbon dioxide was excluded, and of two

flasks (A and B) containing 40 c.c. of dilute formaldehyde, one also contained a film of 0.1 gm. of chlorophyll (A). When this was bleached the filtrates were evaporated to dryness on a water-bath, weighed, and then further heated to drive off the paraformaldehyde.

	First dry residue.	Final dry residue.
A	1.24	0.15
B	1.25	0.052

The final dry residue from A gave a strong reduction with Fehling's test. Since the bleached residue in A still weighed 0.085 gm., there is apparently evidence here of a feeble polymerisation of formaldehyde by chlorophyll.

The Solid Products of the Photo-oxidation of Chlorophyll, Carotin and Xanthophyll.

In all three cases the solid residues consist of a portion soluble in water, and of a white waxy solid insoluble in water but melting when heated. The latter appears to consist, in the case of carotin, partly at least of phytosterin.*

The white insoluble residue from the bleaching of chlorophyll is a waxy solid with a smell of beeswax. In one experiment 0.4 gm. yielded 1.99 per cent. of magnesium oxide (pure chlorophyll should yield 4.5 per cent.), so that apparently after decomposition some of the magnesium can be removed by washing with water. The white "chlorophyll wax" melts gradually on warming, like sealing wax, beginning about 80° C., and is rather brittle in mass when cold. Possibly it consists of a mixture of substances. It dissolves readily in hot alcohol, very sparingly in cold alcohol, and is moderately soluble in petrol ether.

The watery extract from fully bleached chlorophyll contains no aldehydes. It has a bitter taste, turns turbid on boiling or on adding copper sulphate, but becomes clear on adding sodium hydrate, and gives a strong reduction with Fehling's test. On warming with a drop of sulphuric acid, the white precipitate thrown down forms an oily solid clinging to the tube, and on filtering the clear solution gives a strong reduction with Fehling's test. In one experiment 0.61 gm. of chlorophyll yielded 0.64 gm. of dry bleached residue after full oxidation in moist air deprived of carbon dioxide, of which 0.23 gm. was removed by cold water, and 0.41 gm. remained as an insoluble film. If the film is properly prepared and the contact with water made gradually, none of the film breaks away from the glass or mica.

The tendency to turbidity on the part of the watery extract prevents

* See Czapek, 'Biochemie der Pflanzen,' p. 172.

examination with the polariscope. Accordingly large films on mica and glass sheets were placed vertically in gas cylinders filled with air and kept saturated with water vapour. The lower pointed end of the sheet rested in a small beaker. After some days' exposure to sunlight a few drops of a gummy liquid collected in the beaker. This has a sweetish taste, a faint brown colour, forms a clear solution with water, and gives a strong reduction from Fehling's. The liquid gave the glucosazone test, and with phenylhydrazin, sodium acetate, and alkali, a red colour with a faint violet tinge on warming. The last test must always be done with a blank control, since on long standing a slight red colour may be given in the absence of sugar. Distinct traces of reducing sugar were also given by the watery extracts from the white residues from xanthophyll, but no reduction or a doubtful trace with the watery extract from bleached carotin, bleached in moist CO₂-free air. No sugar at all could be obtained from similar films kept in darkness.

Apparently both dextro- and lævo-rotatory sugars are formed. Thus after bleaching 0.72 grm. of chlorophyll in moist CO₂-free air, the watery extract gave a doubtful dextro-rotation indicating not more than 0.003 grm. of sugar, whereas a Fehling's estimation on Soxhlet's method indicated 0.18 grm. of reducing sugar. During the oxidation of xanthophyll films, as much or even more sugar appears to be formed as during the oxidation of chlorophyll. If to an alcoholic solution of xanthophyll half its volume of water is added, and the solution evaporated to dryness after bleaching in the presence or absence of carbon dioxide, the filtered watery extract gives a strong reduction with Fehling's. The bleaching may be hastened by the addition of hydrogen peroxide, but not if a subsequent test is to be made for sugar.

The Photo-oxidation of Xanthophyll, C₄₀H₅₆O₂.

In a preliminary experiment the alcoholic extract from the dried separation residues was used and lined 18 feet of glass tubing. Full bleaching took two days; moist air free from CO₂ was used. The water in which the gas was collected smelt strongly of formaldehyde and gave a strong pink with decolorised magenta. It contained no carbon dioxide. The dry residues in the tubes weighed 0.427 grm. (from 0.46 grm. of dry xanthophyll). The watery extract from the residue gave a strong reduction with Fehling's test but contained no formaldehyde or other aldehydes. The white waxy residue insoluble in water appeared to resemble the residue from the oxidation of carotin.

Subsequent tests showed, however, that xanthophyll prepared in this way may contain a trace of sugar and apparently some phytosterin. Accordingly the alcoholic extract was evaporated to dryness, dissolved in a minimum of

cold absolute alcohol, filtered, again evaporated to dryness, and dissolved in petrol ether, in which the xanthophyll is more soluble and keeps better than in water or even in alcohol.

Using 18 feet of tubing lined with 0.38 gm. of xanthophyll, the bleaching was completed after 12 hours' exposure to sunlight. The water in the collecting tube contained 0.155 gm. of formaldehyde. Some of the formaldehyde may escape solution, however, since the collecting tube always smells stronger of formaldehyde than does a solution of equal strength as indicated by colour tests. The colourless residue in the tubes weighed 0.37 gm., and the whole of it came away in water, forming a slightly turbid liquid, which gave a dense white precipitate with a drop of sulphuric acid. On boiling a pale yellow oily solid separated out on the sides of the tube, the liquid filtering clear. It showed a doubtful feeble dextro-rotation equivalent to 0.001 gm. of glucose (0.01 per cent. in 10 c.c.), and gave a strong reduction with Fehling's equivalent to 0.24 gm. of glucose.

If these numbers are correct they would indicate an absorption of 38 per cent. by weight of oxygen, or of oxygen and water vapour. Haas and Hill quote (p. 238) an absorption value of oxygen of 36.55 per cent. for xanthophyll. In my own experiments the weight and surface of the glass was necessarily relatively so great as to preclude very exact weighing.

The Photo-oxidation of Carotin.

This, according to Arnaud, has the formula $C_{26}H_{38}$, and absorbs up to 21 per cent. by weight of oxygen, whereas Willstätter gives it the formula $C_{40}H_{56}$ and an absorption value for oxygen of 34.3 per cent.* Carotin oxidises much more rapidly than either xanthophyll or chlorophyll. Thus, using thin films of equal weight spread over the same area and exposed to full sunlight, carotin took one hour, xanthophyll 12 hours, and chlorophyll 20 hours to bleach completely. With very thin films, however, there is less relative difference in the rate of bleaching, while with thick films the difference in the relative rates increases. A thick film of chlorophyll may take months to bleach fully. This is largely a matter of the permeability of the oxidised surface layers to oxygen. During the oxidation of chlorophyll the blue-green colour passes to green, then yellow (or brown if a thick layer), then colourless. During the yellow stage xanthophyll appears to be set free, and subsequently to be oxidised, but no carotin.

In one experiment with carotin obtained during the purification of chlorophyll, 0.3 gm. films of carotin were exposed in tubes to light in a current of moist CO_2 -free air. The dry colourless transparent residue

* For literature, see Haas and Hill, as well as Czapek's 'Biochemie.'

weighed 0.26 gm. It turned white in water, but was wholly insoluble in water and contained no sugar. The gas from the tubes was passed into water, which gave a distinct pink with Schiff's test. Apparently oxidised carotin produces formaldehyde but no sugar or carbon dioxide. The amount appears to be less than with chlorophyll or xanthophyll, but further quantitative investigations are necessary on this point. The supply of carotin obtained from the chlorophyll extraction was soon exhausted as the yield is very small, but carotin extracted from carrots may be used in its place. It also appeared to give off appreciable quantities of formaldehyde gas during bleaching, while the bleached residue was practically insoluble in water, and the watery extract either gave no reduction at all, or a doubtful trace with Fehling's test.

The Reduction of Xanthophyll to Carotin.

Although the former differs from the latter simply in containing two atoms of oxygen, it is less readily oxidised, and no oxidising agency tried was found to convert carotin into xanthophyll. According to Palladin, however,* carotin is converted by an oxidase into xanthophyll, while a reductase enzyme carries out the reverse change. Using a variety of plant oxidases, including those of the carrot, apple, potato and parsnip, I have not been able to produce any conversion of carotin into xanthophyll using finely divided carotin and watery or glycerine oxidase extracts. Under water in fact carotin only oxidises slowly and with difficulty even when exposed to sunlight.

The conversion of xanthophyll into carotin is, however, readily produced by adding magnesium dust or zinc dust to a watery solution of xanthophyll in darkness. In the first case nascent hydrogen acts as the reducing agent and the action is rapid, being completed in one to a few hours. The zinc acts slowly (2—3 days). On filtering, a clear liquid comes through, and after washing the residue with absolute alcohol, petrol-ether dissolves out the carotin, which when evaporated is insoluble in water, and has the usual properties.

The Combination of Carbon Dioxide with Chlorophyll.

Tubes were lined with dry chlorophyll, sufficient water added to cover the film, and a current of carbon dioxide passed through until the water was saturated. The tubes were then drawn out and sealed while the gas was passing through, leaving half the tube filled with gas. On exposing to sunlight the rise of temperature causes bubbles of gas to separate on the film, distorting it and making it form an irregular network adhering to the glass. The bright green colour is lost. After a week the gas above had decreased

* 'Ber. d. D. Bot. Ges.,' vols. 26A and 27 (1908 and 1909).

38 per cent. in volume to 80 c.c. It contained 79.7 c.c. of carbon dioxide and 0.3 c.c. of nitrogen.* On adding cold alcohol to the film it formed a pure yellow solution of xanthophyll, leaving a slight white insoluble portion on the glass. The water from the tube contained no perceptible amount of formaldehyde or of reducing sugar.

A similar experiment was repeated with a large tube with exactly similar results, the residual gas being less in amount and consisting of 99.8 CO₂ and 0.2 per cent. nitrogen. The yellow film when dried weighed 0.44 gm., whereas the original chlorophyll weighed 0.34 gm.

Evidently, therefore, chlorophyll, when exposed to sunlight in the presence of carbon dioxide and water, increases in weight and forms xanthophyll and a colourless waxy solid, but liberates no free oxygen.

The same change takes place in darkness, but more slowly, and in the same way the oxidation of chlorophyll takes place slowly in darkness, although it is very greatly accelerated by exposure to light.

In a second experiment a film of dry chlorophyll weighing 0.174 gm. was exposed to air in darkness for 10 days. It lost 0.006 gm. of weight, and, during the next seven days, 0.004 gm. The next week it was kept in a mixture of dry air and carbon dioxide. It lost a further 0.003 gm. in weight but was still quite green. During the next week it was kept still in darkness, but in water saturated with CO₂. The film became dull, contained mainly xanthophyll with a trace of chlorophyll at one point only, and on drying showed a bright yellow colour and weighed 0.165 gm. The water contained no perceptible formaldehyde, and, although it gave no distinct reduction with Fehling's test, left a residue of 0.052 gm. In spite of its long exposure, therefore, in the presence of an excess of CO₂ dissolved in water, the chlorophyll combined with carbon dioxide forming xanthophyll and a colourless, waxy residue dissolving readily in petrol ether.

Water alone has no effect on chlorophyll. Thus, films kept for one week in darkness in water deprived of all carbon dioxide were still quite green and apparently unaltered. On keeping the water saturated with carbon dioxide, the films after two weeks' darkness became dull yellow, and gave a pure yellow solution of xanthophyll soluble in cold alcohol, and leaving a colourless, waxy skin, soluble in petrol ether. This waxy material in all cases prevents the xanthophyll from dissolving in the water, which remains colourless, but when the xanthophyll is isolated it has the same properties as that obtained directly in chlorophyll extraction.

If oxygen is present as well as carbon dioxide, the water in the beaker

* This nitrogen may have come from the chlorophyll, but more probably remained dissolved in the water when the tube was sealed.

may show a faint trace of formaldehyde, but no reduction with Fehling's test.* Apparently, in darkness, a feeble oxidation of xanthophyll may take place as well as of chlorophyll. Chlorophyll, therefore, combines with carbon dioxide when present in a saturated watery solution in darkness, forming xanthophyll and a colourless waxy solid. In light, the same action takes place more rapidly and readily, and even under negative pressure. The rapid yellowing of grass leaves when cut fresh and heaped in masses is due to this action of carbon dioxide on chlorophyll. If a quantity of grass leaves is boiled and kept in a dense mass, in a few hours the *post-mortem* production of carbon dioxide has destroyed nearly all the chlorophyll, and large amounts of xanthophyll can be extracted, but very little chlorophyll.

Xanthophyll, Carotin, and Carbon Dioxide.

Experiments were made by exposing (a) dry films of xanthophyll and carotin in dry carbon dioxide, (b) watery solutions of xanthophyll, and watery emulsions of carotin in tubes sealed after saturating and filling with carbon dioxide. After a week's exposure the colour was unaltered, no evidence of combination could be seen, no new products appeared, and the enclosed gas consisted of pure carbon dioxide with occasionally a trace of nitrogen

General Conclusions.

The foregoing results indicate that the assimilation of carbon dioxide is not a simple process, as represented by the equation $\text{CO}_2 + \text{H}_2\text{O} = \text{CH}_2\text{O} + \text{O}_2$, in which chlorophyll merely absorbs the energy required, but is a very complex one, in which two pigments at least and their derivatives take part, and in which the equilibrium between the products and reacting substances determines the direction in which the reactions may take place, while light influences this equilibrium and strongly accelerates the tendency to oxidation on the part of all the pigments concerned.

According to Willstätter, amorphous chlorophyll is the methylphytyl ester of the tricarboxylic acid, $\text{C}_{31}\text{H}_{29}\text{N}_4\text{Mg}(\text{COOH})_3$, or chlorophylllic acid, and has

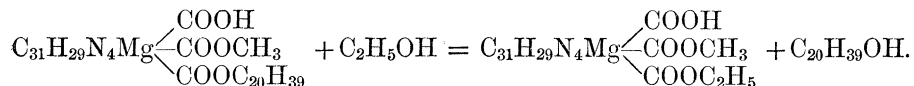
the formula $\text{C}_{31}\text{H}_{29}\text{N}_4\text{Mg} \begin{matrix} \text{COOH} \\ \text{COOCH}_3 \\ \text{COOC}_{20}\text{H}_{39} \end{matrix}$.

The phytyl radicle can be displaced. Thus Willstätter and Stoll† found

* Although a strong solution of formaldehyde gives a reduction with Fehling's test just like a reducing sugar, with a weak solution the slight excess of alkali on warming converts the formaldehyde into methyl alcohol and sodium formate. Hence dilute solutions of formaldehyde give Schiff's test and other sensitive tests, but do not give Fehling's test.

† 'Annalen,' vol. 378, p. 18 (1910).

that chlorophyll in the presence of the enzyme chlorophyllase and of ethylic alcohol forms methyl ethyl chlorophyllide or crystalline chlorophyll and the alcohol of phytol (phytol)



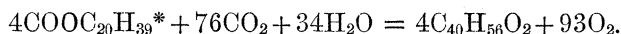
The same enzyme appears also to be able to produce the synthesis of chlorophyll from phytol and methyl ethyl chlorophyllide.

In any case, the phytol radicle of chlorophyll is easily displaced, and there appears to be some evidence that the chlorophyll may be re-formed from xanthophyll and the colourless waxy residues of bleached chlorophyll. Thus, if in the first separation of chlorophyll a slight excess of water is added and the liquid kept in darkness in a separating funnel for one or more days, at the top is impure petrol ether chlorophyll, on the surface of the watery layer is an emulsion containing chlorophyll, impurities, and solid carotin, well below this the liquid is yellow with a white turbidity (A) and at the bottom is a clear yellow liquid (B). If B is evaporated to dryness and digested with cold alcohol, it yields xanthophyll. If an equal volume of A is run off, no chlorophyll can be extracted from it by petrol ether, or detected in it by the spectroscope. On evaporating it (in very feeble light), when nearly all the alcohol has been driven off, green skins of chlorophyll form. On filtering, the liquid is brown but turbid and again forms fresh chlorophyll on evaporating. If filtered as often as any green pigment separates, the ultimate dry residue may contain little or no xanthophyll as compared with the residue from B. The amount of chlorophyll recovered in this way may be sufficient to tinge the original volume quite green, and to mask the yellow colour when dissolved in alcohol and added to a fresh portion of the original liquid.

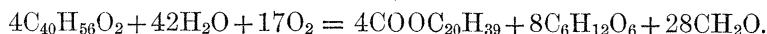
A large number of attempts were made to obtain chlorophyll from the colourless products of its oxidation and xanthophyll or carotin. In one experiment freshly bleached chlorophyll dissolved in hot alcohol was poured into water, the alcohol partially evaporated, and a watery solution of xanthophyll added. A little zinc dust was added, and the tube was sealed so as to enclose half its volume of air, so that on exposure to diffuse daylight oxidation took place at the surface, reduction at the bottom. After three days, on opening, partially evaporating, adding alcohol, filtering, and then adding first petrol ether and then water, the petrol ether showed a distinct green colour and red fluorescence and the spectrum of chlorophyll, although no chlorophyll was present originally. This result was, however, quite capricious. Out of some 20 experiments about three in four were

failures, although apparently repeated in exactly the same way. Possibly the stage of bleaching may be of importance, but in any case these results cannot be taken as establishing a fact but rather as suggesting a possibility, namely, that a reconstruction of chlorophyll may be possible from the products of its oxidation and from xanthophyll. There is no doubt, however, as to xanthophyll being one of the first decomposition products of chlorophyll, especially in the presence of CO_2 and sunlight. Although not claiming any exact quantitative accuracy for the following equations, they may express the probable process of carbon dioxide assimilation in the plant.

Stage 1.—Carbon dioxide and water combine with the phytol base of chlorophyll, forming xanthophyll and oxygen. This will take place slowly in darkness but is accelerated by light.



Stage 2.—A portion of this oxygen is used in the oxidation of the xanthophyll into phytol, hexose sugars and formaldehyde, and the remainder is excreted from the chloroplastid. This takes place in light only.



Stage 3.—The phytol retakes its place in the chlorophyll molecule, the oxygen remaining (76O_2), which is equal in volume to the carbon dioxide absorbed, is exhaled, and the surplus formaldehyde is polymerised to hexoses, either by contact with the chlorophyll or with dilute alkali in the protoplasm around the chloroplastid.

The one point which cannot so far be repeated outside the living chloroplastid is the evolution of oxygen. Possibly with extracted chlorophyll, any oxygen set free in its substance at once combines with it. It is possible that in the living plastid some special separation is effected, or the magnesium of the chlorophyll may reduce some of the xanthophyll to the very readily oxidisable carotin, which might protect the chlorophyll from oxidation. Further, carotin and chlorophyll, being insoluble in water, oxidise much less rapidly when suspended in water than does a solution of xanthophyll. This gives a further possibility for the separation of oxygen at the surface of the chloroplastid. The separation of oxygen can hardly be produced by the action of a reductase reducing xanthophyll to carotin, for this would only give one of oxygen for every 19 of CO_2 , and in addition, when magnesium (or zinc) reduces xanthophyll to carotin, no oxygen is set free.

The exact means by which the oxygen formed escapes without wholly

* For simplicity the rest of the chlorophyll molecule is omitted from the equation.

combining with the readily oxidisable chlorophyll needs further investigations, but the views put forward seem to give a reasonable temporary working hypothesis of the function of chlorophyll, xanthophyll, and carotin in the assimilation of carbon dioxide. A continuous liberation of oxygen would only be possible when stages 2 and 3 were carried out. The chemical reactions involved form a series of chemical changes which in part at least are reversible, and whose continuance in a definite direction is determined by light and by a continued supply of carbon dioxide at one end of the equation and by a continued removal of the sugar, formaldehyde, and surplus oxygen at the other end of the equation.

Summary.

No peroxides, organic or inorganic, are produced during the photo-oxidation of chlorophyll, xanthophyll, or carotin, but these substances, when exposed to light in the presence of an abundant supply of oxygen, may act as oxidases not only to themselves but also to substances with which they may be in contact, such as hydriodic acid, litmus, or guaiacum. Hence arises the "iodoxidase" reaction of bleaching chlorophyll, carotin, xanthophyll, and other pigments oxidising in light.

Chlorophyll and xanthophyll decompose during photo-oxidation into (a) solids and (b) a gas. The solids consist of colourless waxy substances and hexose sugars. The waxy solids are relatively small in amount in the case of xanthophyll. The gas is formaldehyde gas. With dry films in dry air free from carbon dioxide, relatively more formaldehyde is produced and less sugar, and the bleached residue weighs much less than the original solid. In moist air more sugar is formed and the residue may weigh nearly as much as the original solid.

Carotin oxidises more rapidly than xanthophyll or chlorophyll, and yields a little formaldehyde and a large amount of a colourless waxy solid, which may be a form of phytol or of phytosterin.

Carbon dioxide combines with chlorophyll forming xanthophyll and a colourless waxy solid. The combination only takes place actively in the presence of water, and is accelerated by sunlight. Portion of the oxygen liberated by this reaction may oxidise the xanthophyll in the presence of sunlight to formaldehyde, sugar and phytol, the latter retaking its place in the tricarboxylic chlorophyll grouping. No oxygen is set free when extracted chlorophyll is used. Some special means must exist in the chloroplastid of liberating the remaining oxygen without its oxidising the chlorophyll. Carotin may aid in protecting chlorophyll from photo-oxidation, and the reductase action of magnesium may be of importance.

There is some evidence suggesting the possibility that chlorophyll may be built up not only from ethyl chlorophyllide and phytyl alcohol, but also from xanthophyll and the products of the photo-oxidation of chlorophyll. The assimilation of carbon dioxide involves a complex series of chemical changes which are reversible in part at least, in which chlorophyll and xanthophyll play a direct chemical part, and in which light acts as an accelerating and possibly as a directive agency.

On Forms of Growth Resembling Living Organisms and their Products Slowly Deposited from Metastable Solutions of Inorganic Colloids.

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(Received February 6, 1915.)

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[PLATE 1.]

Graham, in his classical papers on colloids, draws attention to the remarkable dynamic properties possessed by matter in the colloidal form, whether as a hydrosol or a hydrogel. He states that "another and eminently characteristic quality of colloids is their mutability. Their existence is a continued metastasis. A colloid may be compared in this respect to water, while existing liquid at a temperature under its usual freezing point, or to a supersaturated saline solution. The colloidal is, in fact, a dynamical state of matter; the crystalloidal being the statical condition. The colloid possesses energia. It may be looked upon as the probable primary source of the force appearing in the phenomena of vitality. To the gradual manner in which colloidal changes take place (for they always demand time as an element), may the characteristic protraction of chemico-organic changes also be referred."*

It is only within recent years that the importance of these slow metastable variations in colloids so closely simulating the changes in living organisms, which are themselves metastable colloidal complexes, have been appreciated by a few authors, as thus clearly expressed by Graham over 50 years ago.

A metastable colloidal solution is, as Graham states, comparable to a

* 'Phil. Trans.,' vol. 151, pp. 183-224 (1861).