Quantitative Experiments on the Effect of Formaldehyde on Living Plants.¹

BY

SARAH M. BAKER,

Quain Student at University College, London.

With Plates XXX and XXXI and four Figures in the Text.

THERE are no chemical problems so fascinating or so elusive as those which are continually presenting themselves in connexion with the metabolism of living plants and animals. The complexity of the reactions involved, and the microscopic dimensions of the tissues in which they take place, render an exact study of any one process an exceedingly difficult task. In general, it is only by the accumulation of a mass of more or less indirect evidence, obtained by many observers working from different stand-points, that any indication can be obtained of the course of a reaction as it occurs in living tissues.

The present investigation was undertaken in the hope that some light might be thrown on the problem of photosynthesis by a careful and detailed study of the action of formaldehyde on living plants. These hopes have only in part been realized, as the process appears to be much more complicated than has often been supposed.

Previous Work on the Subject.

Almost all the chemical theories which, from time to time, have been brought forward to explain the process of carbon assimilation in green plants, begin from the assumption that formaldehyde is the first product in the reduction of carbon dioxide.

From a purely chemical point of view, much evidence is available that, if a suitable source of energy is provided, carbon dioxide can be converted into carbon monoxide or formaldehyde in the presence of water, and thence in alkaline solution to ketoses and aldoses. The recent work of Stoklasa²

¹ Thesis approved for the Degree of Doctor of Science in the University of London.

² Stoklasa and Zdobincky : *Biochem. Zeit.*, 1911, xxx. 433-56; and Stoklasa, Sebor, and Zdobincky : *Biochem. Zeit.*, 1912, xli. 333-72.

and Berthelot¹ and their collaborators on the effect of ultra-violet light on carbon dioxide and water seems in some ways closely analagous to natural photochemical reactions. Besides this, Usher and Priestley² have brought forward evidence of the production of formaldehyde and hydrogen peroxide from carbon dioxide and water by means of chlorophyll in sunlight.

Direct evidence as to the presence of formaldehyde in plant tissues³ or its effect on living plants has, however, been meagre. The well-known toxicity of formaldehyde precluded its application in aqueous solutions, except at very great dilutions.⁴

Th. Bokorny⁵ was the first to try the effect of formaldehyde vapour on plants. He found that cress seeds could be grown, in an air-space containing formaldehyde vapour, for thirty days, while those without formaldehyde came to grief much sooner. But Viktor Grafe⁶ considerably elaborated and extended Bokorny's methods, and tried the effect of formaldehyde and other vapours on seedlings of *Phaseolus vulgaris*. He found that in light formaldehyde vapour could be tolerated up to a large percentage in the air, but its toxic effect at once became pronounced in darkness, or on colourless parts of the plant. He also found a noticeable increase in the size of the formaldehyde cultures in light (as compared with controls free from carbon dioxide), but no sign of increase in the dark. He tried several other compounds: acetic, salicylic, and benzoic aldehydes, and also acetic and benzoic acids, but found no increase in these cases. Although these results indicate the possibilities of using formaldehyde in vapour form as a source of carbon for higher plants, they are all purely qualitative, and it seemed very desirable to have a definite quantitative determination of its effect on living plants in order to test the value of the chemical theories, involving the production of formaldehyde, in the course of photosynthesis.

General Aim of the Experiments.

The quantitative treatment of living material is a process involving many difficulties. In the experiments to be described the idea has been to subject plants to the action of formaldehyde vapour, and to estimate the gross effect of the reagent by weighing the cultures dry before and after treatment. Dry seeds have been used as the starting-point for every experiment, and, in order to grow these in an atmosphere of known composition and under as nearly as possible natural conditions, a somewhat complicated apparatus was used, which it is necessary to describe in detail before proceeding to the actual experiments.

¹ Berthelot and Gaudechon: Compt. Rend., 1910, cli. 395-7.

² Usher and Priestley: Proc. Roy. Soc., vol. lxxxiv, B, p. 101.

³ Bokorny: Chem. Zeit., 1909, xxxiii. 1141-2 and 1150-1.

⁴ Bokorny: Biochem. Zeitschr., 1911, xxxvi. 83-97.

⁵ Bokorny: Pflügers Archiv für Physiologie, Bd. cxxviii, S. 565, 1905.

⁶ Grafe: Ber. d. Deut. Bot. Ges., xxix, 1911, Heft iv, S. 19.

General Experimental Methods.

The first essential was to grow the plants under as nearly normal conditions as possible. The whole apparatus (shown diagrammatically in Fig. 1) was set up in a sunny conservatory. The cultures, contained in three inverted glass bell-jars, were set side by side at the south end of the house. For each experiment two controls were used, one with normal air containing carbon dioxide, the other with air free from carbon dioxide. A continuous current of air was passed through the whole apparatus, so that no 'damping off' of the cultures occurred. The organic vapour to be tested was introduced into the air-current in minute traces just before it was bubbled into the critical culture. A special method of watering was used, so that the plants could grow undisturbed in the apparatus for four or five weeks.

Desiccated seeds were used as the starting-point for each culture, because their dry weight could be accurately ascertained, and also so that the young plants should become accustomed to the treatment from the first. When first set they were moistened with pure distilled water; their further requirements were supplied from an automatic waterer, set above each culture, in the form of a nutrient solution (containing 0.2 gramme each of KH_2PO_4 , KNO_3 , MgSO_4 , and CaSO_4 per litre).

After three or four weeks' growth, the culture without carbon dioxide showed signs of flagging, and this condition necessarily stopped the particular experiment; for a vigorous growth of mould or bacteria on any of the cultures would have vitiated the weight relations involved. All the cultures were therefore removed from their bell-jars and measured or photographed. Then they were quickly carried to an oven, heated for some hours to 80° – 100° , dried in a calcium chloride desiccator, and weighed. After subtracting the weight of nutrient salts, calculated from the weight of nutrient solution given to the plants, this gave the final dry weights of the cultures. These could be compared directly with the original dry weights of the seeds used.

The important difference between these experiments and others on similar lines is that here the plants are weighed and put into the apparatus as seeds, and growth can continue for some weeks in a continually changing atmosphere containing a given amount of any desired vapour. Also, on account of the air-current, the vapours to be experimented upon can be used in very minute quantities, comparable to the amount of CO_2 normally present in air, and the chances of poisoning effects are reduced to a minimum.

Details of the Experimental Methods.

The Treatment of the Seeds.

The seeds were weighed out—after being kept some time in a calcium-chloride desiccator—on to small tared glass 'Petri dishes', about five or

six centimetres across, filled with dry sand. The sand was then moistened with distilled water, and the cultures set on glass tripods in a large bath of water. Where carbon dioxide was to be excluded, this water contained 10 per cent. of stick potash, and where the air contained carbon dioxide a little calcium chloride was added to the water to prevent algal growths. A weighed waterer containing nutrient solution was set above each culture. Then a glass bell-jar, supported on the edges of a large porcelain 'developing dish', was inverted over each culture (or pair of cultures) so that its rim was covered to a depth of about three centimetres by the water or potash solution in the bath. The only connexion with the outside was now through the air inlet- and exit-tubes, which dipped, through the solution in the bath, under the rim of the bell-jar.

The Watering of the Seeds.—The Automatic Waterer.

The solution to this problem gave the key to the whole investigation. A watering apparatus was required which would give small and regular quantities of water over a considerable period of time, and, if possible, introduce no organic substance, such as rubber tubing, into the gas globe. After experimenting for about eighteen months with various forms of siphon-tube, connected with a reservoir, outside the gas globe, through which water could be introduced by a tap worked by hand, it was found that all such methods were highly unsatisfactory.

An automatic waterer was then devised, of which Text-fig. 2 is a diagrammatic sketch. It is composed of a glass reservoir drawn out into a narrow outlet-tube below. Two glass tubes were sealed into the top of the reservoir. One of these was drawn out into a fine end, which could be opened for filling the apparatus and then sealed again with a blowpipe. The other tube was sealed into a long piece of capillary tubing, conveniently bent to form a stand for the apparatus. The principle upon which its action depends is very simple. The apparatus is filled by putting the outlet-tube under water and applying suction at the open end of the filling-tube. The end of the filling-tube is then sealed over. Now no water can escape from the apparatus until an equivalent volume of air has entered the space above it. There are now only two possible entries for air: one is through the water, of which more later;

TEXT-FIG. 2.

the other is through the long bent capillary tube. By means of a well-known physical formula¹ it is possible to calculate the length and bore of tubing required to allow any given volume of air to enter at any temperature under the driving pressure represented by the height of the column of water in the reservoir. The necessary length is rather unwieldy for the small amounts of water (about 10 grammes per fortnight) required in these experiments. It works out to about 50 centimetres of 0.05 mm. bore capillary. But as the air admitted varies as the fourth power of the radius, and inversely as the length, a small decrease in the bore of the capillary is equivalent to a large increase in length, and so a convenient length of 0.5 mm. tubing was used, and this was then drawn out into exceedingly fine capillary at the end. The adjustment is made by breaking off small pieces of the fine capillary. Then the volume of water expelled is exactly equal to the volume of air admitted. The apparatus works admirably until the capillary tubing becomes blocked by condensed water, and the small bulb leading into the capillary tubing was filled with glass wool to prevent this as long as possible. No doubt this principle could be applied to a larger watering apparatus, the air being admitted through capillary tubing of suitable length, waxed into a well-fitting cork.

In actual practice it was found, however, that the other entry for air, through the solution itself, allowed sufficient water to escape, without the adjustment of the capillary, when the temperature underwent the usual diurnal variations, and so the end of the capillary was sealed over. The air above the water in the reservoir became so rarefied in the cool of evening that air bubbles were sucked up through the liquid, with the result that next day, as the air warmed again, water was expelled. This method of watering was the more convenient because the water-supply was automatically regulated to the needs of the plants; on hot days much water was expelled, on cool days little. But it could not be used to give much more water than about 15 grammes a week in summer unless a huge air-bulb were made at one side, and, of course, in a carefully regulated laboratory, where the temperature could be kept fairly constant, it would prove inadequate even for the needs of mustard seeds.

As a rule, one waterer was set above each culture, one culture occupying each bell-jar, but in one or two of the final experiments it was found convenient to make large waterers—the reservoirs about 4 cm. in diameter and 15 cm. high—which would hold sufficient water to last four or five weeks for two cultures, and have two cultures in each bell-jar. The water

¹ The volume of gas V , passing per second through a tube of length l and radius r , under a difference of pressure $(p_1 - p_2)$ is represented approximately by the equation $p_2 V = \frac{(p_1^2 - p_2^2)}{16 l \eta} \pi r^4$, where η is the coefficient of viscosity of the gas. (See Poynting and Thomson : Properties of Matter, p. 212, London, 1905.)

was then divided equally between the two cultures by allowing it to fall into the opening of a 'divider', i.e. a symmetrically bent glass tube open at both ends, and with another opening blown out at its apex (Text-fig. 1, Divider). The amount of nutrient solution given to the cultures could be accurately determined by weighing the waterers before and after each experiment.

The Air Supply.

A continual stream of air was kept passing through the apparatus, which prevented the 'damping off' of the cultures, so that healthy growth was maintained throughout. The importance of this in physiological experiments has been emphasized by F. F. Blackman.¹ In order to prevent any chance of the entrance of CO₂ through a possible leakage, and also to facilitate smooth running, pressure was used to drive the air through the apparatus, instead of suction. This is easily obtained from an ordinary water-suction pump by allowing the water and air drawn through with it to run through a trebly bored rubber cork into a bottle. The air is then forced out through a glass tube at the top of the bottle, while the water runs out through a siphon-tube reaching to the bottom. The use of a siphon-tube, instead of an outlet-tube worked with a tap, is to allow for variation in the water-pressure. The siphon adjusts the level of the water in the bottle, automatically, through a considerable range of pressures (Text-fig. 1, Pump).

The Purification of the Air-current. (See Text-fig. 1.)

From the pump the air was led first through an empty 'safety' bottle, then through calcium chloride to dry it, then through soda lime, and finally through two bubblers, filled with strong potash solution, to free it from carbon dioxide. As a final precaution, the bell-jars containing the cultures without carbon dioxide were inverted in troughs of dilute potash, and the entering air was bubbled through this. By adjustment of the screw clips next to the two glass T-pieces, the air could be divided equally between the three cultures, and was passed simultaneously through all the bell-jars. At one time, two experiments were running together, using five bell-jars, all worked from the same pump. The pressure required is small, provided the whole apparatus is air-tight.

The Introduction of Small Traces of Formaldehyde Vapour into the Air-current.

This was effected in three ways:

(a) The air was bubbled through ordinary commercial 'formalin', which is an aqueous solution of formaldehyde of about 40 per cent. concentration,

¹ Blackman : Phil. Trans. Roy. Soc., clxxvi, 1895, B, pp. 485-502.

containing certain quantities of methyl alcohol and organic acids as impurities. These impurities were absorbed by the potash through which the vapour was passed, as it entered the bell-jar (Text-fig. 1).

(b) The air was passed over solid paraform, at known temperature. This is a polymer of formaldehyde, which yields the aldehyde in the form of pure vapour on heating.

(c) The air was passed through a 'diffusion bulb' containing formalin. This little apparatus (Text-fig. 3) was used for introducing more volatile

TEXT-FIG. 3.

vapours, such as carbon disulphide, into the air-current when required (see p. 441). The bulb was filled with the liquid (in this case formalin) by the method of heating and cooling, and then the air-current passed along the glass-tube, sealed into the opening of the capillary, T-piece fashion. The air carries with it the traces of formaldehyde vapour which diffuse through the capillary, thus leaving its vapour pressure at the opening of

the capillary always zero. This keeps the amount of vapour diffused out constant at a given temperature, the driving pressure being always equal to the vapour pressure of formaldehyde at that temperature.

The Air Outlet.

The air passed out of the bell-jar through a long bent glass tube, opening above the liquid in the bath and passing under the rim of the bell-jar through this liquid. In the cultures where carbon dioxide was excluded, the air passed out from the bell-jar through a caustic potash bubbler, to prevent possible access of carbon dioxide by a back rush of air; and where formaldehyde or acetic aldehyde was the vapour, this was cleared out of the air by bubbling through aniline. The pump was, however, about seven metres from the bell-jars, so that contamination of the air-stream was not probable.

EXPERIMENTAL RESULTS.

Effect of Formaldehyde Vapour in the Air.

Amount of Formaldehyde in the Air-current.

Determined in a separate experiment by weighing the diffusion bulb, bubbler, or U-tube, containing the formalin or paraform used as the source of formaldehyde, before and after the passage of a known volume of dry

air, and subtracting from this loss in weight the weight of water and alcohol caught in a calcium chloride tube.

It was not thought worth while to make a long series of observations, through a range of temperatures and pressures in order to ascertain the exact percentage of vapour in the air at any time during the experiment (as, of course, both temperature and pressure varied widely during the course of a single day); but these values indicate roughly the concentration of the vapour under mean conditions. They are probably somewhat higher than the actual concentration in the bell-jar, because the caustic potash solution will dissolve and also destroy a certain fraction of the formaldehyde bubbled through it.

Values:

Source of CH_2O .	Properties of the air.			Weight CH_2O in vol. V. gm.	% CH_2O in air by weight.
	Vol. litres.	Press. mm.	Temp.		
Diffusion bulb, cap. 4 cm., 0.5 mm. bore . . .	25.3	762	13°	0.0002	0.0006
Formalin solut. about 40 %	28.316	not obs.	16°	0.1782	0.5303
	56.632	„ „	16°	0.3464	0.5155
Paraform in tube . . .	28.316	757	16°	0.0301	0.0872
	28.316	758	17°	0.0304	0.0880

For comparison, it may be noted that the maximum percentage of carbon dioxide normally present in the atmosphere is only 0.04 %, so that there is an adequate supply of potential carbon for purposes of photosynthesis produced by both the last two methods of producing formaldehyde.

A. EFFECT OF FORMALDEHYDE IN LIGHT.

The plants used in all these experiments were White Mustard—*Brassica alba*, Boiss.—and about sixty seeds were the starting-point for each culture.

EXPERIMENT I. May, 1911.

Source of formaldehyde: formalin in a diffusion bulb.

Amount of formaldehyde in air, about 0.0006 per cent.

Temperature range, 11° to 23°.

Duration, 23 days.

Atmosphere.	Orig. dry weight.	Final dry weight.	Final/orig. weight.
Air - CO_2 + CH_2O	0.4050 gm.	0.2265 gm.	55.9 %
Air - CO_2	0.4015 „	0.2175 „	54.2 %
Air + CO_2	0.3995 „	0.4255 „	106.2 %

Notes.—The slight increase in weight of the formaldehyde culture made it appear possible that, with a greater concentration, assimilation might take place.

EXPERIMENT II. July, 1912.

Source of formaldehyde: solid paraform at room temperature.

Amount of formaldehyde in air, about 0.09 %, or about three times as much potential carbon as in normal air containing 0.04 % of carbon dioxide.

Temperature range, 21.2° to 29.8°.

Duration, 22 days.

<i>Atmosphere.</i>	<i>Orig. dry weight.</i>	<i>Final dry weight.</i>	<i>Final/orig. weight.</i>
Air - CO ₂ + CH ₂ O	0.3865 gm.	0.3105 gm.	80.34 %
Air - CO ₂	0.3855 "	0.2451 "	63.59 %
Air + CO ₂	0.3875 "	0.4446 "	114.74 %

Notes.—A photograph of these cultures is shown in Plate XXX A. The effect of formaldehyde was to make the plants stouter and with somewhat broader cotyledons than those without carbon dioxide. The second leaves were developed, but to a less extent than in presence of carbon dioxide.

In the two following experiments the concentration of formaldehyde in the air was five times as great as in Experiment II; and yet it will be seen that there is only a very slight additional increase in dry weight produced. From this it appears probable that the maximum assimilation of formaldehyde has been obtained.

EXPERIMENT III. June, 1911.

Source of formaldehyde: formalin at room temperature.

Amount of formaldehyde in air, about 0.52 %, or more than fifteen times as much potential carbon as is present in normal air containing 0.04 % of carbon dioxide.

Temperature range, 14° to 25°.

Duration, 24 days.

<i>Atmosphere.</i>	<i>Orig. dry weight.</i>	<i>Final dry weight.</i>	<i>Final/orig. weight.</i>
Air - CO ₂ + CH ₂ O	0.4015 gm.	0.3585 gm.	89.3 %
Air - CO ₂	0.3985 "	0.2590 "	65.0 %
Air + CO ₂	0.3965 "	0.4035 "	102.0 %

EXPERIMENT IV. May, 1912.

Source of formaldehyde: formalin at room temperature.

Temperature range, 14.5° to 20.8°.

Duration, 26 days.

<i>Atmosphere.</i>	<i>Orig. dry weight.</i>	<i>Final dry weight.</i>	<i>Final/orig. weight.</i>
Air - CO ₂ + CH ₂ O	0.3845 gm.	0.3144 gm.	81.79 %
Air - CO ₂	0.3885 "	0.2608 "	67.01 %
Air + CO ₂	0.3860 "	0.4316 "	111.8 %

A fifth experiment was performed to see if a still further increase in the amount of formaldehyde would cause any increase in dry weight. The

percentage of formaldehyde in the air was about four times as great as in Experiments III and IV.

EXPERIMENT V. November, 1912.

Source of formaldehyde: formalin at about 50°.

Temperature range, 5° to 15°.

Duration, 28 days.

<i>Atmosphere.</i>	<i>Orig. dry weight.</i>	<i>Final dry weight.</i>	<i>Final/orig. weight.</i>
Air - CO ₂ + CH ₂ O	0.3780 gm.	0.3715 gm.	98.28 % (?)
Air - CO ₂	0.3840 „	0.1936 „	50.42 %
Air + CO ₂	0.3830 „	0.3645 „	95.17 %

Notes.—A photograph of these cultures is shown in Pl. XXX B. Formaldehyde at this concentration had decidedly toxic effects. The chief symptoms were stunted growth, little heliotropism, and a collapse of the hypocotyls. This last effect caused some of the plants to fall over into the potash solution, and, in spite of washing, some of this was probably retained over the surface, which caused the great increase in dry weight. This has, therefore, no special significance.

B. EFFECT OF FORMALDEHYDE IN THE DARK.

The general methods were the same as before, only the whole apparatus was set up in a dark room and the bell-jars covered with black paper; also the check dark experiment had carbon dioxide in the air.

EXPERIMENT VI. November, 1911.

Source of formaldehyde: formalin at room temperature.

Temperature range, 8.5° to 13.3°.

Duration, 33 days.

<i>Atmosphere.</i>	<i>Orig. dry weight.</i>	<i>Final dry weight.</i>	<i>Final/orig. weight.</i>
Air - CO ₂ + CH ₂ O, dark	0.3895 gm.	0.1498 gm.	38.48 %
Air + CO ₂ , dark	0.3960 „	0.2247 „	56.50 %
Air + CO ₂ , light	0.3840 „	0.3155 „	79.56 %

Notes.—A photograph of these cultures is shown in Pl. XXXI c. The effect of formaldehyde in the dark was not notably toxic. The diminished etiolation and slight development of root-hairs were the most noticeable features of the formaldehyde culture, and these effects were in part produced by deficient water-supply.

A second experiment was performed to see whether a gain in weight would be shown in the dark if chlorophyll had been formed in the leaves first. The two 'dark' cultures were first kept fourteen days in light, without carbon dioxide, and then the bowls were transferred bodily into the dark room. The formaldehyde bell-jar was not lifted at all in the process.

EXPERIMENT VII. November, 1912.

Light without carbon dioxide, and then darkness.

Source of formaldehyde: formalin at room temperature.

Temperature range, 6° to 15°.

Duration, 28 days.

Atmosphere.	Orig. dry weight.	Final dry weight.	Final/orig. weight.
Air — CO ₂ + CH ₂ O, dark	0.3845 gm.	0.2869 gm.	74.62 %
Air + CO ₂ , dark	0.3845 "	0.3175 "	82.52 %
Air + CO ₂ , light	0.3830 "	0.3645 "	95.17 %
Air — CO ₂ , light	0.3840 "	0.1936 "	50.42 %

Notes.—A photograph of these cultures is shown in Pl. XXXI D. The effect of formaldehyde was immediately toxic. The first symptom was a collapse of the hypocotyls just below the cotyledons, and after that no etiolation was produced. The relative large weight of both the 'dark' cultures, compared with the 'light' culture without carbon dioxide, was no doubt due to decreased respiration under the conditions obtaining in the dark room, where the temperature was uniformly lower than in the conservatory.

Discussion of Results.

The cultures in light, with traces of formaldehyde in the air, show uniformly an increase in dry weight compared with the cultures free from all carbon. This increase in dry weight seems to depend also, to some extent, on the amount of formaldehyde present in the air, but does not appear to be proportional to it. That is, after a certain percentage of the vapour in the atmosphere, a greater concentration does not cause a corresponding increase in dry weight. This seems to indicate that the maximum increase in weight possible with formaldehyde has been attained; and that, therefore, the percentage of the gas was no longer a determining factor in the reaction. The increase in dry weight is not so great as the decrease due to respiration, so that plants will not continue to grow indefinitely in formaldehyde. The increase due to formaldehyde is not so great as that due to carbon dioxide, even when the proportion of potential carbon in the air is greater in the former vapour. It would be natural to attribute the apparent gain in weight, under the influence of formaldehyde, to a decrease in the respiratory activities of the plants, but for the fact that the dark cultures show increased respiration due to formaldehyde stimulation.

Benedicenti and De Ton¹ have performed a series of experiments on *Nicotiana*, *Papaver*, and *Pelargonium*, and find that these plants show uniformly an increase in respiration, under the stimulus of formaldehyde vapour in darkness, until poisonous concentrations are reached. The only possible inference is, therefore, that the plant can make use of formaldehyde, for

¹ Benedicenti and De Ton: Atti del Reale Istituto Veneto di Scienze, Lettere ed Arti, 1901-1902, t. lxi, p. 329.

the synthesis of food materials, to a limited extent in light, but not at all in darkness. Bouilhac¹ and V. Grafe,² working by different methods, have previously come to a similar conclusion.

It may cause surprise that there was no greater increase in dry weight, even in the cultures under carbon dioxide. But it must be borne in mind that, during the first half of each experiment, before the cotyledons are unfolded, no assimilation can take place; and even in the latter part of the experiment a comparatively small green area has to supply materials for the respiration of the whole of the root and hypocotyl, besides its own requirements, before any increase in dry weight will be shown. It appears that in practice no actual increase in dry weight occurs until the first true leaves are unfolded; the assimilation due to the cotyledons being only sufficient to compensate for the losses in the first stages of germination. As explained above (p. 413), it was not possible to continue an experiment long after the culture without carbon dioxide showed symptoms of flagging, and so none of the cultures were continued for more than a few days after the leaves appeared in the culture under carbon dioxide.

The Products of Photosynthesis with Formaldehyde.

Qualitative experiments, on cultures grown simultaneously with Experiment V, in the light, confirmed Grafe's statement that no starch is formed from formaldehyde either in light or darkness, though the cotyledons contained some sugar. This need not necessarily lead to the conclusion that the process of photosynthesis is abnormal with formaldehyde, but merely that the reaction is not sufficiently vigorous to warrant the storage of starch in the corpuscles. Grafe also states that formaldehyde inhibits the action of amylase, the enzyme supposed to be the agent concerned in the synthesis of starch from sugar. This was not investigated.

Poisonous Action of Formaldehyde.

Grafe and Bokorny have already shown that traces of formaldehyde in the air do not poison plants in the light. The poisonous effect is, however, shown when the concentration of formaldehyde is sufficiently high, even in light (see Experiment V, light). The present experiments also show that plants grown from seed in the dark, under formaldehyde, show no symptoms of poisoning, provided always that the formaldehyde is purified by bubbling through caustic potash solution. Grafe's idea that the presence of chlorophyll acts as a protection against formaldehyde poisoning does not explain the case, for symptoms of poisoning were shown immediately after passing formaldehyde into a green culture in the dark (Experiment VII). The general conclusion to be drawn from the results appears to be that the light, or high temperature resulting from it, makes it possible for plants to

¹ Bouilhac et Gustiniani : *Comptes Rendus*, cxxxvi, 1903, pp. 1155-7.

² Grafe : *loc. cit.*

endure a larger dose of formaldehyde without poisonous effects than they otherwise would ; also, that the plants can become accustomed to a certain amount of formaldehyde if reared from seed in presence of the vapour. The question has not been further investigated.

Theoretical Deductions from Results.

The basis for further inquiry was the observation that formaldehyde may function, to a limited extent, as a source of food-material for plants in light, but not in darkness. This shows that the plant is possessed of some agency by means of which it is able to absorb formaldehyde. More than this cannot be assumed, for there are several explanations of the way in which formaldehyde might be used by the plant after its absorption. These may be expressed under two main headings : either formaldehyde is the first step in photosynthesis, and its further elaboration requires light energy ; or it is the last step in respiration, and is only used by the plant for photosynthesis after being converted by the respiratory processes into carbon dioxide. In either case there is a further possibility that the agency in question, which may be an enzyme, does not attack formaldehyde specifically, but is a general reagent for the aldehydic grouping, used to polymerize or oxidize quite a different compound in the plant tissues.

This last question led to a few experiments on acetic aldehyde, to see whether the aldehyde group would again be attacked.

Effect of Traces of Acetic Aldehyde in the Air.

Amount introduced into the Air-current.

It was almost impossible to estimate this accurately, especially in water solution, because of the extreme volatility of the aldehyde, which caused the concentration to change continually. The method used was the same as for formaldehyde (see p. 419).

Values.

<i>Source of Aldehyde.</i>	<i>Properties of Air.</i>			<i>Wt. of $\text{CH}_3\text{CH}_2\text{O}$ evolved.</i>	<i>% $\text{CH}_3\text{CH}_2\text{O}$ by pressure in air.</i>
	<i>Vol.</i>	<i>Press.</i>	<i>Temp.</i>		
	<i>litres.</i>	<i>mm.</i>		<i>grms.</i>	
Pure aldehyde in bubbler.	2.36	773	13°	3.8435	52.1
	1.18	773	12°	1.8800	51.3
40 % water solution of aldehyde	4.72	772	15.5°	0.7159	4.9
	4.72	773	15°	0.2584	1.8

Besides the uncertainty of these values the potash solution destroys a large proportion of the aldehyde vapour, forming a bright orange solution. However, the results serve to show that a large proportion of aldehyde could be obtained in the air. Probably the diffusion-bulb method, with pure aldehyde in the bulb, would be the best method of introducing it, but this was not used in the experiments.

Effect of Acetaldehyde in the Air, in Light.

EXPERIMENT I. December, 1912.

Source of aldehyde: pure liquid aldehyde.

Temperature range, 8° to 14°.

Duration, 14 days.

<i>Atmosphere.</i>	<i>Orig. dry weight.</i>	<i>Final dry weight.</i>	<i>Final/orig. weight.</i>
Air - CO ₂ + CH ₃ CH ₂ O	0.3845 gm.	0.3326 gm.	86.50 %
Air - CO ₂	0.3850 "	0.3111 "	80.83 %
Air + CO ₂	0.3845 "	0.3279 "	85.28 %

Notes.—The effect of acetaldehyde on the plants, in this concentration, was strongly toxic. Most of the seeds germinated, and in some the cotyledons were unfolded, but they remained colourless, and the roots showed no geotropism.

The high value merely means inhibition of respiration.

EXPERIMENT II. February to March, 1912.

Source of aldehyde: 10 % water solution.

Temperature range, 9.5° to 11°.

Duration, 19 days.

<i>Atmosphere.</i>	<i>Orig. dry weight.</i>	<i>Final dry weight.</i>	<i>Final/orig. weight.</i>
Air - CO ₂ + CH ₃ CH ₂ O	0.3910 gm.	0.2915 gm.	74.54 %
Air - CO ₂	0.3885 "	0.2545 "	65.53 %
Air + CO ₂	0.3860 "	0.3530 "	91.45 %

Notes.—The increase in weight shown in presence of acetic aldehyde was probably due to retardation of respiration and general growth, as the cotyledons were unfolded a day later in aldehyde than in the 'control' cultures. There were no other symptoms of poisoning. The temperature and light conditions were so poor that the experiment was not considered conclusive.

EXPERIMENT III. July to August, 1912.

Source of aldehyde: 40 % water solution.

Temperature range, 17.5° to 23.5°.

Duration, 28 days.

<i>Atmosphere.</i>	<i>Orig. dry weight.</i>	<i>Final dry weight.</i>	<i>Final/orig. weight.</i>
Air - CO ₂ + CH ₂ O	0.3870 gm.	0.2446 gm.	63.20 %
Air - CO ₂ + CH ₂ O	0.3875 "	0.2615 "	60.67 %
Air - CO ₂	0.3865 "	0.2455 "	63.52 %
Air - CO ₂	0.3860 "	lost	—
Air + CO ₂	0.3845 "	0.4325 "	112.40 %
Air + CO ₂	0.3850 "	0.3775 "	98.95 %

Notes.—The experiment was run with two cultures in each bell-jar, the first of the two cultures being nearer the light in every case; unfortunately the second culture without CO₂ was lost by an accident. The experiment

was continued until the two cultures without CO_2 showed signs of flagging (the ones with aldehyde were the first to do so). They may be taken as showing conclusively that acetic aldehyde cannot be used by the plant for photosynthesis. The plants showed no symptoms of poisoning under acetic aldehyde.

Conclusion from Results.—Acetic aldehyde cannot be used for photosynthesis, though its poisonous action tends to retard respiration, especially under unfavourable temperature conditions. Hence the aldehyde group is not the sole reason for the reaction in the case of formaldehyde, but the agency which can make use of formaldehyde in the plant is specific for that compound.

THE FORMALDEHYDE QUESTION.

The question remains (see p. 424): Is formaldehyde the first step in a complicated series of reactions requiring light energy in photosynthesis, or is it the last step in respiration, and hence converted by the plant into carbon dioxide? Without an answer to this primary question, the results with formaldehyde have no significance, as we cannot tell their real meaning. The obvious method of attacking this problem was to determine, quantitatively, the ratio between the weight lost and carbon dioxide evolved by the plants in respiration, with and without formaldehyde in the atmosphere. For, if formaldehyde is absorbed by the plant and converted into carbon dioxide, there will be more carbon dioxide evolved for a given loss of weight in the plant than under normal conditions. Before attempting to determine this, it was, however, necessary to find whether there was any definite relation between the weight lost and the carbon dioxide evolved during normal respiration, and to determine this relation if it was sufficiently definite.

Quantitative Determination of the Ratio between the Loss in Weight of the Plants and the Carbon dioxide evolved during Respiration in Darkness.

The only experiments in which a direct determination has been made of the ratio obtaining between the loss in weight of plants and the carbon dioxide of respiration were made some years ago by T. C. Day.¹ Day undertook an elaborate quantitative study of the chemical processes taking place during the earlier stages of the germination of barley. He found that a certain proportion of oxygen was absorbed and retained by the seeds, in excess of that oxidized to form carbon dioxide; but that, having allowed for this, the proportions between the loss in wet weight and dry weight of the seeds and the carbon dioxide evolved in respiration approximated very closely

¹ T. C. Day : Experiments on Germinating Barley and Influence of Temperature on Germinating Barley. Journ. Chem. Soc. Trans., 1880, pp. 645-58, and 1891, pp. 664-77.

TEXT-FIG. 4. Diagram of apparatus used to estimate the quantitative effect of formaldehyde on plants in the dark. In the last experiments the position of the paraform tube and the KOH:CaCl₂ tube before the formaldehyde culture were reversed, so that the paraform tube was next to the culture.

to those represented by the chemical formula for the complete oxidation of a glucose :—

As there is no reason to believe that there is a definite or general chemical process always concerned in the complex oxidation reactions included under respiration, it was essential to determine these weight relations for mustard seeds germinating under the experimental conditions. For this purpose, several modifications of the original apparatus were necessary, and finally a somewhat complicated apparatus was used.

General Experimental Methods.

The apparatus used was similar to that shown diagrammatically in Text-fig. 4, except that the paraform tubes and the absorption apparatus for formaldehyde were omitted. The aim of the experiments was to find the loss in weight of the cultures, and also the total weight of carbon dioxide and water evolved during growth. The seeds, which had been desiccated over calcium chloride, were grown, as in the former experiments, on tared Petri dishes filled with dry sand. A separate experiment with three weighed amounts of the same seed, heated to 80° – 100° , gave the loss in weight on heating and hence the true dry weight of the seeds. A second experiment gave the total gain in weight of the seeds on hydrolysis, by hydrolysing them with dilute acid. When first set, the seeds were moistened with a weighed amount of pure distilled water, and their later requirements were supplied by means of nutrient solution from an automatic waterer (as described on p. 415) of suitable size. The waterer could also be accurately weighed, before and after the experiment, which gives the amount of nutrient solution given to the culture. The seeds, supported on the base of the waterer, were contained in a 'vacuum' desiccator, with two glass tubes sealed through its glass stopper, through which a continual current of air was passed over them. Light was carefully excluded from the desiccator with a black cloth shade. The air-current was produced by pressure from a water-jet pump (as described on p. 417), freed from CO_2 (as in the former experiments) by means of soda lime and strong caustic potash, and dried by calcium chloride.

After from seven to fourteen days the plants had grown to the height of from 3 to 7 cm. They were then weighed, first wet and then dry. The drying was effected by heating to 80° – 100° for some hours, and cooling in a calcium chloride desiccator.

The Absorption of Carbon Dioxide.

This was effected by means of a Liebig's absorption bulb, filled with very concentrated potash solution and with a calcium chloride tube sealed on to it to prevent loss of water from the bulb; the air-current was passed

through this bulb after leaving the cultures and the drying-tube. To prevent a possible backrush of air containing carbon dioxide, another potash bubbler was connected on to the exit-tube of the weighed Liebig bulb. It was found that the potash solution in the Liebig bulb had to be absolutely saturated, at room temperature, and the calcium chloride fresh and perfectly dry, or water escaped. If these precautions were observed, and the weighed apparatus was kept covered from dust, results could be obtained quite as accurate as the weighings of the seeds with which they were to be compared.

The Absorption of Water.

The quantitative determination of the water relations of the plants was a very fascinating problem, but the practical difficulties involved were so great that no really satisfactory method has so far been found. The observations are, however, sufficiently accurate to indicate the general nature of the relations.

The first important point is to keep the same water-absorbing medium throughout the apparatus, so that the air should not be more efficiently dried at one point than another, and so take up water from a reagent which is supposed to dry it, as is the case, for example, when air dried in sulphuric acid passes over calcium chloride. Calcium chloride was chosen as this drying reagent, not because it is the most efficient, but because it is clean and convenient, and gives no possibility of acid gases, even when acted upon by organic vapours.

Before commencing each experiment every precaution must be taken to exclude water from the whole apparatus. The desiccators, tap grease, tubing, &c., must all be dried with calcium chloride before use.

The first absorbent for water is a calcium chloride U-tube next to the desiccator containing the culture. This was kept open towards the desiccator, even when no air-current was passing, in order to keep the exit-tube dry.

In a few preliminary experiments it was found that while the U-tube absorbed about 1.5 gm. of water, about 2 gm. were lost in some way. The only possibility of loss was by condensation on the walls of the desiccator. To obviate this a second absorbent for water was made. This was a small 'house' of asbestos board, made to fit into the desiccator, which held the waterer and culture and had a hole in the roof for the air-tubes. The 'house' was varnished over the outside with shellac varnish, so that it could be weighed before and after each experiment. The rough inner surface of the 'house' absorbed much of the water of transpiration.

A third measure of water was by the original and final wet weighings of the seeds. For these weighings, in which a free evaporating surface of water was present, special methods had to be employed.

In the earlier experiments the method of timed weighings was employed. Allowance was made for the amount of water lost during weighing, by weighing the loss in the next interval of the same time. The weighings were done as rapidly as possible, but could not be very accurate, because of the continually changing values.

The second method used was to weigh the wet cultures, or 'house' inside a small paper hood which completely protected them from the outside air, and which could be weighed before or after the weighings. Even by this method a small loss, due to evaporation or condensation on the hood, occurred, but its amount was not great and its estimation could not lead to serious errors.

Besides these losses, a small loss always occurs in transferring the wet cultures to or from the desiccators. This was reduced as much as possible by speed in manipulation.

Precautions to render the Apparatus Air-tight.

It is of the utmost importance, in these experiments, that the whole apparatus should be absolutely air-tight. A considerable number of experiments were made before this result could be attained. The first essential is to have all rubber connexions irreproachable; absolutely sound, well-fitting corks, and thick pressure tubing, closed with double screw-clips, for all connexions. The second essential is to have air-tight ground-glass joints round the stoppers and lids of the desiccators. This last was not easy to secure. It was found that, though every joint was carefully greased, and then sealed over with paraffin wax, after about a week little bubbles of air worked their way through the grease round the desiccator lids, collected under the wax sheath, and finally cracked the wax and sprung a leak. After several experiments had been spoiled in this way, it was found that the desiccator lids could be kept air-tight by applying a little pressure from the outside to counteract the internal air-pressure. For this purpose my brother—Mr. G. R. Baker—designed and made a wooden clip to hold the lids tightly together. This consisted of a wooden frame fitting under the flange of the desiccator, with four wooden clips screwed down from it on to the upper edge of the desiccator lid, by means of metal thumb-screws. Two of these are shown in section in Text-fig. 4. When these were screwed down, and the lids greased and waxed round, no sign of leakage could be detected in any part of the apparatus during the whole time.

Finally, to prevent the movement of air from one part of the apparatus to another when the air-current was not passing, the screw-clips at each side of the weighed apparatus and the desiccator were closed, immediately after stopping the pump. Only one of these was kept open, that between the desiccator and the calcium chloride U-tube, in order to prevent the air exit- and inlet-tubes from condensing moisture.

EXPERIMENTAL RESULTS.

Determination of the true Dry Weight of Desiccated Seeds.

The seeds were weighed out on to tared watch-glasses, heated for several hours to 80°–100°, cooled in a calcium chloride desiccator and weighed.

Results:—

	<i>Expt. 1.</i>	<i>Expt. 2.</i>	<i>Expt. 3.</i>
Original weight	0.4010 gm.	0.4010 gm.	0.3980 gm.
Final weight	0.3860 "	0.3855 "	0.3830 "
Water lost	3.74 %	3.62 %	3.77 %

Mean amount of water lost = 3.71 %

This mean value was used in calculating the dry weight of the seeds in the respiration experiments. In the later experiments two or more weighed quantities of ground seeds were heated, simultaneously with the cultures, to give their loss in weight on heating. This was necessary because of the impossibility of exactly regulating the temperature of the oven, and also because, after prolonged heating, some charring was produced.

Determination of the Gain in Dry Weight due to Hydrolysis.

In order to find the true loss in dry weight due to respiration, it was necessary first to know the total gain in weight due to hydrolysis of sugars, &c., during germination. This was not easy to determine, and even when concordant results were finally obtained, they can only roughly indicate the real value in the plant, because an inorganic hydrolysing agent was used whose action may differ widely from that of the enzymes in the germinating seeds.

The mustard seeds were found to contain no starch, but a considerable reserve of fats and proteins, together with the glucoside of mustard oil.

On hydrolysing them in the ordinary way with 10 % HClAq and evaporating off the acid over a water-bath, so much charring of the sugars occurred that it was impossible to obtain concordant results. Besides this, the uncharred sugars were so deliquescent that they formed syrupy drops even in the desiccator. Experiments were made in which the HClAq was distilled off from a weighed retort *in vacuo* at temperatures of 20° to 30°. Here less charring occurred, but it was necessary to dry the weighed vessel for some hours in a current of dry air at 50°, and the action of the remaining HCl vapour on the seeds at this temperature seemed to induce further charring. The results were more nearly concordant, but were not considered sufficiently satisfactory.

The final method employed was to digest the seeds, previously ground to powder in a dry pestle and mortar, with 10 c.c. of 2 % HClAq for five to eight hours, and then to allow the mixture to stand for two or three weeks in a vacuum desiccator over stick potash until all signs of moisture

were gone. Then they were heated to 80°–100°, cooled in a calcium chloride desiccator, and weighed. A blackening over the surface was still produced, but this may have been rather an oxydase action than due to true charring.

	<i>Expt. 1.</i> <i>gm.</i>	<i>Expt. 2.</i> <i>gm.</i>	<i>Expt. 3.</i> <i>gm.</i>	<i>Expt. 4.</i> <i>gm.</i>	<i>Expt. 5.</i> <i>gm.</i>	<i>Expt. 6.</i> <i>gm.</i>
Original weight	0.4715	0.3917	0.4767	0.4084	0.3998	0.4259
Original dry weight	0.4540	0.3772	0.4590	0.3826	0.3745	0.3989
Final dry weight	0.4801	0.3968	0.4853	0.4062	0.3954	0.4234
Gain in dry weight	0.0261	0.0196	0.0263	0.0236	0.0209	0.0245
% gain due to hyd.	5.75 %	5.21 %	5.73 %	5.78 %	5.23 %	5.75 %

Mean value for gain in hydrolysis = 5.58 %

The mean of these results has been used in calculating the loss in dry weight during respiration. The total loss during respiration was taken as the loss in dry weight observed in the respiration experiment plus this value for the gain in dry weight on hydrolysis.

Experimental Results for Respiration.

The first two experiments were running simultaneously from October 7 to 21, 1912. Of the two cultures, Experiment 2 grew far the more luxuriantly, as the waterer of Experiment 1 was not correctly adjusted, and so the plants suffered through insufficient water-supply. At the end of the experiment the plants in Experiment 1 were only 3 cm. high and were of stunted and irregular growth. In Experiment 2, however, the plants were 10 cm. high and very healthy in appearance, but they grew out at the top of their 'house' before it was possible to stop the experiment, and so reached the 'dead' space between the air inlet- and exit-tubes, and no doubt a certain amount of the water of transpiration was lost on the sides of the desiccator, and perhaps also some of the carbon dioxide was 'dead-locked' between the air-tubes.

The third experiment was running from October 24 to November 4, 1912. The plants were well grown, about 3 cm. high; the same waterer was used as for Experiment 2.

The wet weighings in all these experiments were done by the method of 'timed' weighings. The balance used was by Becker, London, but only sensitive to 0.0005 gm.

QUANTITATIVE RESULTS ON RESPIRATION IN DARKNESS.

	<i>Expt. 1.</i>	<i>Expt. 2.</i>	<i>Expt. 3.</i>
	<i>grm.</i>	<i>grm.</i>	<i>grm.</i>
Orig. wt.	0.4020	0.4020	0.4000
Orig. dry wt. . . .	0.3870	0.3870	0.3850
Final dry wt. . . .	0.3670	0.3426	0.3865
Change in dry wt. . .	-0.0200	-0.0444	+0.0015
Gain on hydrolysis . .	0.0224	0.0224	0.0223
Total loss in resp. dry	0.0424	0.0668	0.0208
Dist. water given . .	4.7225	3.3590	3.2235
Nut. soln. given . . .	0.5985	7.3965	5.0335
Total water given . .	5.3210	10.7555	8.2570
Water on seeds . . .	1.5810	6.6020	5.1320
Water in CaCl ₂ . . .	0.6735	0.4925	1.3955
Water in 'house' . . .	3.0510	3.3260	1.7185
Total water caught . .	5.3055	10.4205	8.2460
Loss in wet wt. . . .	0.0155	0.3350	0.0110
H ₂ O evolved in resp. .	0.0269		0.0098
CO ₂ evolved in resp. .	0.0590	0.0885	0.0285
<i>Theoretical Values.</i>			
CO ₂ calc. from dry wt.	0.0620	0.0981	0.0303
CO ₂ calc. from wet wt.	0.0568		0.0403

The last two sections show the values for carbon dioxide, calculated from the found loss in dry weight and wet weight of the plants respectively, on the assumption of the quantitative formula for respiration representing the complete oxidation of a glucose :—

Parts by weight . . . 30 32 44 18

(The proportions obtaining when a polysaccharide, such as saccharose or starch, is oxidized are sufficiently close to these to be within the limits of experimental error.) It will be seen that, except in the 'wet' values of Experiment 2, which (as explained above, p. 432) are probably low because the plants grew out of their 'house', these calculated values approximate very closely to the found weights of CO₂. This shows that in mustard seeds, under the experimental conditions, respiration is, in the main, represented by a reaction in which carbohydrate is converted quantitatively into carbon dioxide and water.

There is, therefore, a somewhat surprising agreement with the results of Day (previously cited, p. 426) for germinating barley. This is particularly remarkable when it is remembered that mustard is an oily and barley a starchy seed. Besides this, Day's experiments were only concerned with the very first stages of germination, before the plumule had appeared; while in the present experiments it has been found that by far the greater

proportion of carbon dioxide (about 80 per cent.) is evolved after the cotyledons have emerged. In the present investigation no constant gain in weight of the seeds has been detected, which could be attributed to absorption of oxygen; this may be due to the comparatively prolonged experiments. Day, on the other hand, did not take into account the gain in dry weight due to the hydrolysis of his seeds, which has been found to be such an important factor in these experiments.

QUANTITATIVE EXPERIMENTS ON THE FORMALDEHYDE PROBLEM (see p. 426).

Having found the relation between the loss in weight of the plants and the amount of carbon dioxide evolved by them in respiration under normal conditions, it was possible to proceed to investigate the question: Is formaldehyde a step in respiration and converted by the plant into carbon dioxide, or is it merely a step in photosynthesis, requiring light energy for its further elaboration?

General Experimental Methods.

The apparatus used is shown diagrammatically in Text-fig. 4 (p. 427). It consists fundamentally of the ordinary respiration apparatus, with arrangements for introducing small traces of formaldehyde into the air and for removing it again quantitatively.

In these experiments two exactly similar cultures, with desiccators, &c., of the same dimensions, were grown simultaneously. In one culture the formaldehyde was introduced into the air before it passed over the cultures; in the other 'check' experiment it was introduced immediately after the air had passed the calcium chloride tube. When the air had been quantitatively freed from formaldehyde and any other volatile substance used to condense this, the 'check' experiment should show exactly the same weight relations between the loss in weight of the plants and the carbon dioxide evolved during respiration as was found in the earlier respiration experiments.

The general method of procedure was exactly the same as in these experiments (see p. 428 et seq.).

A great many experiments were made before the quantitative absorption of formaldehyde could be satisfactorily accomplished. A short account of these may perhaps be given before proceeding to the final method used, as they not only indicate some of the difficulties involved, but also some of the capabilities and dangers of the gravimetric method of plant physiology.

Experiments in which Chemical Absorbents for Formaldehyde were used.

There are two general chemical methods for the estimation of formaldehyde. One depends on its oxidation to formic acid. This is obviously

unsuitable for the present experiments, as formic acid would be even more readily absorbed by the potash bulb used to absorb carbon dioxide than the aldehyde itself. The other depends on its absorption by ammonia to form formamide. Ammonia itself was too volatile for convenient use in the present experiments, so a modification of the method was used and aniline was chosen as the absorbent for formaldehyde. This works fairly efficiently, but the great drawback to its use was the difficulty of reabsorbing the aniline vapour carried away by the air-current.

The first absorbent used was fused calcium chloride, but this did not absorb the vapour quantitatively.

The second absorbent was calcium chloride and then concentrated sulphuric acid, but the sulphuric acid dried the air so much more efficiently than calcium chloride that the potash bulbs lost water, and their change in weight gave no measure of the carbon dioxide absorbed by them.

The results show one or two interesting points, however, and so they are recorded here.

Expt. 1.	Source of CH_2O	.	.	Formalin solution.
	Absorbents	.	.	Aniline, then CaCl_2 .
Expt. 2.	Source of CH_2O	.	.	Paraform.
	Absorbents	.	.	Aniline, then CaCl_2 .
Expt. 3.	Source of CH_2O	.	.	Paraform.
	Absorbents	.	.	Aniline, then CaCl_2 , then H_2SO_4 .

Results:—

	<i>Expt. 1.</i>		<i>Expt. 2.</i>		<i>Expt. 3.</i>	
	+ CH_2O .	− CH_2O .	+ CH_2O .	− CH_2O .	+ CH_2O .	− CH_2O .
	<i>gram.</i>	<i>gram.</i>	<i>gram.</i>	<i>gram.</i>	<i>gram.</i>	<i>gram.</i>
Loss in dry wt. .	0.0188	0.0304	0.0131	0.0108	0.0193	0.0231
Gain on hyd. . .	0.0223	0.0223	0.0225	0.0223	0.0223	0.0223
Total loss in resp.	0.0411	0.0527	0.0356	0.0331	0.0416	0.0454
Gain of KOH bulb	+ 0.0770	0.0000	+ 0.0927	+ 0.0652	+ 0.0023	− 0.0105
CO_2 calculated .	0.0603	0.0774	0.0524	0.0484	0.0612	0.0576

These results serve to show the inadequacy of the chemical methods of absorbing formaldehyde. In Experiment 1 the water solution of formaldehyde, after the culture without formaldehyde, absorbed the whole of the carbon dioxide as well as the aniline. In Experiment 3 the sulphuric acid used to absorb aniline caused a loss of water from the potash bulbs. In Experiment 2 the whole of the aniline was not absorbed. But, as a slight increase in the weight of carbon dioxide was shown by the formaldehyde culture in all these experiments, it was thought probable from these results that formaldehyde was used as a source of carbon dioxide; but as the amount of aniline passing through would depend on the speed of the air-current, which was hard to regulate uniformly, no real conclusion of any kind can be drawn from the experiments.

The variations in weight between the two check cultures are chiefly due to differences in the water-supply, which is a very important factor in respiration in the dark.

Physical Methods for the Absorption of Formaldehyde.

The experiments detailed above led to the final abandonment of chemical methods for absorbing formaldehyde. The physical method used was as follows. The formaldehyde was condensed out of the air by cooling it to the temperature of solid carbon dioxide. Formaldehyde polymerizes at 0° to form its polymer, the solid paraform, whose vapour pressure at this very low temperature would be negligible.

Experimental Methods.

The condensing apparatus is shown in Text-fig. 4. The air was first passed through an empty Walther's condenser, which was cooled in carbon dioxide snow contained in a vacuum cylinder. At this temperature the formaldehyde should condense and the traces of carbon dioxide in the air pass on. The air was then warmed in a water bath at about 50°, and passed through a long calcium chloride tube (not shown in the diagram) to bring it to room temperature, and the humidity of air dried by calcium chloride, in order that no evaporation of water may take place from the potash bulb. It was necessary to have the glass apparatus sealed together with ground-glass taps because of the low temperature. The taps were greased with a mixture of vaseline, rubber, and paraffin-wax, and were air-tight.

The carbon dioxide and water were estimated in the same way as in the former experiments (p. 431 et seq.), and the plants were treated similarly.

Notes.—In Experiment 1 the apparatus was newly set up and was not dried sufficiently long before the experiment, hence the irregular wet weight values.

In Experiment 4 a certain quantity of water condensed in the air exit-tubes, and so escaped estimation.

The excess of water, apparent in all the formaldehyde cultures, is partly due to partial condensation of the formaldehyde from the air-current into the water on the cultures and also into the calcium chloride tube.

After the first two experiments were completed, it was found that only a very small trace of formaldehyde had actually been passing over the formaldehyde culture, as a large proportion of the vapour was absorbed by the concentrated potash and the calcium chloride, which intervened between the culture and the source of formaldehyde. For this reason, in the last two experiments the positions of the paraform tube and potash

bubbler were reversed, so that the paraform tube came next to the culture. In the Experiments 3 and 4, therefore, the full percentage of formaldehyde yielded by paraform at room temperature (about 0.0875 %; see p. 419) was present in the air which passed over the formaldehyde culture.

In all these experiments (except the 'check' without formaldehyde of Experiment 4, which suffered from excessive water-supply) growth was healthy and the plants vigorous and well developed. No toxic symptoms, except the sparse production of root-hairs, were shown by any of the formaldehyde cultures.

Each experiment lasted from ten to fourteen days, and the plants varied in height from 3-10 cm., according to the prevailing temperature conditions.

The Balance used was by Oertling, London, sensitive to 0.0001 grm. and taking a 250 grm. load.

Quantitative Results on the Effect of CH₂O in Darkness.

	<i>Expt. 1.</i>		<i>Expt. 2.</i>		<i>Expt. 3.</i>		<i>Expt. 4.</i>	
	+ CH ₂ O.	- CH ₂ O.	+ CH ₂ O.	- CH ₂ O.	+ CH ₂ O.	- CH ₂ O.	+ CH ₂ O.	- CH ₂ O.
	<i>grms.</i>	<i>grms.</i>	<i>grms.</i>	<i>grms.</i>	<i>grms.</i>	<i>grms.</i>	<i>grms.</i>	<i>grms.</i>
Orig. wt.	0.4006	0.4003	0.3998	0.4000	0.4036	0.4040	0.4090	0.3996
Orig. dry wt.	0.3470	0.3467	0.3690	0.3692	0.3734	0.3738	0.3940	0.3857
Final dry wt.	0.3333	0.3296	0.3577	0.3378	0.3456	0.3357	0.3784	0.3796
Loss in dry wt.	0.0137	0.0171	0.0113	0.0314	0.0278	0.0381	0.0156	0.0261
Gain on hydrolysis . . .	0.0223	0.0223	0.0223	0.0223	0.0225	0.0225	0.0227	0.0223
Total loss in resp. dry	0.0360	0.0394	0.0336	0.0537	0.0503	0.0606	0.0383	0.0284
Dist. water given . . .	4.4993	3.7722	4.5016	5.1525	6.4749	6.0366	6.1381	5.5325
Nut. soln. given	2.3957	2.3994	3.1317	3.1672	3.8469	7.7656	5.3754	5.7964
Total water given . . .	6.8950	6.1716	7.6333	8.3197	10.3218	13.8022	11.5135	11.3289
Water on seeds	1.9101	1.7110	1.9563	2.7190	5.4852	8.3693	8.5885	8.3411
Water in CaCl ₂	0.4517	0.6334	0.6988	0.6376	0.3217	0.2857	0.4513	0.2676
Water in 'house'	5.2175	4.0922	5.0845	4.9417	4.6569	5.1136	2.3814	2.5856
Total water caught. . .	7.5793	6.4366	7.7396	8.2983	10.4638	13.7686	11.4212	11.1943
Loss in wet wt.				0.0214		0.0336	0.0923	0.1346
H ₂ O evolved				0.0323		0.0270		
CO ₂ evolved	0.0495	0.0561	0.0511	0.0718	0.0655	0.0805	0.0576	0.0365
<i>Theoretical Values.</i>								
CO ₂ calc. from dry wt.	0.0528	0.0576	0.0493	0.0787	0.0739	0.0889	0.0559	0.0404
CO ₂ calc. from wet wt.				0.0788		0.1232		

Conclusions.—The quantitative results show that in presence of formaldehyde vapour the carbon dioxide evolved during respiration is exactly the same in amount as in normal respiration. Hence formaldehyde is not converted by the plant into carbon dioxide, nor can it be utilized for the synthesis of food materials in the dark, for any gain in weight on either of

these two accounts would alter the proportion obtaining between the loss in dry weight of the plant and the amount of carbon dioxide evolved.

This result is the reverse of that obtained from a consideration of the former results (with aniline as absorbent for formaldehyde, see p. 435), which were comparative but not strictly quantitative. This illustrates the great danger in drawing definite conclusions from purely comparative data.

Theoretical Deductions from Results.

This leads to the conclusion that formaldehyde, as it can be assimilated to some extent by plants in light (p. 422), is probably a step in photosynthesis, but that the further steps in this process also require light energy. In this case, the next step after formaldehyde must be a substance still more unstable than formaldehyde itself. Recent work on this subject, e. g. Usher and Priestley's¹ investigations on the extra-cellular production of formaldehyde from carbon dioxide in light, as well as the theories of Baeyer, Erlenmeyer, and Bach, are only concerned with the first step in photosynthesis, the assumption being that the subsequent processes—carried out, according to Usher and Priestley's work,¹ by the protoplasm—consist merely in the polymerization of formaldehyde. The electrical theory of photosynthesis, announced by J. Harvey Gibson,² also only accounts for the production of formaldehyde from carbon dioxide. The experiments which have been described seem to indicate that, if formaldehyde is part of the natural scheme of photosynthesis, the subsequent processes are much more complex than has often been supposed. It should, however, be noticed that such an authority as Meldola³ seems to lean to the less simple explanations of the phenomenon.

Various suggestions have been brought forward at different times as to the nature of the substances formed from formaldehyde in photosynthesis. Fischer⁴ suggested glycerose, and Piloty⁵ glyceric aldehyde with dihydroxyacetone, as steps in building up the hexose molecule. These substances have not been found in plants, nor would the processes require light energy.

In my opinion the most plausible suggestion is that due to Collie,⁶ that keten, or some similar compound, is the first step in the process, after the production of formaldehyde. This substance keten, whose formula is $\text{CH}_2=\text{CO}$,

is the first member of the extraordinarily reactive series of compounds, of the same general formula, known as polyketides. From substances of this

¹ Usher and Priestly : Proc. Roy. Soc., lxxxiv, B. p. 101.

² Gibson : Ann. of Bot., 1908, xxii, p. 117.

³ Meldola : Trans. Chem. Soc., lxxxix, p. 749 et seq.

⁴ Fischer : Ber. d. Deutsch. Chem. Ges., 1890, xxiii, 2138.

⁵ Piloty : Ber. d. Deutsch. Chem. Ges., xxx, 3166.

⁶ Collie : Trans. Chem. Soc., 1907, xci, 1806.

series, Collie and others.¹ have been able to produce representatives of almost all the groupings present in plants and animals (including many nitrogen compounds), by means of such reactions as hydrolysis, in ammoniacal or faintly alkaline solutions, at ordinary temperatures. This suggestion practically combines the formaldehyde hypothesis with the ingenious and suggestive multiple-photosynthesis hypothesis,² and it has moreover much chemical evidence to support it. The formation of the exceedingly unstable $\text{CH}_2=\text{CO}$ molecule from formaldehyde would probably be endothermic, and so require light energy.

The action of certain of these polyketides on plants is at present under investigation, but it would be useless to go into a further discussion of this theory until more experimental data are available.

SUMMARY.

Experiments have been described in which seeds were grown in an atmosphere containing known quantities of formaldehyde vapour in light and darkness. A comparison of the change in dry weight with that of control cultures with and without carbon dioxide revealed the fact that formaldehyde could be used for the synthesis of food materials to some extent in the light. The gain in dry weight produced was about half the loss due to respiration, and an increase in the percentage of formaldehyde in the air did not produce a corresponding increase in dry weight after a certain concentration. An excess of formaldehyde was toxic.

In the dark formaldehyde was not assimilated, but seemed to stimulate respiration. Its poisonous effect was more marked than in light.

Experiments with acetic aldehyde showed that formaldehyde was not assimilated in light merely by virtue of the aldehyde group; for acetic aldehyde could not be taken up by the plants.

The results were capable of two interpretations: either formaldehyde is a step in respiration, and converted by the plant into carbon dioxide before it can be assimilated; or it is the first step in photosynthesis, and its further elaboration by the tissues requires light energy.

In order to decide between these two possibilities, quantitative experiments were made in which the change in dry weight of the cultures could be directly compared with the carbon dioxide evolved during respiration. It was found that this ratio agreed closely with that calculated for the complete oxidation of a carbohydrate. When formaldehyde was passed over the cultures in the dark, there was no change in the quantitative relations between the loss in dry weight of the cultures and the carbon dioxide of respiration. Hence formaldehyde was not converted into carbon dioxide by the plants, nor used as a source of food materials in the dark.

¹ Cf. chapter on Polyketides in Stewart: *Recent Advances in Organic Chemistry*, London, 1908, p. 44.

² Brunner and Chouard: *Ber. d. Deut. Chem. Ges.*, 1886, xix, p. 595.

Probably, therefore, formaldehyde may function as a stage in photosynthesis; but the production from it of sugars and other food materials requires light energy. This is in contradiction to most chemical theories on the subject; but the results tend to confirm such a hypothesis as that of Collie, which postulates the production from formaldehyde of a still more unstable substance, keten ($\text{CH}_2=\text{CO}$), before it is further elaborated into food materials.

In conclusion, I have to acknowledge my obligation to Mr. T. G. Hill, whose advice and criticism have been of the utmost assistance throughout the investigation.

EXPLANATION OF PLATES XXX AND XXXI.

Illustrating Miss Baker's paper on the Effect of Formaldehyde on Living Plants.

A. Plants grown under formaldehyde in light, Expt. II. Source of formaldehyde: solid paraform at room temperature. Centre: $\text{Air}-\text{CO}_2+\text{CH}_2\text{O}$. Right hand: $\text{Air}-\text{CO}_2$. Left hand: $\text{Air}+\text{CO}_2$.

B. Plants grown under formaldehyde in light, Expt. V. Source of formaldehyde: formalin at 50° . Centre: $\text{Air}-\text{CO}_2+\text{CH}_2\text{O}$. Left hand: $\text{Air}-\text{CO}_2$. Right hand: $\text{Air}+\text{CO}_2$.

C. Plants grown under formaldehyde in darkness, Expt. VI. Source of formaldehyde: Formalin at room temperature. Centre: $\text{Air}-\text{CO}_2+\text{CH}_2\text{O}$ dark. Left hand: $\text{Air}+\text{CO}_2$ dark. Right hand: $\text{Air}+\text{CO}_2$ light.

D. Plants grown for fourteen days in light without CO_2 and then transferred to dark room and grown 14 days with formaldehyde, Expt. VII. Source of formaldehyde: Formalin at room temperature. Centre: $\text{Air}-\text{CO}_2+\text{CH}_2\text{O}$ dark. Left hand: $\text{Air}+\text{CO}_2$ dark. Right hand: $\text{Air}+\text{CO}_2$ light.

APPENDIX.

In the course of the above investigation a few experiments were tried with other organic vapours, not aldehydes. The results obtained were all negative; but, as they may serve to indicate the applicability of the method, they are appended here.

The apparatus and experimental details were the same as those described for formaldehyde (pp. 413-18).

EXPERIMENTS ON THE QUANTITATIVE EFFECT OF CARBON DISULPHIDE AND ACETYLENE ON MUSTARD AND CRESS PLANTS IN LIGHT.

1. Effect of Acetylene in the Air-current.

The acetylene was produced by bubbling the air through pure water at known temperature, and then passing the saturated air over calcium carbide. A quantity of acetylene was produced equivalent to the amount of water vapour in saturated air at the temperature.

Amount of Acetylene in the Air.

Temperature of water varied from 1° to 9°.

Partial pressure of water = from 4.9 mm. to 8.6 mm. = p. press. C_2H_2 .

From this it can at once be calculated by simple proportion that at ordinary atmospheric pressure of 760 mm. the partial pressure of acetylene is 0.64 % to 1.13 % of the total atmospheric pressure.

Results.—Effect of C_2H_2 in Light.

Plant used : Cress (*Lepidium sativum*).

Several unsuccessful experiments were devoted to working out the general method, from March to June, 1910.

Final Experiment, June, 1910 :

<i>Atmosphere.</i>	<i>Orig. dry weight.</i>	<i>Final dry weight.</i>	<i>Final/orig. weight.</i>
Air — CO_2 + C_2H_2	0.2015 grm.	0.1002 grm.	49.7 %
Air — CO_2	0.2010 „	0.1387 „	60.0 %
Air + CO_2	0.1996 „	0.2520 „	158.0 %

Notes on the Morphology of the Cultures.

The effect of acetylene on the plants was to render them rather stout in appearance, about half their normal height, and non-heliotropic.

Conclusions.—Even from this one result it is immediately obvious that there is no assimilation of acetylene. The loss in weight, in excess of the culture without carbon dioxide, is interesting as indicating that the gas stimulated the respiratory activities of the plants.

2. Effect of Carbon Disulphide in the Air-current.

Amount of Carbon Disulphide introduced into the Air.

Determined in a separate experiment by weighing the diffusion bulb before and after the passage of a known volume of dry air.

Values :

<i>Dimensions of Capillary.</i>		<i>Properties of Air.</i>			<i>Time t</i>	<i>Weight given off</i>	
<i>Length.</i>	<i>Bore.</i>	<i>Vol.</i>	<i>Press.</i>	<i>Temp.</i>	<i>in hours.</i>	<i>in t hours.</i>	<i>in 1 hour.</i>
		<i>litres.</i>	<i>mm.</i>			<i>grm.</i>	<i>grm.</i>
4 cm.	0.5 mm.	28.31	760	14°	6.0	0.0045	0.00075
4 cm.	0.5 mm.	14.26	767	13°	2.5	0.0029	0.00125
10 cm.	0.5 mm.	3.54	not obs.	16°	1.125	0.0002	0.00018

As 1 litre of air weighs about 1.32 grm., the mean per cent. by weight of carbon disulphide in the air is about :

With 4 cm. capillary	.	.	0.00135
„ 10 cm. „	.	.	0.00042.

Experiments with Carbon Disulphide in Light.

EXPERIMENT I. February–March, 1911. Plants used: Cress.

Length of capillary in diffusion bulb, 10 cm.

Temperature ranged from 10° to 18°.

<i>Atmosphere.</i>	<i>Orig. dry weight.</i>	<i>Final dry weight.</i>	<i>Final/orig. weight.</i>
Air + CS ₂ – CO ₂	0.1982 gm.	0.1145 gm.	57.8 %
Air – CO ₂	0.1972 „	0.1105 „	56.0 %
Air + CO ₂	0.1977 „	0.1180 „	59.7 %

Notes.—As the weather was dull and cold throughout, none of the plants were very vigorous.

EXPERIMENT II. March–April, 1911. Plants used: Cress.

Length of capillary in diffusion bulb, 4 cm.

Temperature ranged from 9.5° to 18°.

<i>Atmosphere.</i>	<i>Orig. dry weight.</i>	<i>Final dry weight.</i>	<i>Final/orig. weight</i>
Air + CS ₂ – CO ₂	0.2005 gm.	0.1255 gm.	62.6 %
Air – CO ₂	0.2015 „	0.1385 „	69.2 %
Air + CO ₂	0.2000 „	0.1575 „	78.8 %

Notes.—Plants under this concentration of carbon disulphide showed signs of poisoning. The chief symptoms were an entire absence of heliotropism and geotropism, and a curled appearance. Besides this, there was often a reversal of the usual sequence in the emergence of root and shoot from the seed, and in many cases the cotyledons were bright orange in colour.

EXPERIMENT III. April–May, 1911. Plants used: White Mustard (*Brassica alba*, Boiss.).

Length of capillary in diffusion bulb, 10 cm.

Temperature ranged from 11° to 27°.

<i>Atmosphere.</i>	<i>Orig. dry weight.</i>	<i>Final dry weight.</i>	<i>Final/orig. weight.</i>
Air – CO ₂ + CS ₂	0.3990 gm.	0.2455 gm.	61.5 %
Air – CO ₂	0.3995 „	0.2525 „	63.2 %
Air + CO ₂	0.3990 „	0.3400 „	85.4 %

Notes.—Both cultures without CO₂ were beginning to shrivel, when the experiment was stopped; the other was vigorous and showing second leaves.

Conclusions.—As in the experiments with acetylene, there is no assimilation with carbon disulphide. The slight apparent increase in the first experiment was due, no doubt, to some irregularity in the lighting. It was also performed at an unfavourable time of year. Again, in this case the vapour has the effect of increasing respiration, though not to the same extent as acetylene, a fact which has been observed before by other methods.¹

¹ Cf. H. E. and E. F. Armstrong: Proc. Roy. Soc., 1910, lxxxii, B, p. 588.

A

B

BAKER—FORMALDEHYDE

C

D

BAKER—FORMALDEHYDE

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