

Presence of Formaldehyde in Rain and Dew and its Formation by Photo-oxidation of Organic Compounds and the Problem of Carbon Assimilation.

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Recently we have shown (*Nature*, 1932, 130, 313) that all samples of freshly collected rain-water contain formaldehyde. The amount of formaldehyde in rain-water varies from 0·00015 to 0·001 g. per litre. We have ascribed the presence of formaldehyde in rain-water to its photo-formation from carbon dioxide and water vapour present in the air. We have also advanced the view that the first stage in this photo-formation of formaldehyde and in photosynthesis in plants is the photolysis of water into H and OH. The carbon dioxide is subsequently reduced by the atomic hydrogen formed by the photo-decomposition of water.

It might be argued that the formation of formaldehyde present in rain-water may be due to the electric discharge in the atmosphere during thunder storms. During the months of January, February, March and April, rain-water was collected and analysed every time there was a shower. On some of these days, electric discharge and thunder storms were observed. The amounts of formaldehyde present in rain-water are as follows :

Date.	Amount of formaldehyde per litre of rain-water.	Remarks.
Jan. 13, 1933	0·00055 g.	Accompanied with thunder storm
15	0·00055	No thunder storm
22	0·0004	Accompanied with thunder storm and hail storm
Feb. 25	0·0006	No thunder storm
Mar. 12	0·00045	Do
(2nd shower)		
13	0·0005	Accompanied with thunder storm
25	0·00075	Do
Apr. 12	0·0001	No thunder storm
14	0·0007	Accompanied with severe thunder storm and frequent lightning discharge
15	0·001	No thunder storm

From the foregoing observations it appears that the incidence of thunder storm does not increase the amount of formaldehyde present in rain-water. We have observed that the amount of formaldehyde present in rain-water is greater when the rainfall is preceded by some clear sunny days. Hence we are inclined to the view that formaldehyde in rain-water is obtained as a result of its photo-formation from carbon dioxide and water vapour.

Recently we have found that dew also contains formaldehyde. The amount of formaldehyde in dew is appreciably greater than that in rain-water. The amount of formaldehyde in most of the samples of dew collected so far was approximately 0.0015 g. per litre. The formaldehyde in dew appears to come from the air in contact with grass, leaves, soil, etc.

It is well known that many organic substances on exposure to light forms formaldehyde. The production of formaldehyde by exposing chlorophyll under various conditions to light has been investigated by several plant physiologists.

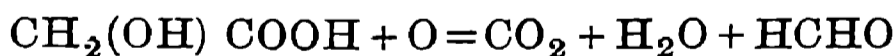
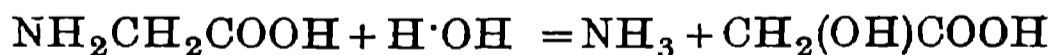
The origin of this formaldehyde has not yet been satisfactorily explained. In order to throw light on this problem we have started a systematic investigation on the formation of formaldehyde by exposing solutions or suspensions of various organic substances to sunlight in presence of air. Dilute solutions of organic substances are exposed in beakers to sunlight for six hours and after exposure the solutions are distilled and the amount of formaldehyde estimated by the iodine method or by Schryver's reagent.

The organic substances investigated so far can roughly be classified into the following three groups according to the amount of formaldehyde formed from 100 c. c. of the solution.

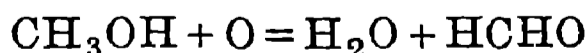
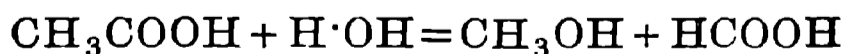
A	B	C
Formaldehyde.	Formaldehyde.	Formaldehyde.
(0.0015—0.0007 g.)	(0.0006—0.0001 g.).	(0.0001—0.000015 g.).
Acetic acid	Pyruvic acid	Congo red
Glycine	Tartaric acid	Safranine
Methyl violet	Phloxine	Oxalic acid
Methylene blue	Aurine	Glucose
Acridine orange	Gallamine blue	Formic acid
Crystal violet	Laevulose	Arabinose

A	B	C
Malachite green	Galactose	Starch
Gentian violet	Aniline green	Inulin
Citric acid	Butyric acid	Cane sugar
Malic acid	Propionic acid	Stearic acid
Lactic acid	4:5'-Dihydroxyfuran	Pot stearate
Glycerol	4-Hydroxy-2'-methyl-5'-isopropylfuran	Alanine
Acetone	4-Hydroxy-2'-isopropyl-5-methylfuran	Gelatine
Chlorophyll	4-Hydroxy-3':4'-phenylene(<i>p</i> -hydroxy)furan	Cholesterol
Glycogen	Sodium acetate Sodium formate Sodium butyrate Sodium propionate Sodium oleate	Leucine Histidine Phenylalanine Ergosterol

In the first group, the substances on exposure to light and air yield formaldehyde readily. By carrying on blank experiments in the dark and in the absence of air we have not observed any formaldehyde. When solutions of these substances are exposed to light and air, formaldehyde is formed mainly as a direct product of photo-oxidation. Thus in the case of glycine in presence of light and air the following reaction takes place:



In the case of acetic acid the probable mechanism of the reaction may be as follows:



We are of the opinion that with other substances of this group formaldehyde is a direct product of the photo-oxidation.

In the second group of substances the amount of formaldehyde formed from photo-oxidation is less than in the first class. It is difficult to state definitely whether the formaldehyde is a direct product of photo-oxidation or formed photochemically from carbon dioxide and water which are the products of oxidation of organic substances.

The formation of formaldehyde with the third class of substances is likely to be entirely due to the photosynthesis from carbon dioxide and water, produced in the photo-oxidation of the organic substances. From the researches of Palit and Dhar (*J. Phys. Chem.*, 1925, **29**, 925; 1928, **32**, 1263 ; 1930, **34**, 993 ; *Z. anorg. Chem.*, 1930, **191**, 150), it is evident that the energy-rich organic substances when exposed to light and air are partially oxidised to carbon dioxide and water. These freshly formed substances appear to be energy-rich and can undergo photosynthesis to formaldehyde in presence of light more readily than the ordinary carbon dioxide and water present in the atmosphere. That is why formaldehyde is more easily detected in the photo-oxidation of organic substances which liberate energy in their oxidation than in the case where ordinary carbon dioxide or bicarbonate solutions are exposed to sunlight. It will be of interest to note that almost all samples of acetic acid, glycerol, lactic acid, pyruvic acid, glycol, methyl alcohol, butyric acid, propionic acid and several other organic compounds kept in the store of our laboratories contain small amounts of formaldehyde formed from their slow photo-oxidation, and when exposed to air and light directly, the amount of formaldehyde is greatly increased.

It seems likely that the energy generated in the photo-oxidation of these organic substances applies a part of the energy for the photo-formation of formaldehyde. We are of the opinion that in nature, the photosynthesis that is taking place in the plants is aided by the energy obtained in plant respiration, which goes on as long as the plant lives. The ease with which formaldehyde or other energy-rich compounds are formed in plants is partly due to their getting a constant supply of energy from the oxidation of the food materials present in the plant. We have postulated that the most important chemical change in the formation of carbohydrates in plants and in the formation of formaldehyde in nature from carbon dioxide and water is the photolysis of water into H and OH. The amount of energy required to decompose a gram molecule of water into H and OH is the same as that necessary for the formation of a gram

mole of formaldehyde from carbon dioxide and water. These are highly endothermal changes requiring radiations of wave-length 2550\AA (1,12,000 calories). In nature, however, photosynthesis takes place in visible light especially the red. We are of opinion that the energy derived from respiration in the plants already supplies a part of the energy necessary for the photosynthesis and thus rendering the photo-decomposition of water possible by longer wave-lengths. Although the adsorption of carbon dioxide and water by the chlorophyll of the leaf may partially activate these substances, just as the adsorption of hydrogen and oxygen on a platinum or palladium surface renders them active, it appears to us that this activation of carbon dioxide and water by their adsorption on the leaf surface is less important than their activation by the adsorption of energy from respiration. These observations explain the difficulty of obtaining formaldehyde or carbohydrates directly from carbon dioxide, bicarbonates and water *in vitro*. In the presence of an exothermal chemical change, such as the photo-oxidation of carbohydrates or other organic substances, the photo-formation of formaldehyde from carbon dioxide and water vapour appears to be greatly facilitated. We have carried on numerous comparative experiments on photosynthesis *in vitro* by exposing to sunlight aqueous chlorophyll suspensions saturated with either carbon dioxide alone or a mixture of carbon dioxide and oxygen. Moreover, similar experiments have been performed with potassium bicarbonate solutions instead of carbon dioxide. In the majority of cases, slightly greater amounts of formaldehyde were obtained with a mixture of carbonic acid or bicarbonate solutions and oxygen than in the cases where the chlorophyll suspensions were exposed to light and air in the absence of carbonic acid or bicarbonate solutions. In some of his experiments Warner (*Proc. Roy. Soc.*, 1914, **87 B**, 378) obtained more formaldehyde by exposing chlorophyll films to light in presence of a mixture of air and carbon dioxide than in presence of air alone. From comparative experiments on the photo-oxidation of organic acids, Palit and Dhar have shown that under identical conditions, oxalic acid is more readily oxidised in presence of light than tartaric or citric acid. On the other hand, by exposing solutions of these acids, very small quantities of formaldehyde are obtained in the case of oxalic acid, whilst the amount of formaldehyde formed from citric acid is much greater. It appears, therefore, that formaldehyde obtained by exposing oxalic acid solutions to sunlight and air is not a product of direct

oxidation, as in the case of citric acid, but is produced photosynthetically from energy-rich carbon dioxide and water, which are products of its photo-oxidation. It is rather peculiar that formic acid and sodium formate solutions yield very small amounts of formaldehyde when exposed to light and air. Dilute solutions of malachite green, crystal violet, methylene blue, gentian violet, acridine orange and methyl violet yield formaldehyde readily when exposed to light and air. Most of these dyes also behave as antiseptics. It will be interesting to investigate this problem further in order to find out whether all antiseptic dyes yield formaldehyde on photo-oxidation and the formaldehyde produced is the real antiseptic.

In this connection it will be worth while noting that formaldehyde is obtained even in the dark by treating carbonic acid or bicarbonate solutions with metals like magnesium, cerium, iron, etc. (Compare Dhar and Atma Ram, *Nature*, 1932, 129, 203). Recently we have been able to show that small amounts of formaldehyde are obtained by treating bicarbonate solutions with yellow phosphorus. In these cases the amount of formaldehyde formed is greater in light than in the dark. It appears that the energy-rich hydrogen produced by the action of the metals on water is capable of reducing the bicarbonate ion or carbonic acid to formaldehyde even in the dark, aided by the energy produced by the reaction of the metals on carbonic acid and bicarbonate solutions.

Now the question arises, can carbohydrates be obtained in plants even in the absence of light? It seems to be definitely settled that protein synthesis in plants can take place even in the dark in presence of carbohydrates, which undergo metabolism. It appears, that the synthesis of protein is possible when energy is supplied from the oxidation of carbohydrates without the addition of solar energy. From experiments carried on in these laboratories *in vitro*, by exposing solutions of formaldehyde and ammonia to sunlight in presence of different catalysts, we have come to the conclusion that there is less likelihood of the production of amino-acids and alkaloids than the formation of pyridine, piperidine, etc., due to the absence of carbohydrates, which are formed very slowly from formaldehyde *in vitro* even in presence of light. Recently we have been able to obtain evidence of the formation of amino-acids by exposing solutions of formaldehyde, glucose and ammonia or nitrite and catalysts like TiO_2 , ZnO , etc., to sunlight. Without using any catalyst we have been able to obtain evidence of amino-acids by the "ninhydrin"

test on exposing solutions of glucose, galactose or laevulose with potassium nitrate to sunlight.

Moreover, it appears that protein formation in plants is likely to be facilitated by the presence of fats, which yield glycerol readily. It has been shown by Dhar and collaborators that reducing sugars and formaldehyde are obtained by exposing glycerol to sunlight. Recently we have observed that reducing sugars are obtained by exposing solutions of tartaric acid and other hydroxy organic acids to light. Hence the presence of tartaric acid or other hydroxy organic acids may also favour the formation of proteins in plants, and these proteins appear to undergo oxidation very readily in plants, animals and *in vitro* in presence of light with the liberation of energy. Many plant physiologists have attempted to connect respiration with photosynthesis. Thus Spoehr ("Photosynthesis," 1926, p. 143) states "Also, the light green varieties had a lower rate of respiration than the normal plants, though there was no direct parallelism between respiration and chlorophyll content. A closer relationship seems to exist between the rate of photosynthesis and that of respiration." Again on page 164, it is stated "Respiration is an important internal factor affecting photosynthesis, in addition to the chlorophyll content and the number of chloroplasts. The exact manner in which these two processes of respiration and photosynthesis may be linked is still an open problem." "Thus, while it is evident that there is no parallelism between chlorophyll content and rate of photosynthesis, the quotient respiration-photosynthesis is more constant. This quotient for the light green varieties was found to be as follows :

$$\begin{aligned} \text{Ptelea} &= 1.77. \text{ Catalpa} = 1.72, \text{ Mirabilis} = 2.0. \\ \text{Ulmus} &= 2.0, \text{ Populus} = 2.1. \end{aligned}$$

A correlation between respiration and photosynthetic rates was also noticed by Miss. Henrici (Inaug. Diss. Basel, 1918). She has observed in a study of alpine and lowland plants that those plants which had a high photosynthetic rate also had a high velocity of respiration and *vice versa*. Boysenjensen (*Bot. Tidsskrift*, 1918, 36, 219) has also reported that plants which have a high rate of respiration also have a high velocity of photosynthesis. Similar results have also been obtained by Spoehr and McGee (*Carnegie Inst. Wash. Publ.*, 1923, No. 325, pp. 76-98).

From the view points discussed in this paper, it will be evident that the greater the respiration in a plant, the greater is the possibi-

lity of a supply of energy for photosynthetic purposes. In a recent communication (Dhar, *Bull. Acad. Sci. U. P.*, 1933, 2, 141) the importance of respiration in plant life has been emphasised and it has been concluded that respiration appears to be the more fundamental reaction in plants and is more important to plant life than photosynthesis, which predominates in plants only under restricted conditions of temperature and light intensity. The factor which really controls plant life is its respiratory or metabolic activity. As all plant processes depend on the energy available from respiration, which is the most vital reaction in plant life as much as in animal life, photosynthetic activity can not proceed without respiration taking place in the plant and hence without the presence of oxygen, which supports respiration in both the plant and animal kingdom. Because want of oxygen is detrimental to respiration, it is also harmful to photosynthesis. Consequently the classical experiments of Bous-singault (1868) and Pringsheim (1887), showing that in an atmosphere of hydrogen, nitrogen, carbon dioxide or methane, plants lose the power of photosynthesis, are easily explained, because in presence of these gases, oxygen respiration stops and the supply of energy, necessary for the partial activation of carbonic acid and water is not available for the plant. It appears, therefore, that besides light, carbon dioxide, moisture and chlorophyll, energy from respiration is also necessary for photosynthesis.

Just as photosynthesis *in vitro*, that is, the formation of formaldehyde from exposing solutions of carbonic acid to sunlight, is exceedingly difficult in the absence of an exothermal chemical reaction taking place along with it, carbon assimilation in plants is also practically impossible in the absence of a supply of energy available from respiration. Moreover, it is well known that atmospheric nitrogen can only be fixed by some bacteria when supplied with energy from carbohydrate oxidation or another suitable exothermal oxidation reaction. In experiments *in vitro*, very little formaldehyde should be expected to form from carbonic acid or bicarbonate solutions on exposure to sunlight, because in sunlight short wave ultraviolet rays exist in small amounts. On the other hand, in presence of manganese and ferrous salts, we have been able to detect small quantities of formaldehyde frequently. This is because of the fact that these salts absorb visible light and also they are oxidised to the trivalent condition in presence of air and this exothermal chemical change supplies a part of the energy required for photosynthesis.

Hence it is easier to obtain formaldehyde or any other energy-rich compound from carbonic acid or bicarbonate solutions on exposure to light when a suitable exothermal reaction is made to take place in the same system along with the photosynthetic reaction.

In the case of the presence of formaldehyde in rain-water, we have explained its generation from photosynthesis of carbonic acid in higher altitudes where ultraviolet radiations of wave-length nearing 2550\AA are available. The following appears to be the important steps in carbon assimilation :

1. Partial activation of carbon dioxide and water at the leaf surface due to their adsorption by chlorophyll and other plant pigments. It seems that chlorophyll and carotinoids present in the leaf act as photosensitisers and as reducing agents in the photo-reduction of carbonic acid.

2. Further activation of the adsorbed carbon dioxide and water by the absorption of a part of the energy available from respiration and the oxidation of carotin and the formation of activated carbon dioxide and water as products of respiration.

3. Absorption of light by chlorophyll and other pigments and the dissociation of activated water molecules in the leaf surface into H and OH and the reduction of activated carbon dioxide molecules to formaldehyde by the atomic hydrogen produced from the photolysis of water. The amount of energy required to decompose a gram mole of water into H and OH is the same as that necessary for the formation of a gram mole of formaldehyde from carbon dioxide and water.

4. The polymerisation of formaldehyde to reducing sugars.

5. The formation of hydrogen peroxide from OH and the rapid decomposition of H_2O_2 into water and oxygen on the leaf surface.

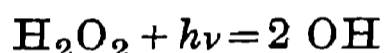
The polymerisation of formaldehyde *in vitro* to reducing sugars is an exceedingly slow process even in presence of light. We have shown that it is accelerated by ferric salts. Moreover, it is known that in presence of alkali, reducing sugars are formed from formaldehyde. Light accelerates this reaction. How the formaldehyde formed on the leaf surface undergoes rapid polymerisation is still unknown.

Usher and Priestly (*Proc. Roy. Soc.*, 1906, 77 B, 369), Thurnberg (*Z. physikal. Chem.*, 1924, 106, 305), Weigert (*Z. physikal. Chem.*, 1923, 106, 313; *ibid.*, 1924, 109, 79) and others have postulated the formation of H_2O_2 in photosynthesis and its decomposition by

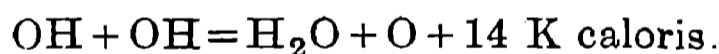
enzymes like catalase, etc. By exposing chlorophyll to light and air *in vitro*, Wager (*Proc. Roy. Soc.*, 1914, **87 B**, 386), Warner (*loc. cit.*) and others have produced evidence in favour of hydrogen peroxide formation.

In recent years, the problem of the formation of hydrogen peroxide from OH radicals has been investigated. Frankenburger and Klinkhardt (*Trans. Faraday Soc.*, 1931, **27**, 431) conclude that two water molecules are produced for each H_2O_2 molecule formed in the combination of hydrogen and oxygen initiated by photochemically generated hydrogen atoms, and that the formation of H_2O_2 is independent of temperature and the OH radicals must be relatively stable and require no energy for their activation in hydrogen peroxide.

From the measurement of the ultraviolet absorption spectrum of hydrogen peroxide, Urey, Dawsey and Rice (*J. Amer. Chem. Soc.*, 1929, **51**, 1371) have concluded that for every absorbed light quantum two hydroxyl radicals are formed as follows:



On the other hand, Bonhoeffer and Person (*Z. physikal. Chem.*, 1931, **14 B**, 1) are of opinion that free OH radicals react chiefly according to the following equation:

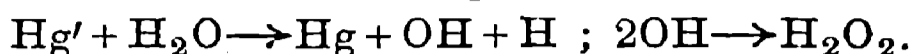


The life period of the free OH radicals is of the order of 10^{-3} second. Moreover, von Elbe (*J. Amer. Chem. Soc.*, 1933, **55**, 62) does not support the reaction mechanism of Frankenburger and Klinkhardt involving the recombination of two OH radicals to form hydrogen peroxide. According to Bates and Salley (*ibid.* p. 110) hydrogen peroxide is the sole product of the mercury sensitised hydrogen oxygen reaction.

Ultraviolet light causes the decomposition of water into its elements. According to Tian (*Compt. rend.*, 1913, **156**, 1063) water is photochemically decomposed by extreme ultraviolet radiations. Coehn and Grote (*Nernst-Festchrift, Halle, 1912*, p. 136) have reported that a stationary state is reached between water and its elements by the action of ultraviolet light.

Senftleben and Rehren (*Z. physik.*, 1926, **37**, 529) have obtained only hydrogen as the gaseous decomposition product in the photosensitised decomposition of water vapour in presence of mercury and

have suggested that the reaction proceeds in the following steps:



Taylor and Bates (*J. Amer. Chem. Soc.*, 1927, **49**, 2438) have reported the production of 73% H_2 and 27% O_2 in the mercury-sensitized photo-decomposition of water.

It appears that the photolysis of the activated water molecules into H and OH on the leaf surface due to the absorption of light by chlorophyll and carotinoids resembles the cases of the photo-sensitized decomposition of water investigated *in vitro*.

SUMMARY.

(1) The amount of formaldehyde in rain-water does not increase with the incidence of thunder storms. Hence electrical discharge in the atmosphere does not seem to form formaldehyde from carbon dioxide and water vapour.

(2) Dew contains appreciable amounts of formaldehyde. The quantity of formaldehyde is greater in dew than in rain-water.

(3) When solutions of organic substances like acetic acid, citric acid, glycine, malic acid, lactic acid, glycogen, acetone, etc., are exposed to sunlight and air, formaldehyde is readily formed.

(4) Dyes like malachite green, methyl violet, methylene blue, etc., also form formaldehyde readily on photo-oxidation. Tartaric acid, butyric acid, propionic acid and some dyes form smaller quantities, whilst oxalic acid, formic acid, glucose, cane sugar, starch, histidine, etc., produce very small amounts of formaldehyde from photo-oxidation.

(5) It is believed that the formaldehyde formed in the first group of substances is a direct product of the photo-oxidation, whilst with oxalic acid, starch, glucose, etc., formaldehyde is obtained from the photosynthesis of the energy-rich carbon dioxide and water produced by photo-oxidation.

(6) It seems likely that the energy generated in the photo-oxidation of organic compounds supplies a part of the energy required for the photo-formation of formaldehyde. In nature, the photosynthesis that takes place in plants is aided by the energy obtained in plant respiration.

(7) There is an intimate relation between respiration and photosynthesis in the plant kingdom, because photosynthesis can not proceed without the energy available from respiration for the partial

activation of carbon dioxide and water vapour. The need of the presence of oxygen in photosynthesis is also explained from the same point of view.

(8) It is easier to obtain formaldehyde or any other rich compound from carbonic acid or bicarbonate solutions on exposure to light when a suitable exothermal reaction is taking place in the system along with the photosynthetic reaction.

(9) A theory of carbon assimilation in the plants is advanced from the view of the partial activation of carbon dioxide and water by the absorption of a part of the energy available from respiration. Absorption of light by chlorophyll and other pigments leads to the dissociation of the activated water molecules on the leaf surface into H and OH and this appears to be the most important photochemical reaction in carbon assimilation. The atomic hydrogen produced reduces the carbonic acid.

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