THE PRINCIPLE OF PHOTOCATALYSIS, ITS BASIS AND APPLICATION.

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In submitting the following to the Faculty of Science as a thesis in fulfilment of the requirement for the degree of Doctor of Philosophy, I wish to express my deep and heartfelt thanks to Professor E.C.C.Baly, under whose guidance I have worked, for his help and advice, both of which have been constantly available.

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The Einstein Law of Photochemical Equivalence.

It has been known for a considerable period of time that while many optical phenomena are explainable on the idea of the principle of equipartition of energy, this latter fails in many cases to lead to any explanation of certain other phenomena. Such phenomena are the photoelectric effect, fluorescence and phosphorescence, atomic and molecular heats, etc.. These require the assumption of a discreteness of light and of radiation generally. By the adoption of the quantum theory of Planck much progress has been made in all fields connected with radiation. This theory assumes that energy is radiated or absorbed by a body only in certain definite integral units which have a magnitude equal to the product hv, in which h is a universal constant having the value 6.57×10^{-27}, and v is the frequency of the radiation in question.

The Planck concept has been applied to the problems connected with photochemical reactions in the form

$$N = Q / hv$$

by A.Einstein[1]. In this relation N represents the number of

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[1] Einstein.-Ann.d.Phys.(4) 37, 832; 38, 881, 888, (1912).

molecules changed by absorption of light of a frequency v, and Q represents the energy necessary for the production of the chemical action. There has been much contention as to the validity of this relation as it fails to agree with facts observed in connection with many photochemical reactions. The most important consequence of Einstein's argument is that the absorption of one quantum of energy at a frequency characteristic of the molecule is necessary before a molecule of reactant will react, the number of molecules reacting being equal to the number of energy quanta absorbed. When this relation is being applied to photochemical reactions it must be remembered that it is only applicable to primary light reactions, which can very seldom be really brought under observation. This primary process has been differently interpreted by different observers. Some have assumed that this primary process consists in the photoelectric separation of an electron, but this is an hypothesis which has received no confirmation. Another suggestion has been that the primary process consists in a splitting up of the molecules into free atoms. This point of view was taken up by Warburg[1] and later by Nernst[2], experimental evidence in its favour being adduced.

It is convenient to set out here in tabular form some comparison of facts observed in connection with photochemical reactions and to show values for the number of molecules reacting per quantum absorbed.

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[1] Warburg.-Series of papers in Sitzb.Berlin Akad.Wiss. 1911--.
[2] Nernst.-Zeitsch.fur Elektrochem.21,335,(1918).

Reaction.	Observer.	hv/No.of mols.reacting.
$3O_2 = 2O_3$	Regener[1].Warburg[2].	1 for $2O_3$
$2NH_3 = N_2 + 3H_2$	Regener[1].Warburg[2].	4
Anthracene → Dianthracene.	Luther & Weigert[3].	1 - 0.7
$S_\lambda \rightarrow S_\mu$	Wigand[4].	4 - 5
$H_2 + Cl_2 = 2HCl$	Bodenstein & Dux[5].	$1/10^6$
$2O_3 = 3O_2$	Regener[1]. Fr.E.von Bahr[6]. Weigert[7].	$1/10^2 - 10^3$
Hydrolysis of Acetone.	Henri & Wurmser[8].	1/200
$4HI + O_2 = 2I_2 + 4H_2O$	Plotnikow[9].	$1/10^6$
$CO + Cl_2 = COCl_2$	Wildermann[10]. Chapman & Gee[11].	1/100
Photobromination of Toluene.	Bruner & Czernecki[12].	$1/10^6$

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[1] Regener.-Ann.d.Phys.(4)20,1033,(1906).
[2] Warburg.-Sitzb.Berlin Akad.Wiss.1911,746; 1912,216.
[3] Luther & Weigert.-Zeitsch.Phys.Chem.,51,297; 53,385,(1905).
[4] Wigand.-Zeitsch.phys.Chem.,77,423,(1911).
[6] Frl.E.von Bahr.-Ann.d.Phys.,(4)33,598,(1910).
[7] Weigert.-Zeitsch.phys.Chem.,80,78,(1912).
[8] Henri & Wurmser.-Compt.rend.,156,1012,(1913).
[9] Plotnikow.-Zeitsch.phys.Chem.,58,214,(1907).
[10] Wildermann.-Zeitsch.phys.Chem.,42,257,(1903).
Proc.Roy.Soc.London,199A,337,(1902).
[11] Chapman & Gee.-J.C.S.,99,1726,(1911).
[12] Bruner & Czernecki,-Bull.Acad.Cracovie A.,1910,576.
[5] Bodenstein & Dux.-Zeitsch.phys.Chem. 85. 297.(1913).

An inspection of the foregoing data renders it obvious that the evidence in direct support of the Einstein Law is far from being considerable. It has been definitely proved to hold for one instance only-i.e.,the photochemical formation of ozone. There has been consequently,a large amount of contention as to the applicability or otherwise of the law.

While the above data do not afford satisfactory confirmation of its validity,the work and theory of Lewis[1] have afforded a certain additional amount of confirmatory results. Lewis's work has been connected with thermal reactions and their velocity,and is based on the expression deduced by Marcelin[2], and later more exactly by Rice[3]. Marcelin treated the effect of temperature on the velocity of a reaction as a physical one dependent on the increase in internal energy of the reacting molecules,and arrived at the expression

$$\frac{d \log K}{d T} = \frac{E}{RT^2}$$

E is the amount of energy which must be supplied to a gram-molecule in order to bring it into a reactive state,and is called by Lewis the critical increment. It signifies that energy which must be absorbed in excess of the average energy possessed by all the molecules. Rice's formulation of the above expression has been used by Lewis in the development of his radiation theory of chemical action and catalysis. Lewis takes the view that this energy increment is supplied to the molecules as quanta of infra-red radiation,and that for every one of these quanta absorbed,one molecule is activated.The function of a catalyst is to absorb the infra-red radiation and to transfer it to the reactant molecules. This conception of catalysis is supported

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[1] Lewis et alii.-J.C.S.1914 et seq.

[2] Marcelin.-Compt.Rend.158,161,(1914).

[3] Rice.-Rep.Brit.Ass.,(1915),397.

by the theories of Trautz[1], Krüger[2], Perrin[3], and Tolman[4].

The radiation theory of chemical reaction was the subject of a very severe recent criticism by Langmuir[5], who set down the following conclusions as regards the theory:-

(1). It has not been demonstrated in a satisfactory manner that the radiation required for a given reaction - as calculated from the temperature coefficient - actually falls within an absorption band of the system.

(2). The total radiation absorbed is not sufficient to account for the activation actually found to occur, the radiation hypothesis being consequently untenable.

The experimental results to be described immediately following would seem to be of very considerable interest and importance in that they give an explanation of the many deviations that have been observed from the Einstein Law. They would also seem to render void the criticisms made by Langmuir and cited above.

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[1] Trautz.-Zeitsch.Wiss.Phot., 4, 160, (1906).
[2] Krüger.-Zeitsch.für Elektrochem., 17, 453, (1911)
[3] Perrin.-Ann.de Physique, (9) 11, 5, (1919).
[4] Tolman.-J.Am.Chem.Soc. 42, 2506, (1920).
[5] Langmuir.-J.Am.Chem.Soc., 42, 2190, (1920).

A Study of the Photochemical Reaction between Hydrogen and Chlorine and of its Variation with the Intensity of the Light.

This work was undertaken with the object of obtaining experimental evidence in support of the theory of chemical reaction recently put forward by Baly in a series of papers which appeared in the Philosophical Magazine[1]. In the last paper of the series, Baly put forward the view that reabsorption of the energy radiated in a reaction by reactant molecules might very easily account for the fact that Einstein's Photochemical Law does not apparently hold in many cases.

Supposing we have a body A, which can absorb energy E, being thereby brought into a reactive state A'. These activated molecules then react giving resultant molecules B, K being the heat effect. We can write this as

$$A + E = A' = B + K$$

The energy E will be absorbed in quanta at a frequency characteristic of the body A. The activated molecules A' then react, giving molecules of the resultant B, while the energy K will be radiated as quanta which correspond to a frequency characteristic of the molecule B.

Now Baly has shown (loc.cit.) that the characteristic frequencies of any molecule are exact integral multiples of the frequencies of its component atoms. Since the molecules A and

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[1] Baly.-Phil.Mag., 39, 565; 40, 1, 15, (1920).

B have common atoms, it follows that there will occur some frequencies which are characteristic of both, so that if B were to radiate at any of these frequencies, this radiation would be absorbable by A in quanta, an integral number of which would be required to activate it. If A was to absorb enough of this radiation to bring it into the reactive state A', then the result would be that more molecules of A would react than correspond to the number of quanta initially absorbed at the original characteristic frequency of A.

Let us consider apart the two cases in which the reaction is endothermic or exothermic. In the case of an endothermic reaction the amount of radiated energy will be small, so that the probability of any one molecule of A being able to absorb a sufficient number of the smaller quanta to activate it is small. Consequently Einstein's law should hold for such a case. It is noteworthy that the only photochemical reaction for which Einstein's law has shown to hold accurately is the formation of ozone from oxygen, an endothermic reaction. As many data as possible connected with the heat effects of the reactions listed on page 3 have been collected, and are presented in the following table.

Reaction.	hv/mols.activated.	Heat Effect.
$3O_2 + 2O_3$	1 for $2O_3$.	Endothermic, -34,100 cals.
$2NH_3 = N_2 + 3H_2$	4	do. , -11,000 cals.
Anthracene → dianthracene.	1 - 0.7	do., since the reaction is an association.
$S_\lambda \rightarrow S_\mu$	4 - 5	Endothermic, $S_\lambda = S_\mu - 15.8$ cals. per gram

Reaction.	hv/mols.activated.	Heat Effect.
$H_2 + Cl_2 = 2HCl$	$1/10^6$	Exothermic, 44,000 cals.
$2O_3 = 3O_2$	$1/10^2 - 10^3$	do., 34,100 cals.

It is at once obvious that those reactions for which the law most nearly holds are endothermic.

On the other hand, considering an exothermic reaction, the density of the radiated energy will be much greater, so that conditions are favourable to the accumulation of the smaller quanta in a molecule. In this case therefore, more molecules of A will react than would be expected from the actual energy absorbed, calculated on the basis of the Einstein law. Also, ceteris paribus, the more exothermic the reaction, the bigger will be the divergence from the Einstein law.

The amount of reaction due to reabsorption of the radiated energy by reactant molecules will depend on two factors, namely (1), the concentration of reactant molecules under constant intensity of activating light, and (2), the radiation density under constant concentration of reactant molecules. Obviously the greater this latter is, the larger will be the number of reactant molecules which will have absorbed sufficient small quanta to bring them up to the active state. This radiation density will depend upon the number of resultant molecules being formed in unit time, i.e., upon the number of reactant molecules being activated, this in turn being dependent on the intensity of the incident radiation of the required type.

Whether or not these two factors do really govern the degree of divergence from the Photochemical Law should be experimentally determinable.

In the case of the first factor, its effect upon the divergence has already been shown by Henri and Wurmser[1], who showed that the divergence was proportional to the molecular concentration of the reactants, the intensity of the illumination being kept constant. The work of Bodenstein and Dux[2] on the photochemical union between hydrogen and chlorine leads to the same conclusion. These authors found that, under constant intensity of illumination, the velocity of the reaction varied approximately as the square of the chlorine concentration, which reactant is the only one activated by the light. The hydrogen concentration had no effect upon the velocity, provided that it was kept within certain limits.

In connection with the influence of the concentration of the photoactive component in a photochemically reacting system it is of very considerable interest to note that in the case of the strongly exothermic photodecomposition of ozone Warburg[3] has observed that in dilute mixtures where the total pressure is only one atmosphere the equivalence law is valid and accurate. At greater ozone concentrations however, as many as 1000 molecules of ozone are decomposed per quantum of energy absorbed into the system. This observation is strongly in support of the Baly view that reabsorption of radiated energy is possible.

To determine the effect of the second factor, it was necessary to investigate an exothermic photochemical reaction, and to study especially the variation of the rate of reaction

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[1] Henri & Wurmser.-Compt.rend., 156, 1012, (1913).
[2] Bodenstein and Dux,-Zeitsch.phys.Chem., 85, 297, (1913).
[3] Warburg.-Sitzb.Berlin Akad.Wiss., 1913, 644.

with the intensity of the incident light. If the divergence from Einstein's law is constant, the rate of the reaction will be proportional to the intensity. Reasoning, however, in the above manner, on the basis of Baly's theory, the divergence from proportionality will be the greater, the greater the intensity of the light.

The reaction selected for observation was the photochemical union of hydrogen and chlorine. This is a strongly exothermic reaction, the mixed gases are fairly readily obtained by the electrolysis of hydrochloric acid solutions, while the rate of the reaction in the presence of water can be conveniently followed by the contraction in volume, as the hydrogen chloride is absorbed by the water as fast as it is formed. Maintaining the gases at a constant pressure by following up the contraction, the molecular concentration of both the hydrogen and the chlorine can be kept constant.

History and Literature of the Reaction.

The first to notice the fact that hydrogen and chlorine combine in light was Cruickshank[1], who also noted that the volume of a mixture of these gases in equilibrium over water did not begin to diminish until exposure to the light had been continued for several seconds. Later workers on the same reaction observed the same phenomenon, which was more closely investigated by Bunsen and Roscoe[2]. These experimenters gave to this initial inactive period the term "Photochemical Induction

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[1] Cruickshank.-Nicholson's Journal, (1)5, 202, (1801).

[2] Bunsen and Roscoe.-"Photochem.Untersuch." Ostwald's Klassiker No.34.
Also in Poggendorf's Annalen.

Period",and they regarded the phenomenon as a specific property of the action of light in a chemical reaction. Between Cruickshank's original discovery of the phenomenon in 1801 and the classical investigations of Bunsen and Roscoe (1855),observations on the induction period were made by Dalton[1],Seebeck[2],Gay-Lussac and Thenard[3],and Draper[4]. Draper's work has considerable historical importance,as he constructed a certain apparatus-the so-called "thitonometer" with which he measured the intensity of "thitonic" or chemically active rays. This apparatus was the first form of actinometer. Draper showed that no hydrogen chloride was formed in a freshly prepared mixture of hydrogen and chlorine until the gases had been exposed to light for a measurable period of time. He also showed that once the reaction started,the rate of the formation of hydrogen chloride gradually increased until a constant maximum was reached.

The first really thorough researches on the kinetics of the reaction were those of Bunsen and Roscoe. Their experimental apparatus is shown in the following sketch.

In a glass bulb i was a mixture of carefully purified chlorine and hydrogen ; the vessel was about half filled with the water whose function it was to absorb the synthesised hydrogen chloride. A water index moved up the capillary tube tt. The rate of reaction was determined by the decrease in volume of the

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[1] Dalton.-"A new System of Chemical Phiosophy".1809.
[2] Seebeck.-Goethes Farbenlehre(1810).
[3] Gay-Lussac & Thenard.-Rech.phys.chim.Paris.I,311;2,128,(1811).
[4] Draper.-Phil.Mag.,19,195;(1841).23,401;25,207;26,473;
27,327,(1845).

~~of the~~ gaseous contents of i on exposure to light-(i.e.,from the motion of the water index up the capillary tube). The only portion of the apparatus exposed to the light was the vessel i which contained the gaseous mixture. The water in i was previously saturated with chlorine,and the exposure to light was not commenced until equilibrium had been attained within the apparatus. The experiments gave the following results:-

At first the reaction proceeded very slowly,and then the reaction rate gradually rose until it had attained a certain maximum value at which it remained constant. This maximum value was dependent on the intensity of the light,and was,as far as Bunsen and Roscoe were able to tell,proportional to it. It was this observation which supplied the fact for the statement of what has been known as the Bunsen and Roscoe Law,which affirmed the proportionality between photochemical effect and light intensity. When exposure to light was stopped, the reaction ceased; in the case of a further exposure to light,the process repeated itself,but the length of the induction period depended very much on the time interval between any two consecutive exposures. The quicker the second followed on the first,the shorter was the period of induction and vice-versa. This phenomenon was designated by Bunsen and Roscoe as the "Deduction Period". They further stated that the light absorption of chlorine was greater in a reaction mixture than that of chlorine by itself,and suggested that this increase in the amount of energy absorbed by the chlorine in the reaction mixture was due to some of the energy being transformed into chemical work. This phenomenon was denoted as "Photochemical Extinction" by Bunsen and Roscoe. It must be noted that their statements as regards the difference in the light extinctions of chlorine in the reaction mixture and of chlorine by itself were contradicted after many years by Burgess and Chapman[1], who failed to observe this difference.

Bunsen and Roscoe further found that different impurities were possessed of great influence on the reaction rate - oxygen having a very definite and considerable retarding influence.

Considering the time at which they were carried out, Bunsen and Roscoe's experiments were very complete, and long remained as a model for photochemical investigations. Their method of experiment has been adopted by the majority of subsequent observers of the reaction, and as will be seen, an adaptation has been used in the investigation to be described in the following pages.

According to several observers(Pringsheim[2], Baker[3], Mellor and Russell[4], Bevan[5], Wildermann[6].) water vapour must play an important rôle in the reaction. Baker for instance found that perfectly dry "chlorknallgas", to use the convenient German term, is stable to light. On the other hand Bodenstein and

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[1] Burgess and Chapman.-J.C.S., 89, 1399, (1906).
[2] Pringsheim.-Ann.d.Phys.u.Chem.N.F.27.32, 387, (1887).
[3] Baker.-J.C.S., 65, 611, (1894).
[4] Mellor and Russell.-J.C.S., 81, 1272, (1902).
[5] Bevan.-Proc.Roy.Soc.Lond., 72, 5, (1903).
[6] Wildermann.-Zeitsch.phys.Chem.-41, 87, (1902). 42, 257, (1903).

Dux[1], who published the results of a rather thorough investigation of the whole problem in 1913, found, in contradiction to the results of the earlier observers mentioned, that moisture played no special role in the reaction, and exerted no influence on the rate when present at a partial pressure of more than 0.004 mm.

The phenomenon of the induction perion has been responsible for the many investigations of this apparently simple photochemical reaction. Bunsen and Roscoe's idea that this phenomenon was a real characteristic of a photochemical change seems apparently to have been generally accepted until about 20 years ago, when the work of Burgess and Chapman(loc.cit.) cleared the matter up very largely.

This work supported van't Hoff's contention that the induction period was not a real and general characteristic of a photochemical reaction, but merely an indication of the omission of some necessary experimental precaution. Burgess and Chapman showed that the observed delay in the combination of hydrogen and chlorine was due to the circumstance that the gases contained impurities which were capable of preventing the formation of hydrogen chloride, and which were themselves gradually destroyed by the light. They found that ammonia was one such impurity, and that if great care were taken to exclude the minutest traces of ammonia, the gases began to combine almost at once, the rate rapidly increasing to a constant maximum.

Before closing this summary of the literature of the reaction, reference must be made to the experimental work of Bodenstein and Dux, and to Bodenstein's suggestions as regards

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[1] Bodenstein & Dux.-Zeitsch.phys.Chem., 85, 297, (1913).

the mechanism of the reaction. Bodenstein and Dux's work established the following points:-

(1). The combination of hydrogen and chlorine in light is a reaction of the second order, the velocity being proportional to the square of the chlorine concentration. Hydrogen is without influence so long as it is present to the extent of at least ¼ of the amount of chlorine. At small hydrogen concentrations the velocity decreases slightly.

(2). The hydrogen chloride formed is without influence on the reaction rate.

(3). Water vapour, within certain limits of concentration is also without influence.

(4). Oxygen retards the reaction, such that the velocity at all stages is inversely proportional to the oxygen concentration.

Bodenstein[1] assumes that chlorine is activated in light, and combines with ordinary hydrogen molecules to form activated hydrogen chloride molecules, which are capable of imparting their activity to fresh chlorine molecules and to oxygen (to explain the dissipation of activity by negative catalysts), but not to neutral gases like nitrogen or hydrogen. Bodenstein regarded the activation of chlorine as consisting in the photoelectric separation of an electron, this electron functioning as a sort of catalyst for the transference of activity in the manner described. In this way, owing to the function of an electron as a catalyst, an energy quantum should be in a position to bring about the combination of a large number of reactant molecules.

Bodenstein was later forced to drop the idea of free electrons being responsible for the transference of activity

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[1] Bodenstein.-Zeitsch.phys.Chem., 85, 330, (1913).

owing to the work of Leblanc and Vollmer[1], who showed that an illuminated mixture of hydrogen and chlorine contained no ions, as an electroscope was not discharged. This observation confirmed a previous statement of Thomson's[2], and showed that results of Kümmel[3], who claimed to have detected electrification in the insolated mixture, were erroneous.

Experimental.

The first month or more of work was devoted to the fitting up and testing of various types of apparatus. The apparatus eventually evolved and fitted up is shown in the sketch on the following page. Pure aqueous hydrochloric acid was electrolysed in the vessel A. The two electrodes were of carbon 5 mm. in diameter, and projected for about 2cm. above the rubber cork, through which also came the tube for admitting and withdrawing the electrolyte. This was introduced by means of the tube B, and, when exhausted, was withdrawn by applying suction to C. The whole vessel was kept cool by immersion in a thermostat through which cold water was constantly circulating. The electrolysis vessel first used held about 100 c.c. of electrolyte only. It was found, however, that by the time the acid was saturated with chlorine, and really equimolecular quantities of hydrogen and chlorine were being evolved, the electrolyte had become too weak, with the result that oxygen contaminated the mixture. Accordingly a much larger electrolysis vessel, holding about 1500 c.c. of acid was later substituted.

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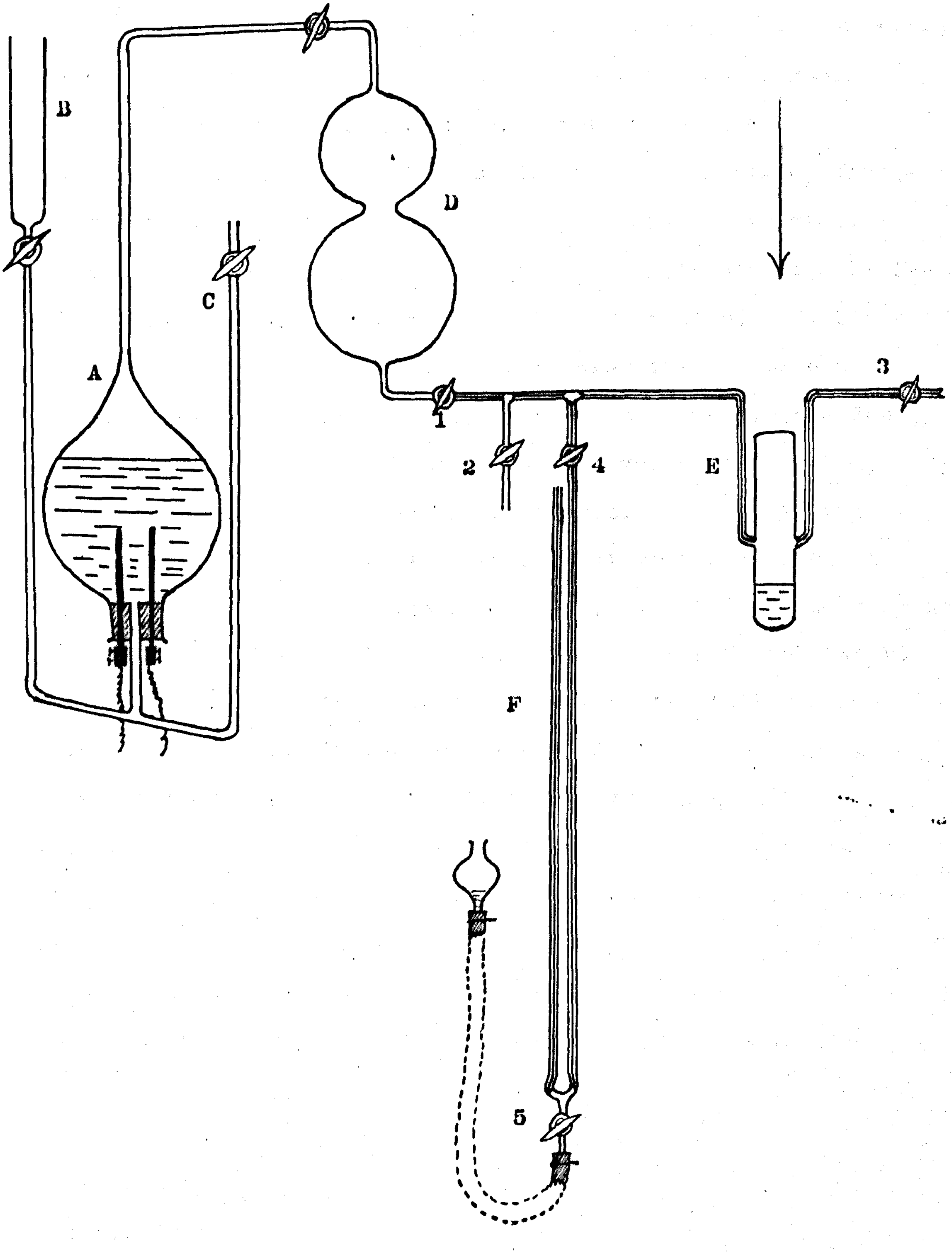
[1] Leblanc & Vollmer.-Zeitsch. für Elektrochem. 20, 494, (1914).
[2] Thomson.-Conduction of Electricity through gases. 2nd. Ed. p. 229.
[3] Kümmel.-Zeitsch. für Elektrochem. 17, 409, (1911).


B
C
A
D
1
2
4
E
3
F
5

The bulbs D were for ensuring a further supply of gas of exactly the same composition as that in use in the insolation vessel E. This was made out of glass tubing 4 - 5 cm. in diameter, sealed at both ends. The upper end through which the light was admitted had been rendered as flat as possible by pressing it, while hot, against a flat surface. Experiments were also made on an insolation vessel which had as a window a quartz plate, fixed n on by various cements, such as Faraday, and Chatterton, etc.. It was found, however, that these were all gradually attacked by the chlorine, so that the quartz window had to be abandoned. Other types of glass insolation vessel were also tested, the one described giving most satisfactory results. The insolation vessel was connected to the electrolysis vessel and to a manometer F by very fine capillary tubing, a way out of the same material being provided. The insolation vessel could be isolated by closing the taps numbered 1, 2, 3 and 4 on the diagram. About 15 c.c. of specially purified water(description later) was placed in the insolation vessel, before the latter was connected up. The insolation vessel was also kept cool and at constant temperature by being immersed in a large tank of running water. F was a very delicate water manometer which could at will be connected with, or isolated from the insolation vessel by means of the tap 4. The contraction could be followed by allowing water to flow into the manometer by way of tap 5. In this way, the gases in the insolation vessel were always at the atmospheric pressure.

The tubing from which the manometer was made was fairly heavy capillary. Particular care was taken in selecting a length uniform in bore, and the average volume contained in one linear millimetre was carefully determined in two ways.

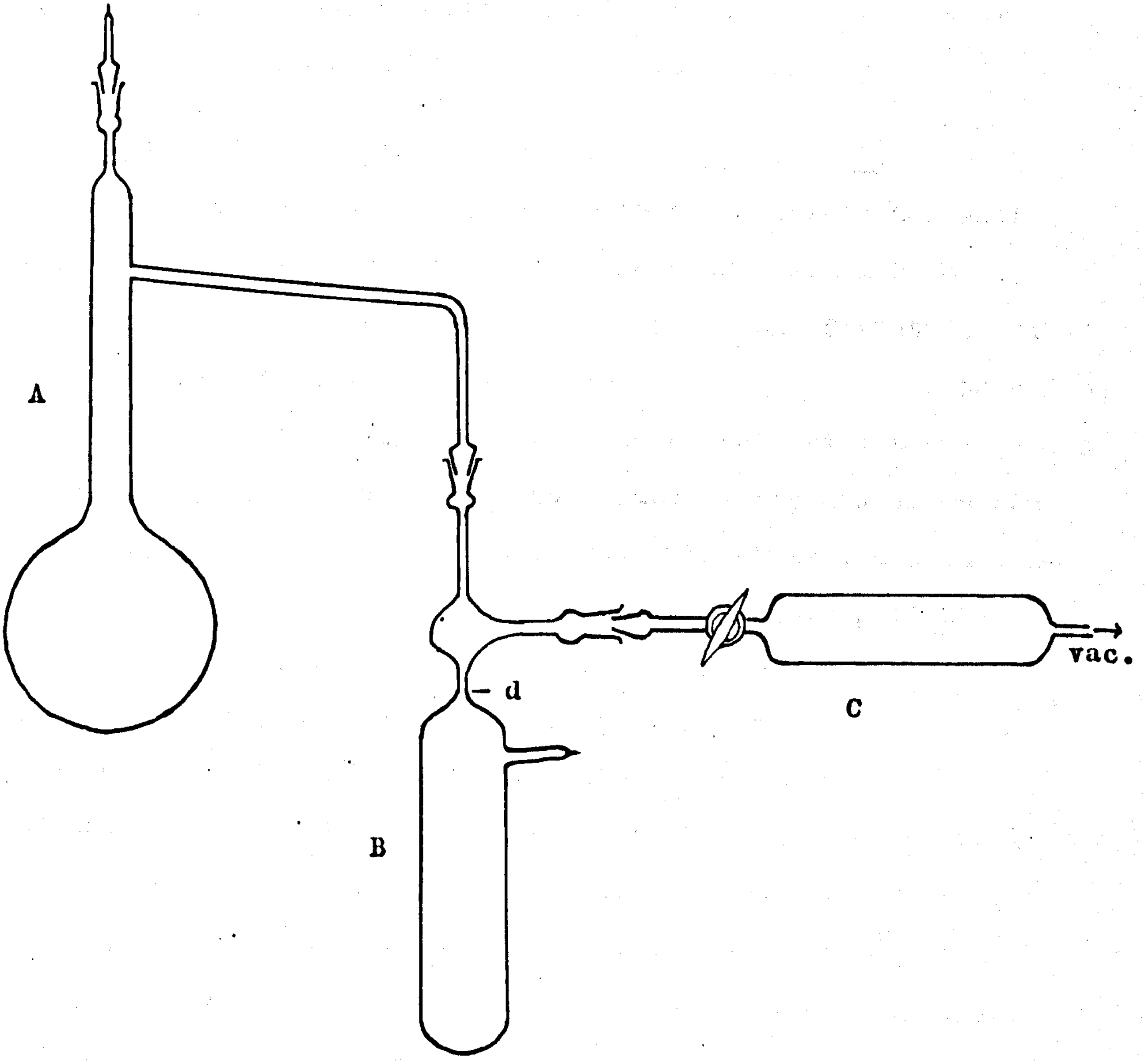
A thread of mercury was introduced into the capillary and its length carefully measured. These measurements were made at different places along the length of the tube, the average length of the thread being taken. The thread was then removed from the tube to a weighing bottle, and the mercury carefully weighed. In this way the result was arrived at that 1 linear millimetre of the capillary had a volume of 0.00477 c.c..

The movement of a water index corresponding to a definite volume was also measured. The volume per linear mm. was less, i.e., 0.00473 c.c. when determined in this way, owing to the wetting of the glass by the water.

The whole apparatus, with the exception of the small window at the top of the insolation vessel was painted a dead black, and all the tubes not under water were well lagged with cotton wool, the whole being finally screened from stray light by a covering of black cloth.

Preparation of the Electrolyte.

18 parts by volume of the purest hydrochloric acid obtainable (specially redistilled) were mixed with 10 parts by volume of pure distilled water. Pure chlorine was bubbled through this mixture for at least an hour. The solution was heated for half an hour on the steam bath, and then submitted to another shorter treatment with chlorine, followed by warming on the steam bath. The acid was well cooled, and introduced into the vessel A by means of the funnel B, as little time as possible being taken over these operations. A potential difference of 4 volts was maintained between the electrodes by a couple of


A
B
C
d
vac.

accumulators, and the gases allowed to stream through the whole apparatus for many hours until gas of constant composition was being evolved and until equilibrium between chlorine and water had been attained in the insolation vessel. It was easy to tell when equilibrium had been attained in the insolation vessel by watching the movement of the water in the manometer.

Preparation of Pure Water, for the Insolation Vessel.

Attention was first drawn to the absolute necessity of using pure water, free from all traces of ammonia by Burgess and Chapman. The apparatus used, a modification of that described in their paper(loc.cit.), is shown in the diagram on the preceding page. A was filled with about 8 grams of potassium permanganate in about 500 c.c. of distilled water, and gently heated on the water bath continuously for 12 hours. The flask was then connected with the receiver B by a ground glass joint, and the apparatus exhausted by means of a Fleuss pump. C was a tube containing phosphorus pentoxide, to prevent the distillation of water into the Fleuss. B was cooled in a freezing mixture, and the water distilled from A without the aid of heat. When sufficient water had collected in B, it was sealed off at d. The water was thus kept isolated until ready for use.

Owing to the fact that this method proved rather slow and laborious, another way of preparation was eventually adopted. Pure conductivity water was saturated with chlorine, boiled, resaturated and so on for several times. In this way all volatile nitrogen compounds were decomposed. The water obtained was immediately transferred to the insolation vessel.

Sources of Light, and Methods of varying the light intensity.

The first method that suggested itself as a means of varying the light intensity, and of relating any two intensities was the application of the Inverse Square Law. In order that the Inverse Square Law shall hold, it is necessary that the light shall emanate from as small a source as possible, i.e., from a point source. Accordingly a small lamp of the half watt type was procured which worked on 12 volts and which was credited with giving about 48 candle power. The lamp was of the type used in automobile headlights, and the filament, of a V shape, was very small, the V being only about 1/8" across. The lamp was fixed vertically above the window, the distance of the filament from the window being measured by a piece of cotton which served as the suspension of a plummet of glass rod.

This method of varying the intensity was discarded for several reasons, although the results obtained by using it on the whole showed a substantiation of the hypothesis which was deduced above. As already mentioned, the filament of the lamp was of a V shape, so that obviously the intensity of the light falling on the window would vary with the orientation of the V with respect to it. If the filament was "broadside on", the light intensity would probably be almost double what it would be when the filament was "end on". It was extremely difficult, almost impossible, to keep the V in the same position with respect to the window while the alterations and measurements of the lamp distances were being carried out.

The next method of varying the intensity tried was by the interposition between light and window of more or less opaque screens. These were made by exposing plates for various times, developing, fixing, and selecting uniform portions for use. The amount of reduction obtained by any screen was measured by means of the spectrophotometer. Mean values for the relative intensities of the light on the window, with various screens interposed were obtained in this way which were within about 3% of the actual values. The lamp was fixed in one position vertically over the window, a constant current maintained through it, and the screens interposed as desired. Many measurements of the rate of the reaction were carried out, using this screen method. This was a very discouraging period, as it was almost impossible to obtain reproducable results, those obtained holding out very little hope of being of any use. The cause of this lack of uniformity in the results was a lack of uniformity in the density of the screens. Examining these by looking at a light, or at the sky through them, they appeared to be perfectly homogeneous. It was not until, happening to look through one of them with the eyes focussed on the bars of a window, and happening to move the screen across the field of vision, that a perfectly appalling lack of uniform density was observed. This discovery of course necessitated the rejection of this method.

The third method tried proved to be the most satisfactory, and it was by using this method that good results were obtained. The intensity was varied by passing the beam of light through two Nicol prisms, and changing the angle between these. The source of light used in this case was much more powerful a 500 c.p.Pointolite lamp. The light emanated from a small Tungsten ball, not more than 1/3" in diameter, and was kept constant by keeping the current consumed always the same by means of a suitable adjustable resistance in series. Great care was taken always to keep the glass globe of the lamp perfectly clean and free from dust. The Nicol prisms used were taken from a Hilger Spectrophotometer, and were mounted suitably on a wooden base. One Nicol was fixed, and the rotation of the other was controlled by an accurately graduated circle. By the aid of a vernier, readings correct to 0.5 of a minute were obtainable.

The arrangement was calibrated before being placed in position, and it was found that all the light was cut off when the reading on the disc was 89° 23'. The maximum of light passing through would consequently be obtained when the angular reading was either 0°37' or 179° 23'. If this maximum intensity is denoted by I_0, and the intensity corresponding to an angular reading θ (observed), by I, then it is known that

$$I/I_0 = \cos^2(\theta + 37').$$

I_0 was taken as unity, as only relative intensities were to be observed.

The Nicols were then placed vertically over the window, and an image of the source focussed on to the latter, after which everything was well covered with black cloth.

Proceedure of an Experiment.

Before every experiment performed it was necessary to determine a creep, i.e., to notice the motion of the water in the manometer when no light at all was being admitted. The chief factor causing the creep was temperature, the creep being noticeably large in the morning, and falling off to zero or thereabouts later in the day. The rates of movement during consecutive intervals of time, generally either three or five minutes, were observed. Having obtained a constant creep this was either added to, or subtracted from the reaction rate in the experiment following, according as it was in the reverse or the same sense, i.e., an expansion or a contraction.

Having obtained the creep the shutter was removed and readings of the manometer taken at definite intervals, the contraction being followed up by admitting water by means of tap 5(diagram p.17). The levels in the manometer were thus always the same at the time of reading. It was found possible to obtain readings for the rate correct to within about 0.2mm. contraction per minute. The rates were generally expressed in cm. contraction per minute.

Experimental Results.

These definitely establish three facts, all independently supporting the hypothesis of the absorption by chlorine molecules of energy radiated by freshly synthesised hydrogen chloride molecules. In the first place they confirm the observation several times previously made on the attainment of a constant maximum rate. For instance Burgess and Chapman observed that, if volatile nitrogen compounds are absent, there is no period of induction, i.e., no period of absolute inactivity. The reaction starts slowly, and the rate gradually accelerates for a time and then remains constant. This increase in rate is shown in almost all Burgess and Chapman's tables. A typical example of this acceleration might be given here.

Illumination. Small 48 c.p. V filament lamp, running on 1.8 amps. lamp distant 134.7 cm. from the window.

Creep. .035 cm. per min. to be added.

Time. (mins.)	Intervals.	Scale readings.	Increment.	Rate (obs.)	Rate(corr.) cm./min.
-	-	92.18	-	-	-
5	5	91.87	0.31	0.062	0.097
10	5	90.78	1.09	0.218	0.253
15	5	85.95	4.83	0.966	1.001
17	2	83.39	2.56	1.28	1.315
19	2	80.8	2.59	1.295	1.320
24	5	74.15	6.65	1.33	1.365
26	2	71.42	2.73	1.365	1.400
28	2	68.7	2.72	1.36	1.395
30	2	65.85	2.85	1.425	1.460
32	2	63.1	2.75	1.375	1.410
34	2	60.3	2.8	1.4	1.435
36	2	57.55	2.75	1.375	1.410
38 x	2	54.75	2.8	1.4	1.435
40	2	54.5	0.25	0.125	0.16
42	2	54.6	-0.1	-0.05	

x - light cut off.

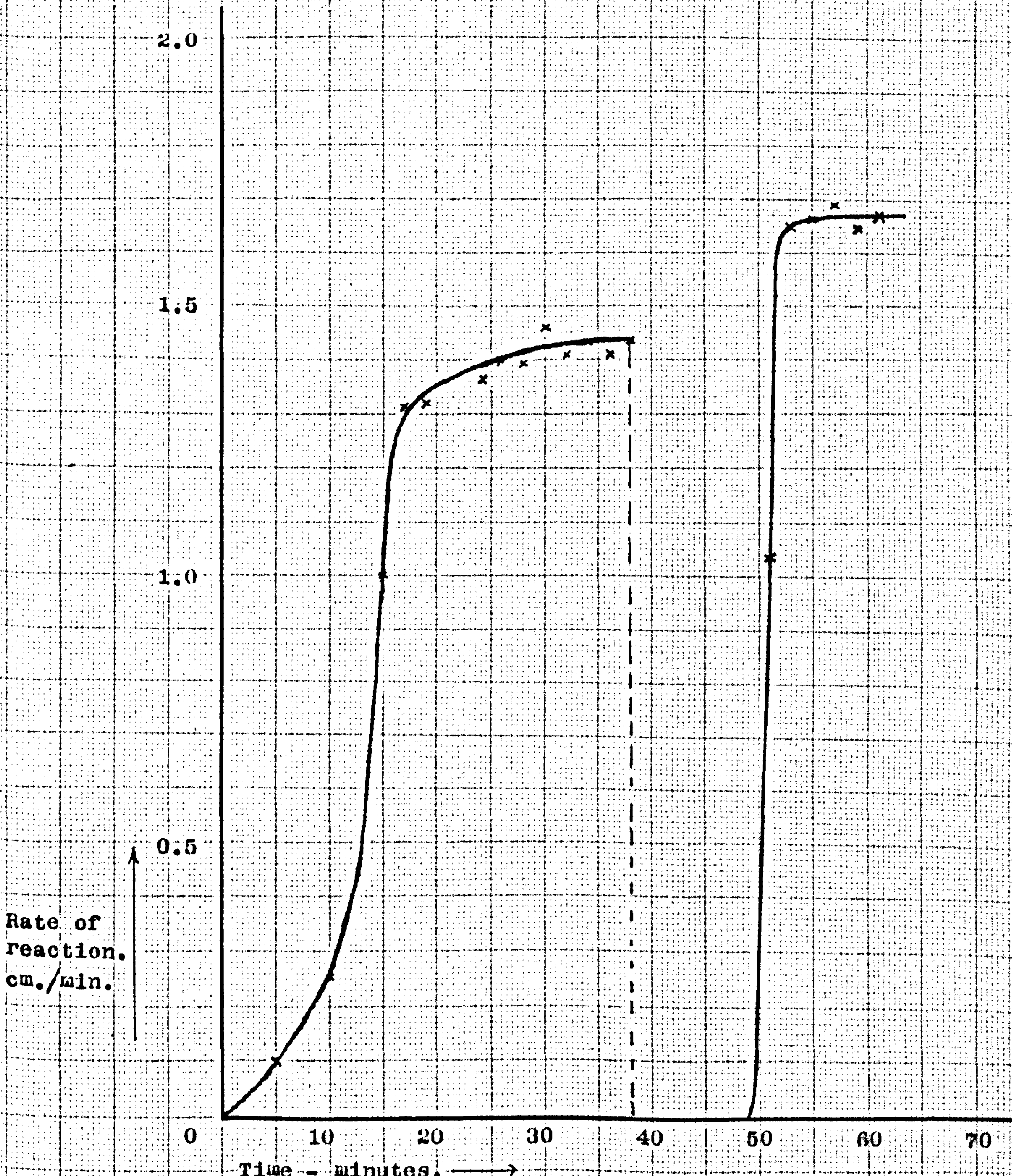
It will be seen that when the light is cut off, equilibrium is very rapidly established. If the light is now turned on again, it is found that the same phenomenon will occur, but that both the initial rate and the acceleration will be greater, with the result that the maximum rate will be attained in very much less time. 11 minutes after turning off the light in the above experiment the light was turned on again. Following is the continuation of the above table. It will be noticed how very much more rapidly the maximum is reached.

(Creep. 0.04, to be added).

49	-	104.14	-	-	-
51	2	102.15	1.99	0.995	1.035
53	2	98.93	3.22	1.61	1.65
55	2	95.68	3.25	1.625	1.665
57	2	92.38	3.3	1.65	1.69
59	2'	89.17	3.21	1.605	1.645
61	2	85.91	3.26	1.63	1.67

These results are plotted on the following graph. (p.27).

Reaction Rate - Time.



Before the original curve can be approximately followed, it is necessary for the gases to remain for at least half an hour in the dark. In the example just quoted it will be seen that the constant maxima are not quite the same. This example serves to show the difficulty that was experienced in obtaining reproducable results, which were not obtained until the Pointolite was used as the illuminant, and the Nicols were fitted into position. Even after that , as will be shown, some difficulties were still encountered.

Both the above phenomena, i.e., the shortening of the time necessary for the attainment of the constant maximum rate and the fact that a maximum rate is not attained at once, are capable of very easy and natural explanation on the basis of the reabsorption hypothesis.

In the first moments of illumination, the reaction follows the photochemical equivalence law, the only source of radiation being that radiation incident on the window. Radiated energy will then begin to be available, with the result that reabsorption will take place, and the reaction velocity increase. That is to say, the reaction rate under constant illumination will first of all be small, and will increase up to a constant maximum, the magnitude of this latter being dependent upon the proportion of radiated energy absorbed.

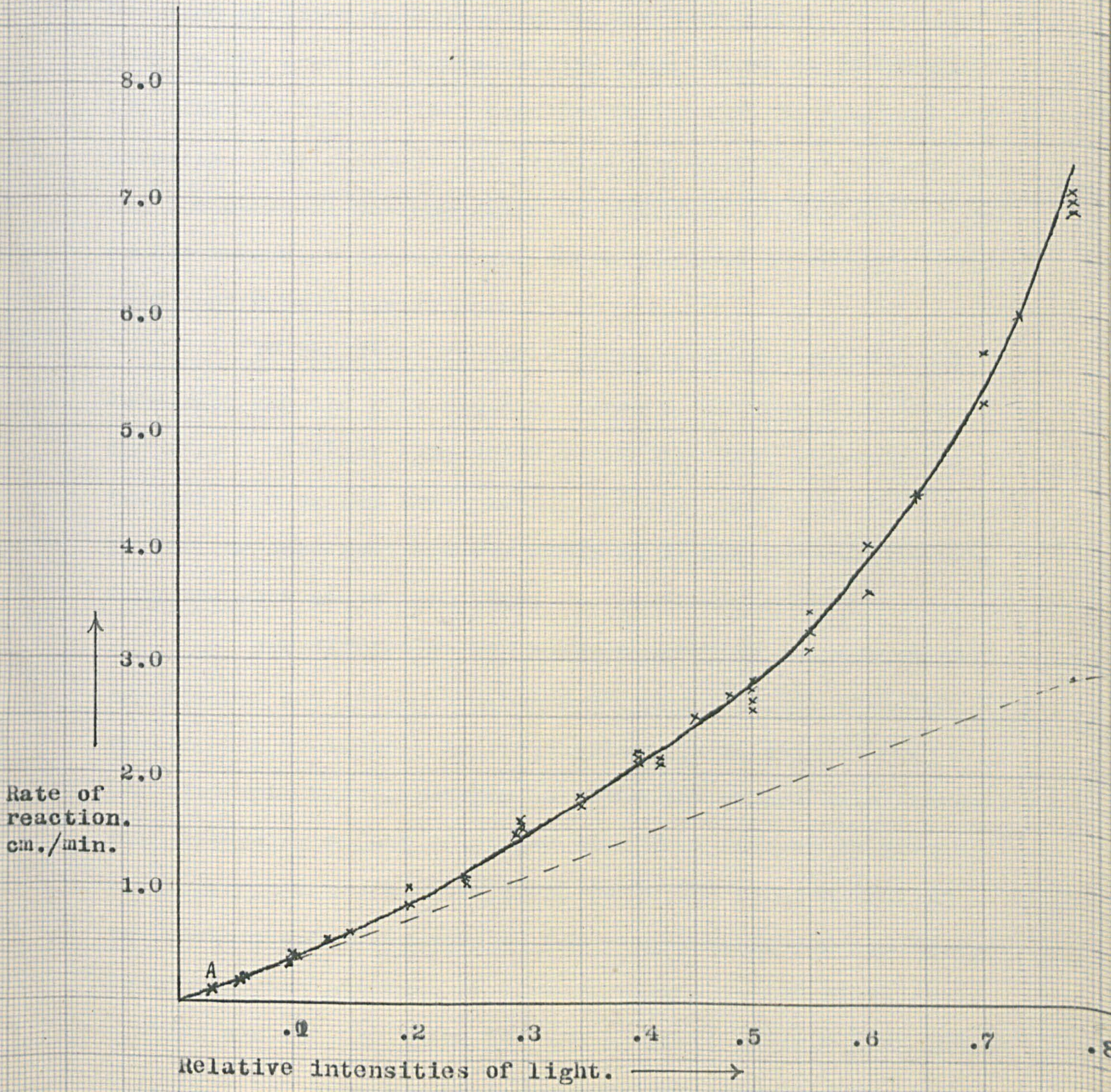
When the light is turned off, some molecules of chlorine will remain which are part of the way towards activation. If left for a time these will radiate all this acquired radiation away. If, however, the light is turned on again not very long after it has been turned off, these molecules will not require so much energy to bring them up to a reactive state, with the result that the maximum rate will be attained sooner than if a start were made with totally inactive molecules. As far as can be seen , partially activated molecules must be recognised as existing before this phenomenon can be adequately and clearly explained.

Turning now to the variation of the rate of the reaction with the intensity of the light, it has been found that the former increases very much more rapidly than the intensity. The maximum rates were those compared, since these alone could be regarded as comparable.

The following table shows the variation of the constant rate with the intensity.

Relative Intensity.	Maximum Rate. (Average of various successful exp'ts.)	Maximum Rate. (Calc'd. from rate at $I/I_o = 0.03$.)
.78	7.0 (3)	2.88
.73	6.0 (2)	2.60
.70	5.5 (2)	2.59
.64	4.45 (1)	2.37
.60	3.8 (2)	2.22
.55	3.26 (3)	2.03
.50	2.70 (5)	1.85
.48	2.70 (1)	1.77
.45	2.50 (1)	1.64
.42	2.24 (2)	1.55
.40	2.16 (3)	1.48
.35	1.76 (2)	1.28
.30	1.55 (3)	1.10
.294	1.45 (1)	1.07
.25	1.09 (4)	0.92
.203	0.93 (2)	0.75
.15	0.61 (3)	0.55
.13	0.55 (1)	0.48
.105	0.42 (3)	0.38
.099	0.42 (3)	0.37
.066	0.26 (3)	0.24
.06	0.25 (3)	0.22
.03	0.11 (8)------------	0.11

Reaction Rate - Intensity.



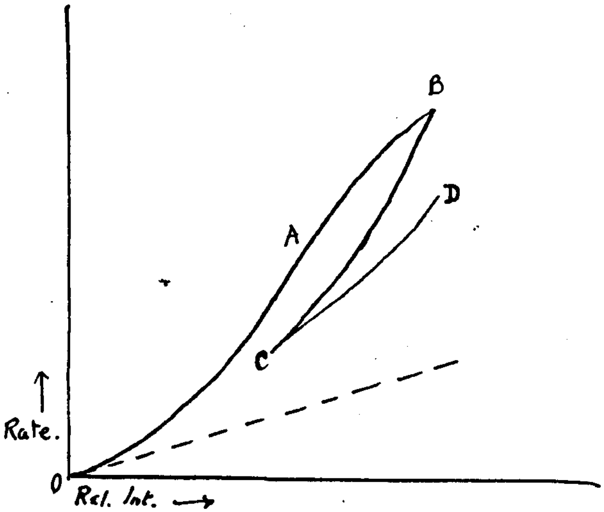
The rate of the reaction at the relative intensity of 0.03 was determined very carefully, at least eight reliable observations being made. These gave values for the rate of

0.12, 0.11, 0.11, 0.10, 0.09, 0.12, 0.11, and 0.11 cm./min. . The mean of these is 0.11 cm./min., and this point is denoted by A in the graph on the preceding page, on which is plotted the relation between the light intensity and the reaction velocity. The relative intensities are plotted as abscissae, and the constant maximum rates as ordinates -(expressed in cm. on the manometer scale. 1 cm. corresponds to an actual volume change of 0.0473 c.c.).

If the Einstein Law held, the relation between the intensity and the rate would be linear. Through A is drawn the dotted straight line which would have been expected had the law held, or had there been a constant deviation from it. It will be seen that all the rates at higher intensities than 0.03 lie well above this line.

One difficulty met with in carrying out the above experiments was the accumulation of oxygen in the reacting system, due presumably to the interaction of some of the chlorine with water, hydrogen chloride and oxygen being formed. The retarding effect of oxygen was first noticed by Bunsen and Roscoe, and has been referred to in the section dealing the history and literature of the reaction. Bunsen and Roscoe found that the presence of 0.5% of oxygen in the reaction mixture changed the value for the constant maximum from 100 to 9.7, while the presence of 1.3% of oxygen took it down as far as 2.7. The hindering effect of oxygen is also marked in other chlorine reactions, and has been the subject of study by Luther and Goldberg[1], and by Weigert[2].

On carrying out a series of experiments, starting with low intensities, and working up to higher ones, points were obtained which lay upon a curve, say OA on the following diagram.



On reaching intensities of the magnitude 0.6 - 0.7, the curve began to fall off a little (AB). Now, on going back to measurement of the rates at lower intensities again, it was found that the points obtained this time all lay below OAB, a curve like BC being followed. Then, on using higher intensities once more a curve was obtained like CD. The oxygen gradually accumulated in the insolation vessel and lessened the rate. This fact caused much confusion initially. It must be emphasised however that all the values obtained lay well above the straight line drawn through the origin and the point showing the magnitude of the rate at the relative intensity 0.03. The oxygen effect was finally rendered negligible by fresh gas being blown through the whole apparatus after each single experiment. On the following page is a curve showing constant

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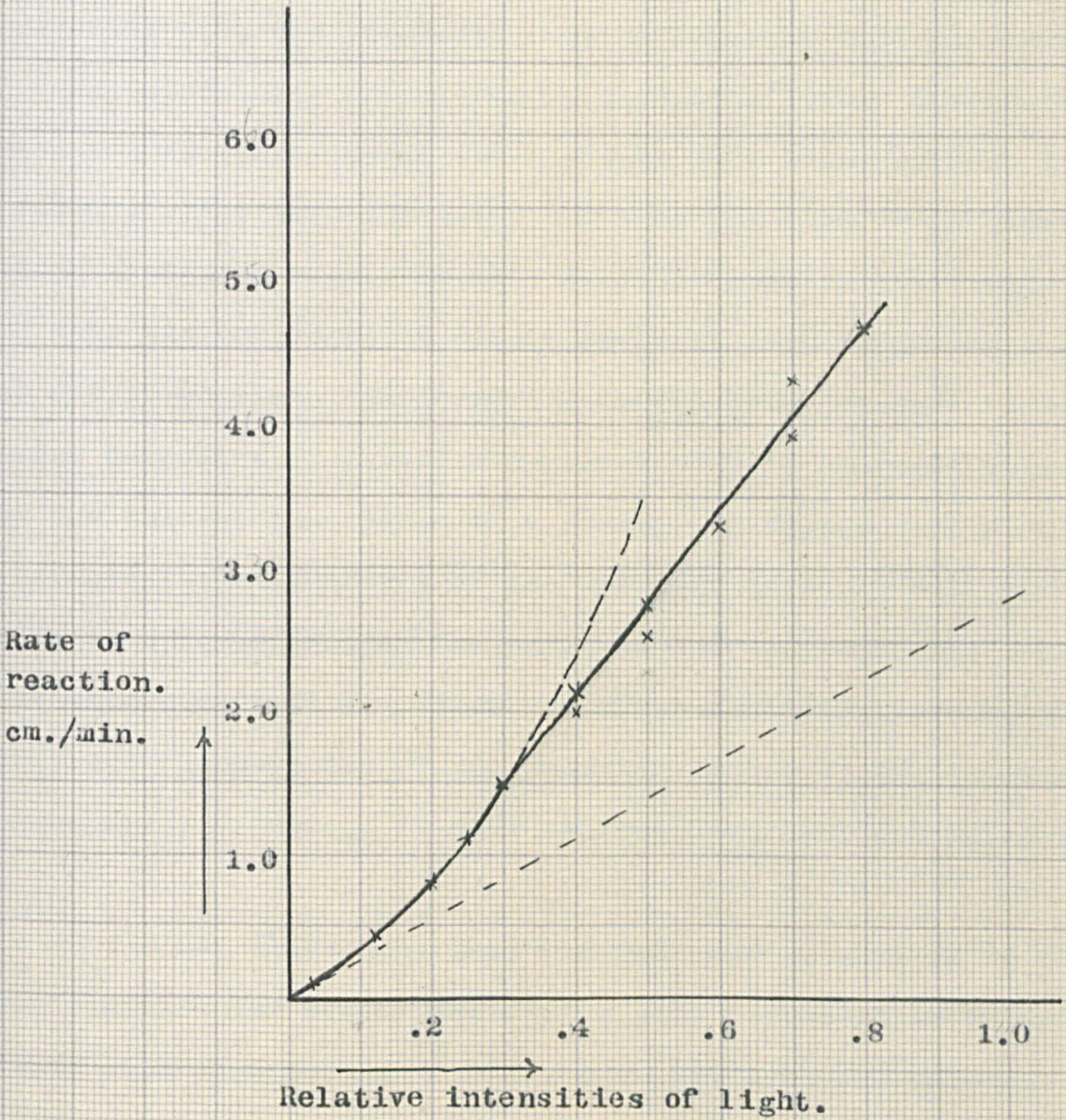
[1] Luther & Goldberg.-Zeitsch.phys.Chem.56, 43, (1906).
[2] Weigert.-Ann.d.Phys.24, 55, (1907).

Gas mixture slightly
contaminated with Oxygen.

Reaction Rate - Intensity.



maximum rates obtained at various relative intensities with a gas mixture contaminated with oxygen. Although the slope of the curve is less than the normal, the same deviation from a linear relation is indicated.

The shape of these curves shows very clearly how the proportion of energy reabsorbed by molecules of chlorine increases with the intensity. It is apparent that the curves are asymptotically approaching a certain critical intensity, at which the velocity of the reaction will become almost infinite, i.e., at this intensity the reaction will be propagated as an explosion wave. As the intensity goes on increasing so does the reabsorption become the more complete, until a limit of almost complete reabsorption is reached. At this limit a few molecules of chlorine reacting to form hydrogen chloride, by the initial absorption of a quantum of energy each at the characteristic frequency of the chlorine molecule, will act as a sort of trigger, causing the explosion of the whole mass. Baly has before defined the criterion of an explosion wave as "the complete reabsorption of the radiated energy by the reactant molecules surrounding freshly synthesised resultant molecules".

The third fact that has been established is in connection with the Draper Effect, i.e., the expansion in volume which occurs as soon as a mixture of hydrogen and chlorine is illuminated. The Draper Effect is not noticed when there is a pronounced induction period, for instance when nitrogen compounds are present. When a previously insolated mixture of hydrogen and chlorine is illuminated, there occurs a sudden expansion - the Draper Effect - followed by the accelerating contraction already described.

Budde[1] has shown that chlorine alone expands in an analogous manner to a mixture of hydrogen and chlorine, under the influence of light rays of high refrangibility. He also showed that the temperature of the chlorine rises about 1°. This is not a direct heat effect, for it takes place after all the infra-red has been cut out by a screen of water. Richardson[2] not only established the reality of the Budde effect, but also noted that the magnitude of the expansion was proportional to the intensity of the more refrangible light rays. The expansion is independent of the temperature between the limits 14° and 138°. It also occurs, according to Richardson when the chlorine is mixed with air, hydrogen, and carbon monoxide.

Budde has suggested that the expansion can be explained in the following ways:-

(1). As a dissociation of chlorine molecules into free atoms.

(2). That the expansion is caused by the fact that the light does work, which is converted into heat.

The most probable explanation would seem to be that the Budde effect is due to the fact that chlorine molecules fluoresce some of the energy absorbed in the visible as heat, this causing the expansion. Mellor[3] adopts the same explanation of the Budde effect. Mellor concludes from his experiments that there is no rise in temperature of dry chlorine when it is exposed to the light, and that the rise observed in the temperature of improperly dried chlorine is due to some chemical reaction

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[1] Budde.-Phil.Mag., (v)42, 290, (1871). Pogg.Ann.6, 477, (1873).
[2] Richardson.-Phil.Mag., (v)62, 277, (1891).
[3] Mellor.-J.C.S., 81, 1230, (1902).

between chlorine gas and moisture. This reaction he formulates as $2H_2O + 2Cl_2 = 4HCl + O_2$

Returning to the Draper effect, Mellor and Anderson[1] seem to explain this as nothing more than a small hydrogen chloride- forming explosion. The expansion is due to the heating of the gas by the reaction heat. The effect is stronger when the vessel containing the active water is shaken before exposure to light. In this case a mist is formed inside the insolation vessel, The effect seems therefore to be also dependent on the heat of solution of the hydrogen chloride. When condensation occurs on the little drops of the mist, absorption is more rapid, with the result that the temperature rise is more pronounced.

On conducting observations on~~x~~ the magnitude of the Draper effect at different light intensities, it has been found that this is proportional not to the light intensity, but to the reaction rate. In addition, when the light is cut off, there is a rapid contraction,- the reverse of the Draper effect, This also is proportional to the reaction rate. Thus whatever explanation be given to the Draper effect, whether it be a dissociation, or whether it be due to a degradation of the light absorbed, there is no doubt that it is increased by the absorption by chlorine of some of the energy radiated by the hydrogen chloride.

The graphs following (page 37) show clearly this deviation from proportionality. The first graph shows the relation between the observed Draper expansion and the relative

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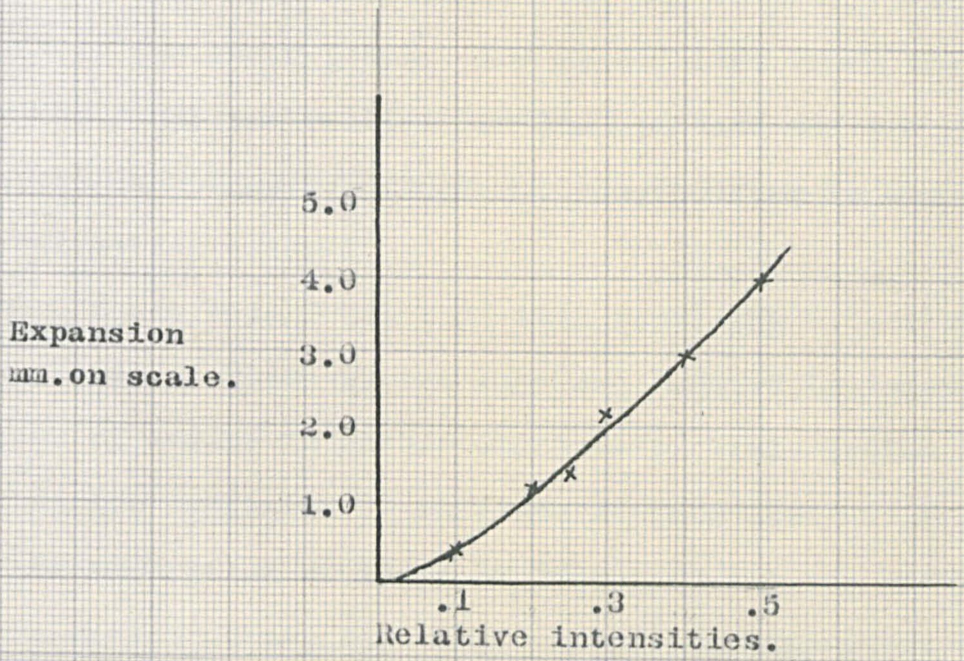
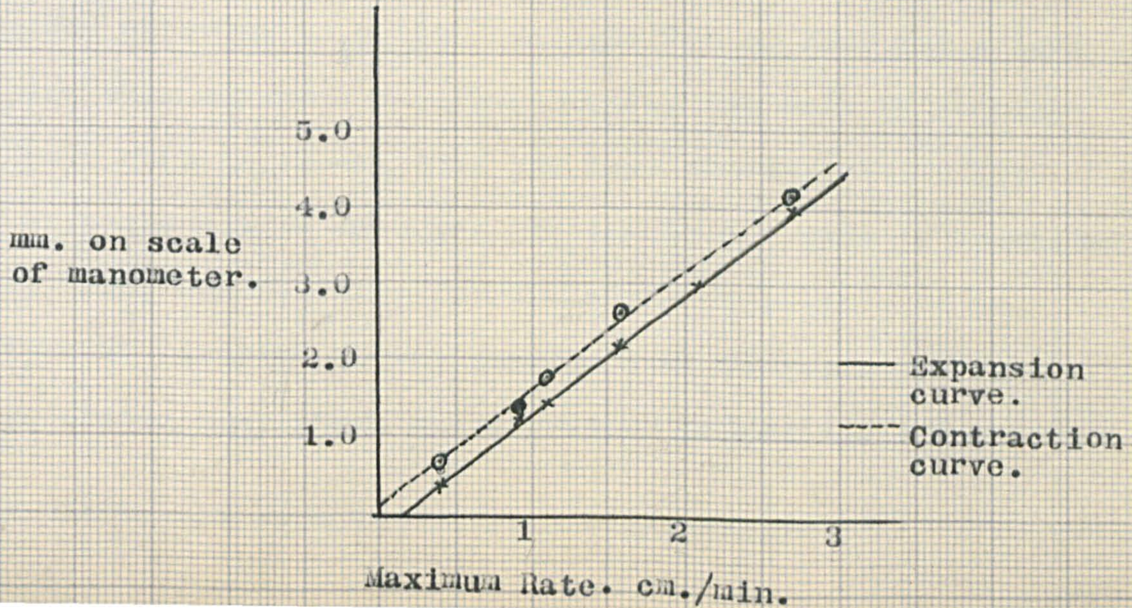
[1] Mellor & Anderson.-J.C.S., 81, 414, (1902).

Draper Expansion - Relative Intensity.



Draper Expansion - Maximum Rate.
Final Contraction - Maximum Rate.



light intensity. It is obvious that this relation is not linear in character, but that a curve similar in shape to the intensity rate curve is obtained. This at once suggests the plotting of the expansion against the constant maximum velocity, which has been done in the lower graph. The dotted line shows the relation between the final contraction and the constant maximum rate. (The curves do not pass through the origin owing to the action of a contraction creep.)

It has been found that this expansion is not observed when the blue and ultra-violet rays are removed by interposing a solution of iodine in carbon disulphide. This proves beyond doubt that the expansion of chlorine and its activation are due to the absorption of light of frequencies lying within its ultra-violet absorption band. Draper was the first to show that only those rays which are absorbed are photochemically active and it was this observation of his that first led to the conception of active molecules. Favre and Silbermann[1] claimed to have definitely established the existence of active molecules of chlorine by measuring the heats of combination of insolated and non-insolated chlorine with caustic potash. They exposed chlorine to bright sunlight for an hour and a half or more, and then measured calorimetrically the heat of combination. Their results showed that the heat of combination per gram of chlorine was 40 calories greater in the case of insolated chlorine. They consequently formulated the existence of an active type of chlorine having this heat of formation per gram. They also stated that there was a greater proportion of chlorate

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[1] Favre & Silbermann.-Ann.Chim.Phys., (3)<u>37</u>, 497, (1853).

formed when insolated chlorine was used. Berthelot[1] contradicted Favre and Silbermanns' conclusions, and later still Mellor[2] showed that neither with this reaction, nor with that between chlorine and potassium iodide was the observed heat effect greater in the case of insolated chlorine. Mellor's results show that there is no evidence at all for the existence of active molecules after insolation. The fact that wholly activated molecules do not exist after the activating light has been cut off was further shown by some work of H.S.Taylor[3]. Taylor allowed light to shine on to chlorine contained in a glass vessel, and then immediately passed this insolated gas into a darkened bulb containing hydrogen. He was able to detect no traces of combination whatever.

During the period of insolation however, many chlorine molecules will be active, there being a definite dynamic equilibrium between active and non-activated molecules for any given intensity of light, and concentration of chlorine.

Absorption measurements were carried out with the object of determining the magnitude of the activating quantum.

The ultra-violet absorption spectrum of chlorine.

The absorption of chlorine at atmospheric pressure was investigated by means of a Hilger ultra-violet spectrophotometer. The cells used were made of glass tubing about 1 cm. in diameter, each cell having two side tubes blown on, so as to allow of the passage of a continuous stream of chlorine through

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[1] Berthelot.-Ann.Chim.Phys.,(5)5,322,(1876).
[2] Mellor.-J.C.S.,81,1280,(1902).
[3] see Bodenstein.-Zeitsch.für Elektrochem.pp.202,255,(1916).

the cell. The ends of the cell were ground parallel with carborundum and then two quartz plates were cemented on with secotine. Three different cells were made and used, measuring respectively 60.5 mm., 22.5 mm., and 5.2 mm..

At atmospheric pressure the band extends from about $\lambda = 400\mu\mu$ to $\lambda = 270\mu\mu$, and the centre lies near $\lambda = 333\mu\mu$. At the centre of the band the extinction coefficient is 2.5. The band is graphed on page 41, while on page 42 is a graph of the band spectrum of chlorine as measured by Ribaud[1]. It will be noticed that Ribaud gives the centre of the band as occurring at $\lambda = 338\mu\mu$.

From the facts given, it will be seen that the evidence very strongly upholds the hypothesis outlined in pages 6 - 10, i.e., that the divergence from Einstein's Law is due to the reabsorption by reactant molecules of energy radiated by freshly synthesised resultant molecules, many more reactant molecules thus being activated than correspond to the number of quanta absorbed from actual incident radiation. It has been shown that the divergence depends upon the light intensity, and this, coupled with the fact that the divergence varies with the concentration of the absorbing molecules, would seem to give a very firm basis for the belief that the explanation advanced is correct.

Slade and Higson[2] have obtained somewhat similar

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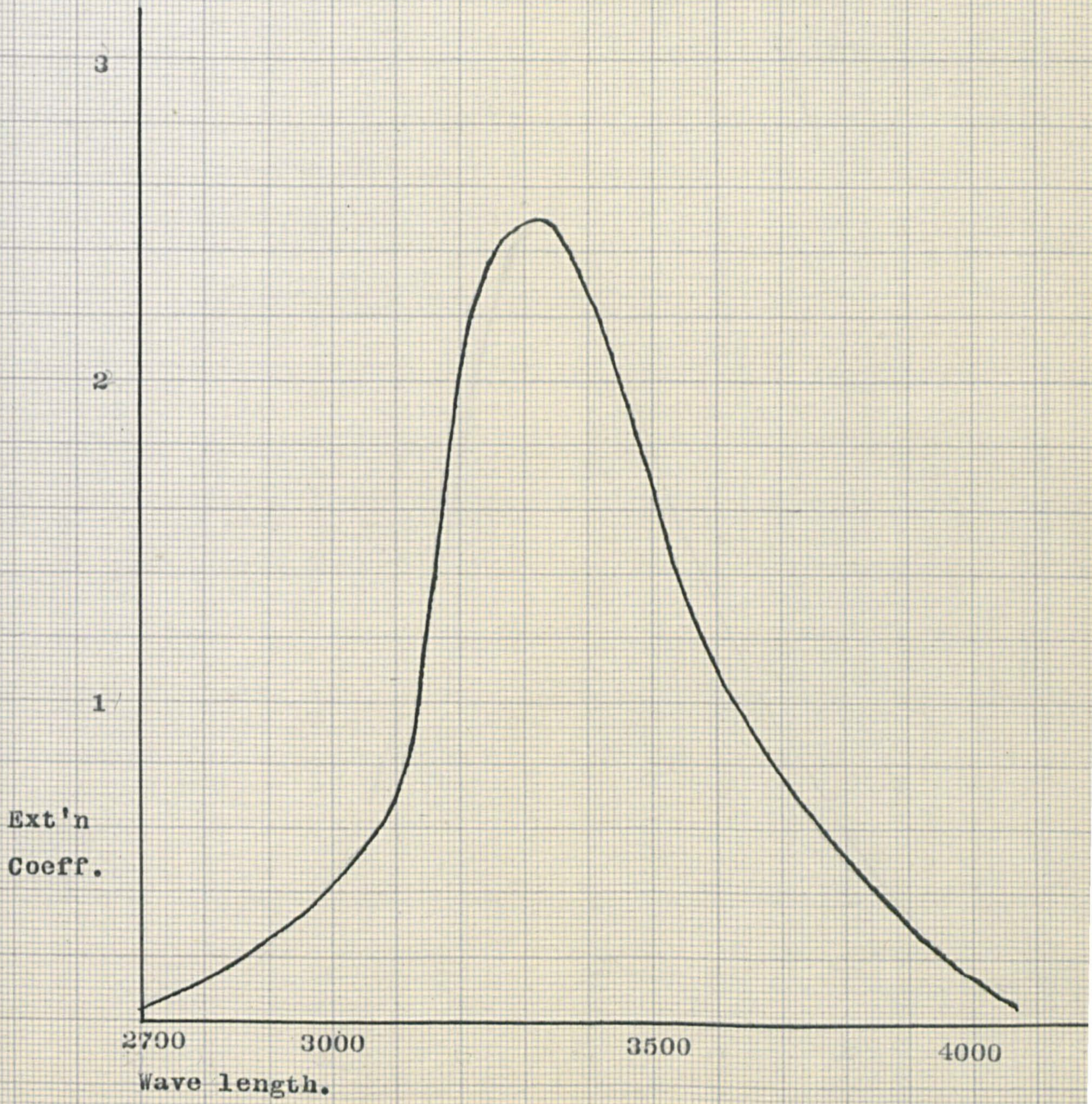
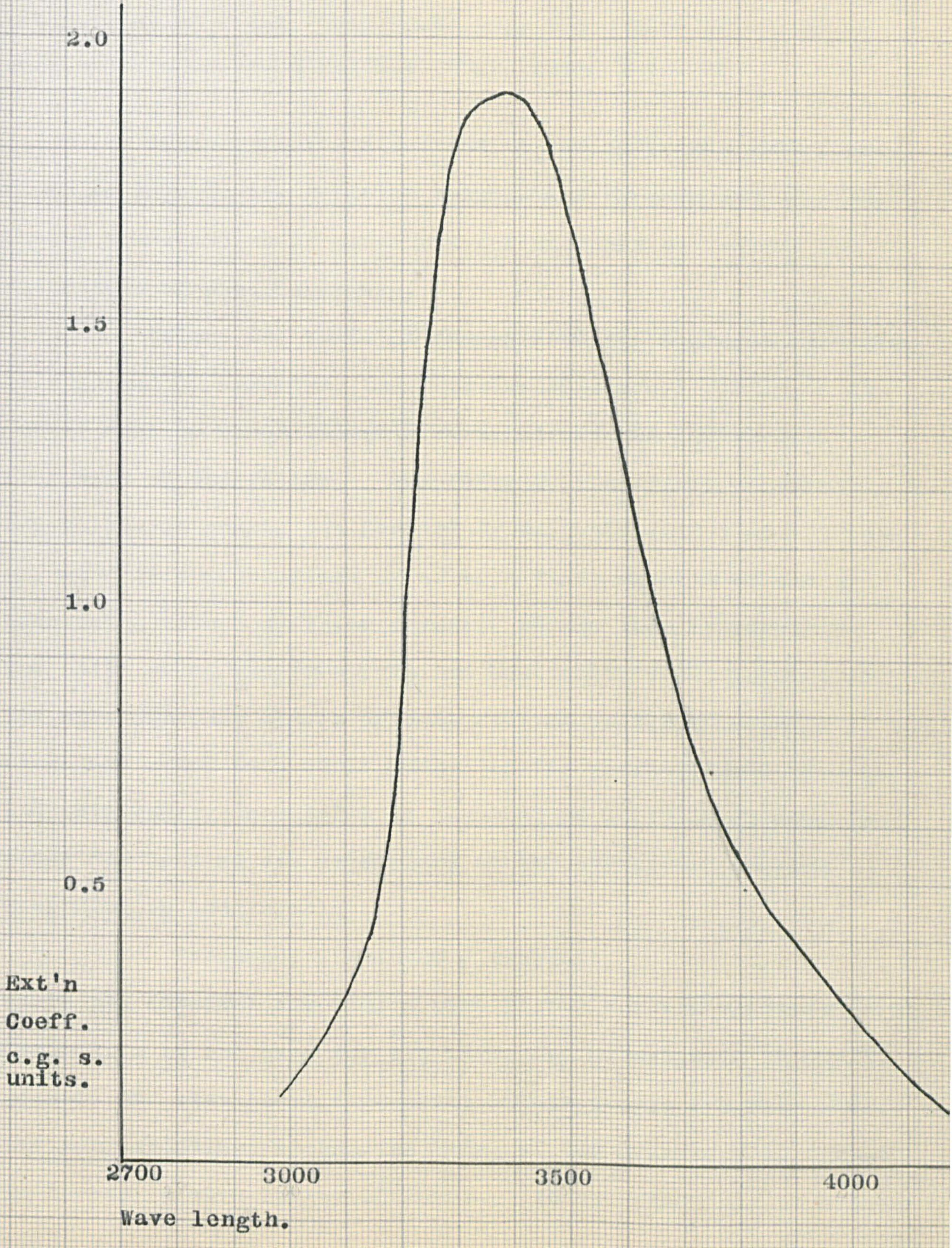
[1] Ribaud.-Ann.de Phys., 9, 12, (1919).
[2] Slade & Higson.-Proc.Roy.Soc.Lond., 98A, 154, (1920).

Absorption Spectrum of Chlorine.



Absorption Spectrum of Chlorine - Ribaud.



results in the case of the photographic plate. Exposing very fine films of silver chloride for the same time to different intensities of light, they found that the amount of silver obtained was not proportional to the light intensity.

"These results also lead to two important deductions, first as regards the relation between the frequencies of elementary atoms and their compounds, and secondly as regards the Phenomenòn of photocatalysis.

"Since the radiated energy from the hydrogen chloride is absorbed by chlorine, the frequencies characteristic of the former must be exact integral multiples of the fundamental frequency of the latter. According to the theory of atomic and molecular quanta put forward by Baly (loc.cit.) an elementary atom is characterised by an elementary quantum of energy associated with a definite physical change, such as possibly the shift of an electron from one stationary orbit to another. The atom can only absorb or emit radiant energy in amounts which are equal to a whole number of its characteristic quanta, and this energy is emitted or absorbed at definite frequencies. These frequencies are determined by the elementary quantum according to the formula

$$\text{frequency} = \text{quantum} / \text{constant},$$

where the constant embodies a time factor for the electron shift, assumed to be the same for all atoms and molecules. It may be pointed out that these elementary atomic quanta are very small, the corresponding frequencies being of the order 3×10^{11}, that is to say, a wave length of 1000μ. It is reasonable to suppose that when a compound molecule gains or loses energy as a whole its component atoms share equally in the process. The smallest quantity of energy therefore that a molecule can absorb or radiate as a whole will be equal to the least common multiple of the quanta characteristic of its component atoms. Dr.Norman Campbell[1] has, however, very rightly pointed out that the least common integral multiple of twp or more atomic quanta can have no physical significance unless the quanta are integral multiples of a fundamental unit of energy. It may, however, be suggested that all elementary atomic quanta are integral multiples of the quantum characteristic of the atom of hydrogen. Whether this suggestion is true or not, the proof that the frequencies of a compound molecule are exact integral multiples of the frequencies characteristic of its component atoms would undoubtedly afford strong support to the existence of the fundamental unit associated with matter, and hence would justify the principle of the least common integral multiple. The results now communicated show that the frequencies characteristic of the hydrogen chloride molecule are at any rate exact integral multiples of the fundamental frequency of chlorine. It is not possible directly to state that they also prove that the hydrogen chloride frequencies are exact multiples of the atomic frequency of hydrogen since the reaction between hydrogen and chlorine is catalysed by light of frequencies equal to those of chlorine, light for which hydrogen seems to possess no measurable absorptive power. On the other hand, the probable mechanism of the reaction between hydrogen and chlorine may be considered, and it is interesting to note that no attention has been paid to this.

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[1] Campbell.-Phil.Mag.41,707,(1921). and Baly.-ibid.711.

A mixture of hydrogen and chlorine undergoes no measurable reaction when screened from light, but when exposed to rays of frequencies comprised within the known ultra-violet absorption band of chlorine the two gases at once begin to unite. The chlorine molecules, inactive in the dark, are activated by the absorption of energy, the minimum amount absorbed per molecule being

$$9 \times 10^{14} \times 6.56 \times 10^{-27} = 5.91 \times 10^{-12} \text{ ergs,}$$

where 9×10^{14} is the central frequency of the absorption band. These activated molecules then react with the inactive hydrogen molecules, and it would seem that this process must be accompanied by a transference of energy from the former to the latter. Whatever may be the actual mechanism of the process, the net result is the dissociation of the hydrogen molecule to form two molecules of hydrogen chloride, and the energy necessary for this dissociation must have been derived from the activated chlorine molecules. This transference of energy can only be possible if the hydrogen and chlorine have frequencies in common, that is to say, either the frequencies of chlorine must be exact integral multiples of the fundamental atomic frequency of hydrogen, or the frequencies of both must be exact integral multiples of a universal fundamental unit. It may be stated therefore that the evidence now brought forward is strongly in favour of the exact integral relationship between the molecular frequency of hydrogen chloride and the atomic frequencies of both hydrogen and chlorine. The simplest possible relation is that the molecular frequency of hydrogen chloride should be the least common integral multiple of the atomic frequencies of its component atoms, and it has been shown in previous papers that this relation finds very considerable support in the constant frequency differences between the absorption bands shown by a compound molecule and also between the constituent lines of these bands. (Baly & Barker, J.C.S., 119, 659, (1921)).

Photocatalysis.

The results outlined in the preceding pages seemed to have opened up a very large field for research in the realm of photochemistry, since it is now possible to predict a new type of photocatalysis. The first results of the application of the principles arrived at have been very gratifying and will be described in later pages.

Let us consider any reaction $A + B = AB$, which is known to take place under the influence of light of a frequency characteristic of A or B. Then it should be possible to induce this reaction to proceed under the influence of a body, which we will call the photocatalyst, which absorbs light at other frequencies than those characteristic of A or B, and which fluoresces this radiation at a frequency which is characteristic of A or B as well as of the photocatalyst itself. For this to be possible it must contain the same elementary atoms as A and B, for this is the condition which must be fulfilled before the photocatalyst and A and B can have any common infra-red frequencies.

The mechanism of the reaction is then as follows:- The photocatalyst absorbs light of its characteristic frequency in the visible or ultra-violet regions of the spectrum, and fluoresces in the infra-red at frequencies, some of which are characteristic of A or B as well as of the photocatalyst itself. Infra-red radiation will be absorbed by A and B, and if there is a sufficient radiation density from the photocatalyst to allow of enough of these smaller quanta accumulating to activate A or B, the reaction will proceed on account of the light absorbed by the photocatalyst, even though this cannot be capable of direct absorption by either A or B.

It will thus be seen that there are two conditions which must be fulfilled before any substance can act as a photocatalyst for a given reaction. In the first place it must have some infrared frequencies in common with one of the actual reactants, and secondly it must have a different characteristic frequency in the visible or ultra-violet from that of the reactant.

A very typical example of photocatalysis has quite recently been described by Daniels and Johnston[1]. These authors show that nitrogen pentoxide has no absorption whatsoever in the region 460 - 400$\mu\mu$, and that it is not decomposed on exposure to light of wave length between these limits. Nitrogen peroxide however is known to be able to absorb light of these wave lengths - it is a reddish gas which has an absorption band there. If now a mixture of nitrogen pentoxide and nitrogen peroxide is exposed to light of this wave length, the nitrogen pentoxide undergoes decomposition. Their explanation of this phenomenon, which explanation will be seen to be almost the same as that which has been suggested, is quoted following:-

> "The following hypothesis is suggested as a possible explanation of the auto-catalytic effect of nitrogen dioxide. It absorbs blue light over a wide range and through fluorescence passes it out in the infra-red region where its absorption lines overlap those of nitrogen pentoxide and causes decomposition of the latter. Two assumptions, which have as yet no experimental support, are involved in this hypothesis; first, that nitrogen pentoxide is decomposed by the energy which it absorbs in the infra-red, and second,

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[1] Daniels & Johnston. - J. Am. Chem. Soc., 43, 72, (1921)

that the dioxide fluoresces, i.e., that light corresponding to its absorption lines in the infra-red is emitted when blue light is absorbed.....

"If this hypothesis proves to be correct, it will lend valuable support to the radiant energy theory of catalysis. It will offer experimental facts in a gas-phase system to show that a substance can act as a catalyst by absorbing radiant energy, transforming it through fluorescence and giving it out at a particular frequency which is effective in bringing about a reaction. It suggests that other chemical reactions may be brought about by catalysts which absorb radiant energy over a wide range and emit it in a narrow region where it is effective. It suggests also that a catalyst of this type may be used together with radiation from an external source to bring about reactions which could not be brought about in a reasonable time by the catalyst alone or by the photochemical action alone."

They point out that the absorption bands of the two gases overlap in the short wave infra-red. Nitrogen peroxide has bands at 3.43μ and 6.12μ, while nitrogen pentoxide has bands at 3.39μ and 5.81μ. Following is a criticism of their assumption:-

"This assumption is not satisfactory for two reasons. In the first place, the absorption by the pentoxide of energy radiated by the peroxide cannot take place, on the quantum theory, unless the frequencies possessed by the two are exactly equal, since otherwise a gain of energy by the system from the surroundings must occur, because the quanta characteristic of nitrogen peroxide at $\lambda = 6.12\mu$ and 3.43μ are smaller than those characteristic of nitrogen pentoxide at $\lambda = 5.81\mu$ and 3.39μ. In the second place it is well known that at low temperatures the breadth of absorption bands decreases until they become a single absorption line. At low temperatures therefore it becomes necessary to postulate that the photocatalysis cannot occur below a certain temperature. Such a postulate would mean that the reaction at ordinary temperatures is not purely photochemical, but partly photochemical and partly thermal. On the other hand, since both these oxides contain the same atoms they must possess frequencies in the long wave infra-red which are exactly equal, and doubtless the transference of energy takes place at these frequencies." (Baly & B.).

Another instance of the same phenomenon occurs in the decomposition of carbonyl chloride. Weigert[1] has pointed out that carbonyl chloride is photochemically decomposed in glass vessels when chlorine is present. Carbonyl chloride only absorbs in the far ultra-violet, none of which radiation can penetrate the glass, so that the dissociation must depend on the light absorption of the chlorine.

It was determined to attempt to apply the above principle of photocatalysis to the problem of phytosynthesis. It is well known that carbon dioxide and water react to form formaldehyde under the influence of ultra-violet light of very short wave length. It should, however, be possible to catalyse this reaction by the use of a substance containing carbon, hydrogen and oxygen, which can function as a photocatalyst by

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[1] Weigert.-Ann.d.Phys., 24, 55, (1907).

virtue of its power of selectively absorbing some of the visible or ultra-violet light from sunlight. The plant uses carbon dioxide and water and from these substances synthesises within itself all the starches and tissues necessary for its growth. The problem of discovering the mechanism of this synthetic process in the living plant has for many years excited much interest, a large number of experimental results having been published. Up to the present, however, it has not been possible to bring forward any definite experimental proofs that the hypothesis generally accepted is the correct one.

Consequently it was thought that the application of the new ideas developed in the foregoing pages on the part played by radiation in chemical reactions might very probably result in some light being thrown on this problem.

The Conversion of Carbon Dioxide and Water into Formaldehyde and Carbohydrates.

The photochemical changes which result in the conversion of carbon dioxide and water into carbohydrates are not only of the greatest importance in the sustenance of vegetable life, but also to animal life, for the carbohydrates are extremely important animal foodstuffs. Indeed this photochemical change, effected by vegetation, is one on which the continuance of all life on this planet depends more than on any other. It has been the means by which all the usual sources of energy tapped by man have been accumulated, and by which the bulk of his raw materials have become available. In addition to its action in effecting a fixation of some of the energy radiated from the sun, growing vegetable matter is also the means of removal from the air of the carbon dioxide derived from the animal metabolism.

The action of growing plants on impure air in restoring it to its original purity was first discovered by Priestley[1]. Priestley also later identified as oxygen the gas which was evolved from the surface of the leaves of growing plants. Ingenhousz[2], eight years later, showed that the correction of the bad air by vegetation was due directly to the influence of the sun on the plant. This observation was rapidly followed by one by Th.de Saussure[3], noting the simultaneous formation of starch

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[1] Priestley.-"Experiments and Observations".vol.i.p.86.1771.
[2] Ingenhousz.-"Experiments on Vegetables".London,1779.
[3] de Saussure.-"Recherches chimique sur la Végétation".1804.

and by another by Engelmann showing that the presence of chlorophyll was a necessity.

It was only much more recently that problems suggested by this phenomenon were investigated, and of course the first problem presenting itself is that having to do with the nature of the chemical changes involved in the conversion of the carbon dioxide into starch. It can at once be stated that this conversion has not been reproducable in the laboratory. A second outstanding problem is the relation of the kind of light most effective in bringing about assimilation to the kind of light absorbed by the chlorophyll,while a third has to do with the chemical nature of chlorophyll,and by the changes,if any, undergone by it during assimilation. This last problem has been the subject of much study by Willstätter,who deserves special mention from among a number of other workers,and whose researches have given a very clear idea of the character of the chlorophyll.

The second problem was early answered,although occasional papers on the subject still continue to be published, it being shown that assimilation proceeded most rapidly under the influence of the light of the red-yellow region,which is absorbed by the leaf green.

It is the first problem which has shared with the ~~first~~ third the greatest interest of the chemist,and very many experimental results have been published without having led to any definite conclusion. Baeyer[1] was the first to suggest the hypothesis that the first stage in the process was the reduction of the carbon dioxide and water in the plant,formaldehyde and

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[1] Baeyer.-Ber.deut.chem.Ges., 3, 66, (1870).

oxygen being produced. The formaldehyde was then supposed to polymerise, yielding a carbohydrate. The series of reactions suggested by Baeyer can be represented as follows:-

1. $CO_2 \xrightarrow[\text{(in plant)}]{} CO + O$

   $H_2O \longrightarrow H_2 + O$

2. $CO + H_2 \longrightarrow CH_2O$

3. $6\ CH_2O \longrightarrow C_6H_{12}O_6$

There are many seemingly adequate criticisms to the main idea of this theory. In the first place it does not seem definitely to have been established that formaldehyde is actually the first stage in the synthetic process. One of the difficulties met with is the absence of conclusive proof that formaldehyde exists in growing leaves as a definite inter mediate stage. Work published by Kimpflin[1], Usher and Priestley[2], and Curtius and Franzen[3] seems to support the view. These authors claim to have established the presence of formaldehyde in leaves. Usher and Priestley obtained it by subjecting green leaves to steam distillation. Curtius and Franzen considered that the presence of formaldehyde in green plants had not been established with certainty by other workers owing to the fact that the tests used were inconclusive since they were also given by other aldehydes. They claimed to have definitely proved the presence of formaldehyde by working with 1500 kilos of hornbeam leaves, and preparing formic acid. All these results, however, do not demonstrate the presence of formaldehyde in the actual growing leaf. In any case the intermediate product must be of a very

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[1] Kimpflin.-Compt.rend., 144, 148, (1907).

[2] Usher & Priestley.-Proc.Roy,Soc.Lond., 77B, 369, (1906).

[3] Curtius & Franzen.-Ber.deut.chem.Ges. 45, 1715, (1912).

transient character, for the formation of carbohydrate is a very rapid process, oxygen being evolved almost immediately on exposure to light, and the carbohydrate appearing very soon after. In addition, formaldehyde is poisonous to plants, and must, therefore be polymerised before it has time to do any injury to the protoplasm. This is the basis of the criticism of the Baeyer hypothesis by Moore and Webster, to be dealt with later. Several authors have shown that under certain conditions plants can make use of formaldehyde as a food. For instance Bokorny[1] has shown that Spirogyra make starch when supplied with the sodium bisulphite compound of formaldehyde. Treboux[2] has found that Elodia, Sinapis, and certain Algae can form starch when supplied with very dilute formaldehyde solutions. Then again Grafe[3] has recorded the fact that seedlings grown in an atmosphere containing formaldehyde vapour (less than 1.3%) and no carbon dioxide show a greater increase of growth and dry weight than controls grown under the same conditions without the formaldehyde.

It has been observed by Fenton[4] that carbon dioxide is reduced to formaldehyde by magnesium. Usher and Priestley[5] were the first to discover that formaldehyde could be detected afterx aqueous solutions of carbon dioxide had been exposed to ultra-violet light from the quartz mercury lamp. This observation has since been contradicted by Moore and Webster[6].

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[1] Bokorny., Biol. Centrbl. 17, 1, (1897).
[2] Treboux.-"Flora", 92, 49, (1903).
[3] Grafe.-Ber. deut. bot. Ges. 29, 19, (1911).
[4] Fenton.-J.C.S., 91, 687, (1907).
[5] Usher & Priestley.-Proc. Roy. Soc. Lond., 84B, 101, (1911).
[6] Moore & Webster.-Proc. Roy. Soc., 87B, 163, 556, (1914). 90B, 168, (1918).

The latter authors take the view that formaldehyde does not form one of the stages in the photosynthesis of the carbohydrates because, they say, if formaldehyde were formed it would combine at once to form stable complexes with the proteins and this would prevent its polymerisation to form sugars. Now they have obtained formaldehyde by passing carbon dioxide into colloidal solutions of uranium and ferric hydroxides, and into solutions of certain inorganic "catalysts". They have also shown that formaldehyde is polymerised to reducing sugars in ultra-violet light. On the other hand they have found that chlorophyll and all organic substances of biochemical origin give formaldehyde on exposure to ultra-violet light, and thus it would seem that the synthesis by light of biochemical substances from formaldehyde does not take place in the plant. Their results are thus in a sense contradictory, and their evidence does not seem to bring the problem much nearer solution.

As a result of work which will be described forthwith, it is now possible to state that carbohydrates have been synthesised from carbon dioxide and water in light, with formaldehyde as the first stage in the photosynthesis.

In the first place it is well known that carbon dioxide absorbs ultra-violet light of very short wave length ($\lambda = 200\mu\mu$). By the absorption of this light, the carbon dioxide becomes activated, and then undergoes reaction with hydrogen to yield formaldehyde[1] .Loeb[2] has also observed that moist carbon

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[1] Berthelot & Guadechon.-Compt.rend., 150, 1890, (1910).

[2] Loeb.-Zeitsch.für Elektrochem., 11, 749, (1905).

dioxide is converted into formaldehyde under the influence of the silent electric discharge - consisting of very short wave length radiation.

Formaldehyde absorbs ultra-violet light of longer wave length than that absorbed by the dioxide, the head of its ultra-violet absorption band lying near $\lambda = 290\mu\mu$. Since the ultra-violet light which will act on an aqueous solution of carbon dioxide has a much shorterx wave length than that which will act on an aqueous solution of formaldehyde, light of two different wave lengths will be required if the photosynthesis of carbohydrates from carbon dioxide and water is to take place.

The first point to be proved was whether an aqueous solution of carbon dioxide gave formaldehyde on exposure to light of very short wave length. In addition to Loeb's observation (above) Usher and Priestley report having obtained formaldehyde by exposing an aqueous solution of formaldehyde to the light of the quartz mercury lamp. Moore and Webster, however, state that no formaldehyde is formed under these circumstances. The following experiments were accordingly carried out with the intention of settling this point definitely:-

(1). Pure water, previously saturated with carbon dioxide was exposed in a quartz test tube at a distance of 6" from a quartz mercury lamp. The presence of formaldehyde was tested for after 5, 10, and 20 hours exposure by means of Schryver's test[1] with negative results.

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[1] Schryver's modification of Rimini's test for formaldehyde. To 10 c.c. of the liquid to be tested add 2 c.c. of a 1% solution of phenyl hydrazine hydrochloride freshly made up and filtered; then add 1 c.c. of a 5% solution of sodium ferricyanide, or of a 6% solution of potassium ferricyanide, also freshly made up, and 5 c.c. of hydrochloric acid.

(2). Pure water, through which was constantly bubbling a slow stream of carbon dioxide from a cylinder, was again exposed in a quartz test tube at a distance of 6" from the lamp. After six hours' exposure, very definite evidence of the presence of formaldehyde was obtained.

Thus it is quite true that if water saturated with carbon dioxide is exposed in quartz vessels to ultra-violet light, no formaldehyde is to be found in the solution. If, however, during the exposure, carbon dioxide is passed through the water, formaldehyde can readily be detected. Thus we have an explanation of Moore and Websters failure to obtain formaldehyde - their solution of carbon dioxide was not agitated, any formaldehyde produced being destroyed as fast as it was formed. When the solution is agitated, some of the formaldehyde is carried to the back of the vessel where it is more protected from the light. The small amount which escapes polymerisation in this way is thus readily detected by means of Schryver's test. In addition to the above two experiments, which, of course have been fully confirmed, experiments similar to (2) in every way, except that glass vessels were used, were carried out. It was found that, carried out in this way, no formaldehyde was formed. This shows that the effective light has a very short wave length.

The action of ultra-violet light on aqueous solutions of formaldehyde has also been investigated. The following are some of the experiments performed:-

(3). A solution of formaldehyde, originally giving a very good

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Schryver's test. (from p.57).
A brilliant magenta colour is produced if formaldehyde is present. Sensitivity: 1 part in 1,000,000.

pink colouration with Schryver's reagent, was exposed for 4 hours at a distance of 6" from the lamp. On testing at the close of the exposure, no trace of formaldehyde could be found.

(4). A similar experiment was set up, the test in this case being carried out after 1 hour had elapsed. Even by that time a very marked diminution in concentration was shown.

(5). A similar experiment was set up again, except that a thin glass test tube was used as a containing vessel in this case. A test made at the end of 6 hours showed a slight diminution in the concentration of the formaldehyde, while after 11 hours this had fallen still further.

(6). A stronger solution of formaldehyde was exposed in a quartz test tube to the light. After 5 hours' exposure a portion of the solution was tested with Benedict's solution, which is reduced by certain carbohydrates, but not by formaldehyde. This test showed the presence of reducing material.

These experiments show that polymerisation to reducing sugars readily takes place. The result of experiment 6 had been previously obtained by Moore and Webster, and is a confirmation of that part of their work. Experiment 5 shows that the effective light has a greater wave length than that required for the synthesis of formaldehyde from carbon dioxide and water. Thus if an aqueous solution of carbon dioxide be exposed to ultra-violet light, and protected from those particular rays which cause polymerisation of the formaldehyde, the amount of this found in the solution should be increased. It is no difficult matter to find a substance which is capable of selectively absorbing these rays, while being transparent to the rays of shorter wave length which are responsible for the synthesis of formaldehyde. After numerous experiments, paraldehyde was the substance chosen to screen the formaldehyde from those rays which would cause its disappearance. Paraldehyde does not interfere in any chemical way with the reaction under study, so that it could be added to the water through which the carbon dioxide was being passed. The following experiments were performed:-

(7). 50 c.c. of a 1% solution of paraldehyde were mixed with 10 c.c. of water and exposed to the light in a quartz test tube. After 5½ hours' exposure, 25 c.c. of the solution were distilled, the first fraction of 5 c.c. being tested for formaldehyde. None was to be found.

(8). The same experiment was carried out in a manner exactly similar, the only difference being that a slow stream of carbon dioxide was passed through the solution during the exposure. In this case the test showed that formaldehyde was present to the extent of approximately 1 part in 50,000.

(9). 50 c.c. of a 1% solution of paraldehyde were mixed with 10 c.c. of a dilute solution of formaldehyde, and the mixture exposed under the same conditions and for the same time as the above. The result of the formaldehyde test was compared with that of a blank that had been prepared similarly, but which had not been exposed. The comparison showed that the concentration of the formaldehyde was slightly, but only slightly less in the case of the solution which had been exposed to the light.

That is, a solution of paraldehyde in water free from carbon dioxide gives no formaldehyde on exposure to light, a solution of formaldehyde containing paraldehyde is quite stable to light, and a good yield of formaldehyde is obtained on exposing to the light a solution of paraldehyde through which a stream of carbon dioxide is passing. In this case therefore, the paraldehyde does not act as a catalyst in the synthesis of formaldehyde but as a protector for the formaldehyde when this has been formed. Similar results are obtained when sodium phenate is used instead of paraldehyde. In the absence of carbon dioxide no formaldehyde is formed, a solution of formaldehyde is not polymerised in the presence of sodium phenate, but a solution of the latter in the presence of carbon dioxide gives a good yield of formaldehyde on exposure to ultra-violet light.

Exactly the same explanation applies to the case of the metallic salts which Moore and Webster considered as catalysts. These do not catalyse the production of formaldehyde but merely protect it when formed from the action of those rays which would cause its disappearance. Moore and Webster claim to have obtained the best results with solutions of uranium nitrate, ferric chloride, and beryllium chloride, while solutions of cupric chloride, manganous chloride and potassium chromate also give distinctly positive results. On solutions of these Moore and Webster "catalysts", three kinds of observations have been made, viz., (a) on the relative amounts of formaldehyde produced on exposure to light, (b), on the stability to light of solutions of formaldehyde containing these "catalysts", and (c), spectroscopic measurements on the light absorption of solutions of the salts. These measurements have established the fact that each catalyst, so-called, selectively absorbs those rays which polymerise formaldehyde. In addition, it was found that while good yields of formaldehyde were obtained when the experiments were carried out in quartz test tubes, none whatever was obtained when a screen of plate glass was interposed between the tubes and the light. This screen removes the very short wave length light which is the cause of the formation of the formaldehyde. Thus it would seem that Moore and Webster have misunderstood the function of their "catalysts", and in a way this is not surprising since they did not observe the formation of the formaldehyde when they were absent. Consequently their ideas as regards the non-participation of formaldehyde in the photosynthetic process seem to need alteration. Moore has propounded the theory that the process depends on the presence of iron, and has proved the presence of iron in the chloroplast. It is evident however, that so far from being a catalyst, the iron is really a sort of anti-catalyst, for in the first place it does not catalyse the formation of formaldehyde, and in the second it prevents the formaldehyde from polymerising to carbohydrates. Two other catalysts were used by Moore and Webster, colloidal uranium hydroxide and colloidal ferric hydroxide, fall into a different category. The explanation of the role played by these substances will be discussed later.

The second stage in the photosynthetic process is the polymerisation of formaldehyde to yield carbohydrates. Reactions by means of which this polymerisation can occur are well known. For instance under the influence of dilute hydrochloric acid or of zinc chloride, acetaldehyde polymerises and yields a body known as Aldol.

$$CH_3CHO + CH_3CHO = CH_3CHOH.CH_2.CHO$$

Formaldehyde acts in an analogous manner under the influence of dilute alkalies. In this way two molecules of formaldehyde yield glycollic aldehyde, according to the equation

$$CH_2O + HCHO = CH_2OH.CHO ,$$

while three molecules may combine yielding glyceric aldehyde.

$$CH_2OH + HCHO + HCHO = CH_2OH.CHOH.CHO$$

The first important synthesis of carbohydrates from formaldehyde was due to Loew[1]. A 4% solution of formaldehyde wsa shaken up with lime water for half an hour, filtered and allowed to stand for some days, until the smell of formaldehyde had disappeared. In this way was obtained a crude mixture of sugars as a sweet syrup having the reducing properties of sugars, but which was unfermentable and optically inactive. From this "formose" true reducing sugars have been separated[2]. Euler[3] later produced Arabinoketose by heating a 2% solution of formaldehyde with calcium carbonate for some time. In smaller quantities glycollic aldehyde and dihydroxyacetone were also found in the reaction mixture.

Moore and Webster (loc.cit.) were successful in obtaining reducing carbohydrates from solutions containing about 5% formaldehyde by the exposure of these to ultra-violet light. These authors found that the formaldehyde solutions acquired the power of reducing a solution of copper sulphate in the presence of sodium citrate and sodium carbonate (Benedict's sol-

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[1] Loew.-Ber., 20, 142, 3039, (1887). 21, 270, (1888). 22, 470, 478, (1889).
[2] Fischer & Tafel.-Ber., 20, 1093, 2566, 3384, (1887).
[3] Euler.-Ber., 39, 36, 39, (1906).

ution) on exposure to light, which power is not possessed by formaldehyde. They showed that this photocondensation did not require the presence of an inorganic "activating agent" such as they had found necessary in the synthesis of formaldehyde, and that it proceeded much more rapidly in quartz vessels than in glass. They also stated that the rate of disappearance of the formaldehyde was greatest when the concentration of this in the solution was about 5%, and was still further increased by the presence of a little sodium carbonate. They attempted to isolate as an osazone and identify the sugar produced, but these attempts were unsuccessful.

I, also, have attempted to determine what sugars are produced as a result of this photocondensation of formaldehyde, with almost as little success up to the present as that which attended the efforts of Moore and Webster. They found, it will be remembered, that the rate of condensation of the formaldehyde was increased if sodium carbonate was present. It was found better, however, to use stronger solutions of formaldehyde containing potassium bicarbonate, made up by mixing equal volumes of formalin and a cold saturated solution of potassium bicarbonate. Quartz test tubes containing this solution were exposed to the light of the quartz-mercury lamp for times varying between 24 and 48 hours. The contents of these test tubes were then transferred to a basin, very carefully neutralised with dilute sulphuric acid, and evaporated down to a small bulk. In this way most of the unchanged formaldehyde was allowed to escape, the rest being destroyed by making slightly alkaline and then carefully adding hydrogen peroxide. Evaporation to a small volume was once more carried out, the residue made up to a known volume and the reducing matter estimated with Pavey's solution. In this way it was found that about 0.5 gram of reducing material had been obtained, the original volume of the contents of the quartz test tubes having been about 500 c.c.. The remainder of the solution not used in the estimation was again evaporated down and an attempt made to prepare an osazone by treating the syrup with phenyl hydrazine and acetic acid. As a result a few oily crystals were obtained, which were, however, too small in bulk and too impure for anything to be done with them. Further progress of this investigation will be reported in part II.

In addition to noticing the polymerisation of formaldehyde solutions under the influence of ultra-violet light, Moore and Webster have also noted the fact that solutions or emulsions of substances of biochemical origin such as mono-, di-, and polysaccharides, glycerol, chlorophyll, egg albumen, and vegetable juices yield formaldehyde on exposure to ultra-violet light for three or four hours. This would seem to contradict the view that these substances can be built up by the plant from formaldehyde, but an addition which has been made to their observations on these decompositions throws a different light on the subject. Experiments in confirmation of their results have been carried out, and these have established the correctness of their work. A very important fact, which Moore and Webster failed to observe, however, is that reducing carbohydrates are produced at the same time.

(10). A 1% solution of pure water, free from carbon dioxide and oxygen was prepared. A test showed the presence of formaldehyde after 3 hours' exposure. An additional hour's exposure increased largely the quantity of formaldehyde.

(11). A 1% solution of ferric chloride in CO_2-free water contain- 0.8% pure glucose was made and exposed for three hours in a quartz test tube. 25 c.c. were then withdrawn, distilled, and the first fraction of 5 c.c. tested in the usual way with Schryver's reagent. A good quantity of formaldehyde was shown - much more than was obtained from a control consisting of a 0.8% solution of glucose without the ferric chloride, which had been exposed simultaneously and treated in a similar manner.

In this experiment the ferric chloride had protected the formaldehyde from those rays which cause its polymerisation, with the result that it was present in larger quantity.

(12). A 5% solution of glycerol in pure water free from carbon dioxide was exposed for three hours in a quartz test tube to the light of the quartz mercury lamp. A Schryver test at the end of this time showed that there was present about 1 part of formaldehyde in 10,000. A portion of the solution was also tested with Benedict's solution, a marked reduction of which was obtained. In fact, after three hours' exposure the total amount of sugar formed was found to be equivalent in reducing power to about 0.04 gm. of glucose.

It was found also that substances of other than biochemical origin often behaved in a similar manner. This the following experiment performed on acetone, amongst others, will show:-

(13). A 1% solution of acetone in boiled water was exposed for six hours, when a concentration of formaldehyde of 2 parts or thereabouts in 10,000 was found. On treatment with

Benedict's solution, a very heavy precipitate of cuprous oxide showed that there had been present a considerable quantity of sugar in the solution.

Now it is well known that acetone is photochemically decomposed, not into formaldehyde, but into formic acid, carbon dioxide, methane, acetic acid etc., the particular products obtained depending on the amount of dissolved oxygen present in the solution. In the absence of oxygen, carbon dioxide and methane are the products. It seems therefore, quite obvious that the formaldehyde has been derived from carbon dioxide by combination with water under the influence of the light,-i.e., it has been photosynthesised. One at once begins to wonder whether this is not the explanation of the production of formaldehyde from chlorophyll, carbohydrates etc., on exposure to ultra-violet light. They very probably decompose, yielding as a product carbon dioxide, and it is this which is responsible for the presence of formaldehyde.

The following reactions have been discussed:-

(a). Carbon dioxide + Water → Formaldehyde.

(b). Formaldehyde → Sugars.

(c). Sugars → carbon dioxide.

These reactions are known to take place in ultra-violet light, and it is almost certain that an equilibrium is set up between sugars, formaldehyde and carbon dioxide, this equilibrium being represented by

$$\begin{tikzcd}
\text{Sugar.} \arrow[rr] & & \text{Carbon Dioxide.} \arrow[dl] \\
& \text{Formaldehyde.} \arrow[ul] &
\end{tikzcd}$$

There is a very considerable amount of experimental support for the existence of this equilibrium, one very noteworthy point being the fact that the formation of formaldehyde is enhanced very considerably if the concentration of the carbon dioxide in the solution is increased by the addition of sodium carbonate(Moore and Webster) or of potassium bicarbonate.

Then again it has been noticed that in no experiment which has been performed has the concentration of formaldehyde risen above a magnitude of about 1 part in 5,000. For instance, if a solution of glycerol is exposed to the light from the quartz mercury lamp, the concentration of formaldehyde increases from zero, eventually becoming more or less constant at between 1 and 2 parts in 10,000. As far as could be seen, this concentratio of formaldehyde is never exceeded.

Another significant fact regarding the equilibrium under consideration is that the difficulty of obtaining a recognisable amount of sugar becomes much greater if more dilute solutions of formaldehyde are used, this in spite of the fact that there is still present in the solution much more than enough formaldehyde to give sufficient sugar for its reducing effect to be observed.

It can thus be taken as true that an equilibrium is established between carbon dioxide, formaldehyde and carbohydrate, but it must be pointed out that the reactions constituting this equilibrium have only been proved to take place in ultra-violet light. In such light the equilibrium lies very far over to the side of the carbon dioxide, the equilibrium concentration of the formaldehyde apparently being of the order of 1/10,000 parts.

It has been shown then, that the synthesis of carbohydrates from carbon dioxide and water is possible, and that it occurs in two stages. It should be possible to accomplish this directly, however, without any intermediate use of formaldehyde. In trying to effect this direct synthesis, some difficulty was encountered owing to the postition of the equilibrium. A measurable quantity is unobtainable directly unless the concentration of the carbon dioxide is much bigger than it is in aqueous solution at atmospheric pressures. The following experiments were carried out:-

(14). Pure water, through which a stream of carbon dioxide was passing was exposed for four hours. At the end of that time no sugar could be detected.

(15). A clear, saturated solution of magnesium bicarbonate was exposed to the light for four hours, a current of carbon dioxide being passed through the solution meanwhile. At the end of the exposure no traces of sugar were detectable by Benedict's solution.

(16). A similar experiment was set up, except that calcium bicarbonate was used. Again no sugar was detected.

(17). A solution of potassium bicarbonate was exposed in a similar manner for six hours. A strong reaction for formaldehyde was obtained with Schryver's reagent. A sugar test showed the presence of a very small quantity of carbohydrate. A small amount of formalin was then added to the remainder and the exposure continued for another two hours. At the end of this period a test with Benedict's solution showed the presence of a relatively large amount of sugar.

(18). A saturated aqueous solution of oxalic acid was exposed for 40 hours to the light. This solution was then carefully neutralised, evaporated to small bulk and tested with Benedict's solution, when a strong positive reaction for a reducing sugar was obtained.

Reference may also be made to the result of Experiment 13, in which reducing carbohydrates were obtained from acetone on exposure to light (via carbon dioxide and formaldehyde). Oxalic acid behaves in a similar manner to acetone when exposed to ultra-violet light. It decomposes into carbon dioxide, and this in turn gives formaldehyde and sugar. The greater ease with which carbohydrate is produced from carbon dioxide in the case of acetone and carbon acid might very possibly be due to the fact that the carbon dioxide has just been produced, and is in a reactive state. With acetone or oxalic acid present, the position of the equilibrium is thus different. This might be due to a shielding by these of the sugar produced from destroying radiation. This point has not been experimentally verified.

Now in the plant, carbon dioxide and water are photochemically converted into carbohydrates, but between this process and that which has been described above there is a great deal of difference. The plant makes use of ordinary light of the visible region for the syhthesis, assimilating at a maximum rate when the light supplied has a wave length of about $600\mu\mu$. It has not been found possible to obtain any formaldehyde at all from carbon dioxide and water under the influence of light which has been transmitted through glass, i.e., light of wave lengths longer than $\lambda = 350\mu\mu$. Again in the case of plants, it is common knowledge that the presence of certain substances, of which chlorophyll is the best known, is necessary before starch formation can take place. Might not the long-sought-for solution of the problem of phytosynthesis lie in photocatalysis ? It is quite possible that one of the functions of the chlorophyll might be to act as the transformer of the energy absorbed in the visible region, i.e., to photocatalyse both stages of the condensation to carbohydrates. It can at once be stated that reducing sugars have been synthesised directly from carbon dioxide and water in two stages by using suitable photocatalysts and light which has been passed through a screen of plate glass over ½" in thickness. That is, the synthesis has been effected in light of wave length longer than $\lambda = 350\mu\mu$, such as is used by the plant.

(a). The synthesis of formaldehyde from carbon dioxide and water in light of longer wave length than $\lambda = 350\mu\mu$.

Suitable caralysts for this reaction were found in coloured organic basic compounds such as malachite green, methyl orange, p-nitrosodimethylaniline, etc.. In this way both of the conditions for a photocatalyst were fulfilled, the substances absorbingl light in the visible region, and allowing of the existence of a suitable "exchange" frequency in the infra-red. (19). A solution of methyl orange in pure water, free from carbon dioxide was placed in each of two test tubes, and through the solution in one a stream of carbon dioxide was passed during exposure. A thick plate of glass was interposed between the mercury lamp and these test tubes. After five hours of exposure to the light, each solution was distilled, and the test for formaldehyde applied to the first 5 c.c. of each distillate. No formaldehyde was found in the case of the blank, but it was found to be present to the extent of about 1 part in 200,000 in the sample from the tube through which the carbon dioxide had been passed.

(20). A solution of p-nitrosodimethylaniline in CO_2-free water was placed in each of two test tubes, and exposed for 6½ hours behind the plate glass screen. Carbon dioxide was passed through the solution in one of the test tubes, the other test tube being kept well corked. After exposure the solutions were acidified and distilled. While there was again no formaldehyde in the solution from the test tube that had been kept corked, 1 part in 100,000 was found in the other.

(21). Experiments performed on malachite green in exactly the same manner gave similar results - the concentration in this case being of the order 1/200,000.

The increased yields of formaldehyde which Moore and Webster obtained when colloidal ferric hydroxide or colloidal uranium hydroxide were present probably owe their occurrence to a similar, and truly photocatalytic reaction. In all probability, when these colloidal hydroxides are present, there is some formation of a loose compound of these with the carbon dioxide. This would fulfil the condition for a photocatalyst, as there would be provided a mechanism for the necessary interchange of radiation. It would seem, therefore, that these were the only true photocatalysts that were used by Moore and Webster. Their contention as to the absolute necessity of an inorganic catalyst falls to the ground, for as is shown above, coloured organic bases act in quite as efficient a manner, quantities of formaldehyde every bit as big being obtained by using solutions of these as are obtained by using the majority of the Moore and Webster "catalysts".

(b).The synthesis of carbohydrates from formaldehyde in the presence of light of wave length longer than $\lambda = 350 \mu\mu$.

In this, the second stage, a coloured substance which is able to form a complex with formaldehyde must be used as a photocatalyst. Such a substance has not been easy to obtain, but an experiment which leaves no doubt of the photocatalysis of this reaction is described following:-

(22).An aqueous solution, containing 17% of sodium citrate and 9% of sodium carbonate was exposed in a quartz test tube to the light of the quartz mercury lamp for two hours. At the end of that period formaldehyde was easily detectable by means of Schryver's test, while clear evidence of the presence of reducing material was obtained by adding an equal volume of a 2% solution of copper sulphate and warming.

A similar experiment was set up and performed in an analogous manner, a thick plate glass screen being placed between the test tube and the lamp, however. In this case neither formaldehyde nor reducing sugars were detected.

Benedict's solution, containing 1% of copper sulphate, 17% of sodium citrate, and 9% of sodium carbonate, was also exposed for two hours behind the screen. At the end of that time the solution was warmed, very marked reduction of the copper being obtained, much more than was obtained in the experiment in which the sodium citrate and sodium carbonate solution was exposed to ultra-violet light, unscreened, for a similar time.

This experiment is considered definitely to establish the photocatalysis, for the formation of formaldehyde and its polymerisation take place within the citrate complex, which has the power of absorbing in the visible region (owing to the presence of the copper). The energy so absorbed is sufficient to cause the polymerisation to take place.

Thus it appears that the two stages in the synthesis of a reducing carbohydrate from carbon dioxide have been carried out photochemically in the laboratory both in ultra-violet light, and, in the presence of a suitable photocatalyst, in ordinary light of the visible region of the spectrum. A photocatalyst which is capable of photocatalysing both stages simultaneously has not as yet been found. If such a catalyst were to be used the separate existence of formaldehyde would not be capable of definite proof, since presumably both formaldehyde and sugar would be formed within the same complex. None of the photocatalysts which have been found effective in catalysing the first stage are capable of forming complexes with formaldehyde, the presence of this latter thus being observable. In the case of the synthesis carried out in the plant, it would would seem that the chlorophyll is ideal as a photocatalyst. By virtue of its structure it seems to be capable of adding on carbon dioxide to form a complex, and also of retaining as another complex the formaldehyde which is formed, the synthesis thush passing through both stages without any intermediate formation of free formaldehyde. In this probably lies the cause of the lack of definite proof of the presence of free formaldehyde in the growing leaf.

Part II. October, 1921 - April 1922.

As a result of the work described in the first part of this thesis it was determined to proceed to investigations with chlorophyll and its companion pigments, and to attempt to apply the principle arrived at to the actual assimilation process as occurring in the plant. The closer acquaintance with the literature of, and the facts connected with, the phenomena of phytosynthesis has resulted in a certain modification of ideas.

This second part has been subdivided into three sections. The first contains an account of the experimental work which has been performed in continuation of that already described relating to the two stages of carbohydrate photosynthesis.

The second section contains a historical and descriptive summary of the investigations of the pigments occurring in the chloroplasts, an account of their chemical nature in so far as it is known, and descriptions of methods for their isolation and estimation.

In the last subsection an attempt is made to indicate the opinions which have been arrived at concerning the functions of the chloroplast pigments. The section is concluded by a short account of the present state of experimental work on the subject.

A Continuation of the Work on the Photosynthesis of Carbohydrates.

Preliminary efforts to determine the nature of the sugars produced by the photo-polymerisation of formaldehyde solutions were described on pp.64-65. These efforts have been continued, but although much work has been done in this respect it is not yet possible to state definitely what sugars are synthesised. This object, however, will most probably be attained after but little more experimental work.

It will be remembered that it was found better, in order to obtain a reasonable amount of reducing matter in a reasonable time, to use fairly strong solutions of formaldehyde containing potassium bicarbonate. Experiment has shown that an optimum effect is obtained by using a solution made by mixing equal volumes of formalin and of a cold saturated solution of potassium bicarbonate. This mixture was exposed in quartz test tubes set up in a semicircular stand at equal distances from a quartz mercury lamp. The following table shows the amount of reducing matter produced in such a solution by a given length of exposure, the lamp being at a distance of about 3" from the tubes containing the solution.

24 hours'exposure	0.096 gm.Cu_2O	0.0162 gm.Glucose Equiv.
54	0.153 gm.	0.024 gm.
80	0.226 gm.	0.936 gm.
126	0.444 gm.	0.070 gm.

The reducing matter obtained by allowing such a mixture of formalin and potassium bicarbonate to be exposed to ultra-violet light was isolated after some trouble as a syrup, free from formaldehyde and from all but a trace of mineral matter. By careful preparation this syrup was obtained of a very light yellow colour, and of a viscosity exceeding that of treacle.

Method for the preparation of a syrup from an exposed solution.

The alkaline liquid is neutralised extremely carefully with 4N sulphuric acid, being slightly warmed at the same time so as to expel carbon dioxide, of which large quantities are given off. The final additions necessary to make the solution exactly neutral are made of more dilute acid. The neutral liquid, strongly smelling of formaldehyde, is concentrated to as small a bulk as possible in a Claisen flask under diminished pressure, the temperature of the liquid not being allowed to exceed 30°. The concentrated syrupy residue is tested so as to make perfectly sure of its neutrality. A quantity of water is added and again removed in vacuo, this procedure being repeated many times, until all smell of formaldehyde has disappeared. Alcohol is now added, the syrup dissolved, and the solvent removed in vacuo. After several such evaporations with absolute alcohol most of the water will have been removed. The syrup is then dissolved in a large volume of dry alcohol, and allowed to stand for about 12 hours. At the end of this period a large quantity of potassium sulphate will have settled out. The alcoholic sugar solution is decanted off, the alcohol removed under reduced pressure, after which the syrup is taken up with fresh alcohol and the process repeated. If any difficulty should be experienced in causing the separation of the potassium sulphate, a few drops of acid will probably effect a rapid precipitation of this.

In this way there is obtained a pale yellow syrup, containing but small quantities of potassium sulphate. The syrup has a slightly acid taste which is apt to mask its sweetness.

This method can be shortened by making some sacrifice of the quality of the syrup. The formaldehyde is driven off from the completely neutral liquid by repeated evaporation in the air with water. The solution resulting is much darker, and needs decolourising with animal charcoal. The bulk of the salt is removed by a twice repeated treatment of the syrup with an excess of dry alcohol.

When a specimen of the syrup prepared in this way was placed over phosphorus pentoxide in a vacuum exsiccator, it commenced to crystallise after several days. The crystals which separated were neither salt nor sugar. The whole mixture was poured on to a porous plate, which took up the syrup, and left behind a white powder, very easily soluble in water. It is intended to proceed to the investigation of the nature of this body at an early date. The syrup can be extracted from the porous pot by repeatedly boiling up the latter with distilled water.

Syrups were also prepared in a similar manner by exposing formalin solutions to the light of the quartz mercury lamp, and removing excess of formaldehyde in the manner described above. The syrups so obtained seemed to be identical in every way with those prepared by the photopolymerisation of formaldehyde solutions in the presence of potassium bicarbonate. They both showed precisely similar reactions. Mention must, however, be made of an observation lately made in connection with the preparation of these "plain formaldehyde polymerisation syrups". When the excess formaldehyde was removed by continued evaporation with water in an open dish, a dark brown solution was obtained. After this had been decolourised, and taken down in vacuo, a viscous residue was left in the flask containing some solid matter in suspension. On treatment with alcohol the solution contained the synthesised sugar, while a white solid remained. This was filtered off and washed. A beautiful specimen of syrup was obtained on working up the alcoholic solution.

It is intended to prepare this white solid in larger quantity and to investigate its nature more closely. From a few preliminary experiments with the small quantity in hand, the body seemed to be very similar to a cellulose. In appearance it was an amorphous white powder, very like starch. It did not melt up to 350°C., and contained no mineral matter beyond a slight trace of sodium. It was quite insoluble in water, alcohol, ether, and dilute caustic soda. It appeared to give a trace of formaldehyde on prolonged boiling with water, but this might have been due to the presence of a little trioxymethylene as impurity. That the body was certainly not an ordinary polymer of formaldehyde is shown by its insolubility in soda. It was soluble in strong acids, but was thrown out on dilution of these with water, or on neutralisation. Attempts were made to hydrolyse it by prolonged boiling with about 40% sulphuric acid, and later by heating to

120° with acid in an autoclave. No sugars were detectable in the solution in either case, but the quantity available for the attempt at hydrolysis in the autoclave was so small that little confidence is felt in the result.

A control portion of the same formalin as was used for the exposure was treated in a precisely similar way, without, of course, having been exposed. Neither syrup nor solid was obtained, so that both of these result from the exposure of the formalin to ultra-violet light.

The nature of the carbohydrates resulting from the polymerisation of formaldehyde under specific conditions has been the subject of investigation at various times from 1861 up till the present. In 1861 Butlerow[1] commenced the history of the synthesis of sugars when he observed that by the addition of lime water to a hot solution of trioxymethylene, a yellow, sweet syrup was formed, which although optically inactive and incapable of fermentation, gave the general sugar reactions. Butlerow called his syrup "Methylenitan". In 1886 Loew[2] announced the discovery of the fact that a sweet syrup was obtainable when formalin was allowed to stand with lime water at ordinary temperatures. This syrup "formose" had the formula of a hexose $C_6H_{12}O_6$. The literature on formose is given below[3]. Loew failed

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[1] Butlerow.-Ann., 120, 295, (1861).

[2] Loew.-J.prakt.Chem., (2)33, 321, (1886).

[3] Formose.- Loew.-J.prakt.Chem., 33, 329; 37, 203; Ber., 20, 142, 3039;
Ber., 21, 270; 22, 470, 478.
Seyewetz & Gibello.-Compt.rend., 138, 152.
Lobry de Bruyn & Van Ekenstein.-Rec.trav.chim. 18, 309.

Chem.Ztg., 21, 242.

to get any acid with six carbon atoms from it on oxidation, whilst no known hexitol was found on reduction with sodium amalgam. The syrup was not fermentable by yeast but gave the α-Naphthol reaction, Resorcinol reaction, Phloroglucinol reaction, and the Orcinol reaction. The principal osazone obtained from a mixture melted at 144°. Loew considered his product as containing a ketose with a branched carbon chain. By a modification of his method of preparing his product Loew later obtained a syrup - methose - which was fermentable.

The work of Fischer and Tafel[1] on the acroses leads to the idea that these sugars probably formed part of Butlerow's and Loew's syrups. Fischer's work has shown the identity of α- and β-acroses, obtained by polymerisation of glycerose, with the inactive ketohexoses dl-fructose and dl-sorbose respectively.

In 1913 a paper was published by Stocklasa, Sebor and Zdobnicky[2] in which the authors claim to have synthesised sugars from carbon dioxide in the presence of nascent hydrogen and potassium bicarbonate under the influence of radium emanation. They obtained a reducing solution and osazones which analysed out as hexosazones. In addition they found pentoses to be present, and the syrup to be optically active, having a specific rotatory power of $[\alpha]_D = 17° 58'$. This last observation seems to be indeed remarkable, as it is difficult to imagine assymetric synthesis under the conditions described. In view of this statement, very careful polarimetric measurements were carried out on the syrups whose preparation by photo-polymerisation of formaldehyde solutions has been described. No traces of optical activity could be detected although exceptionally long polarimeter tubes were used.

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[1] Fischer & Tafel.-Ber., 20, 1093, 2566, (1887).

[2] Stocklasa, Sebor & Zdobnicky.-Compt.rend., 156, 646, (1913).

Ewart[1] has ascribed certain properties to syrups which he has obtained by the polymerisation of formaldehyde solutions under the influence of various alkalies. These syrups according to him are optically active, and contain reducing pentoses and reducing fermentable hexoses. A subsequent paper corrects his statements as regard the optical activity of the syrups.

From what has already been published it thus appears that the sugars obtained by polymerisation of formaldehyde under the influence of alkali are pentoses and hexoses, the latter possibly consisting of the acroses. The tests which have been carried out on the syrups obtained by photopolymerisation of formaldehyde, both in the presence and absence of potassium bicarbonate have shown that these contain no pentose at all, the sugars being exclusively hexoses, apparently ketohexoses predominantly.

This result adds to the conviction that there is being reproduced in vitro what occurs in the plant as a result of photocatalysis. The first observable products in the photofixation of carbon within the plant are hexose sugars - either fructose or glucose. Pentoses are not formed as direct products of assimilation, as G.de Chalmot[2] has shown by experiment. The theoretical speculations to which the nature of this syrup gives rise are outlined very briefly in a recent paper by Heilbron[3]. Some further reference is made to these in the third section of this part.

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[1] Ewart.-Proc.Roy.Soc.Vict.,30(N.S.),178,(1918).
ibid.31(N.S.)379,(1919).

[2] G.de Chalmot.-J.Am.Chem.Soc.,15,618,(1893).

[3] Heilbron.-J.Soc.Chem.Ind.,41,89R,(1922).

Investigation of the nature of the carbohydrates present in the syrups isolated.

(1). On warming the syrups they begin to decompose and char, yielding at the same time the very characteristic odour of burnt sugar.

(2). Sulphuric acid causes a charring to occur, carbon being deposited.

(3). A furfural odour is detectable in the distillate from a solution which has been refluxed with hydrochloric acid.

(4). Tollen's test for pentose sugars is not shown by the syrups. This test is carried out as follows:- The reagent for pentoses is prepared by making a 3 c.c. of a 2% solution of phloroglucinol in alcohol, with 15 c.c. of concentrated hydrochloric acid up to 27 c.c. with distilled water. To 3 c.c. of this reagent three drops of sugar solution are added, and the mixture warmed for a few moments. In the presence of pentoses the solution acquires a cherry red colour, transferrable to amyl alcohol after shaking. On testing the syrups in this way, no colouration was formed, a whitish brown predipitate being thrown down. Amyl alcohol failed to extract any colour from the solution.

(5). Tollen's Orcinol test for pentoses is not shown. Pentoses, when mixed with orcinol and strong hydrochloric acid, give first of all a violet, followed by blue, red, and finally green colours, a bluish green solution being obtained.

(6). The absence of starches or glycogen is shown by the failure of an acid solution of the syrup to give any colouration with iodine.

(7).The reduction of Fehling's solution which is effected on warming shows the presence of any monosaccharide or of lactose,maltose,or dextrin.

(8).The presence of monosaccharides is indicated by the fact that Barfoed's solution is reduced within a minute of boiling. Barfoed's solution is a solution of cupric acetate in very dilute acetic acid.

(9).The presence of a ketose is indicated,the syrup reacting positively to Seliwanoff's test for ketoses. The test is carried out as follows:- To concentrated hydrochloric acid diluted with an equal volume of water, a crystal of resorcinol is added,followed by a few drops of the sugar solution. A red colour and a red precipitate is obtained on boiling if the sugar solution contains a ketose. The precipitate should be soluble in alcohol.

(10).An acetyl derivative is obtained on refluxing the syrup with acetic anhydride and zinc chloride. Charring takes place very rapidly. After about 8 hours refluxing,a blackish brown solid is obtained on pouring the reaftion mixture into cold water.

(11).An orange crystalline mixture of osazones is obtained on treatment according to the following directions:-
About 2 gms. of syrup are dissolved in 20 - 30 c.c. of water, and the solution warmed on the water bath. About 4 - 5 gms. of freshly distilled phenyl hydrazine,dissolved in an equal volume of glacial acetic acid are then added. The turbidity which occurs is cleared up by the addition of no more than just sufficient alcohol. The mixture is heated on the water bath for 1 hour,and then set aside to cool. The clear,dark

orange-red solution becomes turbid as it cools, but scratching and standing result in the separation of a solid, varying from dark orange to light brown in colour, of a nicely crystalline character.

The melting point of the mixture of osazones depends upon the method of its preparation, and upon the crystallisation processes to which it is subjected. The original crystals commence to melt at about 120° - 125°, but it has been found possible by recrystallisation, to obtain a fraction melting at about 190°. Even then the melting point was not sharp. Attempts were made to differentiate between aldose and ketose in the syrups by means of complex osazones (such as methyl phenylosazone, p-nitrophenyl osazone, As. benzyl phenyl osazone, etc.). These attempts failed, however, to achieve any definite result.

(12). It was finally determined to apply the method of attack which Irvine has found so useful in his work on the structure of the starches and celluloses, viz., methylation, followed a fractional distillation in vacuo of the mixture of methyl glucosides so obtained. Experimental work on this line is not yet complete, only a small quantity of syrup having been methylated up to date. Although this was too small in bulk to allow of fractionation, it was distilled at a pressure of 0.1 mm.. The distillate was a perfectly colourless oil, which reduced Benedict's solution after hydrolysis with acid. The details for methylation, according to the method of Haworth[1] follow. It is intended to

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[1] Haworth.-J.C.S., 107, 8, (1915).

fractionate the methyl glucosides in the high vacuum, and to analyse the fractions. Thanks must here be accorded to Principal Irvine of St.Andrews for helpful advice as to the best methods of proceedure, which he freely gave during a recent visit to Liverpool.

Methylation of Syrup.

The syrup, which may contain both aldose and ketose sugars, is dissolved in methyl alcohol, which must be free from acetone, and which contains 0.5% of dry HCl. The solution is allowed to stand at room temperature for 24 hours, subsequently being neutralised with silver carbonate. It is filtered and the solvent removed in vacuo. The syrup is then methylated according to the method of Haworth by means of methyl sulphate and alkali.

The apparatus designed for the process of alkylation with methyl sulphate and sodium hydroxide consists in a wide necked flask fitted with a cork and provided with two dropping funnels, a water condenser, and an efficient arrangement for stirring mechanically. The syrup is dissolved in the minimum amount of water, and the flask surrounded by a water bath, which is maintained at 70°. An excess of the alkylating agents, amounting to three times the quantity theoretically required, is then added slowly through the two dropping funnels, one of which is reserved for methyl sulphate, and the other for a 30% solution of caustic soda. The mixture is vigorously stirred during the operation, which is complete in the course of an hour. The rate of admission of either reagent to the vessel in which the reaction is proceeding is determined by the fact that a slight alkalinity must be maintained throughout. This condition should likewise obtain at the end of the reaction, in order that any unchanged methyl sulphate may be destroyed during the boiling operation, and for this reason the proportion of alkali used should be slightly greater than that of methyl sulphate. The temperature of the water bath is subsequently allowed to rise to 100° for half an hour. The product, on cooling, is extracted twice with chloroform, the extract dried, and the chloroform removed. The methylated syrup is of a nut-brown colour.

The Pigments of the Chloroplast.

History.

Apparently the first written observation on the colouring matter of plants is that of one Nehemiah Grew in a treatise "Anatomy of Plants" which was published in 1682. He noted that a green solution could be extracted from leaves by means of alcohol or oil. In 1770 a method of obtaining the green colouring matter of foliage was described by Rouelle[1]. Rouelle extracted the colouring matter from leaves with alcohol, and considered it to be related to the gummy bodies of flour. The consideration of the leaf green as belonging to the waxes and resins held the field for some 60 or 70 years after its isolation in 1770.

It was in 1817 that Pelletier and Cavantou[2] first applied the name "chlorophyll" to the colouring matter of the leaf. These authors were at that time ignorant of the fact that they were dealing with a mixture of green and yellow pigments, although they later became aware of the complex nature of the leaf extract[3].

The history of the chemical investigation of chlorophyll really begins with the discovery by Berzelius[4], of the decomposition of the pigment by treating the leaf extract with alkali, and also with acid. He was thus the first to obtain

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[1] Rouelle.-Journ.de Med.36,256,(1771).

[2] Pelletier & Cavantou.,Ann.de Chim.et Phys.(2)9,194,(1818).

[3] Ann.de Chim.et Phys.,(2)51,182,(1832).

[4] Berzelius.-Jahresber.,18,381,(1839).

water soluble derivatives of chlorophyll. Brewster[1] was the discoverer of the fluorescence of chlorophyll, and he observed the fluorescence and absorption spectra of its solutions. Work in this region later on by Stokes[2] resulted in the discovery of a further series of very important decompositions. In the meantime results were being published by various observers claiming to have isolated pure chlorophyll. Most of these preparations would be disintegration products of the leaf extract. Amongst others Verdeil[3] claimed to have isolated chlorophyll in the pure state by precipitating a boiling alcoholic extract with lime water and treating the precipitate with hydrochloric acid. It is to this author that credit is due for first hypothecating the similarity of blood and leaf pigments. Several later workers confirmed his statement that chlorophyll contained iron in considerable quantity. The belief that iron was a constituent of chlorophyll was prevalent for about 40 years after its original pronouncement, even being accepted in the work of Schunck[4] in 1891.

Frémy[5] was the first to show that by shaking an alcoholic leaf extract with ether and hydrochloric acid a green-blue pigment, which he called "phyllocyanine" and a yellow pigment , "phylloxanthine" were capable of separation. It remained obscure, however, whether both pigments preexisted in the crude extract, or whether they were formed owing to decomposition of the latter

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[1] Brewster.-Proc.Roy.Soc.Edin., 12, 538, (1834).
[2] Stokes.-Pogg.Ann., 4, 217, (1852).
[3] Verdeil.-Compt.rend., 33, 689, (1851).
[4] Schunck.-Proc.Roy.Soc.Lond., 50, 308, (1891).
[5] Frémy.-Compt.rend., 50, 405, (1860). 61, 180, (1865).

by the acid. Frémy himself first took up the view that chlorophyll was a kind of fat which was hydrolysed by the acid, the phylloxanthine being the part corresponding to glycerine, and the phyllocyanine being the fatty acid. He later altered his point of view, considering chlorophyll as a mixture of two components. The first separation of pigments to which no exception can be taken was effected by G.Kraus[1] in 1872. Kraus obtained his result by a distribution method, his solvents being petrol ether and alcohol. His green petrol-ethereal phase he called "Kyanophyll", and his yellow alcoholic phase "Xanthophyll".

Of very considerable importance in the development of the subject was the work of the physiologist Hoppe-Seyler[2] , which resulted in the furtherance of the analogy between Haematin and Chlorophyll, and also of Frémy's comparison with the fats in that Hoppe-Seyler considered chlorophyll to be a kind of Lecithin. Hoppe-Seyler was the first to prepare in greater quantity crystalline derivatives of the leaf green. He extracted freshly cut grass first of all with cold ether and then with boiling absolute alcohol, thus obtaining a pigment solution as concentrated as possible. On standing in the cold most of a yellow pigment "Carotin" crystallised out. From the residue in solution Hoppe-Seyler prepared a brownish green body which he called "chlorophyllan". This he found to contain ash consisting of magnesia and phosphoric acid. The chromophore group was removed on boiling with alcoholic potash and had acid characteristics. Hoppe-Seyler called this "chlorophyllansäure".

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[1] Kraus.-Untersuch.üb.Chlorophyllfarb. (1872).
[2] Hoppe-Seyler.-Zeitsch.f.Physiol.Chem., 3, 39, (1879). 4, 193, (1880). 5, 75, (1881).

He identified the phosphorus-containing body as glycerine phosphoric acid and also obtained choline from the reaction mixture. As a conclusion from these results Hoppe-Seyler considered that the chlorophyllan might not be merely contaminated with lecithin, but actually a compound of chlorophyllansäure and lecithin, or a lecithin itself. The lecithin hypothesis figured prominently in the literature on chlorophyll until it was shown to be wrong by Willstätter's work.

Of lasting importance, however, in the chemistry of chlorophyll is that portion of Hoppe-Seyler's work which has to deal with the decomposition of chlorophyll by heating to a high temperature with alkali. He obtained in this way a purple - red pigment which he called "dichromatinsäure", and which he found to be very capable of decomposition. With hydrochloric acid there occurred a colour change, owing to decomposition. This decomposition product Hoppe-Seyler likened, on account of its optical properties, to haematoporphyrin, which he had obtained from haematin by the action of concentrated sulphuric acid. It was by this observation that Hoppe-Seyler was able to suggest a definite relationship in molecular structure between chlorophyll and haematin. The porphyrins from chlorophyll and from haematin were very similar to one another, but not quite identical. It was not until 1901 that identical decomposition products were obtained from both pigments. Nencki and Zaleski[1] obtained haemopyrrole from haematin, and Nencki and Marchlewski[2] obtained the same body (or rather mixture of bodies) from a chlorophyll derivative.

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[1] Nencki & Zaleski.-Ber., 34, 997, (1901).

[2] Nencki & Marchlewski., Ber., 34, 1687, (1901).

Attention in Germany seems chiefly to have been directed to the elucidation of the chemical nature of chlorophyll, the important problem as to the homogeneity of the initial material being largely neglected. In England phytochemists had their attention rather more fixed on the application of spectroscopic methods to the province of the differentiation of colouring matters in the leaf extract. It was shown conclusively by Sorby[1] that the leaves of higher plants always contained two very similar green pigments, as well as a number of accompanying pigments. This method of attack of the problem was adopted on the continent by Schunck and Marchlewski[2] and by Tswett[3]. It was this method of control which resulted in the successful achievement of the separation of the single pigments by Willstätter and his co-workers.

Tswett's investigations have shown that the pigments themselves must be regarded as adsorbed with differing degrees of affinity by the stroma or the colloids of the chloroplast. Alcohols such as methyl, ethyl, propyl or amyl alcohols are able to dissipate these adsorption complexes both rapidly and completely. This power is shared by bodies such as acetone, ether, chloroform and similar organic solvents. Petrol ether or benzene, on the other hand are only capable of extracting one of the yellow pigments from either freshly triturated leaves or from dried leaf meal. An extract so obtained contains Carotin exclusively, the other pigments remaining fixed in their adsorption complexes. Should the material have been heated, however, before extraction,

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[1] Sorby.-Proc.Roy.Soc.Lond., 21, 442, (1873).

[2] Schunck & Marchlewski.-for list of refs.see article by M.on chlorophyll in Roscoe & Schorlemmer's text book.

[3] Tswett.-Les chlorophylles dans les Mondes Vegetal et Animal.(1910).

the petrol ether then takes out the green pigments as well as the yellow. Another method of causing the petrole ether to take out the chlorophylls is to add to it just a trace of alcohol. Leaf extract adsorbed on filter paper or talc behaves in a precisely similar manner.

Tswett applied this adsorption phenomenon to the problem of the separation of the leaf pigments. He filtered his extracts through glass tubes filled with calcium carbonate. The carotin remained in solution, while the other pigments were adsorbed - and were found to be distributed in layers. Tswett employed carbon disulphide as his solvent and was able to identify the following zones of adsorption:-

(1). Colourless.

(2). Xanthophyll β - a yellow pigment.

(3). Chlorophyllin β - a dark olive-green pigment, Willstätter and Stoll's Chlorophyll b.

(4). Chlorophyllin α - a dark blue-green pigment -Chlorophyll a.

(5). Two xanthophyll-like pigments.

(6). Colourless.

(7). Xanthophyll α - orange yellow in colour.

The separation of all these pigments was so sharp that Tswett was able to define their spectroscopic differences with certainty. This "chromatographic" method of Tswett's was not applicable for the preparation of larger quantities of single pigments. It is to Willstätter and his co-workers that we owe the knowledge of precise and definite methods of preparation of pure specimens of single leaf pigments. These methods will be described in due course.

The Isolation of the Two Chlorophyll Components a and b.[1]

Willstätter and Isler's method supplied the principle for this more effective and quantitative separation, i.e., partition between petrol ether and aqueous wood spirit. Chlorophyll a gathers in the petrol ethereal phase and chlorophyll b in the alcoholic layer. Willstätter and Stoll recommend the use of the easily accessible, reddy prepared chlorophyll, as this allows of more concentrated solutions being used, and of the proceedure being quantitatively definable. The initial concentration of the chlorophyll in the petrol ether must not exceed 2 grams per litre.

Proceedure.

"8 grams of chlorophyll are dissolved in 150 - 200 c.c. of ether and the Solution poured through a filter into a separating funnel of about 7 litres capacity, containing 4 litres of petrol ether(0.64 - 0.66 s.g.). The chlorophyll generally begins to be precipitated and about 50 - 100 c.c. of methyl alcohol must be added to clear the mixture.

"The ether must be removed before the fractionation by washing with 80% wood spirit, of which about 2 litres in one or two extractions are necessary. These washings are neglected, their sole object being the removal of the yellow pigments and of colourless impurities. If crude chlorophyll is the material being worked up, more washings may be necessary.

"Before the experiment 85% and 90% methyl alcohol are saturated with petrol ether of which they take up 5.5% and 10% respectively. Immediately before use these solvents are acidified

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[1] Willstätter & Stoll.-"Untersuchungen über Chlorophyll."
Springer, Berlin. p.161 et seq.

by the addition of 0.01 gram Oxalic acid per litre(this is to prevent what Willstätter has termed"Allomerisation" of the chlorophyll,i.e.,the rearrangement of the lactam grouping which takes place when chlorophyll stands in ordinary alcoholic solution.).

"The chlorophyll b is sufficiently extracted from the petrol ether by about 14 extractions with 85% methyl alcohol, each of about 2 litres,and the chlorophyll which has passed into these extracts is worked up for the b component solely. The chlorophyll a remains in the petrol ether.

"The first extract,after separation from the petrol ether solution is brought to an alcohol concentration of 90% by the addition of 1 litre of methyl alcohol. It is then thoroughly washed with one litre of petrol ether,is added to 2 litres of ether,and the mixture well shaken with much water.

"The second extract is mixed in the same way with 1 litre of methyl alcohol,and shaken with the wash petrol ether of the first extract,to which 500 c.c.of fresh petrol ether have been added. The purified solution of chlorophyll b resulting is transferred together with 1 litre of ether to the extract containing the compnent b from the first extract. These large quantities of ether are necessary,because the aqueous wood spirit removes much ether,and the petrol ether thrown out on dilution renders more difficult the transference from alcohol to ether. The wash petrol ether from the first two extracts is thoroughly freed from methyl alcohol by a stream of water in a separating funnel,whereupon th pigment is precipitated in a fine condition.(A by-product, chlorophyll rich in b.)

"Extracts 3 and 4 are purified and worked up in an exactly similar manner. The content of b has fallen considerably.

"In the case of the general mixtures having chlorophyll a : b present to the extent of about 2.5 - 2.8 : 1, the sixth extract is treated with 900 c.c. only of methyl alcohol before being washed with petrol ether; the seventh with only 800 c.c.; the eighth with 700 c.c., and so on to the fourteenth which is treated with only 100 c.c..

"The extracts are purified in pairs with the same litre of petrol ether, which on its second application is increased by 500 c.c. of fresh petrol ether. The pigment from the successive extracts is transferred to the same ether solution. This latter has additions beginning with 1 litre each time, falling to about ½ a litre at the tenth extract.

"The solvent in the case of the extracts rich in b - up to about the sixth or seventh - is brought to a concentration of 90%. After this the additions of methyl alcohol are lessened by about 100 c.c. per time. (As above).

"The 15th or 16th extraction of the chief petrol ether solution has the sole object of freeing the chlorophyll a from the last portions of b; this purification is best carried out by shaking the petrol ethereal layer three times with 2 litres each time of 90% wood spirit. The pigment in this 6 litres is transferred to petrol ether, It is rich in chlorophyll a , and is isolated as a by-product, just as is the chlorophyll relatively rich in b which gathered in the petrol etheral washings of the alcoholic extracts.

"The green blue solution of component a, after the separation of b , is washed with water until the chlorophyll is quantitatively precipitated. This latter is taken up by means of talc - about 30 - 100 gms., according to conditions, This allows of filtration under gentle suction through a layer of talc. The petrol ether flows through colourless. The talc layer is washed with a little boiling petrol ether, and sucked at the pump until the smell of petrol ether has disappeared. The pigment is then extracted from the talc by shaking with the least possible quantity of pure ether, the beautiful green-blue solution being filtered off through a small filter. The filtrate is filtered again in order to free it from any talc particles which may have got through the first filter. The ether is finally evaporated almost completely, the concentrated solution rinsed into a dish and the ether allowed to evaporate completely in a vacuum exsiccator.

"The component b, collected from the methyl alcoholic extracts, is in solution in ether - petrol ether. All traces of wood spirit are removed by a thorough washing with water, and the solution, after drying with sodium sulphate, evaporated down to about 500 c.c.. When this volume is reached the boiling point begins to rise to 55-60°, owing to the accumulation of less volatile hydrocarbons. On this account the evaporation is carried out under a diminished pressure at about 40 - 50°. When a volume of 30 - 40 c.c. has been reached the solution is added to about 300 c.c. of volatile petrol ether (30-50° b.p.). Thereupon the chief quantity of the chlorophyll is precipitated, and is filtered off with a little talc. The mother liquors contain an excess of chlorophyll a.

"As the precipitated chlorophyll still contains some chlorophyll a it must consequently be once more precipitated from ether by means of volatile petrol ether. If an initial mixture has been used which was rich in chlorophyll a, this precipitation must be repeated two or three times. The precipitate on talc is washed with volatile petrol ether, the talc sucked dry, and the pigment extracted with ether. The dried solution is avaporated down to about 10 c.c., and precipitated with about 400 - 500 c.c. of petrol ether. It is a characteristic property of chlorophyll b that it is thrown out in a form better suited for filtration than chlorophyll a ; the precipitated particles settle rapidly, and the solvent can be decanted and the particles collected by filtration.

"Chlorophyll a remains behind as a fine blue-black, shining, leaf-like mass. Charateristic tests show it to be free from b.

"Chlorophyll b forms a brittle, green-black mass.

Willstätter obtained as yield from 8 gms. chlorophyll a / b=2.8,

3.7 gms. Chlorophyll a,
1.15 gms. Chlorophyll b,
2.3 gms recovered as by-products.

Purification of Chlorophyll a from Phaeophytin and traces of Chlorophyll b.[1]

"4 gms . of the preparation are dissolved in ether and added to 3 litres of petrol ether containing methyl alcohol.

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[1] Willstätter & Stoll.-"Assimilation der Kohlensäure". Springer, Berlin. (1918). p. 257.

The ether is removed by washing four times with 85% and 90% methyl alcohol. Any admixed chlorophyll b is thereby removed. The pigment is then thrown out of solution by washing away the methyl alcohol. Chlorophyll a is thrown out in the pure state, any phaeophytin remaining dissolved in the petrol ether.

The General Properties of the two Chlorophylls.

Chlorophylls a and b are both microcrystalline when precipitated from ether by means of petrol ether. By slow evaporation of solutions in ether+petrol ether, chlorophyll a crystallises in a characteristic form - bunches of thin, lancet-shaped leaflets. Chlorophyll a forms a blue-black, easily ground powder, which gives green streaks and assumes a steel-blue appearance when rubbed on glass. The powder of chlorophyll b is dark-green to green-black.

In a melting point tube chlorophyll a sinters and between 117 - 120° flows together to a viscous mass. Chlorophyll b sinters between 86° and 92°, becomes viscous between 120° and 130°, and then begins to swell.

The solubility of chlorophyll b is in general a little less than that of chlorophyll a, but the difference is only important in the case of petrol ether. A table showing the solubilities of the two chlorophylls is to be found on p.100.

Solubility of the Chlorophylls.

Solvent.	Chlorophyll a.	Chlorophyll b.
Ether.	Easily soluble. Conc'd sn. absolutely blue, becoming more green on dilution.	v.easily sol. Sol'ns light green comp'd with those of a.
Abs.EtOH.	Easily sol. Sol'n blue-green with deep red fl., Appears red on looking through.	v.easily sol. Fluor'ce brownish red.
95% EtOH.	Easily soluble.	dissolves more difficultly.
90% EtOH.	Soluble.	difficultly soluble.
80% EtOH.	Diff.soluble.	very diff.soluble.
MeOH.	Mod.sol.in cold easily on warming.	in the cold, mod.easily sol. in the warm, app.easily sol.
90% MeOH.	Diff.sol.in the warm.	slightly sol.
80% MeOH.	Almost insol.	v.slightly sol.
Acetone.	Fairly easily sol.	easily sol.
Chloroform.	do.	do.
Carbon disulphide.	do., remarkably yellowish sol'n.	do., yellowish green sol'n.
Benzene.	v.easily sol. Sol'n. unstable to sunlight.	easily sol.
Petrol ether.	Diff. sol. Slight additions of alcohols increase solubility very surprisingly.	Quite insol.
Ligroin.	Rather more sol. than in petrol ether.	Mod.soluble.

By the rapid dilution of concentrated solution of chlorophyll a in alcohol or acetone with much water, a colloidal solution arises, which is stable for about a week. On looking through this solution it appears pure green, and is non-fluorescent. It shows a very beautiful blue-green opalescence. The colouring matter can be obtained from this colloidal solution by shaking with ether, and adding a little calcium chloride.

The colloidal solution of chlorophyll b appears yellowish-green on looking through it, and shows a dark olive-green opalescence.

Tests.

(1). Acid splitting.

Chlorophyll a is decomposed gradually in ethereal solution by shaking with 6% hydrochloric acid. With 20% acid the decomposition is immediate - a beautiful blue colour resulting. Chlorophyll b is decomposed with greater difficulty. With excess of hydrochloric acid in ether a green colour of the Phaeophytin hydrochloride appears immediately.

(2). Phase Test.

Willstätter carries out this test so that an ethereal solution of the chlorophyll is carefully added to methyl alcoholic potash, so as not to mix. At the junction of the two layers there appears a zone of characteristic colour, which on shaking is imparted for a time to the whole liquid. The chlorophyll colour then returns. On the addition of water the ether floats as a colourless layer.

The characteristic zone colour in the case of chlorophyll a is a pure yellow. The green chlorophyll colour returns in a few seconds. In the case of chlorophyll b a red zone appears which returns, more slowly than is the case with chlorophyll a, by way of a brown mixture of colours to the original green colour. Ordinary chlorophyll shows a brown zone.

Composition.

Willstätter's preparations, dried in a high vacuum, were analysed, and gave the following formulae for the two chlorophylls:-

Chlorophyll a. $C_{55}H_{72}O_5N_4Mg + \frac{1}{2}H_2O$ (Hemihydrate).

Chlorophyll b. $C_{55}H_{70}O_6N_4Mg$.

Estimation in the presence of one another.

The only method at present extant is due to Willstätter, and consists in a colorimetric comparison of hydrolysis products. This method will be described later.

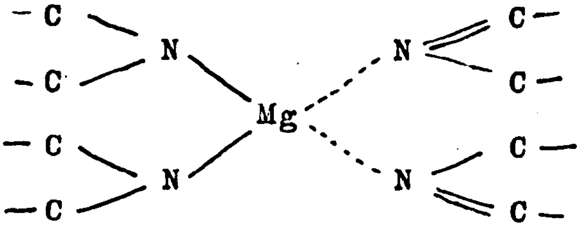
It is hoped, however, that work will be carried out in the near future in these laboratories with the object of perfecting a simpler and more rapid method of estimation of the leaf pigments in the presence of one another by means of the spectroscope.

Chemistry of the Chlorophylls.

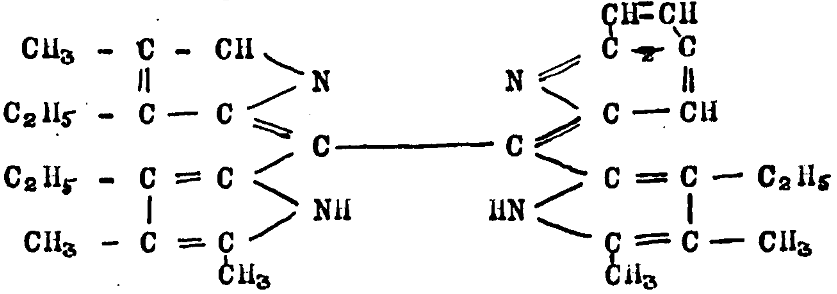
This is a subject which has been dealt with very fully indeed by Willstätter and Stoll in their book "Untersuchungen über Chlorophyll". The following account is included as summarising the chief types of decomposition undergone by the chlorophylls and the results of Willstätter's classical work on the subject.

The two most characteristic decompositions undergone by chlorophyll and its derivatives are those brought about by the action either of alkali or of acid. By the action of alkali the insoluble chlorophyll is converted into soluble salts, which have a green colour. This reaction is an ester hydrolysis, the water soluble salts being the alkali salts of the chlorophyll green acids known as the "chlorophyllins". This hydrolysis is avoided by treating chlorophyll with weak acid, a salt-forming group not appearing under this treatment. The result of this treatment with acid is a colour change to olive-green, and a weakening of the fluorescence. It was by the application of these two types of reaction to chlorophyll derivatives that Willstätter was enabled to go so far in the direction of solving the problem of the constitution of the chlorophylls.

The chlorophyllins, or chlorophyll-green acids, are obtained by the alkaline hydrolysis of an alcoholic leaf extract, and can be separated fairly easily from mixture with other products of hydrolysis. They are magnesium compounds in which the magnesium containing group is uncommonly stable towards alkali and extremely sensitive towards acid. They are tricarboxylic acids, one of the carboxyl groups apparently being bound in a lactam grouping. These chlorophyllins undergo further decomposition when they are subjected to a more vigorous treatment with alkali. By heating with concentrated alcoholic alkali to about 250° C., a series of finely coloured, well crystalline, fluorescent bodies are obtained, which have been called "phyllins". The phyllins are bodies all of which contain magnesium, and three, two or one carboxyl group, and are consequently free acids. By heating with soda-lime all carboxyl groups are removed, there then resulting an oxygen-free body $C_{31}H_{34}N_4Mg$, which has been called "Aetiophyllin". This body is built up of four pyrrole nuclei, the magnesium being bound to nitrogen complexly in this manner:-



By the action of acids all the phyllins lose their magnesium, the carboxylic phyllin acids being thereby converted into corresponding amino-acids displaying amphoteric characters. This series of bodies has the class name "porphyrin". The simplest porphyrin is Aetioporphyrin, $C_{31}H_{36}N_4$, a basic body obtained by the action of acid on aetiophyllin. These porphyrins give rise, on reduction, to certain homologues of pyrrole ("Haemopyrrole") from a consideration of which reactions Willstätter has arrived at the following formula for aetioporphyrin:-

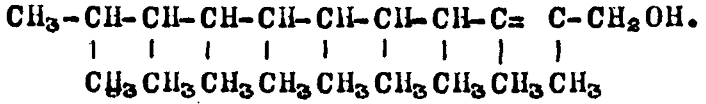


Aetioporphyrin is also obtainable as a degradation product of haematin.

Chlorophyll itself is as sensitive towards the action of acids as are the phyllins. If an alcoholic solution of chlorophyll is treated with oxalic acid, there occurs a precipitation of the magnesium-free body phaeophytin. The splitting off of magnesium is the only change which has occurred, so that phaeophytin is an ester still, and has the basic properties of the porphyrins. It is a wax-like body, of a yellow colour, and its solutions do not resemble at all in colour intensity those of chlorophyll. Phaeophytin very readily forms complexes with many metals to give bodies extremely similar to chlorophyll. For instance, by the action of copper or zinc acetates on an alcoholic solution of phaeophytin, copper or zinc analogues of chlorophyll result. Willstätter found it much more difficult to re-introduce magnesium into phaeophytin, but succeeded in effecting a p partial synthesis of chlorophyll from phaeophytin by the application of the Grignard reaction. The magnesium can be reintroduced into, and the phyllins synthesised from the porphyrins in a similar way, and often more simply in these cases by the action of magnesia and hot alkali.

As has already been mentioned, phaeophytin contains the ester groups of chlorophyll unaltered and behaves as does any ester on alkaline saponification. It yields complex nitrogen-containing acids, one series from chlorophyll a, and another from chlorophyll b, a nitrogen - free alcohol $C_{20}H_{39}OH$, known as phytol, and methyl alcohol.

Phytol is an unsaturated alcohol with a straight carbon chain on to which very many methyl groups are probably attached. It is a colourless, viscous oil, distillable in a high vacuum. Phytol is autoxidisable and easily combines with ozone, adds on a molecule of bromine and can be catalytically reduced by hydrogen in the presence of platinum. Willstätter suggests as a provisional formula



$$CH_3-\underset{\substack{|\\CH_3}}{CH}-\underset{\substack{|\\CH_3}}{CH}-\underset{\substack{|\\CH_3}}{CH}-\underset{\substack{|\\CH_3}}{CH}-\underset{\substack{|\\CH_3}}{CH}-\underset{\substack{|\\CH_3}}{CH}-\underset{\substack{|\\CH_3}}{CH}-\underset{\substack{|\\CH_3}}{C}= \underset{\substack{|\\CH_3}}{C}-CH_2OH.$$

Chlorophyll, therefore, contains two ester groups, one a phytyl ester, and the other a methyl ester group. All carefully extracted chlorophyll contains about 1/3 of its weight of phytol. It was observed, however, that when chlorophyll was only extracted slowly from leaves, and the solvent allowed to remain in contact with the dried leaves for some time, the phytol content of the chlorophyll so obtained was too low. This phenomenon has been shown to be due to the fact that, accompanying chlorophyll in the chloroplast, there is a hydrolysing enzyme, which Willstätter has called "Chlorophyllase". This enzyme is inactive in alcoholic media, but causes a displacement of the phytol in the chlorophyll by the alcohol used for the extraction, alcoholysis of the chlorophyll being thereby effected. The chlorophyll containing an ethyl ester group instead of the phytyl ester group is much better crystalline than ordinary chlorophyll, and has been shown to be identical with the "crystalline chlorophyll" first observed microscopically by Borodin and later separated by Monteverde(1893).

The fundamental acids of the hydrolysis are chlorophyllins a and b. The monomethyl esters are known as "chlorophyllides" a and b. Crystalline chlorophyll is ethyl chlorophyllide, whereas ordinary or amorphous chlorophyll is phytyl chlorophyllide.

Phaeophytin, the magnesium-free compound from chlorophyll is, of course, a mixture of two phaeophytins. On hydrolysis it yields a mixture of amphoteric acids which differ in colour and in basic strength. This difference in basic strength has been utilised by Willstätter in the separation of the mixture, and forms the basis of his method of colorimetric estimation of the two chlorophylls.

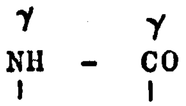
From phaeophytin a there results, on treatment with alkali, the phytochlorins, whose solutions are olive-green. From phaeophytin b are obtained the phytorhodins, whose solutions are beautifully red. By a regulation of the conditions of hydrolysis of phaeophytin Willstätter and Isler were enabled to obviate the formation of any but two well-defined crystalline bodies as decomposition products. It was by this means that they were first able to show chemically that phaeophytin, and consequently chlorophyll was a mixture of two components. These bodies are

Phytochlorin e , $C_{34}H_{34}O_5N_4$, a tricarboxylic acid with two free carboxyl groups, and one bound in a lactam formation. This product is the result of the [illegible] hydrolysis of phaeophytin a .

Phytorhodin g , $C_{34}H_{34}O_7N_4$, a tetracarboxylic acid, only two or three of whose carboxylic groups are found in a free state. This product is the result of the hydrolysis of phaeophytin b.

These bodies possess different basic properties, and Willstätter and Mieg's method for their separation utilises a consequent difference in their distribution between ether and hydrochloric acid.

Finally some mention must be made of the structural significance of the changes that take place on treating the chlorophylls with methyl alcoholic potash. It will be remembered that by the application of this reagent a qualitative distinction can be made between the two components (the Phase Test p.101). In this reaction chlorophyll passes through a brown phase, chlorophylla through a yellow phase, and chlorophyll b through a red phase, the original green colour finally returning. Similar results are obtained with the chlorophyllides and with the magnesium-free phaeophytins. Willstätter has suggested that this phenomenon is connected with the lactam group in which the third carboxyl is bound. This group Willstätter writes as



$$\underset{|}{\overset{\gamma}{NH}} - \underset{|}{\overset{\gamma}{CO}}$$

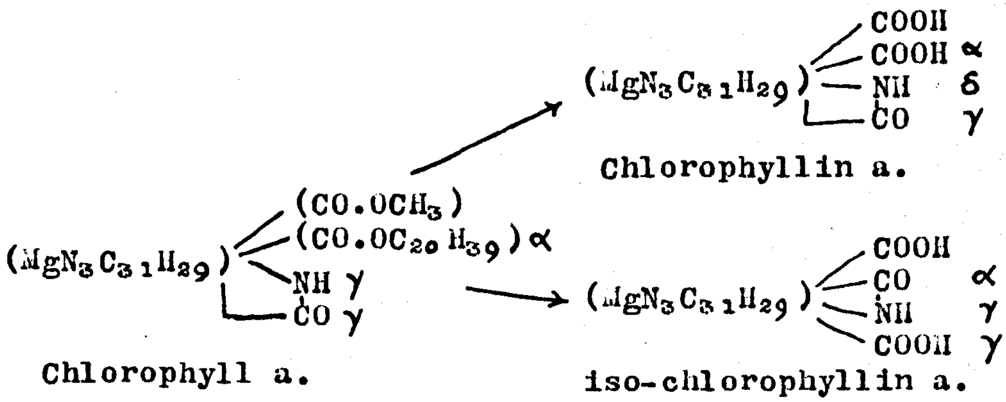
as it occurs in the chlorophylls. By the action of alkali it may undergo hydrolysis and the carboxyl γ enter into combination with another nitrogen atom, to give a new lactam group

$$\overset{\delta}{\underset{|}{NH}} - \overset{\gamma}{\underset{|}{CO}} ,$$

or another carboxyl α may combine with the nitrogen γ , in which case the group is written

$$\overset{\gamma}{\underset{|}{NH}} - \overset{\alpha}{\underset{|}{CO}}$$

Alkaline hydrolysis, it has been already noted, has different results if carried out in the cold or with more concentrated alkali at a higher temperature. A different ~~different~~ chlorophyllin results according as to which condition obtains. The hydrolyses can be represented as follows:-



Each of these two "a" chlorophyllins yield three different constituent phyllins on further decomposition with alkalies. Iso-chlorophyllin a yields cyanophyllin, erythrophyllin, and phyllophyllin. Chlorophyllin a yields glaucophyllin, rhodophyllin and pyrrophyllin. The last two products of both series also result from the decomposition of isochlorophyllin b and chlorophyllin b respectively. Both phyllophyllin and pyrrophyllin give aetiophyllin on being heated with soda-lime.

Change of the lactam grouping is also supposed to be the cause for the changes known as "Allomerisation" which have been observed to take place when chlorophyll or chlorophyllides are allowed to stand in alcoholic solution. Allomerised chlorophyll or chlorophyllides lose their capacity for crystallisation, and give in place of normal hydrolysis products weakly basic chlorins and rhodins. The Phase Test is no longer shown by Allomerised products.

The Yellow Pigments accompanying the Chlorophylls in the Chloroplast.

The discovery that yellow pigments accompanied the green was first made by Kraus[1], who obtained a certain degree of separation by adding petrol ether to an aqueous alcoholic extract of leaves. The petrol ether took up the chlorophylls, while the alcoholic layer was left yellow in colour. After several extractions with petrol ether, Kraus was unable to detect in the alcoholic residue any traces of the characteristic bands of chlorophyll in the red. Kraus called this yellow alcoholic fraction "Xanthophyll". This xanthophyll fraction gave a dark blue colouration with concentrated sulphuric acid and rapidly bleached in sunlight. It is now known that Kraus's "Xanthophyll" was a mixture of several yellow pigments, Carotin amongst others. This latter pigment was isolated earlier by Frémy[2] in 1865. Frémy observed that the green colour was removed from the leaf extract

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[1] Kraus.-Untersuch.üb.Chlorophyllfarbstoffe (1872).

[2] Frémy.-Compt.rend., 61, 189, (1865).

by treatment with aluminium hydrate, a yellow pigment being left in solution. Tswett[1] later showed that this would be Carotin. After Kraus, other authors claimed to have isolated a red crystalline pigment - what we now know as Carotin - from the leaf extract. This was the "Chrysophyll" of Hartsen, the "Erythrophyll" of Bougarel, the "Xanthine" of Dippel, and the "Etiolin" of Pringsheim.

This redly crystalline pigment was shown to be identical with the colouring matter of the ordinary carrot by Arnaud[2]. Arnaud found it to be a hydrocarbon, and assigned to it the formula $C_{26}H_{38}$. This was later corrected by Willstätter to $C_{40}H_{56}$. Spectroscopic evidence soon showed there to be present, besides carotin, at least one other yellow pigment - probably several. Tschirch[3], Schunck[4] and Tswett[1] isolated yellow preparations which were called "Xanthophyll", but which really appear to have been mixtures of what we know as "Xanthophyll" with varying amounts of carotin and certain other yellow pigments which only occur in slight amount and of which nothing seems to be known. Willstätter and Mieg[5] first isolated from the mother liquors from chlorophyll hydrolyses large quantities of a yellow crystalline pigment, present to at least four times the extent of carotin. Crystal form, colour, and solubility differentiated it from carotin as well as the fact that it contained oxygen. Its formula was found to be $C_{40}H_{56}O_2$, that is, its molecule was richer in oxygen than that of Carotin by two atoms of oxygen.

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[1] Tswett.-Ber.deut.bot.Ges.,24,384,(1906).
[2] Arnaud.-Compt.rend.,100,751,(1885); 102,1119,1319,(1886).
[3] Tschirch.-Ber.deut.bot.Ges.,14,76,(1896); Flora,(1905),p.383.
[4] Schunck.-Proc.Roy.Soc.Lond.,63,389,(1898); 65,177,(1899); 72,165,(1904
[5] Willstätter & Mieg.-Ann.,355,1,(1907).

These two yellow pigments must be very similar in constitution, but next to nothing is known about the structure of either. In petrol ether both form yellow solutions, and in carbon disulphide red solutions. It was suggested by Tscirch that carotin passed into xanthophyll on long standing in the air, and by treatment with certain reagents. This suggestion was not confirmed by Willstätter, however. Ewart[1] claims to have succeeded in preparing carotin by the reduction of xanthophyll. His work is described in more detail in section three.

Details for the preparation of the two yellow pigments mentioned follow, together with an account of their properties.

Carotin. - from the carrot.[2]

The powder of the dried roots is extracted in a percolator with petrol ether, and the percolate evaporated under diminished pressure very rapidly at about 40°. The carotin then crystallises out, although mixed with considerable quantities of colourless impurity. It is purified by fractional precipitation from carbon disulphide solution with absolute alcohol.

Willstätter obtained 125 gm. of carotin from 5000 kilos. of carrots. Further remarks on the isolation of carotin are to be found on p.136.

Properties of Carotin.

Carotin crystallises in reddish, copper-coloured, rhombic crystals, which have a blue, almost metallic reflex, and which melt at about 174°. It is a hydrocarbon of the composition $C_{40}H_{56}$, difficultly soluble in boiling ethyl or methyl alcohols, in which it is almost insoluble in the cold. It does not seem to

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[1] Ewart.-Proc.Roy.Soc.Victoria, 30(N.S), 178, (1918).
[2] Willstatter & Stoll.-Chlorophyll, p.240.

dissolve to a very much greater extent in low boiling petrol ether, is difficultly soluble in acetone, but soluble easily in benzene, chloroform, or carbon disulphide. The solutions are of an intense yellow colour, orange when concentrated - almost indistinguishable in colour from aqueous solutions of potassium bichromate; the solution in carbon disulphideb is an exception to this statement,- it is red. Carotin dissolves in concentrated sulphuric acid, a blue colour being formed. It is unsaturated and very easily oxidisable, bleaching on standing in air. It combines with iodine to form a dark violet, crystalline body, $C_{40}H_{56}I_2$.

Xanthophyll. - from leaves[1].

Crude chlorophyll is extracted from 5 kilos. of stinging nettle leaves and hydrolysed in ethereal solution with methyl alcoholic potash. The chlorophyllin salts are precipitated, there remaining over these an apparently pure yellow solution, which is washed with methyl alcoholic alkali and then concentrated to 250 c.c.. About 2 litres of petrol ether are added to this solution, and the resulting precipitate of xanthophyll taken up with talc. The layer on the filter is washed with low boiling petrol ether, and the xanthophyll extracted from the talc by means of 1 litre of ether. The ethereal solution, evaporated down to about 50 c.c., is added to 1 litre of methyl alcohol, and the ether completely removed by a further slight concentration of the solution. Xanthophyll, beautifully crystalline, is obtained in a yield of about 2.2 gm. on allowing the methyl alcoholic solution to stand in the cold.

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[1] Willstatter & Mieg.-Ann., 355, 1, (1907).

Carotin can be obtained from the petrol ether mother liquors from the xanthophyll-talc separation to the extent of about 0.6 gm..

Properties of Xanthophyll.

Xanthophyll forms yellowish crystals with a steel-blue reflex. From alcohol it crystallises in plates, and from methyl alcohol in prisms, and is inclined to take up these solvents for crystallisation. It is obtained free from solvent by precipitation from chloroform solution with petrol ether. Its solutions are of the same colour as those of carotin, and its behaviour towards concentrated sulphuric acid, halogens, and atmospheric oxygen is similar to that of carotin. Ethereal solutions of xanthophyll bleach in air even more rapidly than do those of carotin. The solubility of xanthophyll is generally different from that of carotin. It is insoluble in petrol ether, and fairly difficultly soluble in wood spirit, nevertheless much more soluble than carotin in the latter. It is considerably more easily soluble in ethyl alcohol, quite soluble in ether, and very easily soluble in chloroform. It is soluble in acetone, and only very slightly soluble in carbon disulphide. It melts at about 173-4°.

Its molecular formula is $C_{40}H_{56}O_2$, and as it shows neither carbonyl, alcohol, or acid reactions, Willstätter considers the oxygen to be bound in an ether-like manner.

In addition to the two yellow pigments described, a third carotinoid occurs in brown algae. This is known as "Fucoxanthine", and when this carotinoid occurs the carotin - xanthophyll content is less. The formula of fucoxanthine is $C_{40}H_{56}O_6$, and it is very similar in its behaviour to the two yellow pigments described. A point of difference, however, is the pronounced basic properties of its oxygen atoms. Fucoxanthine forms a characteristic blue hydrochloride.

Two other pigments belonging to the class of the carotinoids might be mentioned in passing. One of them, Lycopin, is the colouring matter of the tomato, and is isomeric with carotin. The other, Lutein, isomeric with xanthophyll, is responsible for the colour of egg yolks.

Quantitative Estimation of the four Chloroplast Pigments.[1]

"The quantitative determination of the four leaf pigments in any given solution resolves itself into three series of operations. First of all there is the separation of the carotinoids from the total chlorophyll, and then, on the one hand, the separation of carotin and xanthophyll, followed by their estimation, and on the other hand, the decomposition and fractionation of the chlorophylls.

"The first operation is based on the differing behaviour towards alkali of the two series of pigments. The chlorophylls are removed from an ethereal solution of the pigments by a treatment of this with methyl alcoholic potash. On washing with water, the chlorophyllins are removed, the carotinoids remaining unchanged in the ethereal layer.

"Carotin and xanthophyll are not separable by chemical means because they are indifferent and cannot be converted into reactive derivatives. Their fractionation is effected by the

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[1] Willstätter & Mieg.-Ann., 350, 1, (1906).

application of a physical method, also suited for preparative purposes,- the distribution between petrol ether and aqueous wood spirit. Carotin only remains completely in the petrol ether layer, the xanthophyll transferring itself to the wood spirit.

"The chlorophylls are converted quantitatively by the action of acid and alkali under certain conditions into phytochlorin e and phytorhodin g. These bodies differ in strength as bases, and are separated by fractionation with ether and hydrochloric acid. The separated solutions of the two bases are compared colorimetrically with standards.

The following directions are for pure solutions containing about .03 - .07 gm. of chlorophyll, and the generally corresponding quantity of carotinoids (.005 - .015 gm.) per 100 c.c. of ether. 100 c.c. of ether solution is used for the chlorophyll analysis, and a second 100 c.c. for the carotinoid analysis.

Separation of the chlorophyll components.

"100 c.c. of the ethereal solution are taken, and the ether removed completely at about 30° under diminished pressure. About 20 c.c. of concentrated methyl alcoholic potash are brought to the boil in a test tube with a broken top, and added to the warm chlorophyll inn the flask by introducing the test tube into the flask and tipping it out. The liquid is maintained in brisk ebullition under a reflux for two minutes, 4 c.c. of water are added, and the boiling continued for a further two minutes. The deep-green and strongly red-fluorescing solution is rendered weakly acid after cooling, the magnesium-free products being transferred to 250 c.c. of ether. This ethereal solution is washed well with about 200 c.c. of water to which about 1-2 c.c. of 3% hydrochloric acid have been added.

"The solution is shaken withc 400 c.c. of 3% hydrochloric acid, in four or five portions, and then several times with 5% acid until this becomes only faintly green. These stronger acid extracts require further fractionation, and are extracted, after neutralisation with 30 c.c. of ether. This ethereal solution is extracted with 3% hydrochloric acid, these extracts being added to the earlier 3% acid phytochlorin fraction, until the combined fraction is brought to a volume of 500 c.c.. The two ethereal phytorhodin fractions are united, and the phytorhodin transferred to acid by treatment with 12% hydrochloric acid four or five times, until the volume of the extract amounts to 500 c.c.. The ether should by this time be coloured only faintly reddish yellow.

Separation of carotin and xanthophyll.

"A second 100 c.c. of the ethereal solution of pigments is hydrolysed with some c.cs. of concentrated methyl alcoholic potash, with vigorous and continued shaking. After a short period of standing the ether is generally of a purely yellow colour, but should it show any red fluorescence, the shaking is continued and more alcoholic potash added if necessary. After complete hydrolysis of the chlorophyll, the ethereal solution is decanted from the potassium salts, and these washed with ether. This washing does not suffice, however, for the complete removal of the xanthophyll from the syrupy residue of chlorophyllin salts. 30 c.c. of ether are added to this, the mixture shaken up, water added and the shaking continued. It is then necessary to wait until an emulsion separates. The alkaline liquid, after running out, is again shaken with ether as a control. The ether should remain colourless, and if it does not do so it must be added to the united ethereal solutions. These are washed with water, again treated with methyl alcoholic potash to remove all traces of chlorophyllins, and finally washed twice with water. Most of the ether is then removed in vacuo at ordinary temperatures from the purified solution. The residue is treated with about 80 c.c. of petrol ether, and the solution brought into a separating funnel, the flask being rinsed clean with a little ether.

"Fractionation of this solution is then effected by successive extractions with 100 c.c. 85%, 100 c.c. 90%, and then with about 50 c.c. of 92% methyl alcohol. If this last does not separate colourless, the extraction with 92% methyl alcohol is repeated. The methyl alcoholic extracts are free from carotin. The first is mixed with 130 c.c. of ether, and the pigment transferred to the ether by slowly adding water. Then the second methyl alcoholic xanthophyll extract and a further 100 c.c. of ether is added to the strongly yellow ethereal solution. Separation is again effected by the slow addition of water. The xanthophyll is collected in the one solution from the later extracts in a similar manner, with the addition of more ether and water. This ethereal solution of xanthophyll, as well as the petrol ethereal solution of carotin are each freed from methyl alcohol by a double washing with water, and poured through dry filters into 100 c.c. measuring flasks. A few drops of absolute alcohol are added, until each solution is clear, upon which the flasks are filled to the mark with ether and petrol ether respectively.

Standard solutions.

(a).For the Chlorophylls. Weights of pure chlorophylls a and b similar to those expected in the "unknown"solution are dissolved in the same portion of ether to about the same concentration as the chlorophylls in the "unknown"(i.e.,so as to carry out the hydrolysis of the standard under as nearly similar conditions to that of the "unknown" as possible).

This mixture of accurately known concentrations is then hydrolysed and fractionated ~~under~~ in precisely the same manner as was the "unknown". In this way are obtained 500 c.c. each of solutions of phytochlorin e in 3% hydrochloric acid, and of phytorhodin g in 12% hydrochloric acid,which represent standard amounts of chlorophyll a and b respectively. Colorimetric comparison of the corresponding "unknown"solutions with these standards allows of the calculation of the concentration of the chlorophylls in the "unknown".

The phytochlorin and phytorhodin solutions are stable for about a week.

(b).For the Yellow Pigments. Owing to the instability of solutions of carotin and xanthophyll,it was found much more convenient and practicable to use as comparison solutions aqueous solutions pf potassium bichromate. These are of precisely the same tint as solutions of carotin in petrol ether or of xanthophyll in ether,although the colour intensities of the three solutions are very different.

Willstätter's comparison figures are as followx :-

Concentration of a standard Carotin Solution. 5×10^{-5} moles per l.

(0.0134 gm.Carotin in 500 c.c. petrol ether containing a little alcohol.)

Concentration of aqueous Potassium Bichromate Solution.0.2% .

These two solutions were compared,with the following results:-

100	m.m.	of the above Carotin solution corresponded to	101 mm.of 0.2% Pot.Bichr.Sol.
50	mm.	""""""""""""""""""""""""""""	41 mm. """"""""""""""""""""
25	mm.	""""""""""""""""""""""""""""	19 mm. """"""""""""""""""""

Concentration of a standard Xanthophyll Solution. 5×10^{-5} m.p.l.

(0.0142 gm. Xanthophyll in 500 c.c. ether.)

This compared with the above bichromate solution as follows:-

100	mm.	of the above Xanthophyll solution corresponded to	72 mm.of 0.2% Bichr.Sol.
50	mm.	""""""""""""""""""""""""""""	27 mm.""""""""""""""""""""
25	mm.	""""""""""""""""""""""""""""	14 mm.""""""""""""""""""""

The Function of the Chloroplast Pigments in the Assimilation Mechanism.

The first hypothesis as regards the functioning of the leaf green was that of von Baeyer[1], put forward in 1870. The absolute indispensibility of the pigment to the process of nutrition had, of course, been much earlier recognised, by Senebier, Ingenhousz, etc. (see p.52). Baeyer was led to formulate his hypothesis as a result of Butlerow's discovery of the formaldehyde condensation. He suggested that the chlorophyll might have the same power of adding on carbon monoxide as has haemoglobin, - this in view of the similarity between the pigments of the leaf and blood. In sunlight, he thought that carbon dioxide situated round the chlorophyll might dissociate, as it does at high temperatures, giving carbon monoxide and oxygen. The former would be bound by the chlorophyll and be released as formaldehyde, which might be polymerised by the cell contents just as it is by alkali.

The first formulation of the hypothesis that carbon dioxide is bound on to chlorophyll, with formation of an addition product seems to have been due to Hoppe-Seyler[2]. Hoppe-Seyler considered this addition product to be capable of decomposition in sunlight, chlorophyll being regenerated, and the first product of assimilation - probably formaldehyde - liberated. The same

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[1] Baeyer.-Ber., 3, 63, (1870).
[2] Hoppe-Seyler.-Physiol.Chem., Berlin, 1881. p.137.

ideas later found independent expression by Hansen[1], Hartley[2], and Luther and Hallström[3].

Emil Fischer[4] has suggested that the synthesis of optically active sugars is the result of combination of formaldehyde with asymmetric portions of the chlorophyll nucleus. This view was elaborated later in the Faraday Lecture which Fischer gave to the Chemical Society in 1907 on "Organic Synthesis and Biology".

Judging from the treatment of the subject in text books of physiology, up to 1914, opinion was divided between two different sets of ideas as regards the means of participation of the chlorophyll. One body of opinion considered the chlorophyll as acting the role of sensitisor, or catalyst, while the other held that chlorophyll participated chemically in the process.

The optical participation of chlorophyll in the photosynthesis has been discussed by Tswett[5] in a paper published in 1911. Tswett considered that the chlorophyll fluoresces the light absorbed from the sun in the red regions, and that this fluorescence is absorbed by carbonic acid, causing decomposition of the latter.

Willstätter and Stoll[6] dispense with Tswett's fluorescence hypothesis, and consider that the absorbed light does work within the chlorophyll molecule itself. They have shown that chlorophyll

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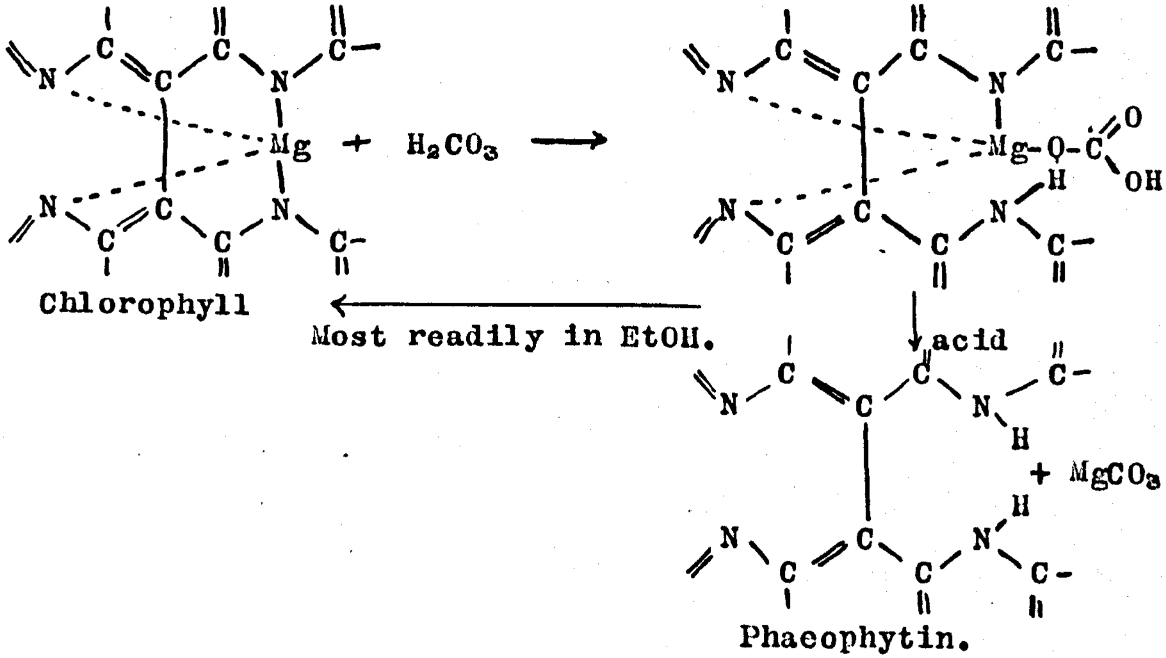
[1] Hansen.-Arb.bot.Inst.Wurzburg. III.426,429,(1885).
[2] Hartley.-J.C.S.,59,106,124,(1891).
[3] Luther & Hallström.-Ber.,38,2288,(1905).
[4] Fischer.-Ber.,27,3189,(1894). J.C.S.,91,1749,(1907).
[5] Tswett.-Zeitsch.physikal.Chem.,76,413,(1911).
[6] Willstätter & Stoll.-Assimilation.p.240.

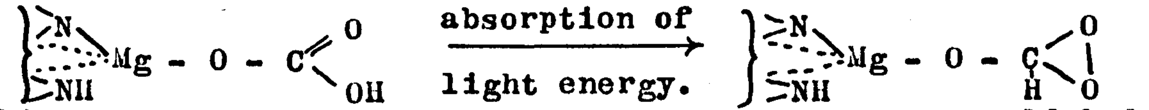
has no affinity for carbon dioxide itself either in light or in darkness, but that with carbonic acid things are different. While chlorophyll in solvents such as ether or benzene does not react at all with carbon dioxide, if a colloidal solution of chlorophyll is taken, it reacts immediately with the carbonic acid. The solution slowly absorbs carbon dioxide, even from the atmosphere. The chlorophyll is, however, destroyed in the reaction, the magnesium being removed from the molecule as carbonate or bicarbonate. The reaction which occurs can be represented by this equation:-

$$C_{55}H_{72}O_5N_4Mg + CO_2 + H_2O \longrightarrow MgCO_3 + \underset{\text{Phaeophytin.}}{C_{55}H_{74}O_5N_4}$$

This reaction does not procedd all in one step, but an intermediate is formed which can, under certain conditions, dissociate and regenerate chlorophyll. Experiment shows that this splitting off of carbon dioxide goes more slowly in alcoholic solution than in water - which hydrolyses out the magnesium. This intermediate addition product is probably formed after this fashion:-

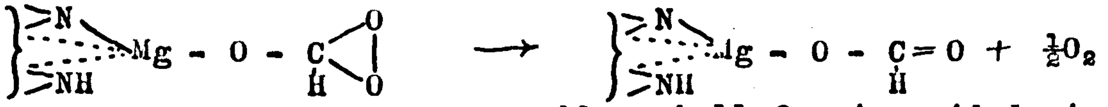


Willstätter and Stoll consider that a bicarbonate complex of the type indicated is formed within the chloroplast, the conditions there being such as to prevent a separation of maghesium. When absorbable rays fall on this complex, the carbonic acid is so rearranged that it is conditioned for decomposition. The addition complex is converted into an isomer of higher energy content to which they give a peroxide structure. This change can be denoted as follows:-



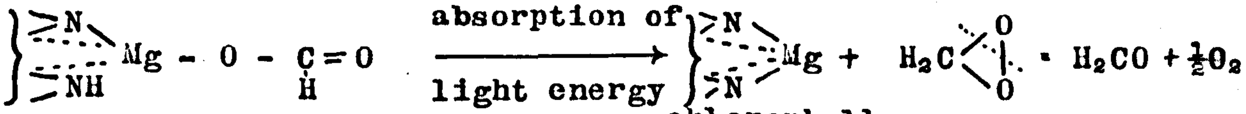
bicarbonate complex. chlorophyll formaldehyde peroxide.

The chlorophyll formaldehyde peroxide is supposed immediately to be decomposed and to split off an atom of oxygen, yielding a formic acid derivative. Thus :-



Chlorophyll formic acid derivative.

This formic acid derivative is supposed, on absorption of more light energy to split up into a peroxide isomer of formic acid which yields formaldehyde and oxygen, the chlorophyll being left unchanged. This series of changes can be expressed as follows:-



chlorophyll.

Willstätter and Stoll consider the peroxide bodies to be decomposed by the agency of some enzyme - just as hydrogen peroxide is decomposed by catalase. The formaldehyde split off is supposed to require no more supplies of energy for its further condensation to sugars.

One point, which seems to the writer to allow of adequate criticism being made of their suggested mechanism as it stands is that free nascent oxygen is considered as being evolved by the chlorophyll molecules. This would seem actually to be impossible in view of the sensitivity towards, and affinity for oxygen shown by leaf pigments in the light. The pigments would be bleached extremely rapidly with oxygen so immediately present. There are present in the chloroplasts not one pigment alone but four, and one would surely think that they must all be necessary in the assimilation mechanism. The questions regarding the rôles played by carotin and xanthophyll in the assimilatory process seem to have received little attention if one judges by what has been published on the subject. Arnaud[1] as long ago as 1889 made the suggestion that carotin and its companion were necessary for the respiration of the plant, but this was soon disproved. Willstätter and Stoll seem to have started their work on assimilation with ideas relating to the participation of the two chlorophylls and the two yellow pigments in some kind of an equilibrium mechanism such as is suggested by the oxygen differences in the formulae. These ideas were given up after they had observed that assimilation by leaves proceeded at an unaltered rate for at least one and a half hours during the interposition of a filter consisting of a 4 cm. layer of a 1% solution of potassium bichromate. This they considered to be efficient in removing all the blue and violet light absorbed by the yellow chloroplast pigments.

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[1] Arnaud.-Compt.rend.<u>109</u>, 911, (1889).

There is normally present in sunlight a great excess of energy of the type utilised by the plant over what is necessary. That this is so has been shown by the work of Brown and Escombe[1]. These observers found that the intensity of light had to be diminished to about a twelfth part of the normal before any diminution at all was observable in the rate of assimilation. In addition to this excess there is in the plant an excess of pigment over what is normally necessary, the excess amounting in some cases to as much as 2000 times that required. One could surely expect a similar excess of carotinoids, and of the light absorbed by them, so that any leak through a bichromate filter, of rays capable of absorption by these pigments might well allow of the ingress of sufficient energy to cause their activation.

Qualitative investigations carried out at Liverpool point to the fact that the filter used was not efficient. It is considered that the only really trustworthy filter would be a thick layer of a solution of the carotinoids themselves. As soon as quantities of these have been isolated it is intended to carry out quantitative spectroscopic tests with the object of clearing up this point.

Opinions do not all agree with those of Willstätter and Stoll with regard to the non-participation of the four chloroplast pigments. This is exidenced by the appearance in 1920 of a paper by Ewart[2], who adheres strongly to the opposite view, and advances a hypothesis involving the decomposition of chlorophyll, carotin and xanthophyll during a cycle. The scheme he suggests, however, is very complicated, and hardly seems to be in accord with

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[1] Brown & Escombe.-Proc.Roy.Soc.76B,29,86,(1905).

[2] Ewart.-Proc.Roy.Soc.Vict.30(N.S.),178,(1918).

what chemical evidence is available.

The views which are at present held by those engaged in the exploration of the problem at Liverpool are based on a firm conviction that all four pigments of the chloroplast are necessary for the complete and smooth-working of the assimilation process. There would hardly be four pigments in the chloroplast if nature could use one and one only , chlorophyll. The pigments <u>are</u> there, and they must be there for a purpose. What other this purpose could be than one connected with the particular cells in which they occur is hard to see. The pigments are all in the chloroplast, the cell in which the plant synthesises and fashions the bricks and mortar of which it is built, and they must consequently be connected with this synthesis - all of them as well as any one.

In green leaves, assimilating under normal conditions, the proportions of the four pigments are generally constant more or less for all plants. Willstätter gives the following values for what are known as the pigment ratios, for normal green leaves:-

$$\text{Ratio}\ \frac{\text{moles.chlorophyll a}}{\text{moles.chlorophyll b}} = \text{Average value } 2.9 \text{ Max.var'n} \pm 0.5.$$

$$\text{Ratio}\ \frac{\text{moles.carotin}}{\text{moles.xanthophyll}} = \text{Average value } 0.6 \qquad \pm 0.1$$

$$\text{Ratio}\ \frac{\text{moles.chlorophyll a+b}}{\text{moles car. + xanthop'l.}} = \text{Generally about 3, but higher in yellowish leaves and in autumn.}$$

Willstätter's experiments, described in his book, show that the ratio of the chlorophylls a and b is hardly altered at all by changing the conditions of assimilation - for instance such a condition as the length of the period of assimilation.

Ont the other hand, the ratio of carotin to xanthophyll commences to alter as soon as assimilation begins, and gradually falls to a value which is determined by the conditions obtaining. This indicates either that carotin is destroyed, or that xanthophyll is formed during assimilation. The ratio of total chlorophylls to total carotinoids shows which of these alternatives occur. Within certain limits, this ratio remains at very much the same value - from 2.8 to 3.5 generally. This would seem to indicate that carotin is transformed into xanthophyll during the assimilatory process, this transformation proceeding until an equilibrium is reached, the position of the equilibrium depending on the experimental conditions. It certainly seems likely that there exists a definite equilibrium between all four pigments of the leaf - an equilibrium of this nature :-

Chlorophyll b and Carotin on the one side, / Chlorophyll a and Xanthophyll on the other.

It will be remembered that in part I of this thesis work was described which showed that it was possible to photocatalyse by the presence of certain types of body the reaction which results in the formation of formaldehyde from carbon dioxide and water, and it was suggested that the pigment of the chloroplast might act as such a photocatalyst. The substances which were found to act successfully as photocatalysts were coloured, basic substances, their basic properties causing the formation of a complex with carbonic acid, such a complex formation being necessary in order to ensure an identity of certain frequencies in the long wave infra-red.

The necessary conditions for a photocatalyst are fulfilled by the chlorophylls. In addition to their power of absorbing energy, they are both capable, as Willstätter has shown, of forming addition complexes with carbonic acid. The necessary conditions for photocatalysis are thus fulfilled.

The first question which arises is the one as to whether both of the chlorophylls are used as photocatalysts by the plant. In connection with this, the view is held that the true photocatalyst is chlorophyll a , and that it is this pigment from which the carbonic acid complex results. In the first place, chlorophyll a is always present in much larger quantities than chlorophyll b, the normal ratio of moles chlorophyll a to moles chlorophyll b being about 3. In brown algae (Phaeophycea) the chlorophyll is almost exclusively chlorophyll a (the accompanying pigment in this case is Fucoxanthine). Secondly, Willstätter and Stoll's experiments on the affinities of the chlorophylls for carbonic acid show that chlorophyll a has a markedly greater affinity for this than has chlorophyll b.

These two considerations, taken in conjunction with the fact that there is always present a great excess of chlorophyll molecules over the normal supply of carbonic acid molecules, render the supposition that chlorophyll a combines with the carbonic acid supplied within the chloroplast[1] very probable. This view is further supported by the literature available on the influence of the wave length of the light on the magnitude of the assimilatory effect. The following are the limits of wave length within which a maximum effect has been obtained by

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[1] Mechanism of the supply and transport of carbon dioxide from the air to the pigment within the chloroplast. p.143.

various authors:-

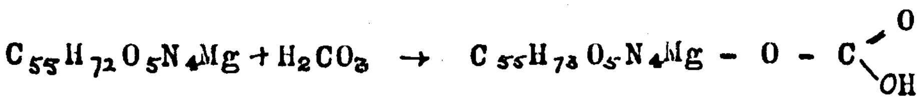
Engelmann.[1]	1882.	685 - 655μμ.	Bot.Ztg.1882,p.419.
Timiriazeff[2].	1890.	685 - 655μμ.	Compt.rend.110,1346,(1890).
Dangeard[3].	1911.	670 - 655μμ.	Compt.rend.,152,277,967,(1911) 1911
Ursprung[4].	1918.	687 - 656μμ.	Ber.deut.bot.Ges.,36,73,86,(18 18

The principal red band of each chlorophyll component has its head situated as follows:-

Chlorophyll a	666 - 657 μμ.
Chlorophyll b	646 - 640μμ.

It is apparent from these figures that the light chiefly absorbed by chlorophyll a is most active in causing assimilation.

It is therefore considered that the first step in the process is the formation of the complex of chlorophyll a with carbonic acid, which can be represented as -



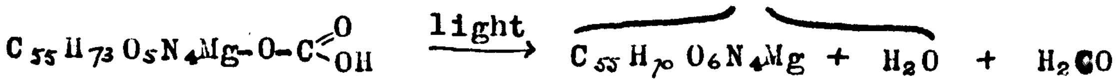
$$C_{55}H_{72}O_5N_4Mg + H_2CO_3 \rightarrow C_{55}H_{73}O_5N_4Mg - O - C\begin{smallmatrix}\nearrow O\\ \searrow OH\end{smallmatrix}$$

chlorophyll a

if we adopt the suggestions of Willstätter as regards the constitution of the chlorophyll carbonic acid complex.

The photocatalysis takes place within this complex, formaldehyde is formed, and splits off in a form of high energy content.

The oxygen is not regarded as splitting off as such - i.e., in the way Willstätter has suggested, but as a result of the process, chlorophyll b is left, and not the a which originally entered into the action. This rearrangement can be depicted in the form of the equation



$$C_{55}H_{73}O_5N_4Mg-O-C\begin{smallmatrix}\nearrow O\\ \searrow OH\end{smallmatrix} \xrightarrow{light} \overbrace{C_{55}H_{70}O_6N_4Mg + H_2O} + H_2CO$$

chlorophyll a - carbonic acid complex.

chlorophyll b

formaldehyde in a reactive state of high energy content.

If a process similar to this actually occurs, then there must be present in the chloroplast some agency for reconverting the chlorophyll b to chlorophyll a, for we know that the ratio of a to b is little altered by changing conditions of assimilation. Such a mechanism would seem to be at hand, for we have two other pigments present, whose molecules differ by just the requisite quantity of oxygen - two atoms. There is not only this difference to give rise to the conception, for we know - Willstätter and Stoll's figures show it - that the ratio of carotin to xanthophyll present is actually decreased on increasing the rate at which assimilation proceeds. Consequently the following is proposed as a further stage in the process:-

$$\overbrace{C_{55}H_{70}O_6N_4Mg + H_2O} + C_{40}H_{56} \rightarrow C_{55}H_{72}O_5N_4Mg + C_{40}H_{56}O_2$$

Chlorophyll b carotin chlorophyll a xanthophyll

This has pushed the difficulty a little further, for although the suggested mechanism, as it stands, allows of the chlorophyll ratio remaining constant, the supply of carotin for the reduction would rapidly vanish unless it were renewed by a conversion of xanthophyll back to carotin. As a result of the changes which effect this, a molecule of oxygen has to be evolved as such, and it is this condition which has rendered the process somewhat difficult of conception.

The conversion of xanthophyll into carotin is apparently an accomplished fact, according to the claims of Ewart[1]. Ewart was unable to effect the conversion of xanthophyll into carotin by the action of either aqueous or glycerolic extracts of plant reductases, but obtained satisfactory results by using metallic "reductases". Magnesium dust rapidly and zinc dust slowly reduce

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[1] Ewart.- Proc.Roy.Soc.Vict.30(N.S.), 178, (1918).

xanthophyll, present in a clear, yellow, aqueous alcoholic solution, to carotin, which is thrown out of solution, leaving the liquid almost colourless. It is thought that just as there is a mechanism for importing carbonic acid to the chloroplast from the leaf surface, there must be some parallel kind of an arrangement whereby the oxygen is carried away from the chloroplast towards the leaf surface, where it can be released without damaging any of the bodies in the chloroplast. The xanthophyll in the presence of light might well be reduced by some colourless constituent of the plasm, the oxygen being loosely bound to this in such a manner that it is easily liberated later, probably by the action of an enzyme present outside the chloroplasts. For example, the enzyme Catalase, which is to be found in all plants, and which has the power of decomposing hydrogen peroxide with the evolution of oxygen, might be responsible for the carrying out of this process.

It must be emphasised that these views are at present only speculative and are unsupported by any direct experimental evidence. They would seem, however, to be very plausible as a tentative effort in the direction of the elucidation of the problem. The hypothesis is in agreement with, and suffers no contradictions from facts as they are known at present. It should be possible, however, to obtain conclusive experimental evidence in its support, work with this aim having commenced. Pure preparations of the four leaf pigments are being made, and when sufficient of these have been obtained it is intended to attempt to demonstrate (1), the existence of an equilibrium such as has been described between the two chlorophylls and carotin, and (2), the possibility of the conversion of xanthophyll to carotin. The present state of this work is described in following pages.

It will be remembered that the formaldehyde which is formed as a result of this first stage of photosynthesis is supposed to be in an activated form of high energy content, and to require no further energy for its polymerisation. Both from a consideration of the products of photosynthesis within the plant, and from the parallel experiments which have been carried out on the photopolymerisation of formaldehyde solutions, it would appear that this polymerisation has as its result the formation of a body with a six-carbon unit, a body which is capable of yielding various hexoses on rearrangement. The nature of the hexose body first produced from formaldehyde has been discussed in a recent paper by Heilbron[1] from which the following quotation is made :-

"It seems as if the activated formaldehyde has the predisposition for polymerisation to the six-carbon unit, even when the experiments are conducted outside the living cell. It follows, therefore, that attempts to look for formaldehyde itself in the green leaf are obviously useless, for this predisposition which exists in the experiments carried out in vitro will undoubtedly be augmented under the ideal conditions existing within the chloroplastid where, moreover, concentration conditions

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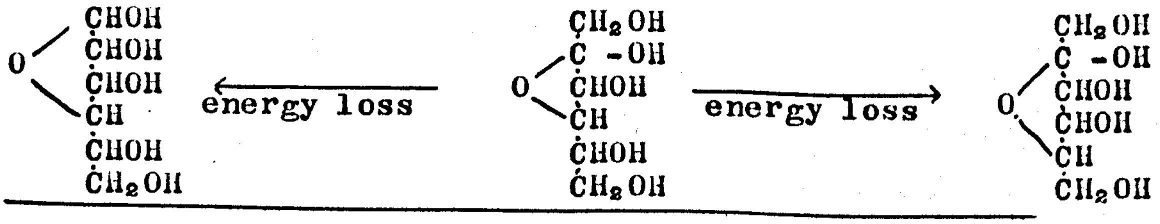
[1] Heilbron.-J.Soc.Chem.Ind., 41, 89R, (1922).

would readily favour maximum condensation. It has often been assumed that trioses might be a definite stage in the condensation of the formaldehyde, but these are not actually found in thev plant, nor are nonoses, which could equally well be anticipated on the assumption that hexose formation produced from the three carbon unit. It is more probable that the three carbon system so frequently met with among plant products results from the disintegration of the six-carbon system, and in this connection the work of C.L.Neuberg and his collaborators is of outstanding interest. Further support to this view, that the freshly synthesised active formaldehyde condenses directly to a hexose is afforded from a consideration of the generally accepted view that the first sugar detected in the leaf is sucrose, whilst at the same time starch formation occurs within the chloroplastid as a temporary reserve material. With regard to the constitution of sucrose, attention must be drawn to the recent work on the structure of this carbohydrate, wherein it has been established that the fructose part of the molecule is present in an active labile form containing either an ethylene oxide ring[1] or a propylene oxide ring[2]. In either case the active sugar would rapidly pass with loss of energy, into the stable form of the ordinary butylene oxide fructose, or, equally possible, into the butylene oxide glucose



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[1] Haworth & Law.-J.C.S., 109, 1314, (1916).
[2] Berseken & Couvert, Rec.trav.chim., 40, 354, (1921).

The latter would thus become the sourse of starch formation within the chloroplastid, whereas cane sugar would be formed between one molecule of the stable glucose and one molecule of freshly synthesised active fructose. The concensus of opinion seems to be that the quantity of fructose in the leaf is almost invariably in excess, which fact is readily explained on the above assumption.that an active ketohexose is the sugar first synthesised. If this view is correct, then the only process in the building up of the innumerable complex substances in plant synthesis must be derived from this source, and hence the function of the chlorophyll is narrowed down to the very definite operation of sugar formation".

With a view to obtaining evidence as to whether an active ethylene or propylene oxide form of sugar is first produced, it is intended to carry out photopolymerisations of formaldehyde in ultra-violet light in the presence of methyl sulphate and alkali. Preliminary experiments have shown definitely that a glucosidic body is formed, but no attempts have as yet been made to differentiate this from the methyl glucosides obtained by subsequent methylation of the isolated syrup.

In conclusion, some reference might be made to hitherto unpublished work which has been carried out by Baly, Heilbron and Hudson, and which sheds considerable illumination on the mechanism of nitrogen photosynthesis. The work has been confirmatory of an original suggestion due to O.Baudisch [1] and goes to show that activated formaldehyde reacts with nitrates and nitrites to yield formhydroxamic acid $HO.CH:NOH$. Quantites of this substance have been prepared, and it has been used as a starting point from

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[1] O.Baudisch.- Ber., 44, 1009, (1911).

which bodies belonging to the classes of the amino-acids and the alkaloids have been obtained. It is considered highly probable that similar reactions take place within the cell through the medium of active formaldehyde, which is the only product really photosynthesised, and the nitrate which is present in the leaf. This question of the formation of nitrogen-containing bodies within the plant comes under somewhat more ample discussion in the paper by Heilbron previously referred to.

Experimental Work.

The isolation of carotin from carrots.

Willstätter in his book on chlorophyll describes the method which he used for the preparation of carotin(see p.111 of this thesis). Judging from hisb results, although ordinary carrots are the most profitable source of this body, the quantities of the latter obtainable are very small. Submitting his carrots to an extraction in the cold with petrol ether, Willstätter succeeded in preparing 125 grams of carotin from 5000 kilos. of carrots. This must have necessitated work on a very karge scale, for which Willstätter must have had ample facilities. The task, therefore, of preparing even a comparatively small quantity of carotin seemed to be rather large, for no equipment was available for working with the large quantities of material that appeared to be necessary. Assuming that it was possible to obtain the same percentage yield of carotin that Willstätter obtained, 40 kilos. of carrots were necessary in order to obtain 1 gram of carotin, i.e., about 90 lbs.. The yield from this quantity seemed likely to be much less than 1 gram for such reasons as inexperience in handling bodies of this kind, the relatively small quantity being worked up, and lack of apparatus such as vacuum driers, percolators, hydraulic presses, etc..

1½ cwt. of carrots were obtained and the first experiments were carried out with the idea of trying to getx a better method of extraction. Small quantities of carrots were minced up and dried in various ways. It was found to be most expedient to dry the minced carrots in the steam oven at about 85 - 90°C. If the drying were carried out much above this temperature, charring occurred, the carrots turning black. At lower temperatures the drying was very tedious and the product apparently little better in quality. It was found necessary to put the carrots to dry as soon as they were minced, as otherwise a slow fermentation of the minced carrots set in, a bad smell being evolved and the carrots drying black.

Preliminary experiments were also performed with various solvents in the hot and in the cold. In the case of the experiments with cold solvents, about 100 gm. of the meal were treated with three or four portions of the solvent in question, and the minute quantities of carotin obtained compared. In the case of hot extraction, 100 gm. of the finely powdered dried carrots were placed in a Soxhlet apparatus, and extracted until the liquid syphoning over was no longer coloured. The quantities of carotin obtained were brought on to a filter paper and compared. As a result of these experiments it was found advisable to extract the ground carrots with about six portions of carbon disulphide in the cold, allowing each portion of the solvent to remain in contact with the meal for from one to two hours. No further portions of solvent were applied when the colour of the extract had changed from red to a yellowish-orange. It was found that a further quantity of carotin could be obtained by drying the cold-extracted meal, finely regrinding in a coffee mill and then extracting with carbon disulphide for some hours in a Soxhlet apparatus. The quantity yielded by this second ~~xx~~ treatment, however, was hardly worth working up with the other extracts because of the relatively large quantities of waxy and resinous bodies which were removed as well as the carotin.

The following method was subsequently adopted as a result of the preliminary experiments carried out :-

The carrots were cleaned and then minced up as finely as possible, the largest size of household mincer obtainable being used, together with the finest cutter provided. The minced carrots were then immediately placed in a shallow layer on the base of a steam oven, kept at a temperature of between 85° and 95° C. The layer was stirred up frequently, and was generally dry after about six or seven hours in the oven. The dried carrot was then ground up to a fine powder in a coffee mill. 100 - 200 gm. of the powder were placed in each of several 3-litre flasks and treated with about a litre of carbon disulphide, the flasks then being put away in the dark for 1 - 2 hours, and being periodically shaken during this period. The extracts were filtered into a large bottle. As stated above, about five or six extractions of about one litre each were necessary. The extracts were rapidly concentrated as far as possible and the concentrated extracts collected in a flask which was kept in the dark.

When all the carrots had been treated in the above manner, the combined extracts were further concentrated in as short a time as possible, and the residue kept in a crystallising dish in a vacuum exsiccator in the dark, flaked paraffin wax being also present in order to absorb some carbon disulphide. The latter was gradually removed by successive evacuations, a very viscous, gummy matter, which deposited some solids, being left. If this was treated with a small quantity of petrol ether this latter tended to dissolve much of the resinous material, as well as some of the carotin. Much of the latter was deposited as minute crystals, which could only be separated by decanting the supernatant solution. For this purpose, the liquid was introduced into a narrow test tube, and the crystals allowed to settle. The clear liquid was then decanted, and the residual crystals washed several times with small quantities of petrol ether, being finally brought on to a small filter paper. As much care as possible was taken throughout all operations to keep the carotin solutions in the dark, and out of contact with air.

It has regretfully to be recorded that all the first preparation of carotin, obtained as a result of two months' work was lost, owing to the collapse of a large vacuum exsiccator, following exhaustion. The preparation of carotin is at present in progress.

The Separation of the Two Chlorophyll Components. (p.94)

The separation is based on a difference in distribution of the two chlorophylls between petrol ether and aqueous methyl alcohol. Chlorophyll a is predominant in the petrol ethereal phase, while most of the b component transfers itself to the alcoholic layer. As can be seen by reference to the description of the properties of the two components, neither are very soluble in volatile petrol ether itself, but are much more soluble in petrol ether containing only very small amounts of methyl alcohol. Consequently a certain initial concentration on the chlorophylls must not be exceeded, so that the pigment may remain dissolved in the petrol ether. This initial concentration of pigment is given by Willstätter and Stoll as 2gm. of chlorophyll per litre of petrol ether. The petrol ether ought to be of a sp.gr.0.64 - 0.66.

The yields that were obtained after a very careful adhesion to the directions fell considerably short of those quoted by Willstätter and Stoll. These moderate yields are attributed to the joint action of some or all of the following three factors:-

(1). The chlorophyll mixture used as a starting point was not of a sufficiently high standard. It was supplied by Merck, but should have been submitted to a purification process before use, this being omitted owing to too optimistic an estimate of its purity.

A preliminary careful purification of the chlorophyll to be used must be urged as highly desirable.

(2). English petrol ether contains more of the unsaturated hydrocarbons than the German, the hydrocarbons in which are all saturated. Doubtless the solubilities of the components will be considerably influenced by the presence of these unsaturated hydrocarbons. In order to push the yields of the pure components as high as possible therefore, the petrol ether used should be as free as possible from unsaturated hydrocarbons.

(3). The methyl alcohol supplied to order was of a very poor quality and besides being very aqueous, contained considerable quantities of acetone and resinous bodies. The alcohol was allowed to stand for a fortnight over lime, and was subsequently refluxed for three hours and distilled over lime three times in succession. The product obtained as a result of these purification operations had a gravity of from .805 to .810 / 15°C. It still contained acetone, which was detectable by smell. A sample was treated with iodine and alkali, a crystalline specimen of iodoform being obtained, whose bulk showed the presence of about 2% of acetone in the purified alcohol. Removal of this from so large a quantity of methyl alcohol (12 gallons) was quite impracticable. As the chlorophylls are very soluble in acetone, it is possible that this 2% in the methyl alcohol caused the latter to take out more chlorophyll a, this being later washed out by fresh petrol ether.

The following list is appended as indicating the lines of work either at present in progress, or contemplated :-

Preparation of Carotin.
Preparation of Xanthophyll.
Investigations on Pigment Estimation.
Investigations on Pigment Equilibrium.
Determination of the efficiency of potassium bichromate
  solutions as screens for the cutting out of the light
  absorbed by the carotinoids.
Analysis of the methylated sugars.
Comparison of methyl glucosides obtained from polymerisations
  in the presence of methyl sulphate and alkali with the
  methylated syrups.
Preparation of, and examination of bodies formed simultaneously
  to sugars on photopolymerisation of formaldehyde solutions.

Literature based on the work described in this Thesis.

The Photochemical Reaction between Hydrogen and Chlorine and its Variation with the Intensity of the Light.
Edward Charles Cyril Baly & William Francis Barker,
Trans.Chem.Soc.,119,653,(1921).

Photocatalysis.Part I. The Synthesis of Formaldehyde and Carbohydrates from Carbon Dioxide and Water.
Edward Charles Cyril Baly,Isidor Morris Heilbron,
and William Francis Barker. ibid.p. 1025.

The Formation of Carbohydrates in Plants.
Edward Charles Cyril Baly and Isidor Morris Heilbron.
J.Soc.Chem.Ind.,40,337R,(1921).

Photosynthesis and the Functions of Pigments in the living Plant.
E.C.C.Baly,C.B.E.,F.R.S.
J.Soc.Dyers & Colourists,38,4,(1922).

The Photo- and Phyto-synthesis of Plant Products.
I.M.Heilbron. J.Soc.Chem.Ind.,41,89R,(1922).

Appendix.

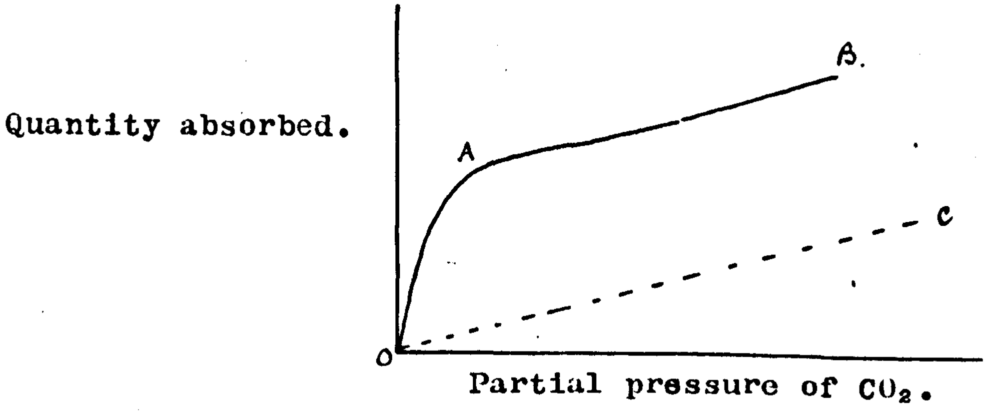
The Mechanism of the Supply and Transport of Carbon Dioxide from the air to the Pigment within the Chloroplast.

The chlorophylls occur in the midst of the chloroplast cells, in which particles of starch often appear after an active period of assimilation. The fact that chlorophyll occurs in the cells negatives any suggestion that this body acts as the direct absorbent of carbon dioxide from the air circulating through the leaf.

It has long been known that many parts of the plant which are totally free from chlorophyll are capable of absorbing carbon dioxide. For instance as long ago as 1804 Th.de Saussure noticed that moist portions of plants absorbed carbon dioxide. In 1876 it was mentioned by Böhm in the Annalen that the absorption of carbon dioxide is not necessarily dependent on the cell juice. Twigs which he had dried for a considerable period at 100° were shown to absorb carbon dioxide in quite considerable quantities. This observation was confirmed by Borodin in 1881, who published detailed data on the behaviour of seeds in the presence of carbon dioxide. In one of his experiments, Borodin placed in a graduated tube 15 seeds which occupied a volume of 17 c.c.. He displaced the air from the tube by means of a rapid current of carbon dioxide, and sealed the tube over mercury. He observed that more than 3 c.c. of the gas were absorbed. A corresponding evolution took place when the carbon dioxide was replaced by ordinary air once more. An absorption of a similar nature would be expected in leaves, and this was first observed by Willstätter and Stoll, who found that slightly moistened leaves rapidly take up carbon dioxide from a gas stream being led over them, which they do to the extent of about 0.3% of their dry weight.

Such an absorption and emission would seem to indicate that there is present a substance which is capable of forming with carbon dioxide an addition product which is dissociable. If this is so, then a dependence of the amount of carbon dioxide absorption and emmission on temperature must be expected as well as a dependence on the partial pressure of the carbon dioxide.

For a case of simple solution of the carbon dioxide, the amount absorbed is proportional to the partial pressure of the carbon dioxide - Henry's Law. Graphically represented the relation would be shown by the dotted line OC



Suppose, however, that we have a substance X present as well which forms a compound with carbon dioxide thus :- $X + CO_2 \rightleftarrows Y$ Naturally more CO_2 will be absorbed than by water alone. The absorption curve will then be like OA, until almost all of X is transformed into Y. From this point the absorption curve will have almost the same slope as the solubility curve of CO_2 in water (portion AB of curve).

Willstätter and Stoll have shown very conclusively by their experiments that there do exist compounds such as X in living leaves, and that these are the means of the conveyance of the carbon dioxide to the active centres of photosynthesis. Their curves all follow the general trend of those expressing the solution of a gas with the formation of a dissociating chemical compound. At higher pressures chemical saturation is reached, and the increase of solubility of the gas in water then makes itself felt. At higher partial pressures the tension curve follows much more closely the solubility curve of carbon dioxide in water.

The difference between the absorptive capacities of the substance of the leaf and of water becomes greater the lower the partial pressure of the carbon dioxide. For instance, while with 5% CO_2 present the absorption of a Helianthus leaf is just over three times the solubility in water, with 1% CO_2 it is about 6 times. Thus the absorption phenomenon is the more noticeable the lower the concentration of the carbon dioxide - that is, the more closely the experimental conditions approach those actually obtaining naturally.

It must be borne in mind that what has just been discussed refers to the carbon dioxide absorption of the unilluminated leaf. No relation has thus far been brought forward between the carbon dioxide absorption of the unilluminated leaf and the assimilatory process. It must be apparent, however, that the affinity of substances in the leaf for carbon dioxide will influence the behaviour of leaves in light.

A further feature of this absorption mechanism is that it can effect a certain regulation in the amount of assimilation in the event of temperature variation. At lower temperatures the velocity of the photosynthetic process is naturally smaller. The absorption effect is, on the contrary, greater, and is able to balance to a certain extent the reduced rate of photosynthesis by supplying an increased concentration of the material undergoing transformation. In this connection it might be mentioned that chlorophyll is always present in a considerable excess, so that if more materials are supplied, more chlorophyll molecules will function as transformers.

The chemical nature of the body or bodies which absorb the carbon dioxide into the leaf is, up to the present, unknown. The substance of dried leaves shows an amphoteric character, a considerable quantity of mineral acid or caustic alkali being used up before the appearance of an acid or alkaline reaction respectively. The dissociation tension values leave out of the question bodies such as the alkali or alkaline earth bicarbonates, for these have too small dissociation pressures. These values, and the chemical conditions in the protoplasm seem to rule out all but organic bodies as the absorbents. Willstätter has compared the conditions of CO_2 absorption by the leaf with those of the absorption of carbon dioxide by the blood, and has shown how very similar are the two phenomena. The dissociation tension curves of the absorbents in blood are very similar to those of the absorbents in leaves. The dissociation values of the carbon dioxide carriers in the blood at 38°-i.e., the normal body temperature of most mammals, and of the CO_2 absorbents in leaves at from 5°- 15°, the temperatures generally experienced by plants, are very nearly the same. He suggests that the same type of body is responsible for the absorption in both cases.

Bodies which could function as such carriers are plasmic bodies containg amino-groups, the CO_2 being absorbed with the formation of carbamates. For instance

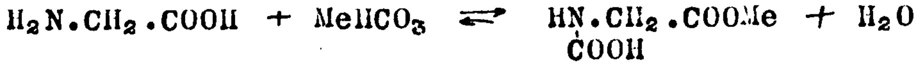
$$H - \underset{|}{N} - H + CO_2 \rightleftarrows H - \underset{|}{N} - \boxed{COOH} \quad ,$$

or bodies containing enolate groupings such as

$$\underset{|}{O} - H + CO_2 \rightleftarrows \underset{|}{O} - \boxed{COOH}$$

forming carbonic esters.

The carbamino reaction is very probable, for there are present both in leaves and in blood many neutral amino-acids or proteins which could so function. Many such bodies cannot exist in the presence of bicarbonates without reacting with them. The bicarbonate adds on in this manner :-



$$H_2N.CH_2.COOH + MeHCO_3 \rightleftarrows \underset{COOH}{HN}.CH_2.COOMe + H_2O$$

The addition product is a monobasic acid. The presence of basic groups, or the saturation of the carboxyl by means of alkali favours the addition reaction, the secondary salt being formed. Further investigations as to the nature of these absorbent bodies should be of considerable interest.