S T U D I E S

ON THE PHOTO-OXIDATION OF CHLOROPHYLL.

A THESIS

for the Degree of Doctor of Philosophy

presented by

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CONTENTS.

GENERAL INTRODUCTION.

Two recent collections of papers[1,2.] concerned in large measure with the reactions of molecular oxygen exemplify the awakened interest in this subject. With the rapid expansion of research in applied science of the war and post-war years the many problems of the immense destructive power of atmospheric oxygen are being widely investigated. Oxygen destroys not only when fire sweeps through warehouse or forest, but in countless more subtle ways, unobtrusively and more slowly away from the heat of the flame, but just as surely. The perishing of plastics and elastomers, natural and synthetic, the tendering of textile fibres and the weathering and fading of pigments and dyestuffs, all processes ascribed in part at least to the action of oxygen, describe the end of usefulness and the need for replacement. In response to the economic demands of users of such materials and possibly in response to more altruistic urges latent in man, technical laboratories seek for substances empirically more resistant while schools of fundamental chemistry tackle the problem at its root in the mechanism of the primary acts between organic

molecules and molecular oxygen.

The current definition of oxidation in terms of electron and proton transfer processes has made necessary a separate word descriptive of those oxidation reactions involving molecular oxygen, the term 'autoxidation' having been chosen.[3].

The autoxidation of organic molecules of moderate complexity is most conveniently studied in systems involving a liquid phase, and under suitable conditions kinetic considerations, classically applied to gas phase reactions, may be readily used in interpreting the experimental observations.

Thermal autoxidation in liquid systems generally proceeds by chain mechanisms initiated by the formation, by removal of relatively labile hydrogen atoms, of organic radicals readily attacked by molecular oxygen.[4]. The participation of a catalytic radical forming species appears to be necessary for the initiation of those chains in saturated compounds, but under certain conditions, involving unsaturated molecules, molecular oxygen itself appears capable of accomplishing the primary radical forming step, by attack upon a double bond.[5].

Commercially the most important autoxidation processes take place in rigid media. The term

'rigid' is perhaps to be preferred to that of 'solid' as the elastomers, plastics and fibres concerned do not markedly display the quality of crystallinity, a usual criterion of the truly solid state. However, the macromolecules of fibres do generally have a degree of orientation along the axis of the fibre[6]. and crepe rubber, an elastomer whose component molecules are normally orientated in a completely random manner, gives a crystalloid X-ray diffraction pattern when fully stretched.[7].

Dark autoxidation processes in such systems are not unknown, but are generally very slow and much less important than those which proceed only on absorption of light. In the pure state these macromolecular species are colourless, absorbing light only in the non-actinic infra-red and in ultra-violet spectral regions, which while able to bring about chemical reaction, are not present in sunlight. Normally, however, there are various 'fillers' and colouring matters present which absorb strongly in the visible or near ultra-violet, actinic spectral regions, present in sunlight.

The most extensively investigated field of this nature is that of the 'tendering' of dyed

textile fibres. All of the normal textile fibres, protein fibres, cellulosic fibres including the various rayons, and the synthetic fibre nylon, are susceptible to tendering. Light energy absorbed by the dyestuff brings about an autoxidative degradation of the textile substrate with loss of valuable physical properties such as tensile strength.[8] While tendering is often accompanied by fading of the dyestuff the processes may be apparently independent, and true photosensitisation can occur, marked degradation of the fibre taking place without detectable change in the dyestuff.

The experimental procedure adopted by many workers has been to determine the "extent of degradation of the dyed fibre exposed under controlled conditions, by measurement of the change in the tensile strength or of the viscosity when dissolved in some suitable solvent.[9] Both of these methods give a measure of the decrease in the average chain length of the molecules of the fibre, and it is apparent that considerable degradation will be indicated when only a small fraction of the total number of residues constituting the molecular chains have been chemically modified.

The supplementary approach by identifi-

cation and estimation of the solid and the gaseous products of the important early stages of degradation would require very delicate analytical techniques and has not been successfully attempted. Hydrogen peroxide has been identified as a gaseous reaction product, in the presence of water vapour, and probably accounts for much, but not all of the degradation observed under normal conditions.[10].

While the destruction of organic systems by molecular oxygen on the largest possible scale is instigated by light energy through the agency of coloured compounds, there is another, and in this instance creative activity of the latter combination of which mention must be made. In green plants radiant energy is utilised, on absorption by the organic pigment chlorophyll, for the synthesis from carbon dioxide and water, of high energy carbohydrate materials. The subsequent reactions of these constitute the whole chemical basis of life, in which the macromolecular species we have considered, the proteins and celluloses, play, in bulk at least, no small part.

The nature of the energy transformations involved in this green plant photosynthesis are such as to make the problems of the subject some of the

most fascinating in chemistry. Light energy is stored as chemical energy, the free energy of the system being increased by the action of oxidation-reduction systems against the normal potential gradient. These processes are understood in principle, and model, in vitro, systems potentially capable of similar energy transformations have recently been investigated,[11.] but as yet a detailed chemical picture is largely lacking, particularly in respect of the mechanism of the primary photochemical acts, and the stabilisation of the resulting high energy products.

For both photosensitised autoxidation and the photosynthetic process much of the information desired, particularly as to the nature of the primary acts between the excited pigment molecule and the other molecular species concerned, cannot be obtained from a study of these systems as a whole. There is, however, a mass of experimental observation on what may be considered as isolated fragments of these systems. The results of studies on fluorescence and phosphorescence and their quenching,[12.] on the magnetic properties of excited dyestuffs,[13.] on the photo-conductivity of solid pigments and pigment solutions,[14.] to mention but a

few of the current lines of research, may all be utilised, with due caution, in the derivation of the final picture of the processes involved. It is in this light that the experimental work and the discussion of this present thesis should be regarded.

The present work is mainly concerned with the photo-oxidation of chlorophyll preparations in the form of thin solid films deposited on thallous bromide crystals and various other supporting solids or substrates. Direct observation was made of the decrease in the oxygen pressure over these deposits on illumination.

Theoretically, certain advantages result from the relative immobility of the main organic reactants and products, as the primary stages of the reaction are less liable to be obscured by possible secondary reactions. On the other hand experimental difficulties may arise from the low quantum efficiencies to be expected from comparison with those obtained in colour fading observations on similar thin films of dyestuffs.[15.] For accurate work an extremely sensitive and reliable experimental method is obviously essential, especially as the use of extremely thin films, with consequent low light absorption, is desirable if complications

due to oxygen diffusion processes are to be avoided.

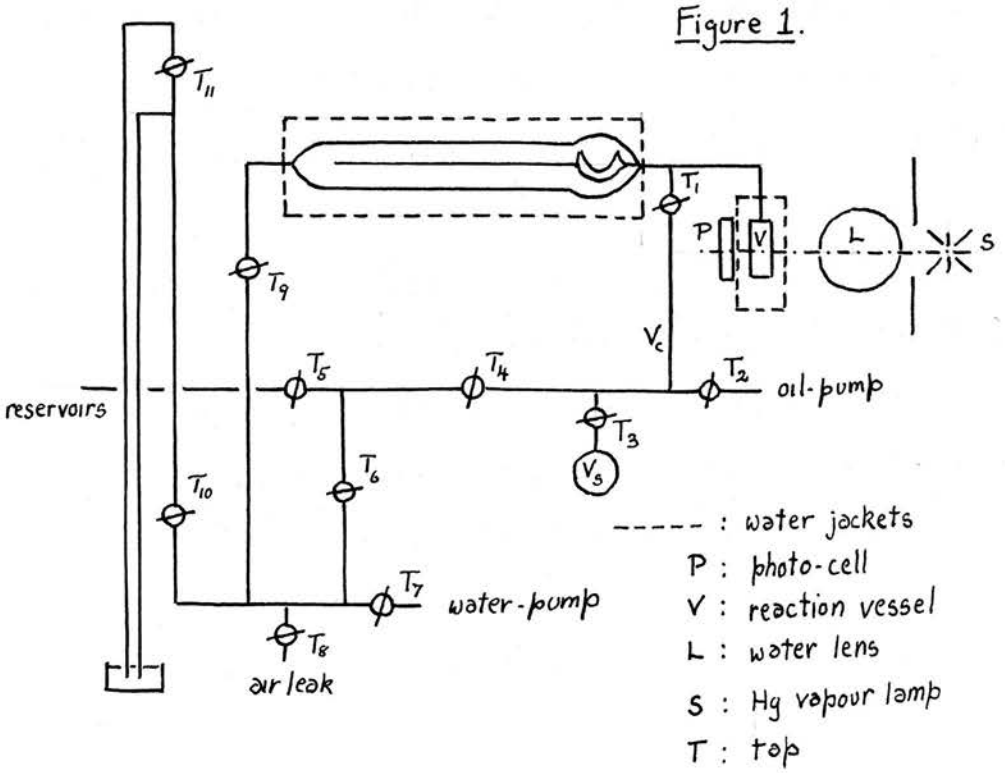
The choice of chlorophyll and the supporting solid thallous bromide as suitable materials for the development of these methods followed from the unpublished observation that chlorophyll acts as a weak desensitiser of thallous bromide gelatin photographic plates. In addition, the instability of the pigment chlorophyll towards light and oxygen has been known for some time,[16.] and its oxidation reactions in solution studied from the point of view of their possible importance in the green plant photosynthetic process.[17.] Such studies provide a background against which the results of the present work may be discussed.

APPARATUS AND GENERAL EXPERIMENTAL TECHNIQUES.

In the experiments of the present work, the pressure change in the gas phase over the solid, system under investigation was recorded by means of a Bourdon glass sickle guage, used differentially. Reaction vessels of soft glass and of silica glass were used.

Illumination was provided by a mercury vapour lamp and a suitable optical train. A photo cell was incorporated in the optical system when measurement of the transmitted light intensity was required. The apparatus finally adopted is sketched in Fig. 1.

Figure 1.



The apparatus was constructed of soda glass throughout. Taps, whenever possible, were of the hollow-barrelled oblique-bore variety. All taps and conical joints were lubricated with Apiezon Grease L.

The Bourdon Gauge. The pressure-sensitive sickle of the gauge was mounted as indicated in Fig. 1. The position of the tip of the gauge pointer was read by means of a telescope with scale, and the sensitivity of the gauge determined, as outlined below, in terms of mm. of pressure change per division of the telescope scale. In general the gauge was used to record only the small changes in pressure occurring during reaction, the absolute pressure of gas within the reaction vessel being measured by means of the mercury manometer. As the maximum pressure that might safely be exerted over the gauge sickle was about 10 mm. care was required in the introduction and removal of gases.

The Calibration of the Bourdon Gauge. To ensure that no change in sensitivity with change in operating pressure takes place the gauge was calibrated for low (0 - 5 mm. Hg) medium (c. 50 mm.) and high (c. 300 mm.) absolute pressures within the apparatus, by the following procedure. The

appropriate absolute pressure of dry air was introduced into the apparatus. The reaction vessel and gauge sickle were separated from the gauge envelope and the manometer by closing tap T_4 (Fig. 1). Careful initial readings of the manometer and the gauge were made. Using the filter pump vacuum line air was withdrawn from the manometer side of the apparatus through Tap T_7 until the gauge reading had risen to as high a value as was intended to be used during experiment (only the central portion of the scale was used in order to ensure a strictly linear relationship between pressure and scale reading). Tap T_7 was closed and this maximum gauge reading noted.

Withdrawal of air from the reaction vessel side of the apparatus through tap T_2 decreased the pressure on that side of the gauge until the minimum permissable gauge reading had been obtained. This reading was also noted.

The process of withdrawing air from each side of the apparatus alternately was continued, a series of minimum and maximum gauge readings being obtained, until the absolute pressure, as measured by the manometer, had fallen by about 5 mm., when final readings of the gauge and of the manometer

were made.

The rise in absolute pressure, in mm. Hg, was divided by the total traverse of the gauge pointer in the direction of minimum to maximum, in scale divisions, to give the gauge sensitivity in terms of mm. per scale division.

The results for a typical gauge calibration are reported below. Two temperatures of the gauge thermostatting jacket, 20 and 25°C. were used.

Table 1.

Temperature of Gauge Jacket °C.	Average Absolute Pressure mm. Hg.	Gauge Sensitivity mm/ scale div.
25°	4.5	0.0237
	53.3	0.0245
	295.6	0.0241
20°	55.6	0.0239

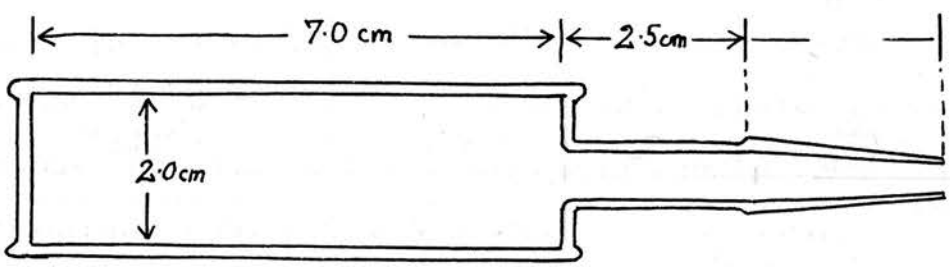
Average Sensitivity 0.024.

It is to be noted that the sensitivity of the gauge is independent of the absolute pressure within the apparatus, and the temperature of the gauge jacket, in the range 20 - 25°C.

Reaction Vessels. In the preliminary experiments spherical soft glass vessels of about 70 ml. capacity, attached to the apparatus by B 10 ground

glass joints, were used, and cylindrical quartz vessels, of the dimensions indicated in Fig. 2, in the later sections of the work. The ultra-violet light transmitting properties of the quartz vessels were not utilised, and soft glass was used at various positions in the optical system, as the lamp envelope, the water-lens flask walls and the window of the vessel thermostat jacket (Fig. 1.)

Figure 2.



For certain of the calculations of later sections of the work an accurate measure of the total gas space within the vessel, the gauge sickle and the capillary lines connecting these, was required. This was determined by noting the fall in pressure occasioned by allowing oxygen in the vessel and gauge sickle at a known pressure and at room temperature to expand, first into the evacuated connecting lines and then into a side-flask of accurately known volume (see Fig. 1). The gauge pointer was maintained in its rest position by

simultaneously lowering the pressure in the gauge jacket, this decrease in pressure being measured on the mercury manometer.

Let V, V_c and V_s be the volumes of the vessel and gauge, the connection lines and the calibrated side flask respectively. At 17°C (room temp.) the pressures for the various volumes in a typical case were as follows:

$P_{(V)}$ = 99.7 mm., $P(V + V_c)$ = 50.3 mm.,

$P(V + V_c + V_s)$ = 33.2 mm.

Volume V_s = 44.7 ml.

then $997V = 503\ (V + V_c)$, $\therefore V_c = V\ \frac{494}{503}$

also $997V = 332\ (V + V_c + V_s)$

$= 332\ (V + V\ \frac{494}{503} + 44.7)$

and $V = 43.7$ ml.

Duplicate experiments gave satisfactory concordant results ($\pm$ 0.1 ml.).

Thermostating. The gauge and reaction vessel were kept at a constant temperature by water jackets replenished from a constant head tank to which water was pumped from a thermostat. The water after passage through the jackets returned to the thermostat by gravity. A 250 watt heating element operated by a large chloroform-mercury

control and Sunvic vacuum switch maintained the temperature in the thermostat tank.

The flow from the header tank to the jackets was controlled by screw-clips on the rubber connecting tubes. The clips were adjusted prior to the commencement of the gauge readings so that about 250 ml. per minute passed through each jacket. The flow conditions were not disturbed during any run, and under these conditions and with a vessel gas space of 80 ml. and 100 mm. pressure of gas the variation in scale reading due to temperature fluctuation was limited to $\pm$ 0.05, corresponding to a pressure change across the gauge of 1.5×10^{-3} mm.

Light Source and Optical System. The light source was a voltage-stabilised 125 W 230 V Osira mercury vapour lamp, having a 'pearl' soft glass envelope. This was placed close to a 4 cm. diameter aperture and shutter. Beyond the shutter a round bottomed flask of suitable diameter filled with water served as a heat-filter and condenser lens. The approximately parallel beam emerging from the water lens passed through any further filter solutions desired, and then through a window in the vessel thermostat jacket to the reaction vessel.

In the majority of the experiments the light used was passed only through the water lens and the soft glass of the lamp envelope, the flask, the thermostat jacket and the vessel. This light is referred to in the work as 'white' light. In some experiments a 1 cm. thickness of 6% cuprammonium sulphate was also interposed, the light in this case being termed 'blue' light. As shown by a spectrum photograph the 'blue' light consisted mainly of the mercury line 4358 A.U., with a little of the near ultra-violet lines 3650-63 A.U. present. The 'white' light contained a proportion of green light of wavelength 5460 A.U. No light of wavelength shorter than 3663 A.U. was transmitted by the soft glass of the optical system.

The oxygen used was taken from a cylinder, passed over soda-lime, calcium chloride and phosphorus pentoxide, and collected in reservoir bulbs attached permanently to the apparatus. In some experiments, such oxygen was further liquefied in a side-bulb by means of liquid nitrogen, and distilled with rejection of initial and final fractions. Neglect of such fractionation did not affect the results obtained.

Cylinder carbon dioxide was passed over

Calcium chloride and phosphorus pentoxide before collection in a reservoir bulb, then frozen out by liquid air, in a subsidiary bulb; uncondensed gas was removed by the pump and the last portion rejected. The process was repeated twice.

Freshly distilled water was 'boiled out' under reduced pressure in a small attached bulb prior to use.

Hydrogen was prepared electrolytically from baryta water, passed over hot copper, platinized asbestos, a white-hot tungsten filament, and phosphorus pentoxide before being colled to liquid air temperature prior to collection.

THE CHARACTERISATION AND SEPARATION OF THE CHLOROPHYLL PREPARATIONS.

Two separate commercial preparations of chlorophyll paste were used in the experiments reported in the present work. Both were obtained from McFarlan & Co.Ltd., of Edinburgh, but were not manufactured by that firm. We designate these preparations as 'chlorophyll Sample 1' and 'chlorophyll Sample 2'.

Chlorophyll Sample 1. was to some extent 'copper substituted.' To quote correspondence with the suppliers, "The chlorophyll we have supplied is alcohol extracted from green vegetable matter .... copper is added as a stabiliser and is present to the extent of 4 to 6 parts per 1000. The exact degree of substitution is not known but it will be far from complete." This figure of c.05% copper has been confirmed by colorimetric analysis.

The dark green paste was completely soluble in chloroform, soluble in methyl and ethyl alcohols, acetone and ether, leaving a slight solid residue, and was almost insoluble in carbon tetrachloride and petrol ether. The solutions in alcohol, acetone or ether were bright green in

colour, and showed no fluorescence in ultra violet light. The Molisch phase test (dil. alcoholic KOH added to an ether solution) was negative, no colour change being detectable.[18.]

Chlorophyll Sample 2. was specially supplied free from copper. Dark green in the paste form it showed very similar solubilities, the solid residue left in the case of acetone solution being approx. 1 % of the original. The colour in solution was yellow-green, and the solutions exhibited a brilliant red fluorescence in ultra violet light. The Molisch phase test was positive.

Left in contact with an aqueous solution of copper sulphate in the dark, an ether or acetone solution of chlorophyll Sample 2. assumed a bright green colour visually very similar to that of Sample 1. solutions. Simultaneously fluorescence disappeared and the phase test became negative, the process as judged by these tests, being complete after two or three days.

Chlorophyll Sample 2. would appear to be a natural mixture of chlorophylls a and b with the impurities, mainly carotene, attendant upon the method of preparation. Alcoholysis, with replacement of the phytol side chain to give ethyl chloro-

phyllide may have taken place to some extent during preparation. The good Molisch phase test indicates the absence of allomerisation, an obscure oxidative process which takes place in alcohol solution only and appears to involve the position 10 of the chlorophyll molecule.[19.]

The modification of the chlorophyll Sample 1. brought about by the partial copper substitution is not known but appears to affect all of the molecules. Addition of quantities of a Sample 1. acetone solution (non-fluorescent) to an acetone solution of Sample 2. diminishes the fluorescence of the latter, as estimated visually, only to an extent readily accounted for by internal filter effect. The absence of fluorescence in Sample 1. solution is not therefore due to quenching by a small proportion of copper substituted molecules but indicates modification of all of the chlorophyll molecules.

The negative phase test given by Sample 1. solutions cannot be taken as indicating extensive allomerisation, as the 'copper substituted' Sample 2. derivative, prepared in ether or acetone solution, wherein allomerisation is said not to take place, also does not give a positive phase test.

While chlorophyll Sample 1. and the

'copper substituted' Sample 2. derivative are similar in colour, non-fluorescent properties and response to the Molisch phase test no claim can be made to have proved them identical.

These chlorophyll preparations contain large amounts of impurities, mainly carotene, and the separation of these was undertaken by both solvent partition and chromatographic methods.

The solvent partition separation procedure adopted was a modification of that outlined by Mackinney.[20]

1.5 g. of the chlorophyll Sample 1. paste was dissolved in a mixture of 100 ml. methanol, 100 ml. acetone and 100 ml. petrol ether (30-70°), by shaking in the cold for several hours. The solution was filtered and the methanol and acetone removed by scrubbing with water in the apparatus sketched (Fig. 3). After repeating the scrubbing process some 5 times, about 90% of the chlorophyll had been precipitated in the petrol ether layer. Any xanthophyll impurities were removed in the aqueous layer while carotene remained in solution in the petrol ether.

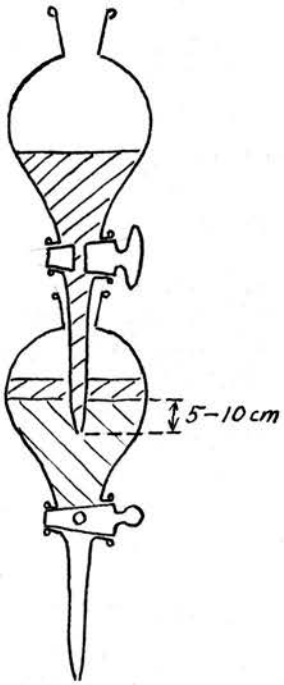


Figure 3.

1 litre separating funnels with tips drawn down to about 1 mm diameter.
400 ml water in each operation.
Taps to be left dry.

The petrol ether layer was centrifuged and decanted, any water in the bottom of the tubes being run off after piercing the chlorophyll crust, the residue being washed and centrifuged twice with fresh petrol ether. The chlorophyll thus obtained was taken up in acetone and stored in the refrigerator.

Such solvent partition methods require much manipulation and are not suitable for the isolation as well as the elimination of impurities. Chromatographic separation methods are better in these respects, but the amounts of material which may be readily processed are limited.

Adsorbents such as alumina, calcium

carbonate and sodium sulphate cause decomposition of the chlorophyll on the column.[21.] Sucrose, inulin and magnesium citrate hexahydrate have been reported as suitable adsorbents in chlorophyll chromatography,[22.] and solvent extracted bone meal has been used in carotene separation.[23.]

Sucrose Chromatography. 90 mesh sucrose was settled from a slurry in 60-80° petrol ether in a half-inch diameter column to a height of 5 inches. 0.082 g. of chlorophyll Sample 2 in petrol ether solution was introduced on to the column. The column was developed with petrol ether until all yellow pigment had been eluted. The green chlorophyll fraction remained immobile at the top of the column. Development was continued with a 9:1 mixture of pet-ether in benzene, when the green zone separated, a pale green zone remaining at the top of the column, and the rest moving slowly down. A proportion of acetone was finally added to the eluant and the mobile green zone washed from the column.

The solutions containing the green and the yellow fractions were evaporated to dryness in weighed vessels at the oil pump at temperatures not exceeding 18°C. Weighings were made to constant weight.

Chlorophyll Sample 2.

Original wt. of preparation	0.082 g.
Wt. of carotene fraction	0.0136 g.
Wt. %age of carotene	16.5%
Molar ratio carotene: chlorophyll	$\frac{16.5}{536} : \frac{83.5}{900} = 38:92$
Molar %age of carotene	29.0%

The chlorophyll fraction thus prepared retained its properties of fluorescence in solution and positive phase test apparently unaltered.

Chromatography on Solvent-extracted Bone Meal. The 100 mesh bone meal was settled from a slurry in 60-80° petrol-ether. About 0.1g. of the chlorophyll preparation in petrol-ether solution was placed on the column, and the carotene fraction eluted with petrol-ether.

The development of the chromatogram was continued with petrol-ether containing a little acetone and a little benzene (c. 5% of each) when the green zone, hitherto immobile at the top of the column was washed down. While slight separation into two zones was obtained with Sample 2. preparation no attempt was made to separate what were presumably chlorophylls a and b.

A small quantity of both Samples 1 and 2 were retained as a green zone at the top of the

column. This could be washed out with acetone.

Chlorophyll Sample 1.	Chlorophyll Sample 2.
Wt. % carotene 24%.	Wt.%age of carotene 17%
Wt. %chlorophyll 76%.	Wt.%age of chlorophyll 83%

The molar percentage of carotene in these preparations (approx. 30%) is so high that they must be regarded as chlorophyll-carotene mixtures and not as chlorophyll preparations containing carotene as an impurity. This is borne in mind in the discussions of results obtained using deposits of the unseparated materials.

THE PHOTO OXIDATION OF CHLOROPHYLL SAMPLE I.

I. PRELIMINARY EXPERIMENTS.

Preliminary experiments indicated that only in the presence of oxygen and light was there any detectable pressure change in the gas phase in contact with the illuminated chlorophyll film, the decrease in pressure then observed being presumably due to oxygen uptake.

In further preliminary studies particular attention was paid to factors which might influence the rate of this oxygen uptake. The effect of the additional presence of gases such as carbon dioxide and water vapour and solid phase factors such as the thickness of the film, the nature of the underlying solid support and the presence of other co-deposited pigments were all experimentally considered.

Chlorophyll on Glass.

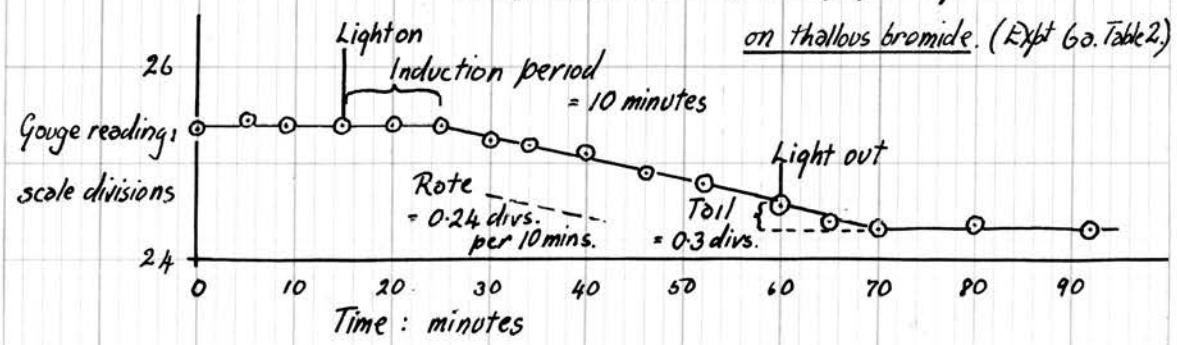
2.48 mg. of chlorophyll Sample I were deposited from alcohol solution on the inside walls of a spherical soft glass vessel of about 70 ml. capacity. The solvent was removed by a gentle air blast introduced into the vessel by a long small diameter jet. Fairly even deposition was obtained by manual rotation of the vessel during the deposi-

tion, the whole process being performed in very dim artificial light.

The vessel was attached to the apparatus and the film exposed to "white" light at 25°C under the conditions noted in Table 2. Fig. 4 is the plot of gauge reading against time for a typical "run", or exposure.

Figure 4.

The photo-oxidation of chlorophyll Sample 1 on thallous bromide. (Expt 60. Table 2.)



There was no pressure decrease in the dark in any experiment. At "light on" the rate of pressure decrease did not immediately reach its maximum value, there being a pronounced induction period, or more properly an acceleration period which may be given a numerical value in minutes, derived as indicated. For the short exposure times of these experiments (30 - 60 mins.) the pressure decreased linearly with time after the acceleration period, and the rate, expressed here as scale

divisions per ten minutes, could be obtained with fair accuracy. On the cessation of illumination the pressure continued to decrease for some minutes, giving a "tail". The extent of this was evaluated as a pressure decrease.

Table 2.

Expt.	CO_2 pressure. (mm.)	H_2O (mm.)	Rate scale divs./ 10 mins.	Induction period mins.	'Tail' scale divs.
1	-	-	0.0	-	-
1a	50.0	3.6	0.0	-	-
2	-	-	0.18	5	0.1
3	-	-	0.27	20	0.0
4	-	-	0.26	5	0.1
4a	489	-	0.22	10	0.0
5	-	7.0	0.18	-	-
6	-	3.8	0.23	6	0.05
6a	49.0	3.8	0.24	10	0.3
6b	49.0	3.8	0.24	-	0.1
7	-	-	0.20	10	0.15
7a	-	6.0	0.25	1	0.2
7b	100.6	6.0	0.20	10	0.05
8	-	7.0	0.22	6	0.1
8a	99.3	7.0	0.20	10	0.05
9	-	-	0.20	20	0.2

Runs 1, 1a were performed in the absence of oxygen.

The oxygen pressure in all other runs was 100 ± 0.5 mms. A change in the number in the first column of Table 2. indicates that an evacuation of the system on the Hyvac oil pump for a period of at least one hour intervened between the exposures. The oil pump, under test at the McLeod Gauge, gave pressures of less than 10^{-3} mm. Hg. A change in the letter suffix of the run indicates that during the preceding dark interval the gas phase conditions were altered only by the addition of gases.

The total illumination time of these runs was about 20 hours, and the rates remained relatively constant over the whole of this period. The rates were apparently unaffected by the presence of CO_2 or H_2O.

Induction periods and tails were present in most cases but their occurrence and magnitude were erratic and cannot be related to the conditions tabulated.

Chlorophyll on various supporting solids.

One half of the inside surface of the spherical reaction vessel was covered with a reasonably uniform layer of the fine granular solid by swirling with a little alcohol. The hemispheric -al coating thus formed was dried by air blast and

evacuation, and a quantity of chlorophyll Sample 1. deposited on it. The system was exposed to Osira lamp 'white' light in the presence of 100±0.5 mm. of oxygen, the deposit being on the side of the reaction vessel remote from the light source.

The rates obtained for the various supporting solids under otherwise identical conditions, are given in Table 3.

Table 3.

2.48 mg. chlorophyll; 0.3 g. supporting solid; Temp. 25°C; oxygen pressure 100 mm.

Substance	Rate: scale divs./10 mins.
Glass (of the vessel)	0.025
Powdered glass (Jena)	0.10
Alumina	0.020
Talc	0.10
Zinc oxide	0.12
Ferric oxide	0.13
Thallous bromide	0.60
Thallous iodide	0.50

Illuminated by themselves in the presence of 100 mm. of oxygen, none of these solids gave detectable pressure changes.

The state of granulation of the thallous bromide and the powdered glass were approximately

equal as determined by microscopic examination. Comparison of the rates on the glass of the vessel, on powdered glass and on thallous bromide or iodide makes it adequately certain that the thallous halides exert a 'catalytic' effect on the rate of oxygen uptake by the chlorophyll, not applicable by the increased surface area presented by the crystals as compared with the smooth walls of the vessel. No acceleration on thallous bromide was obtained on illumination by red light, which is not absorbed by thallous bromide but is absorbed by chlorophyll.

Chlorophyll on Thallous Bromide. Depositions of 2.48 mg. quantities of chlorophyll Sample 1. were made on varying amounts of thallous bromide crystals in the same spherical reaction vessel. The rates obtained on exposure of these systems to 'white' light in the presence of 100 mm. of oxygen are tabulated (Table 4).

Table 4.

2.48mg. chlorophyll Sample 1: 'White' light: $25^{\circ}C$: 100 mm. O_2

Wt. of thallous bromide(g).	Rate:scale divs./10mins.
0.0	0.015
0.2	0.475
0.4	0.79
0.55	0.75
0.82	0.60

The rate of oxygen uptake is at a maximum for a thallous bromide content of the system of about 0.3 g. The rise to this maximum with increasing thallous bromide content may be attributed to the "catalytic" or sensitising effect of the thallous bromide. The final decrease in rate at still higher contents was visibly due to a shading of the rear layers of crystals.

Thallous bromide crystals are pale yellow in colour, absorbing light in the blue-violet and near ultra-violet spectral regions. It is one of a relatively large group of substances which display photoconductivity and is closely related in this respect to the halides of silver. In common with the silver halides, thallous bromide forms a latent image on exposure to light when the crystals are suitably dispersed in a gelatin emulsion, which may be developed to give a visible image.[25.]

The use of dyestuffs as sensitising and desensitising agents in photography demonstrates

the intimate physical contact possible between organic molecules and inorganic crystals.[24].

Chlorophyll as a desensitising agent for thallous bromide emulsions.

Various small amounts of alcoholic chlorophyll Sample 1. solutions were evaporated on thallous bromide emulsion plates, made by the procedure given.[25]. Exposure of the treated plates indicated a reduction of the fogging level of the emulsion, the chlorophyll acting weakly at low levels of illumination, as a desensitising agent.

Variation of Rate with Incident Light Intensity for Chlorophyll on Thallous Bromide.

Under the conditions given in Table 4. a neutral glass filter of transmission factor 0.25 was interposed in the light beam incident upon the system containing 0.4 gms. of thallous bromide.

The rate fell from 0.79 to 0.20 scale divs. per 10 mins., this indicating direct proportionality between rate and incident light intensity.

Remarks on the Preliminary Experiments.

Thallous bromide has been shown to exert a considerable accelerating effect on the rate of oxygen uptake by a given quantity of chlorophyll solid on illumination.

The existence of a conductivity level in thallous bromide, as demonstrated by its photo-conductivity and power of latent image formation under suitable conditions, may in some way be responsible for the effect. The possibility of the utilisation of the light absorbed by the crystal itself cannot be discounted. Thallous bromide differs from the silver halides in its stability to prolonged exposure to light, no visible blackening of the crystal due to the formation of free metal occurring in the absence of a bromine-atom acceptor such as gelatin. There was no pressure change over illuminated thallous bromide, an observation indicating that no significant release of bromine occurs on illumination.

Despite this uncertainty as to the exact mode of action of the supporting solid the advantages of the relatively high reaction rate were such as to encourage more detailed investigation of the photo-autoxidation of solid chlorophyll on thallous bromide as a supporting solid, with a view to comparison with the corresponding results on the photo-oxidation of chlorophyll simply deposited on the glass surface of the containing vessel.

THE PHOTO-OXIDATION OF CHLOROPHYLL SAMPLE 1.

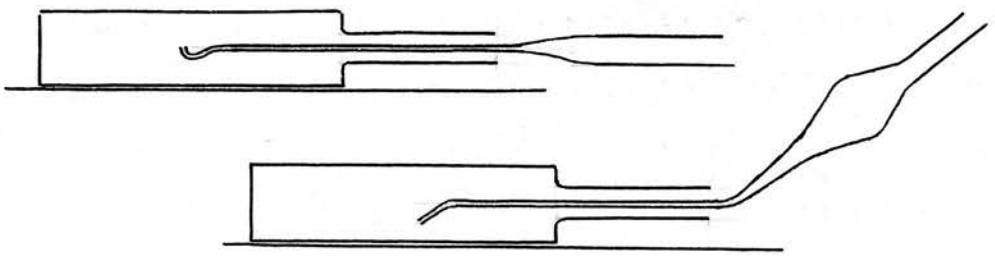
II. THE QUANTUM EFFICIENCY FOR DEPOSITS ON GLASS.

The granular nature of the supporting solid in the systems involving chlorophyll deposited on thallous bromide crystals does not allow ready determination of the light absorption factor to be made. Quantum efficiency determinations were therefore made on chlorophyll Sample 1. deposits spread uniformly on one inner plane face of a cylindrical reaction vessel.

Apparatus. See Fig. 1. Blue light of wavelength 4358 A.U. from the Osira mercury vapour lamp was isolated by means of a cuprammonium sulphate filter.[26] A water-filled roundbottomed flask of about 8 cm. diameter, placed between the lamp and the filter, served as a condensing lens. A circular aperture in the reaction vessel thermostat jacket limited the area of the incident beam so that all of the incident light fell on the rear face of the reaction vessel. A barrier-type photocell, in series with a standard mirror galvanometer and scale was used to measure relative transmitted light intensities.

Deposition of Chlorophyll Films. The deposition of a uniform film of chlorophyll in the vessel used was found to present some difficulty due to slight distortion of the otherwise plane inner surfaces of the cell faces at their junctions with the wall. While some success in correcting this distortion was achieved by coating the surface with a gelatin film, the determinations quoted were made on chlorophyll films deposited directly on the vessel wall as outlined below.

Figure 5.



The deposition was carried out in the dark, the vessel being placed on a photographic levelling table and a measured volume of the chlorophyll Sample 1. solution introduced by means of the pipette as shown in Fig. 5. A gentle current of air, dried over calcium chloride, was passed through the vessel, being directed, as indicated, against the upper face of the vessel in order to avoid stirring of the solution, with resultant uneven deposition. Despite these

precautions there was still a tendency to uneven deposition and before the film was completely dried out the vessel was swirled to wet the whole face, when the last traces of solvent could be removed to leave a reasonably uniform thin film.

An alternative method tried of removing the solvent without disturbing the film was by evacuation on a good water pump. Swirling of the vessel had again to be resorted to in the final stages.

Measurement of Absorption Factors. The absorption factors of the chlorophyll films for the blue light used were measured directly by means of the photocell/galvanometer system. Galvanometer scale readings were made as follows. The zero position of the spot having been noted, the photocell shutter was withdrawn and readings taken after 1 minute and thereafter at 15 second intervals for 1½ minutes. A further zero reading was taken and the average deflection derived. Several such readings were taken for each film exposed. The vessel was then thoroughly cleaned, dried and replaced on the apparatus to give a deflection for the vessel alone.

Rate of Oxygen Uptake. The rates of oxygen uptake by the films on exposure were

obtained in the manner previously described.

Absolute Evaluation of the Incident Light Intensity. Using the reaction vessel as a uranyl oxalate actinometer cell[27], the number of quanta of wavelength 4368 A.U. entering the actinometer solution was determined. A correction for the interface reflections gave the quanta incident on the rear inner face of the vessel when filled with oxygen i.e. incident on the chlorophyll film.

Actinometry. The results and calculation for determination 1. are given in detail. The leading values for this and other determinations are tabulated (Table 5)

Initial titre (unilluminated blank) 50.64ml. 0.1981N. $KMnO_4$

Final titre (after illumination) 50.16 ml. 0.1981N. $KMnO_4$

Oxalate decomposed ≡ 0.48ml. 0.1981 $KMnO_4$

Galvanometer reading (water in cell) G_1 = 28.50 divisions

Galvanometer reading (solution in cell) G_2 = 14.72 divisions

Time of illumination = 2.73×10^4 seconds

$$\text{Quantum efficiency} = 0.6 = \frac{0.5 \times 0.48 \times 0.1981 \times 6.06 \times 10^{23}}{1000 \times I \,(1 - G_2/G_1)}$$

where I = Quanta per sec. entering solution.

Hence I = 3.7×10^{15} quanta per second.

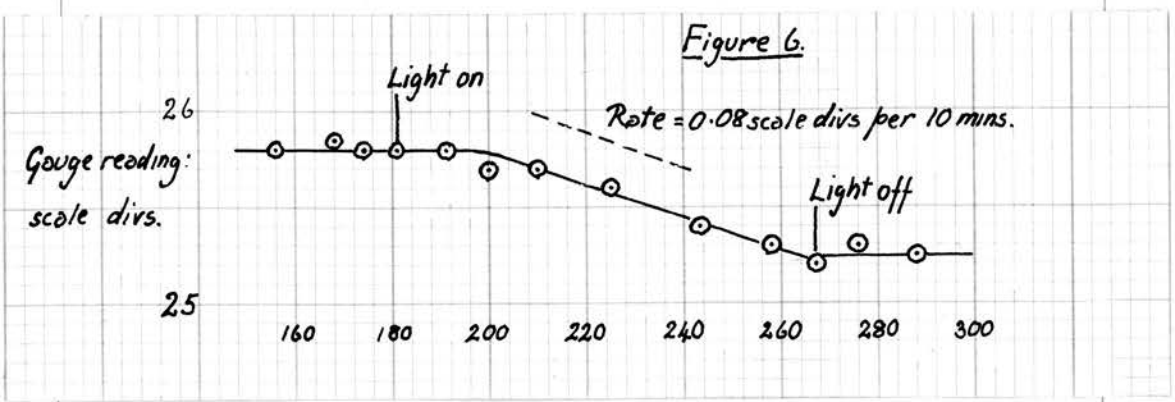
In the course of this series of experiments it was found necessary to alter the optical system, with consequent slight alteration in the light flux. Determinations 1. and 2. were made with the original arrangement and 3. and 4. with the final arrangement.

Table 5.

	Temp-erature °C.	Oxalate equiv. ml. 0.1981N.	Time of Illum. secs.	Absorption Factor $(1-G_2/G_1)$	Quantum Efficiency (given)	I quanta/sec.
1	15	0.48	27300	0.48	0.6	3.7×10^{15}
2	20	0.26	15400	0.46	0.6	3.7×10^{15}
					Average	3.7×10^{15}
		mls. 0.2005N				
3	25	0.75	39600	0.46	0.6	4.2×10^{15}
4	25	0.38	21600	0.46	0.6	4.0×10^{15}
					Average	4.1×10^{15}

Illumination of chlorophyll films. The results based on experimental results given in Figure 6, and the calculations for determination 2. are given in detail. Values for this and other determinations are given in Table 6.

Figure 6.



From Fig. 6.

Rate of oxygen uptake = 0.080 scale divs.per 10mins.

Gauge sensitivity : 1 scale div. = 0.024 mm.

Total gas space = 67.5 ml.

Temperature 20°C

Rate of oxygen uptake

$$= \frac{67.5}{22400} \times \frac{273}{293} \times \frac{0.08 \times 0.024}{600 \times 760} \times 6.06 \times 10^{23} \text{ molecules/sec.}$$

$$= \underline{7.2 \times 10^{12} \text{ molecules/sec.}}$$

Galvanometer readings

(vessel empty) G_1 = 26.20 divisions

(vessel + film) G_2 = 15.85 "

(vessel + water) G_3 = 28.50 "

Absorption factor for film $(1 - G_2/G_1)$ = 0.39

Interface correction for reflection G_1/G_3 = 0.96

$$\text{Quantum Efficiency} = \frac{7.2 \times 10^{12}}{3.7 \times 10^{15} \times 0.39 \times 0.96}$$

$$= 5.2 \times 10^{-3}$$

In the experiments tabulated, O_2 pressure = 100±1 mm.
Temperature = 20.0°C.
Gas space = 76.5ml.

Table 6.

	Chloro-phyll mg.	Abs. Factor $(1-G_2/G_1)$	Rate scale divs. per 10 mins.	Rate molecules per sec.	Light Intensity quanta per sec.	Quantum Efficiency Φ
1	3.51	0.27	0.08	7.2×10^{12}	3.7×10^{15}	7.5×10^{-3}
2	3.51	0.39	0.08	7.2×10^{12}	3.7×10^{15}	5.2×10^{-3}
3	8.68	0.54	0.08	7.2×10^{12}	4.1×10^{15}	3.4×10^{-3}
4	0.231	0.031*	0.03	7.2×10^{12}	4.1×10^{15}	2.2×10^{-2}

* Calculated using the absorption factor $(1 - G_2/G_1)$ of 0.39 obtained for 3.51 mg. chlorophyll (2) and the relationship

$$\log_{10} G_2/G_1 = (\log_{10} 0.61) \times \frac{0.231}{3.51}$$

hence $(1 - G_2/G_1)$ for 0.231 mg. of chlorophyll.

Discussion. In the range of absorption factors 0.27 to 0.54, but under otherwise identical conditions, the observed rate of oxygen uptake by the chlorophyll deposits is constant within the

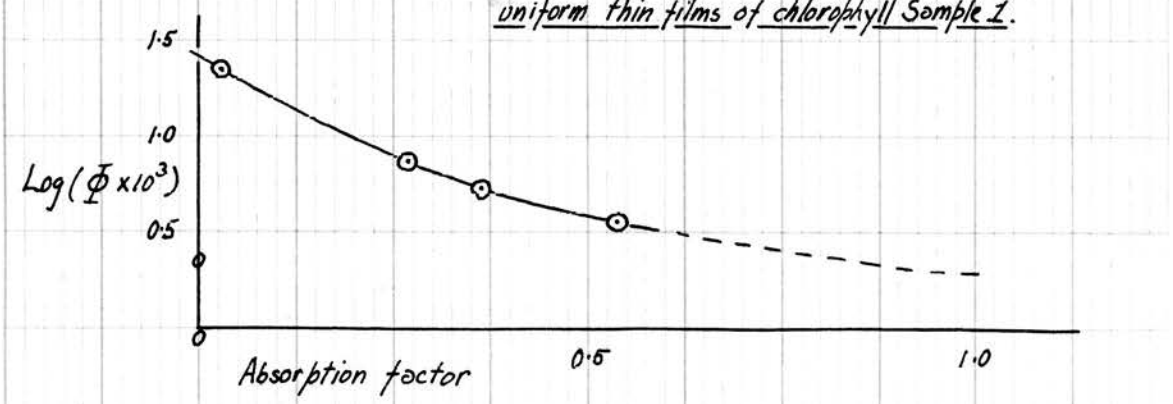
rather wide limits of experimental error ($\pm$ 5%).

The limit of the resulting increase in quantum efficiency from 0.34×10^{-3} to 7.5×10^{-3} with decreasing deposit thickness was investigated by the determination of the quantum efficiency for a very much thinner deposit (containing 2.31×10^{-4} g chlorophyll) the absorption coefficient for the deposit being calculated from the amount of chlorophyll present. A quantum efficiency of 2.2×10^{-2} at an absorption factor of 0.031 was obtained.

The plot of Log ($\Phi \times 10^{3}$) against Absorption Factor for these results is given in Fig. 7.

Figure 7.

Photo-oxidation in blue light (λ 4358 A.U.) of uniform thin films of chlorophyll Sample 1.



The curve obtained is readily extrapolable to zero absorption factor, the limiting value of Φ being 2.6×10^{-2}. This value may be

considered to be that for an infinitely thin film still possessing the properties of the bulk substance. The experimental deposit may be regarded as an infinite number of such thin films in contact.

Let ϕ_x be the quantum efficiency for the thin film of thickness dx situated at a depth x within the deposit. The observed quantum efficiency for a deposit of thickness a may then be expressed as $\frac{1}{a}\int_0^a \phi_x \, dx$

If ϕ_x is constant for all values of x, the observed quantum efficiency should not vary with the deposit thickness, the integral always having the value ϕ_x .

This is not so and the observed quantum efficiency decreases with increasing deposit thickness, the limiting value (for a given light intensity) when complete absorption of the incident light occurs, being approx. 2.0×10^{-3}.

Light absorbed at a depth within the deposit is less efficiently utilised for autoxidation. There are two possibilities of changing condition with increasing depth within the deposit.

(1) Availability of oxygen within the film diminishes with depth.

(2) The uptake of oxygen is inhibited in the deeper layers of the deposit.

The deposits were left in contact with the oxygen (100 mms.) for a period of at least one hour before illumination was commenced, a time presumably sufficient for the concentration of oxygen within the deposit to have become constant, but should this equilibrium concentration of oxygen be sufficiently low a marked concentration gradient would be set up and maintained on illumination. The oxygen concentration gradient within the deposit during illumination would then be determined by the light incident on any particular layer of the deposit, the rate of oxygen consumption in that layer, and the net rate of diffusion of oxygen into the layer, a function of the oxygen concentrations in the layer and the two adjacent ones.

The second suggested explanation arises from the possibility that after the preliminary treatment of the film by evacuation on the oil-pump some solvent, or volatile impurity of the solvent or chlorophyll preparation may remain in the deposit, and will tend to be more concentrated deeper within the deposit. For this to occur the rate of diffusion of such an impurity through the

deposit must be very slow as in general the chlorophyll was pumped out for several hours and left evacuated overnight before runs were commenced.

Low rates of oxygen uptake in deep seated layers would then be due to a catalytic degradation of the energy of the excited chlorophyll or to the diversion of the energy to the oxidation of the impurities, giving rise, because of their low molecular weight, to gaseous products such as carbon-dioxide. The resulting gas exchange could then be zero or even positive.

A third possible explanation involves the transfer of energy from excited chlorophyll molecules deep within the deposit to surface molecules before reaction can take place, the oxygen concentration within the film being in effect zero. The transfer process would not take place without loss, with resulting decrease in quantum efficiency for thicker films. This mechanism is open to the objection that zero rate would be reached when only the surface layer of the deposit had been oxidised, unless supplemented by diffusion processes.

While no determination of the quantum efficiency of the bleaching of solid chlorophyll has previously been made there exist several

determinations of the quantum efficiency of photo-bleaching of chlorophyll in organic solvents saturated with oxygen. Values of 10^{-6} for ethyl chlorophyllide in methanol,[28.] of 4×10^{-5} for chlorophyll in methanol[29.] and 5×10^{-4} for chlorophyll in acetone or benzene[30.] have all been reported. These values are all one or two orders lower than those obtained in the present experiments, but it has been suggested that the primary oxidised product still possesses appreciable light absorption in the spectral range used for concentration measurements, and if this is so, it would render these determinations non-significant.[31.] Further, in the present experiments, the pressure of oxygen was 100 mm., and it will be shown (see later) that the rate decreases with decreasing oxygen concentration, a smaller value in solution being thus not unexpected.

THE PHOTO-OXIDATION OF CHLOROPHYLL SAMPLE 1.

III. THE RATE CURVE FOR DEPOSITS ON THALLOUS BROMIDE CRYSTALS.

Preliminary experiments on the photo-oxidation of chlorophyll Sample 1. deposits in which the illumination periods had been of short duration had indicated the necessity for the study of more prolonged runs. Using the preparative methods and the apparatus previously described depositions of the chlorophyll Sample 1. on thallous bromide crystals were made, and the systems exposed in the presence of oxygen. The resulting fall in the oxygen pressure was observed over a period of several hours.

Experimental. 0.52 g. of thallous bromide crystals were deposited in a uniform layer on one plane face of a cylindrical vessel of 85.5 ml. volume (p.). 2.48 mgms. of the chlorophyll: carotene mixture (Sample 1.) were deposited on the crystals from ethyl alcohol solution by removal of the solvent at the water pump. The deposit was then pumped out for two hours on the Hyvac oil-pump, left evacuated overnight, washed out with a few mm. of oxygen and pumped out for a further two

hours before the introduction of 100.0 mm. of oxygen. The system was exposed to Osira lamp 'blue' light at 20°C, and gauge readings made at five minute intervals for several hours.

Treatment of results. The data obtained was treated as follows. Gauge readings were plotted against time and a smooth curve drawn through the experimental points. At convenient intervals on this smoothed curve rates of change in gauge reading were obtained by direct measurement of the gradient of the tangent to the curve. These rates were expressed in 'scale divisions per ten minutes', units which gave values of the order of unity for the experimental conditions used. Change in gauge reading being directly proportional to change in pressure, a factor, constant for the experiment, and involving the gauge sensitivity, the temperature and the vessel volume, would have rendered these rates in more absolute units. These conversions were made only where necessary for the discussion of the results.

The rates so obtained could be plotted either against time or against the total pressure decrease from the commencement of illumination. From the former plot a curve was obtained in which the rate tended to zero at infinite time. No

information could be obtained visually from this curve.

The plot of rate against the total pressure decrease from 'light-on' gave, however, a curve tending to zero rate after a finite decrease in pressure, and was otherwise more useful in the discussion of the experimental results. Curves of this latter type we have termed 'rate curves'.

Figure 8.

The photo-oxidation of chlorophyll Sample 1. on thallous bromide crystals.

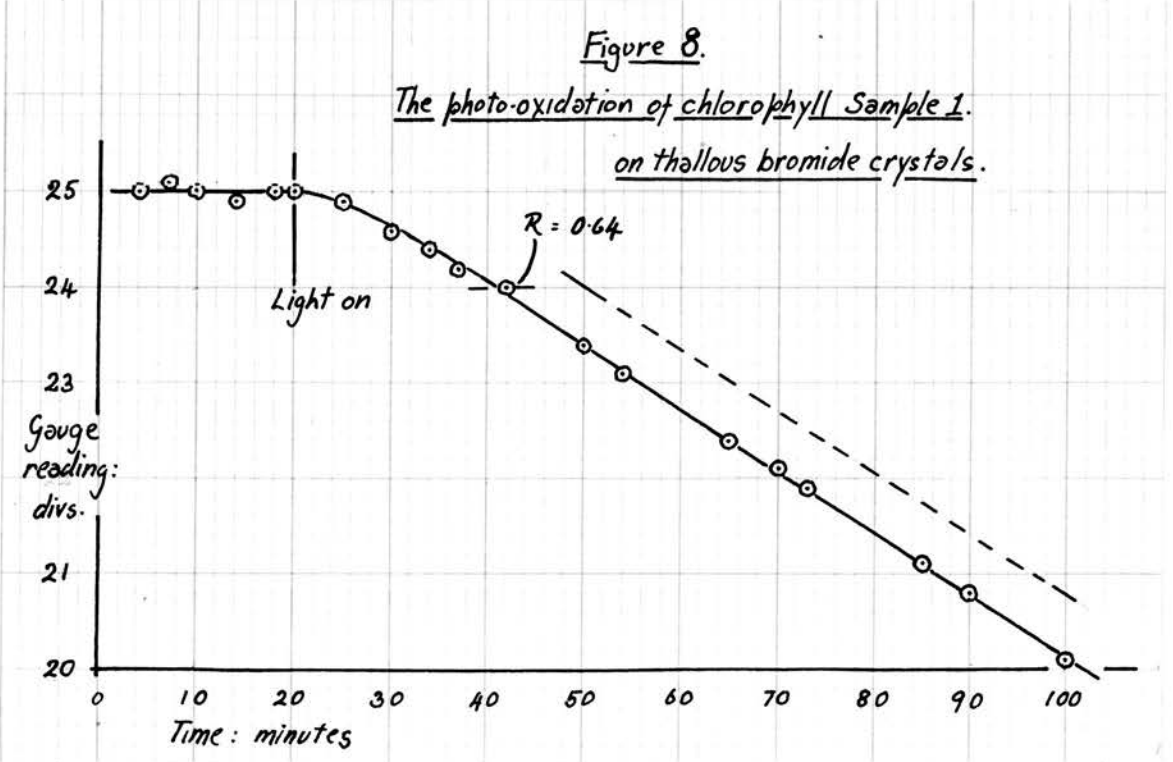


Fig. 8. shows plot of gauge reading against time during the initial period of the illumination of the system described above. The method of making the gradient measurements is indicated.

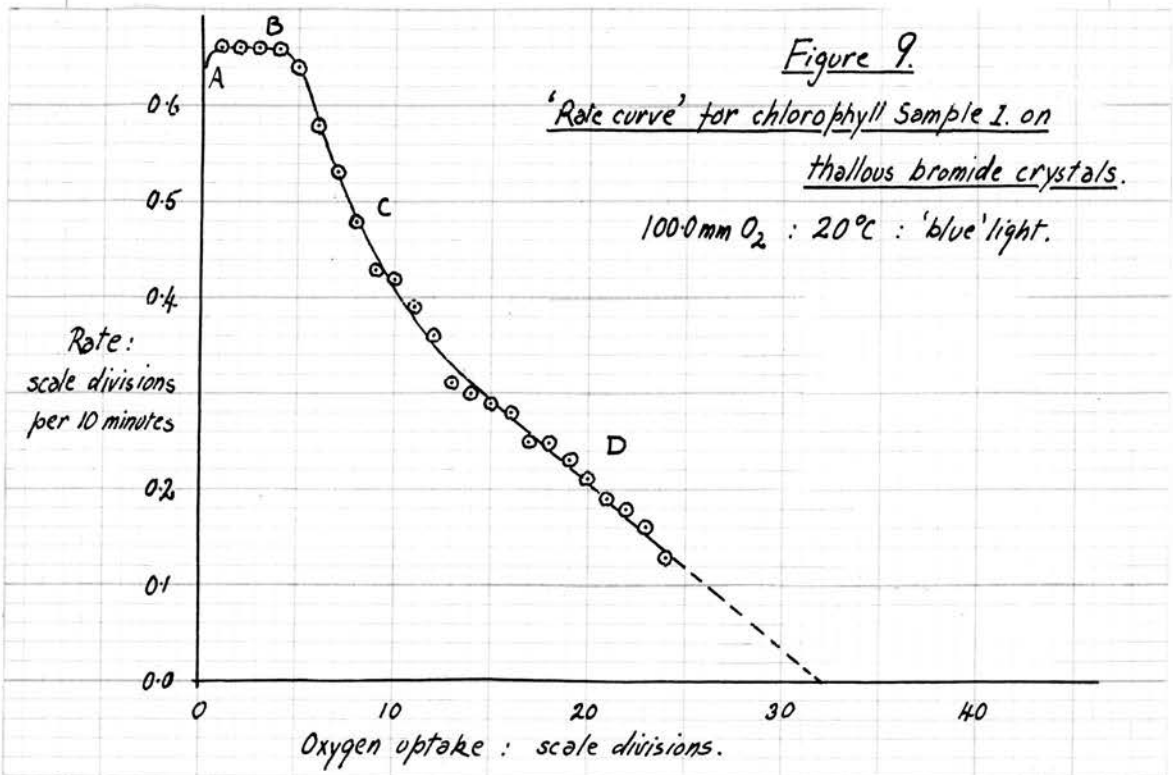


Figure 9.

'Rate curve' for chlorophyll Sample I. on thallous bromide crystals.

100·0 mm O_2 : 20°C : 'blue' light.

Fig. 9. shows the 'rate curve' for the whole observational period and the extrapolation of this to zero rate.

The rate curve of Fig. 9. exhibits features which may be separated conveniently as (A); a short 'acceleration period', followed by (B); a 'period of constant maximum rate', (C); a 'transition period' and (D); a 'period of linear decrease' in rate, maintained to the limit of observation.

Marked bleaching of the chlorophyll green was noted after the illumination in oxygen and it was assumed that decolourisation would have been

complete when the rate of oxygen uptake became zero.

It was further assumed that the uptake of oxygen by the solid chlorophyll:carotene film was the only reaction contributing to the pressure decrease observed.

There was the possibility that the pressure decrease observed was due to the absorption of some gaseous impurity of the oxygen sample used, as under the experimental conditions less than 1% of the total amount of gas present was absorbed before the reaction rate came to zero. Evidence presented in a later section (p. 75) on the effect of admixed gases indicated the non-participation in the reaction of those gaseous impurities most likely to be present. Positive evidence that the absorbed gas was oxygen is presented in the section of the work devoted to the photo-oxidation at low oxygen pressure (p. 92).

That the observed pressure change was due only to the absorption of oxygen and was not the resultant of two or more processes giving pressure changes in opposite senses was more difficult of proof. The results of the work at low pressure may again be adduced in favour of the first hypothesis, as to a first approximation all the oxygen

initially present was taken up, the residual pressure of gas when zero rate was attained being non-significant in this respect.

Direct attempts to detect possible gaseous products were made. Formaldehyde has been reported as a product of the photo-oxidation of chlorophyll, while hydrogen peroxide is a possible reaction product or intermediate.[32.]

A few ccs. of distilled water were frozen in a trap cooled with liquid air. The gases of the exposed system were drawn very slowly through the trap. Air was then admitted, the trap detached from the apparatus and stoppered while the ice and any volatile products trapped melted. Tests for formaldehyde on the resulting 'solution' by means of a sensitive Schiff's reagent[33.] were negative on this and several subsequent occasions. Attempts to detect hydrogen peroxide by its action on a starch-iodide solution, and also by its formation of the yellow oxide of titanium, were equally negative.

On these assumptions the photo-oxidation of the preparation was complete when the observed rate of pressure decrease had fallen to zero. While this condition might not be experimentally attainable, the linear nature of the final section

of the rate curve (Fig. 9).D) enabled an accurate extrapolation to zero rate to be performed. The maximum oxygen uptake by the preparation under the experimental conditions could thus be determined.

The preliminary assumption that the preparation was 100% pure chlorophyll permitted the calculation of the approximate molar ratio of oxygen to chlorophyll for the oxidised preparation.

Molecular weight of chlorophyll = 900

(chlorophyll *a* 893, chlorophyll *b* 907)

Wt. of chlorophyll taken = 2.48×10^{-3} g.

$$= \frac{2.48 \times 10}{900} = 2.76 \times 10^{-6} \text{ moles}$$

Gauge sensitivity = 0.024 mm. per scale division

Temperature = 293°A

Gas space = 67.5 ml.

Extrapolated oxygen uptake = 32.0 scale divisions

$$= \frac{32.0 \times 0.024}{760} \times \frac{273}{293} \times \frac{67.5}{22400} \text{ moles}$$

$$= 2.84 \times 10^{-6} \text{ moles}$$

$$\text{Molar ratio oxygen: chlorophyll} = \frac{2.84}{2.76} = \underline{1.03}$$

Two confirmatory experiments using chlorophyll Sample 1. were made. Deposition upon the thallous bromide crystals was in all cases from ethyl alcohol solution. The leading values for all three experiments are tabulated below (Table 7).

Table 7.

	1	2	3
Wt. of TlBr g.	0.52	0.52	0.30
Oxygen pressure: mm.	100.0	101.4	100.0
Nature of illumination (Osira lamp)	blue	blue	white
Wt. of chlorophyll taken: mg.	2.48	2.48	3.20
Moles chlorophyll present x 10^6 (calcd. as 100% pure)	2.76	2.76	4.33
Extrapolated oxygen uptake: scale divisions	32.0	34.5	35.0
Gauge sensitivity: mm. per scale div.	0.024	0.024	0.031
Gas space: ml.	67.5	67.5	86.9
Temperature A^o	293	293	295
Moles oxygen taken up	2.64	3.06	5.07
Molar ratio oxygen: chlorophyll	1.03	1.11	1.17

These results would indicate that the photo-oxidative bleaching of one mole of chlorophyll is accompanied by the uptake of approximately one mole of oxygen.

The molar ratio figures tend to exceed unity by an average of about 10%, a maximum value of 1.17 being recorded. The presence of carotene

in the preparation was thought to account for this apparent excess, and this hypothesis was investigated in the experiments reported in the following section.

The form of the rate curve of Fig. 9 may be briefly considered at this point. Section B of the curve, the period of constant maximum rate was thought also to be due to the presence of carotene in the preparation. This hypothesis receives fuller treatment in the following section.

The total decrease in pressure recorded during this experiment was about 1 mm., only some 1% of the total oxygen pressure present. The oxygen pressure during the course of a run was therefore regarded as constant. All the carotene present being presumed to have been removed during stages A, B and C, the linear nature of the terminal section of the curve would indicate that the rate of oxygen uptake was directly proportional to the amount of chlorophyll remaining in the system. The mathematical expression of this takes the same form as that for a first order reaction.

i.e. $R = k . [\overline{Chl}]$

where R = rate of oxygen uptake (= rate of disappearance of $\overline{Chl}$) and $[\overline{Chl}]$ = chlorophyll content of the system.

The initial acceleration shown by the rate curve is not unexpected, if the rate of diffusion of oxygen in the film is a limiting factor. At the instant of illumination, oxidation may take place at once at the chlorophyll layer, nearest the sensitising thallous bromide, but this will not show as a decrease in the gas phase pressure until oxygen begins to flow from the gas space through the film.

THE PHOTO-OXIDATION OF CHLOROPHYLL SAMPLE 1.

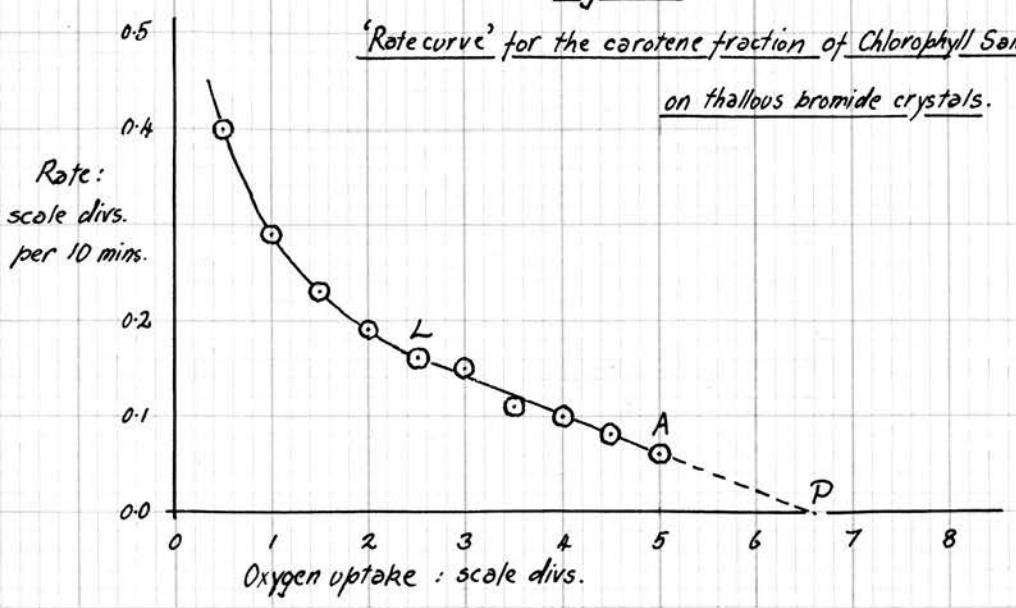
IV. THE RATE CURVES OF THE SEPARATED CHLOROPHYLL AND CAROTENE FRACTIONS.

Chlorophyll Sample 1. has been shown by chromatographic separation to be a mixture of chlorophyll and carotene in the approximate molecular proportions of three to two (pp.24-25). It has already been established[34]. that the yellow plant pigment carotene undergoes autoxidation on illumination in organic solvents and it may therefore be expected to exhibit similar properties when exposed to light and gaseous oxygen in the form of a thin solid deposit. Accordingly, separate deposits of the chlorophyll and carotene fractions, obtained as described previously, were examined as before, with a view to comparison with the results for the initial Sample 1. mixture, recorded in the previous section.

Carotene fraction. A deposit of 0.524 mg. of carotene on 0.3 g. of thallous bromide was prepared as usual, and illuminated in 'white' light at 20°C in 100 mm. of oxygen. The vessel volume was 86.9 ml. and the gauge sensitivity 0.031 mm. per scale division. The rate curve is given in Figure. 10.

Figure 10.

'Rate curve' for the carotene fraction of Chlorophyll Sample 1 on thallous bromide crystals.



This rate curve differs considerably from that previously obtained for the mixture. No induction or acceleration period is to be observed, the rate decreasing rapidly from its initial high value. From the point L, however, the rate curve was again linear and extrapolation to the point P afforded the data necessary for the calculation of the carotene to oxygen molecular ratio. The oxygen uptake was 6.6 scale divisions, giving an oxygen to carotene ratio of almost exactly unity, namely, 0.98. In this calculation the molecular weight of the carotene fraction was taken as that of carotene itself, 536. Repetition of the experiment gave similar values, viz. 0.97, 1.03.

Chlorophyll fraction. The rate curve obtained for the chlorophyll is given in figure 11, with conditions otherwise as before.

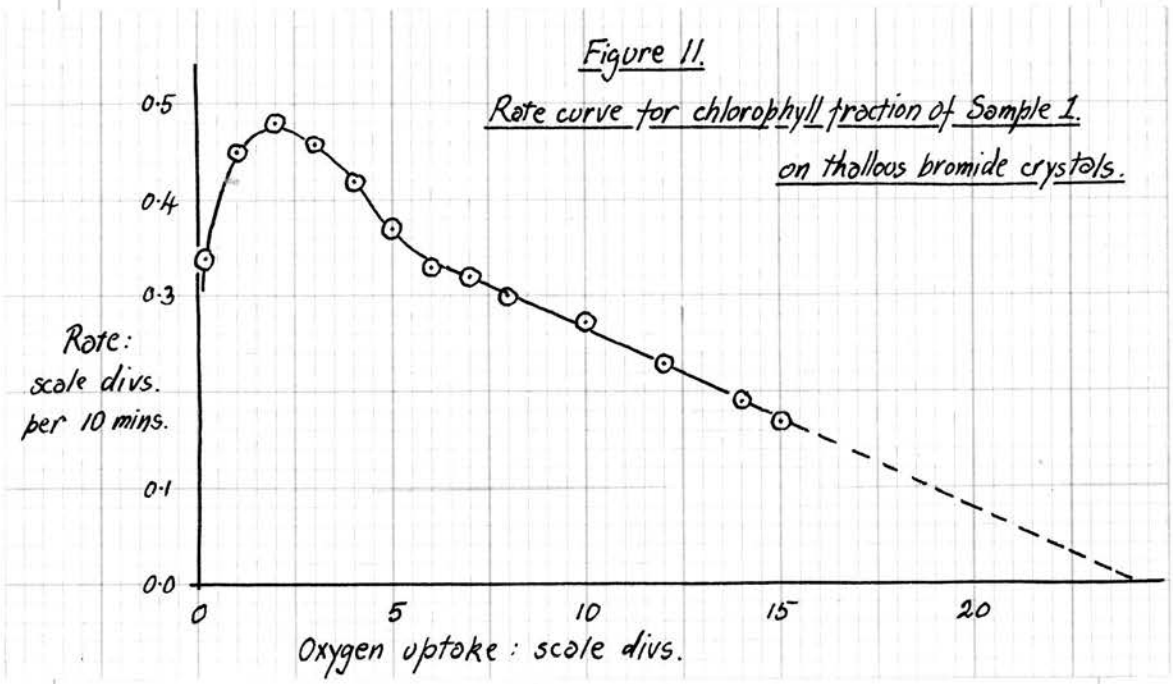


Figure 11.
Rate curve for chlorophyll fraction of Sample 1. on thallous bromide crystals.

A pronounced acceleration period is now in evidence. No constant rate period is obtained but again a final linear section is obtained which allows satisfactory extrapolation. With 3.08 mg. of chlorophyll and an extrapolated oxygen uptake OP of 24.1 scale divisions, volume and gauge sensitivity as before, the calculated oxygen to chlorophyll molar ratio is 1.02 (molecular weight of chlorophyll taken as 900), again indicating that under such conditions one molecule of oxygen is consumed per molecule of chlorophyll oxidised.

On the assumption that these unit molecular ratios are preserved in the mixture represented by chlorophyll Sample 1, it is obvious that the proportions of carotene and chlorophyll in the mixture can be calculated from a knowledge of the total weight oxidised (W) and the total oxygen uptake (OP). If w_1 is the weight in g. of chlorophyll and w_2 is the weight in g. of the carotene, then

$$w_1 + w_2 = W$$

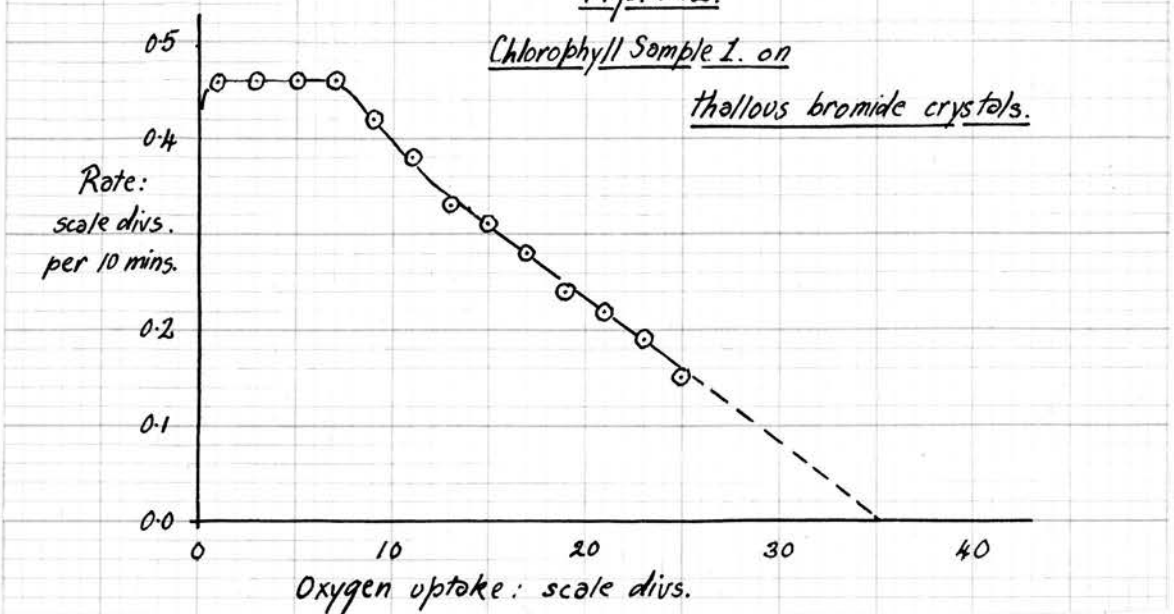
and

$$\frac{w_1}{M_1} + \frac{w_2}{M_2} = (OP)$$

where M_1 and M_2 are the respective molecular weights and OP is expressed in gram molecules of oxygen. The experiments of table 7 will admit such a calculation but the results as shown by the estimated final ratios of 1.0, 1.10 and 1.17 are somewhat erratic and these experiments were repeated with all precautions. A typical example is given in figure 12. The total weight of sample was 3.84 mg. With a vessel volume of 86.9 ml. and a gauge sensitivity of 0.031 mm. per scale division, OP was 35.0 scale divisions.

Figure 12.

Chlorophyll Sample I. on thallous bromide crystals.



By the above calculation, it is then found that the percentage of carotene in the original mixture was 28.2, a figure in reasonable agreement with the 24 per cent given by the chromatographic techniques, when allowance is made for the possible errors involved, especially in view of the fact that the chromatographic separation will not be completely efficient by reason of residual adsorption in the columns.

The constant rate portion of the rate curve for the mixture suggested the possibility of an oxidation of carotene photosensitised by the chlorophyll, as opposed, for example, to simultaneous oxidation of the two species. An attempt was

made to investigate this by using artificial mixtures of greater carotene content. Deposits of 2.62 mg. of chlorophyll and 2.93 mg. of carotene were examined separately and as a mixture. The corresponding rate curves are given in Figures 13, 14 and 15.

Figure 13.
Chlorophyll fraction of Sample 1.

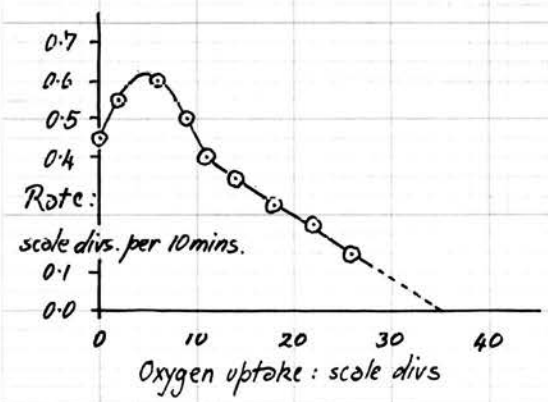


Figure 14.
Carotene fraction of Sample 1.

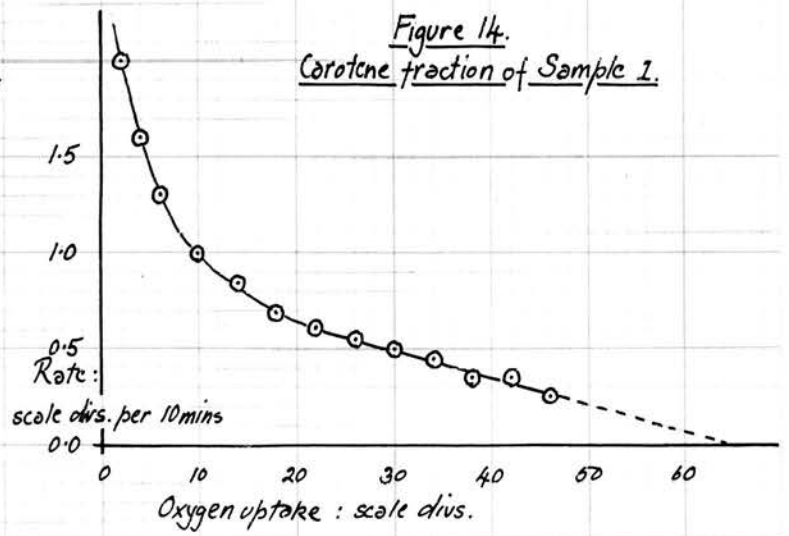


Figure 15.
Mixture of chlorophyll and carotene fractions of Sample 1.

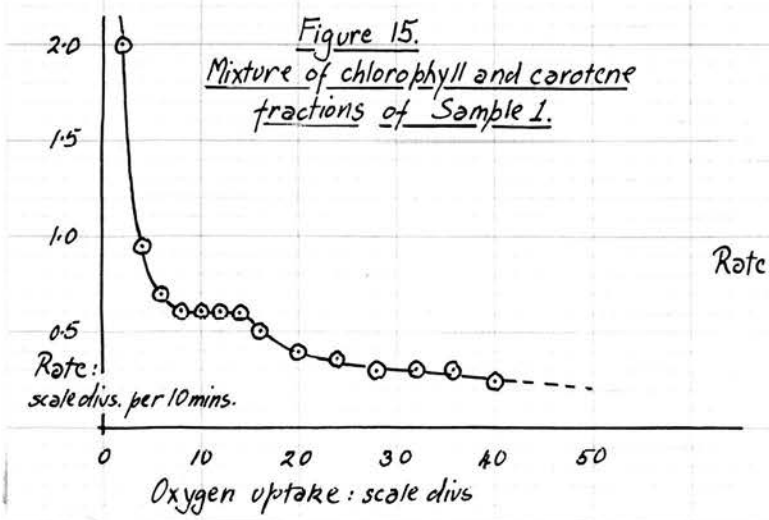
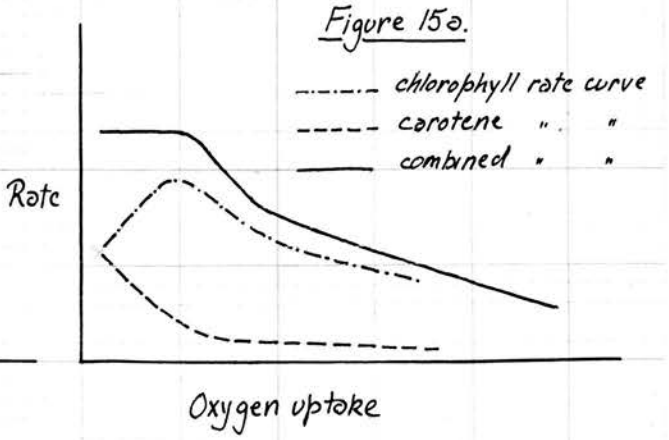


Figure 15a.



The relative amount of carotene is here much greater than in the original chlorophyll Sample 1, and the fact that tue curve of figure 15, that of the artificial mixture, shows an initial uptake rate characteristic of the carotene alone and not a prolonged constant rate section as might be expected from a sensitised oxidation is here in favour of simultaneous oxidations. The rate curve for the mixture can then be represented by a summation of the two separate rate curves. Figure 15a is a qualitative graphical representation of such a summation which does not contradict such a hypothesis. Further evidence is considered in a later section.

THE PHOTO-OXIDATION OF CHLOROPHYLL SAMPLE 1.

V. THE VARIATION OF RATE OF OXYGEN UPTAKE WITH OXYGEN PRESSURE.

The continuous change in the rate of oxygen uptake during the photo-oxidation of a chlorophyll deposit made impossible the direct comparison of the rates obtained at different oxygen pressures, determinations necessarily made consecutively. The comparison of the rates of oxygen uptake by separate chlorophyll deposits identically prepared and exposed under conditions differing only in oxygen pressure was also precluded by a failure to obtain reproducible results from apparently identical preparations and exposures. This lack of reproducability was due to the accumulated effects of small differences in the amounts and distribution of the thallous bromide crystal substrates and the extent of the 'creep' of the chlorophyll on the vessel walls during deposition. These differences were unavoidable with the methods used.

The linear nature of the final section of the rate curve, however, made possible the comparison of the results obtained by varying the oxygen pressure during a single run as this section of

the rate curve for an initial pressure of, say, 100 mms. of oxygen could be extrapolated to provide a standard rate curve with which the rates obtaining at other pressures later in the run could be compared (Figs. 16, 17). These later sections of the experimental rate curve were found also to be linear and, extrapolated, came to zero rate at the same total oxygen uptake as the initial curve. This allowed the more convenient method of comparison discussed below.

Figs. 16 and 17 record the results of two runs in which the oxygen pressure was varied after the establishment of the final linear descending section of the rate curve in each case.

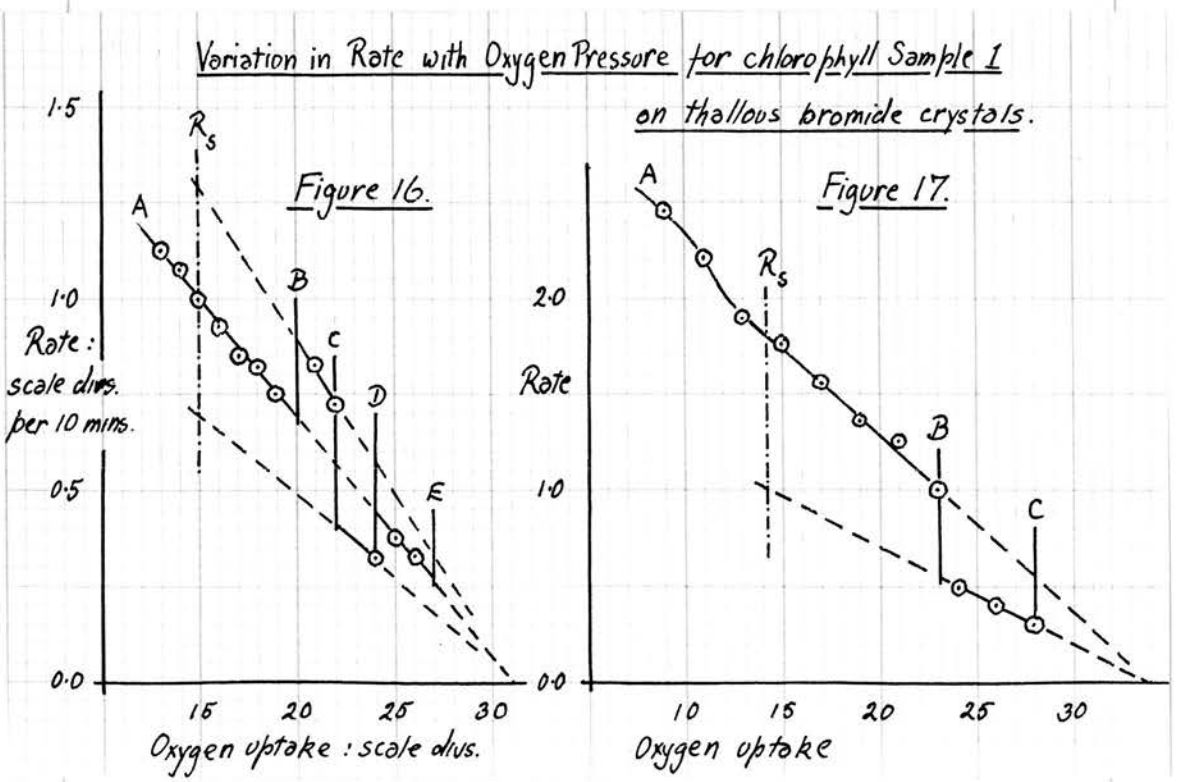


Fig. 16. Chlorophyll Sample 1. (purified) on thallous bromide: 'white' light: 25°C : oxygen pressure AB, 100.0 mm.: BC, 150.0mm.: CD, 50.0. mm. DE, 100.0 mm.

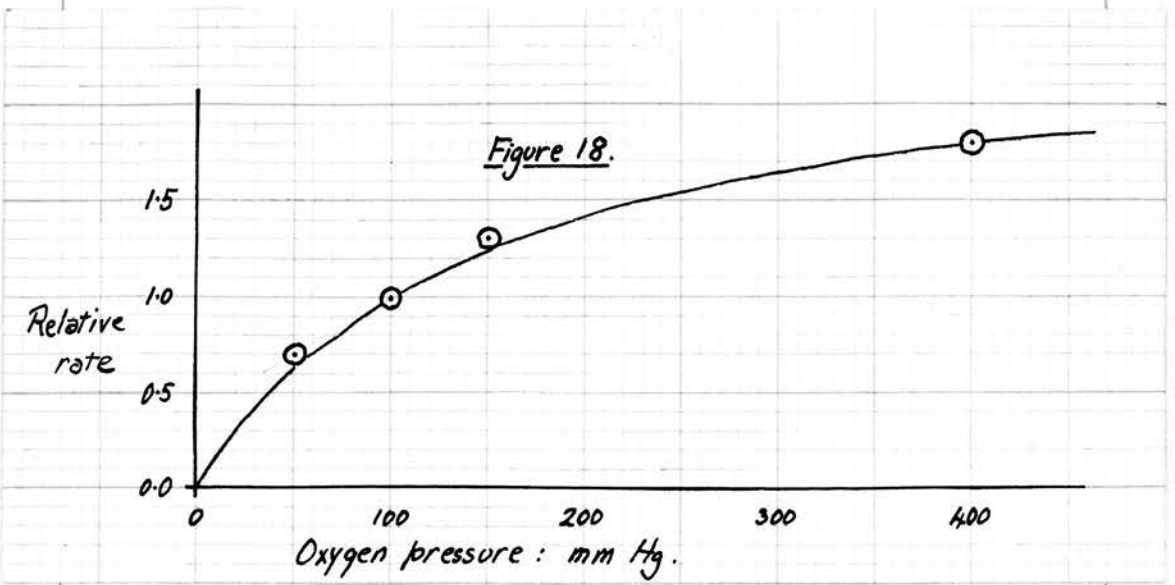
Fig. 17. Chlorophyll Sample 1. (purified) on thallous bromide: 'white' light: 25°C : oxygen pressure AB, 400.0 mm.: BC, 100.0mm.

The condition of the system giving a rate of oxygen uptake of 1.0 scale divisions per 10 minutes at an oxygen pressure of 100 mm. was chosen as the standard at which rate comparisons were made, the rate curves for other pressures being suitably produced to give the rate at the standard condition. The values of R the rate at the standard condition, and the corresponding oxygen pressures derived from Figs. 16, 17, are tabulated (Table 8).

Table 8.

Oxygen pressure mm.	R Scale divs./10 mins.
50	0.7
100	1.0
150	1.3
400	1.8

These values are plotted in Fig. 18.



The curve of Fig. 18. is considered to represent the variation in the rate of oxygen uptake with oxygen pressure for the photo-oxidation of a system of constant chlorophyll content. The rate rises with increasing oxygen pressure, at first rapidly, and then more slowly, a maximum rate being reached at an infinitely high pressure of oxygen.

Empirically the rate oxygen pressure curve fits an expression of the type

$$R = \frac{a\,[O_2]}{(1 + b\,[O_2])}$$, where $[O_2]$ represents oxygen pressure and a and b are constants.

At low oxygen pressures $b[O_2] \ll 1$ and in the limit $R = a[O_2]$ At the other extreme $b[O_2] \gg 1$ when $R = a/b$ and is independent of the pressure.

$\underline{a}$ and $\underline{b}$ may be evaluated for the standard condition of the system. Taking R in the arbitrary units of scale divisions per 10 minutes and $[O_2]$ in mm., the values (Table 8) for R at 100 and 400 mm. of oxygen give the equations:

$$R_{100} = 1.0 = 100\ a/\ (1 + 100\ b)$$

and $$R_{400} = 1.8 = 400\ a/\ (1 + 400\ b)$$

whence $\underline{a} = 1.69 \times 10^{-2}$ and $\underline{b} = 6.88 \times 10^{-3}$.

The curve of Fig. 18 is drawn using these values, and agrees closely with the other experimental points.

For convenience R has been plotted in the 'rate curves' and in Fig. 18 as a positive quantity, whereas the reaction is a disappearance of oxygen.

THE PHOTO-OXIDATION OF CHLOROPHYLL SAMPLE 1.

VI. THE REVERSIBILITY OF THE OXIDATION.

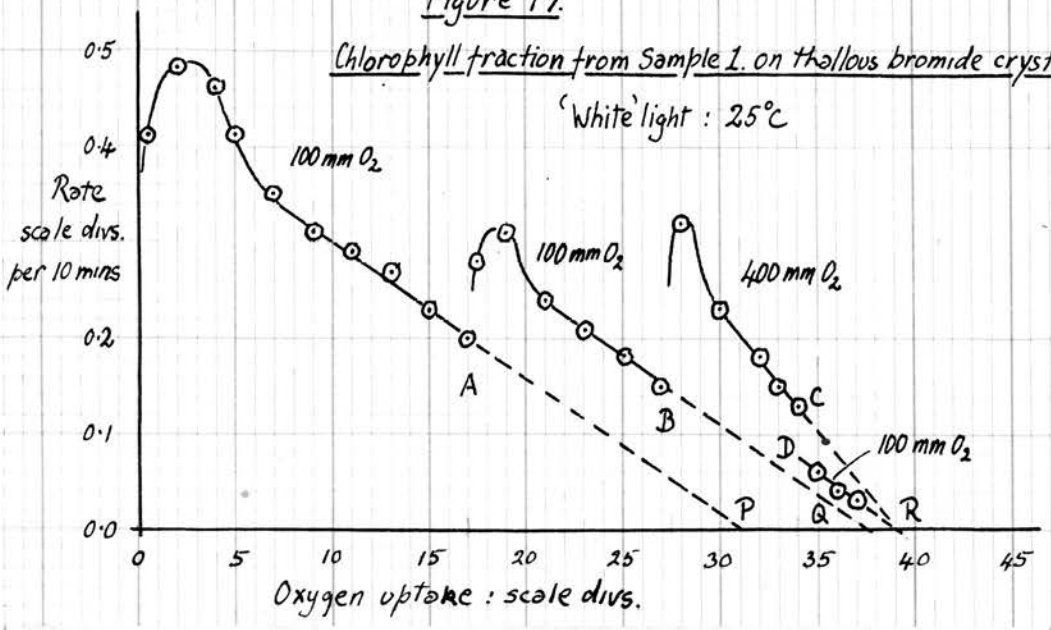
Mention has already been made of the difficulty of obtaining reproducible rate results with different preparations, but, in addition, preliminary experiments with one preparation had shown altered rates when a period of darkness intervened or when the system had been evacuated between illuminations, subsequent conditions being otherwise the same. In particular, the total "oxygen uptake" in such an interrupted illumination rose to values considerably greater than those expected on the basis of the unit molar ratio. The possibility of a reversibility of the oxidation was therefore investigated.

Chlorophyll fraction. Fig. 19. shows the rate curves for a deposit of the chlorophyll fraction of Sample 1, prepared by chromatographic separation on bone meal. The conditions of illumination are indicated in the figure.

Figure 19.

Chlorophyll fraction from Sample 1. on thallous bromide crystals.

'White' light : 25°C



The initial illumination was continued until the linear section had been established, from which extrapolation to the point P gave the expected oxygen to chlorophyll molecular ratio of unity. At the point A the light was cut off and the system thoroughly evacuated for half an hour (pressure less than 10^{-3} mm. Hg) and left to stand overnight (15 hrs.). Oxygen was then introduced to a pressure of 100 mm. as before, and illumination recommenced. A rate curve similar to the first was obtained, but the rates were all higher than expected. A linear decrease in rate was again established and extrapolated to the point Q: the lines AP and BQ are parallel.

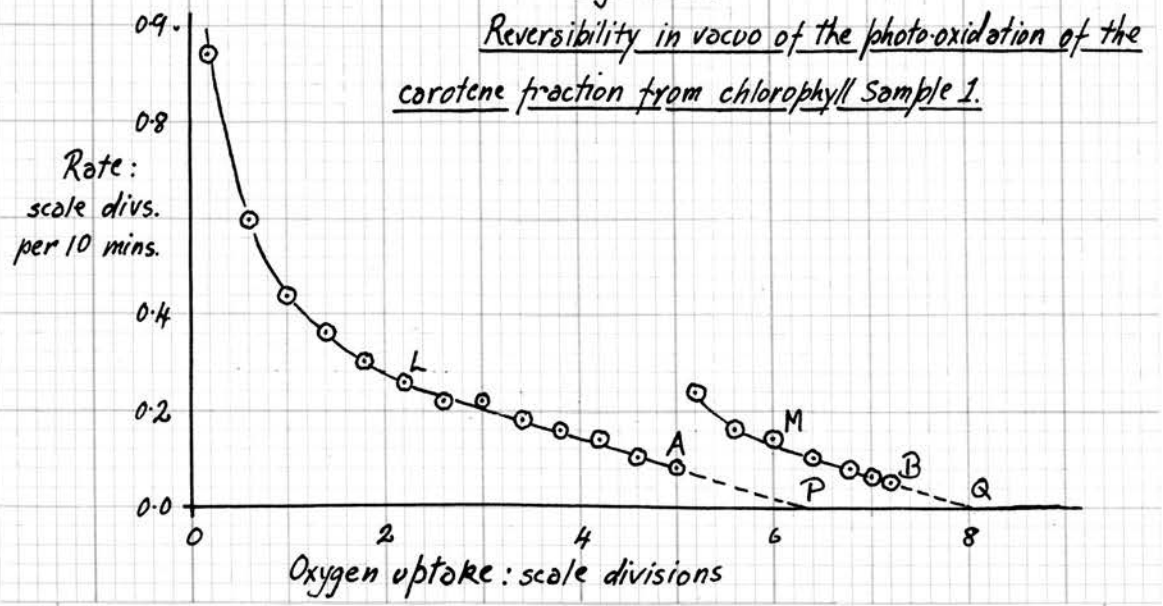
At B evacuation was again given overnight and the experiment repeated with 403 mm. of oxygen present. At the point C the pressure was reduced to 100 mm. without interrupting the illumination, this allowing the extrapolation DR to be made. It will be observed that DR is again parallel to AP and BQ. In addition CR and DR intersect the horizontal axis in the same point, indicating again that the oxygen uptake as obtained by such methods is independent of the pressure.

Such results suggest that the oxidation has been reversed by the evacuations. The fact that the extrapolated rate curves at 100 mm. of oxygen are parallel indicates that no other complicating circumstances arise. The reversibility is in neither case complete, the rates in the earlier stages of the initial exposure never being reattained.

Carotene Fraction. Fig. 20 shows the application of a similar evacuation technique to the carotene fraction of Sample 1.

Figure 20.

Reversibility in vacuo of the photo-oxidation of the carotene fraction from chlorophyll Sample 1.

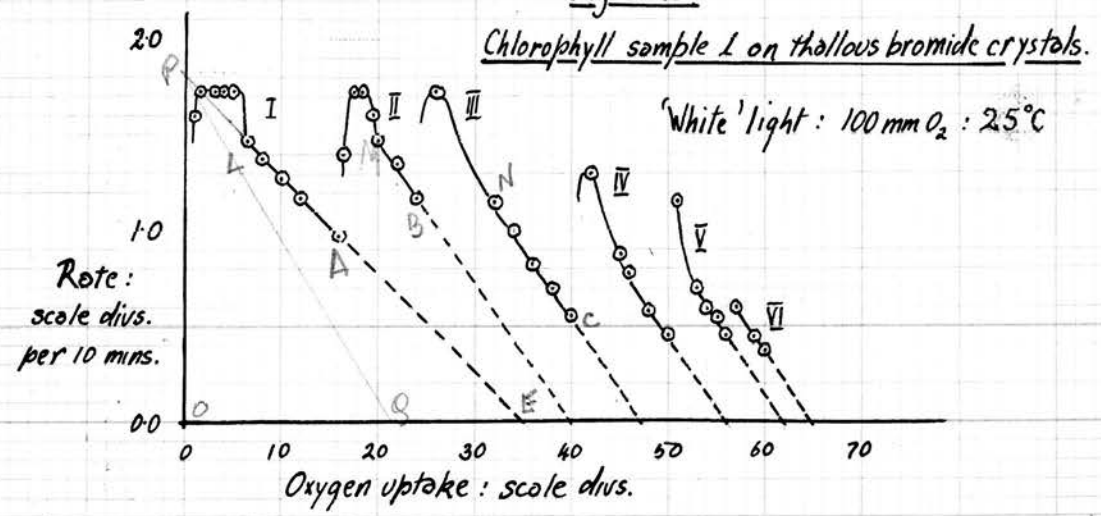


The initial extrapolated oxygen uptake as represented by OP again corresponds to unit molecular ratio. The evacuation gives s subsequent increase in rate. The linear sections of the curves are again parallel. The reversibility is much smaller than in the case of the chlorophyll fraction and evidently carotene is more readily converted into a stable photo-oxide than is chlorophyll.

Chlorophyll Sample 1. The original chlorophyll Sample 1, (4.95 mgm.) containing both carotene and chlorophyll fractions, was then examined in a similar way. Results are shown in Fig. 21.

Figure 21.

Chlorophyll sample 1 on thallous bromide crystals.



It will be observed that the rate at A returns after evacuation to the original maximum value although the constant rate portion is, in terms of oxygen uptake, much shorter than before. Evacuation at B and at C does not restore the original rate, in agreement with the conclusion that such oxidation is not completely reversible; the final product is a stable non-reversible oxygenated compound.

The slopes of the linear portions MB, NC, etc., of curves II to VI are all the same, but differ from that of curve I. This may be interpreted on the basis of the different reversibilities of carotene and chlorophyll in the sense that curves II to VI refer to chlorophyll alone, practically all the carotene having been irrever-

sibly oxidised in the course of curve I. Since the slopes are different, all the carotene cannot have been oxidised in a reaction sensitised by chlorophyll in the constant rate portion of curve I; at least part of the carotene must be oxidised simultaneously with the chlorophyll in the section LA.

If LA be projected to cut the rate axis at the point P and a line be drawn through P parallel to MB to cut the oxygen uptake axis at Q, the quantity OQ corresponds to unit oxygen molecular consumption with respect to the amount of chlorophyll in the sample. This in fact affords an easy way of determining the chlorophyll content of such a sample. No knowledge of the sample wt is required, nor indeed of the sensitivity of the measuring system: the ratio OQ/QE no matter what the units employed, is the molecular ratio of the chlorophyll to the carotene contents.

The above results of Fig. 21 were obtained by the use of a sample of chlorophyll deposited on thallous bromide from a solution in ethyl alcohol. Similar results were obtained when an acetone solution was employed, although the constant rate portion of curves I and II were shorter than before.

The Effect of Carbon Dioxide, Water Vapour and Hydrogen on the Oxidation.

Fig. 21 also shows that carbon dioxide, water vapour and hydrogen have no appreciable effect on the rate of oxidation or on the molar ratio of oxygen in the final product. The last three points on curve I and the last four of curve IV were obtained with 100 mm. of oxygen, plus 50 mm. of carbon dioxide added rapidly without discontinuing the illumination. The last two points of curve V were obtained with 100 mm. oxygen and 50 mm. hydrogen, while curve VI was obtained with 100 mm. oxygen plus 4.3 mm. water vapour.

The Reversibility of the Photo-Oxidation of Chlorophyll Sample 1: The Effect of the Dark Pressure of Oxygen.

The experiments of the preceding sections on the photo-oxidation of chlorophyll Sample 1. deposits on thallous bromide showed that an interruption of the photo-oxidation by a dark period in vacuo brought about an apparent restoration of the partially oxidised system, as judged both from the increase in rate of oxygen uptake and from the increase in the extrapolated total

Figure 22.

Chlorophyll fraction from Sample 1 on thallous bromide.

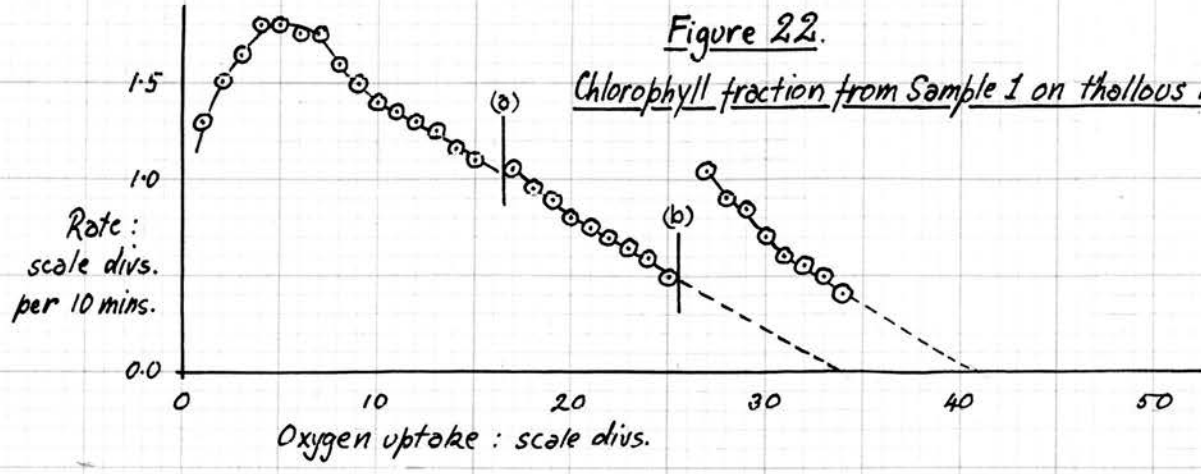


Figure 23.

Chlorophyll fraction from Sample 1. on thallous bromide

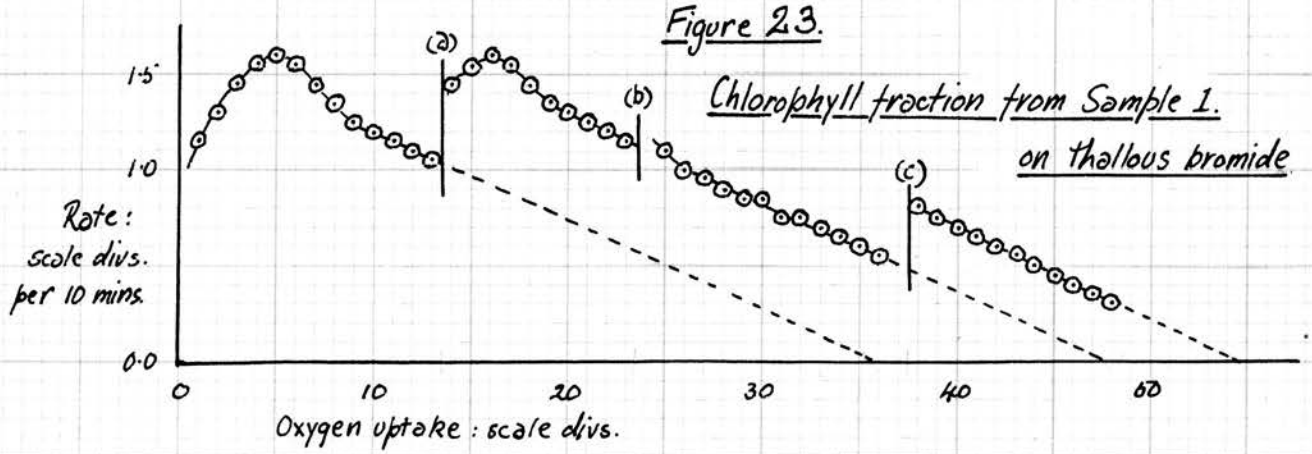


Figure 24.

Chlorophyll fraction from Sample 1. on thallous bromide.

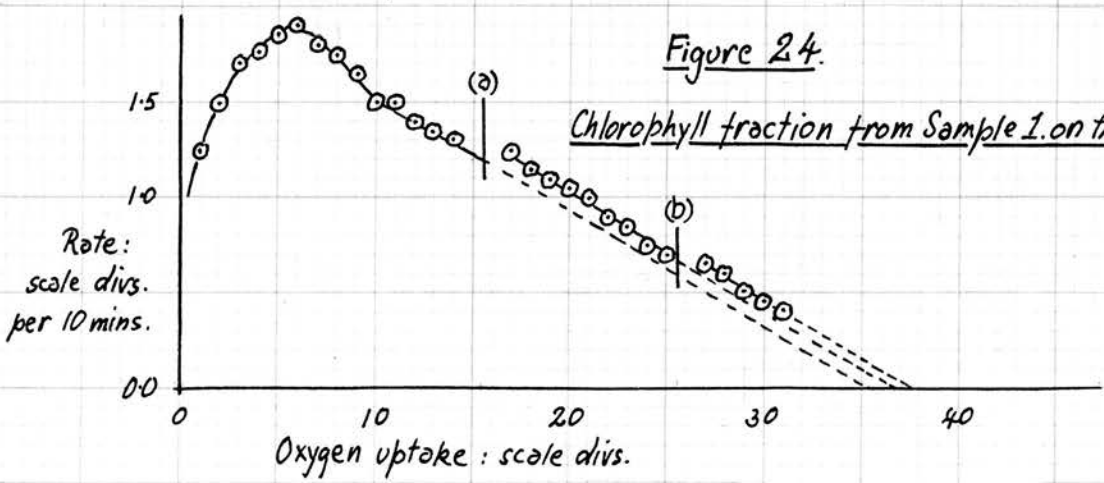
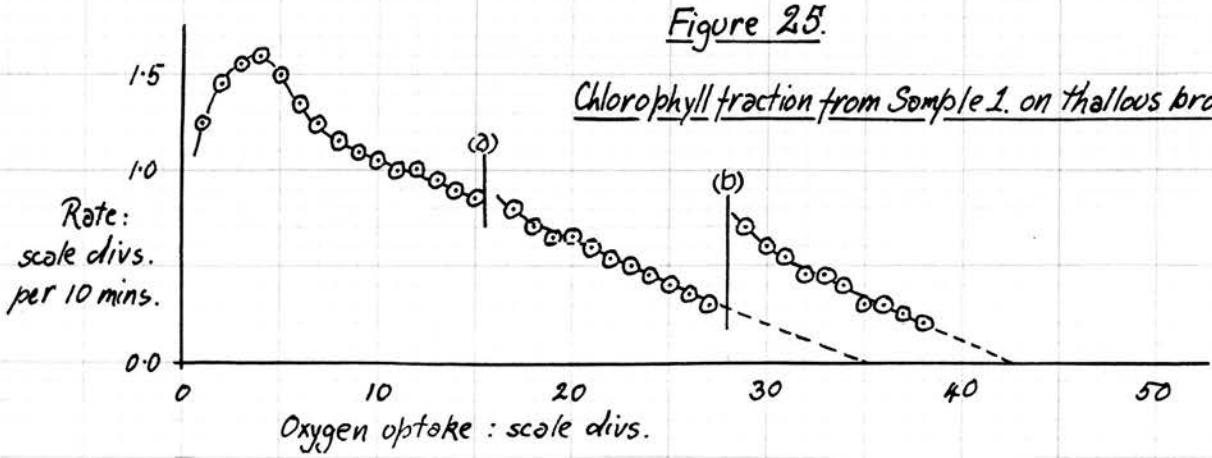


Figure 25.

Chlorophyll fraction from Sample 1. on thallous bromide.



oxygen uptake over the dark interval. These results have been interpreted as a reversibility of the photo-oxidation in vacuo. The following series of experiments was designed to ascertain the extent of this dark reversibility under various conditions.

Four separate depositions of chlorophyll (Sample 1: Sucrose chromatogram purified) on thallous bromide crystals were made and the deposits treated as detailed below. The results are recorded in the appropriate figures.

Fig. 22: 2.96 mg. chlorophyll were deposited from acetone solution on 0.46 g. thallous bromide. Illumination of the system (Orisa lamp 'white' light: 100 ± 1 mm. O_2: 25°C) was interrupted by dark intervals at :-

A; 18 hrs. in contact with 350 mm. O_2

B; 18 hrs. in vacuo (at Hyvac for 15 mins.)

Fig. 23: 2.96 mg. chlorophyll: 0.41 g. thallous bromide; dark intervals at:-

A; 18 hrs. in vacuo.

B; 18 hrs. in contact with 200 mm. O_2

C; 18 hrs. in vacuo.

Fig. 24: 2.96 mg. chlorophyll: 0.45 g. thallous bromide; dark intervals at:-

A; 18 hrs. in contact with 50 mm. O_2

B; 66 hrs. in contact with 100 mm. O_2

Fig. 25: 2.96 mg. chlorophyll: 0.65 g. thallous bromide 300 mm. O_2: dark intervals at :-

A; 18 hrs. in contact with 300 mms. O_2

B; 18 hrs. in vacuo.

The molar ratios of oxygen to chlorophyll in these runs are given in Table 9.

Table 9.

Temp. 25°C : Gas space 67.5 ml.: Guage sens. 0.024 mm. per scale div.

Wt. chloro-phyll.	Extrap. O_2 uptake (divisions).	Molar ratio O_2/Chl.	Fig.
2.96 mgm.	34.0	0.90	22
2.96	35.5	0.94	23
2.96	35.5	0.94	24
2.96	35.0	0.92	25

The general form of the rate curves for this chlorophyll purified by sucrose chromatography agrees well with that obtained for chlorophyll purified by solvent partition methods (p. 59).

The molar ratios for the initial runs in

each experiment (Table 9) are also in agreement with those obtained for other purified chlorophyll preparations. The values tend to be less than the value of unity expected by some 7%, a result explained by the use of a cylindrical vessel in these experiments when a certain amount of 'creep' of the chlorophyll up the walls of the vessel during deposition was unavoidable. The chlorophyll so deposited would not be in contact with the thallous bromide crystals and would therefore not be subject to rapid oxidation. Some oxidation may also have occurred during the purification operations.

The molar ratio obtained for a run at 300.0 mm. of oxygen (Fig. 25) confirms the constancy of this ratio over a wide range of oxygen pressures.

The effect of the dark pressure of oxygen.

In the above series of experiments deposits of chlorophyll, partially oxidised, were subjected to dark intervals under the following conditions of oxygen pressure.

in vacuo (10^{-3} mm.) : 50 mm. : 100 mm. : 200 mm. : 300 mm.

At the two highest pressures there was

no change apparent in the system over the dark interval (Fig. 23.B, Fig. 25.A). At 50 and 100 mm. during the dark interval slight reversion is detectable (Fig. 24A, B) while in vacuo reversion is obtained amounting in some cases to almost complete restoration of the initial conditions (Fig. 22B, Fig. 23A, C. Fig. 25B).

Quantitative treatment of the results required definition both of the condition of the deposit prior to the dark treatment and of the extent of the reversion during the dark treatment. Since the molecular oxygen to chlorophyll ratio was unity, the percentage oxidation on the interruption of illumination,

$$\text{i.e. } \frac{\text{Observed oxygen uptake}}{\text{Total (extrap) oxygen uptake}},$$

was used as a measure of the former, while the reversion was taken as the ratio

$$\frac{\text{Increase in total (extrap.) oxygen uptake}}{\text{Observed oxygen uptake (previous)}}$$

Dark Reversibility in Vacuo.

The reversion, as defined above, obtained for a dark interval in vacuo was taken as a measure of the maximum possible reversibility of the photo-oxidation from the condition of the system

immediately previous to the dark interval. Table 10. gives these values for a number of dark intervals in vacuo.

Table 10.

Dark Interval in vacuo: Fig.	22B	23A	23C	25 B
Observed Oxygen uptake (divs.)	25.6	13.3	25.3*	28.2
Total (extrap.) oxygen uptake (before)	34.0	35.5	35.5	35.0
Total (extrap.) oxygen uptake (after)	41.0	47.5	42.5	42.5
Increase in total oxygen uptake	7.0	12.0	7.0	7.5
% oxidation	75.5	37.5	71.5	80.5
% reversion	27.5	90.0	27.5	26.5

* Where reversion had taken place in a dark interval in vacuo earlier in the run the 'observed oxygen uptake' quoted makes allowance for the oxygen 'lost'.

The values for %age oxidation and reversion are plotted in Fig. 26.

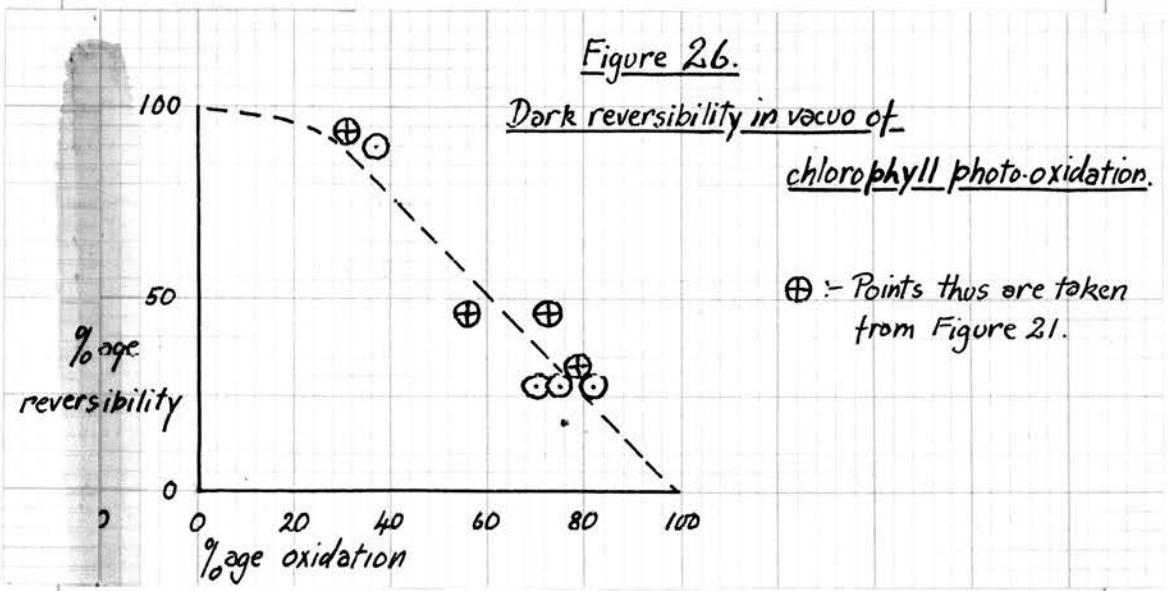


Figure 26.

Dark reversibility in vacuo of chlorophyll photo-oxidation.

The number of results plotted in Fig.26 is small and the possible error in each result high, but it would nevertheless appear that up to about 20% oxidation of the deposit, the maximum possible reversibility approaches 100%, while at oxidations exceeding this value the possible reversibility decreases, a dark interval in vacuo at near 100% finally producing little reversal.

There are thus two oxidised products, one reversible and the other irreversible. The possibility of near-complete reversibility during the early stages of the photo-oxidation would perhaps indicate that the irreversible product is a further stage in the oxidation process, and is not formed by a separate reaction. The process whereby the reversible product changes to the irreversible

product may be either thermal or photochemical, as the possibility exists that the primary reversible oxidation product is still coloured, and capable of absorbing the incident radiation.

The results of a single experiment, in which appreciable increase in the rate of oxygen uptake was observed over a dark interval of 5 days at 100 mm. of oxygen followed by evacuation at the oil-pump for several hours, while indicating the photochemical mechanism, are not considered sufficiently conclusive to decide this point, and further work along these lines is required.

The dark reversibility at various oxygen pressures. Table 11. gives the reversions obtained at various pressures of oxygen for the several dark intervals indicated.

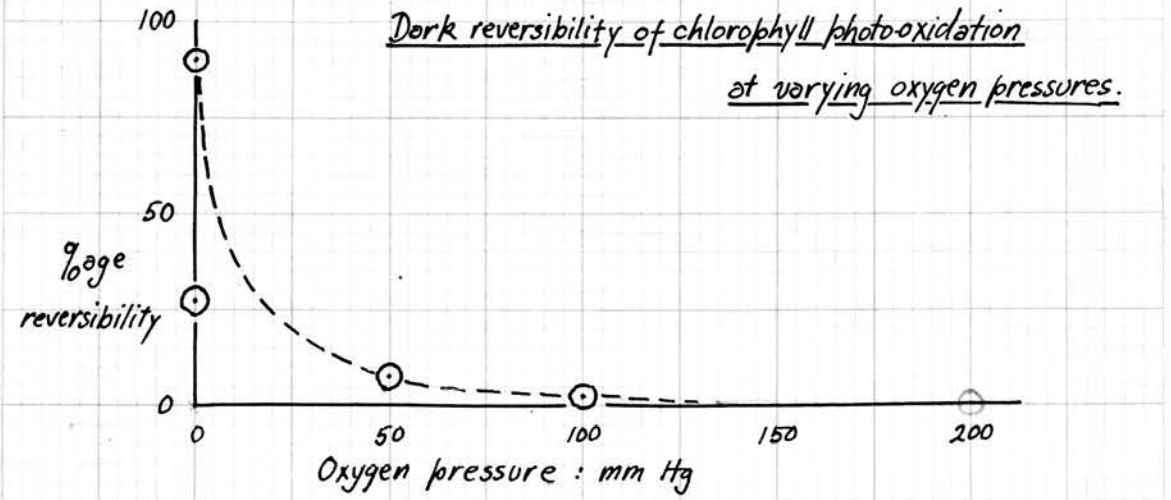
Table 11.

Dark Interval (Fig.)	24.A	24.B	23B	25A
Observed oxygen uptake (divs.)	15.7	24.0	-	-
Total (extrap.) oxygen uptake (before)	35.3	35.5	-	-
Total (extrap.) oxygen uptake (after)	37.0	36.0	-	-
Increase in total oxygen uptake	1.5	0.5	-	-
Oxygen pressure of the dark interval (mm.)	50	100	200	300
%age oxidation	44.0	68.0	-	-
%age reversion	9.5	2.0	None	None

Fig. 27 is the plot of %age reversion against oxygen pressure of the dark interval, the value of the reversion for a dark interval in vacuo being assumed to be 100%.

Figure 27.

Dark reversibility of chlorophyll photo-oxidation at varying oxygen pressures.



The plot of Fig. 27 takes no account of the state of oxidation of the system from which the reversion was obtained, but the curve indicates that only at low oxygen pressures is marked reversal of the photo-oxidation obtained. A further investigation of the reversibility in the region of 0 - 50 mm. at a strictly controlled degree of oxidation of the system would be valuable.

THE PHOTO-OXIDATION OF CHLOROPHYLL SAMPLE 2.

VII THE RATE CURVES AT HIGH OXYGEN PRESSURES FOR DEPOSITS ON THALLOUS BROMIDE CRYSTALS.

Chlorophyll Sample 1, the subject of the experiments reported above, contained copper, added, by the suppliers of the preparation, as a stabiliser (see p. 18). It was considered advisable to repeat much of the work, using a chlorophyll free from copper contaminant. Such a preparation, chlorophyll Sample 2, was obtained from the same source, specifically free from added copper. The differences in the properties of the two preparations have already been reported (p. 19).

A deposit of the chlorophyll component obtained by sucrose chromatography (p. 23) from Sample 2 was exposed to 'white' light at an oxygen pressure of 100 mm. For comparison two deposits of the untreated Sample 2 were similarly exposed. The depositions were made from acetone solution. Other experimental conditions are given in Table 12.

Table 12.

Temp. 25 C : Gas space 67.5 ml. :

Gauge sens. 1 div. = 0.024 mm.

a :- Chlorophyll fraction of Sample 2.

b, c:- Sample 2, unseparated.

	Wt. of deposit	Wt. of TlBr	Oxygen uptake : scale divisions	Molar ratio O /Chlorophyll
a	2.08mg.	0.16g.	49.0	1.85
b	2.16mg.	0.11g.	49.0	1.78
c	2.16mg.	0.30g.	49.0	1.78

Rate curves are plotted in figure 28.

The rate curves of Fig. 28 differ markedly from those for deposits of chlorophyll Sample 1.

The maximum rates obtained are greater by a factor of about three than maximum rates observed for Sample 1. deposits under similar conditions.

The form of the rate curve also differs and now follows the following general description. On the commencement of illumination the rate, initially low, rises to a maximum and then decreases,

the descending portion of the curve becoming linear and apparently readily extrapolated to the uptake axis. This linear decrease is continued to a point where there is a fairly abrupt break in the curve. The curve becomes linear again but with a slope much less steep. This final linear section was followed experimentally to very low rates but no further change in slope was observed.

Using the final extrapolated oxygen uptake obtained for the chlorophyll component (Fig. 28a), the molar ratio, on the usual assumption of molecular weight as 900, of oxygen to chlorophyll is 1.85. As it is highly improbable that the experimental error in this value is greater than 10%, it appears that the final oxidised product in this case contains nearly two molecules of oxygen per molecule of chlorophyll originally present.

The molar ratios for the chlorophyll-carotene mixtures of Fig. 28b and c are both 1.78. The relatively small change in the molar ratio occasioned by the presence of a molar percentage of 26 (see p. *25*) of carotene in the deposit may be due to the chance that while the molecular weight of carotene (536) is approximately half that of chlorophyll (900), the carotene oxidises with the uptake of oxygen corresponding to a molar ratio of

unity, as compared with the chlorophyll (Sample 2) molar ratio of two. Hence the oxygen uptake by a given weight of deposit is not greatly affected by the relative proportions of chlorophyll and carotene present.

The presence of carotene would appear however to have a considerable effect on the position of the break in the descending portion of the rate curve. Good separation was obtained in the preparation of the pure chlorophyll component of Sample 2 so that the rate curve of Fig. 28a is considered to represent carotene-free conditions. The presence of carotene would therefore appear to modify the rate curve but not to play any essential part in determining its form.

It is an attractive hypothesis to link the two linear sections of the curve with the entry of the two molecules of oxygen present in the final oxidised product. These oxygen molecules may react with the excited chlorophyll molecule either independently or consecutively, in the latter case the entry of the second oxygen molecule being dependent on the previous entry of the first.

The hypothesis of independent entry requires, to give a uniform final product, the reaction steps.

$$\overline{\text{Chl}} + O_2 \xrightarrow{h\nu} \overline{\text{Chl}}.O_2^{I} \quad \ldots\ldots k_1 \quad \ldots\ldots\ldots (1)$$

$$\overline{\text{Chl}} + O_2 \xrightarrow{h\nu} \overline{\text{Chl}}.O_2^{II} \quad \ldots\ldots k_2 (< k_1) \ldots\ldots (2)$$

$$\overline{\text{Chl}}.O_2^{I} + O_2 \xrightarrow{h\nu} \text{Chl}.2O_2 \ldots\ldots k_3 (k_3 \simeq k_2) \ldots\ldots (3)$$

$$\overline{\text{Chl}}.O_2^{II} + O_2 \xrightarrow{h\nu} \text{Chl}.2O_2 \ldots\ldots k_4 \text{ (small)} \ldots\ldots (4)$$

Where $\overline{\text{Chl}}.O_2^{I}$ and $\overline{\text{Chl}}.O_2^{II}$ are the monoperoxidic forms having the higher and the lower rate of formation respectively, and $\overline{\text{Chl}}.2O_2$ is the diperoxidic end product.

It is unlikely that the monoperoxidic forms will possess the same reactivities towards oxygen as did the original chlorophyll molecule. The species $\text{Chl}.O_2$ being formed more readily, might be expected to entail less modification of the original molecule. We have therefore taken $k_3 \simeq k_2$. The form $\overline{\text{Chl}}.O_2^{II}$ might be colourless (non-absorbing) and therefore unreactive, k_4 being then very small, unless the internal rearrangement $\overline{\text{Chl}}O_2^{II} \rightarrow \overline{\text{Chl}}O_2^{I}$ ..... k_5 were possible.

The alternative reaction mechanism involving stepwise entry of the oxygen as the main reaction path, may be formulated

$$\overline{\text{Chl}}. + O_2 \xrightarrow{h\nu} \overline{\text{Chl}}.O_2^{I} \quad \ldots\ldots k_1$$

$$\overline{\text{Chl}}.O_2^{I} + O_2 \xrightarrow{h\nu} \overline{\text{Chl}}.2O_2 \ldots\ldots k_3$$

Reaction (2) may well proceed as a side reaction to a small extent, and if further reaction of the form $\overline{Chl}.O_2^{II}$ were impossible, this side reaction might account for part of the observed deficiency in the oxygen uptake requirements for a molar ratio of two in the final product.

Both mechanisms appear capable of yielding rate curves of the type obtained experimentally, providing suitable assumptions about the relative magnitudes of the rate constants are made. With $k_1 > k_3$ the first linear section would be determined mainly by the exponential decrease in $\overline{Chl}$, modified slightly by the simultaneous uptake due to reactions (2) and (3). The second linear section would appear when the exponential decrease in $\overline{Chl}.O_2^{I}$ became the main rate-determining factor.

It is to be noted that the rate curves of Fig. 28 do not exhibit the constant rate section which were a characteristic of chlorophyll Sample 1 deposits containing carotene.

These experiments having shown that there are possibly two positions at which oxygen can enter the chlorophyll (Sample 2) molecule under the prevailing conditions, the fact that the copper containing Sample 1. chlorophyll shows only one might be interpreted in two ways.

In the copper containing sample the first position might already have been completely oxidised. This would require a very selective oxidation of the first position with negligible attack at the second for the observed oxygen uptake ratio to be so precisely unity for the second position oxidation.

Alternatively the modification of the chlorophyll molecule brought about by 'copper substitution' may effectively block one of the two positions, this modification also leading to non-fluorescence and failure to give the Molisch phase test (see p. 19).

The establishment of the correct alternative requires further study of the differences between chlorophyll Sample 1. and chlorophyll Sample 2. and the conversion of the latter into the former under controlled conditions, particularly in respect of oxygen present in the conversion solvent.

A dark interval in vacuo brings about considerable reversal of oxygen uptake, the rate curve following the interruption exhibiting the same characteristic break in the descending portion of the curve. It is not possible to determine

whether the oxidation at both positions is reversible from the available data and further investigation of this point is required.

It is further possible that the fundamental assumption above is incorrect, namely that two molecules of oxygen are taken up by each chlorophyll molecule. Chlorophyll preparations of this kind may contain pheophytin, in which the magnesium atom is replaced by two hydrogen atoms. It is known that such positions are also generally oxidisable, in which case the oxygen molecular ratio might be three. The experimental value of 1.85 would then depend on the relative amounts of chlorophyll and pheophytin, and the closeness of the value to two would be fortuitous. A possible line of investion of this would be the accurate determination of the adsorption spectrum over certain wavelengths.

THE PHOTO-OXIDATION OF CHLOROPHYLL SAMPLE 2.

VIII THE REACTION RATE AT LOW OXYGEN PRESSURES FOR DEPOSITS ON THALLOUS BROMIDE CRYSTALS.

Both chlorophyll preparations having shown that their oxidation is partly reversible by simple pressure reduction, it was considered possible that a critical oxygen pressure might exist below which no photo-oxidation would occur, the forward rate of formation of the oxygenated product being then counterbalanced by the reverse dissociation into reactant and oxygen. Preliminary experiment showed that such a limit must lie at very low pressures, of the order of 0.1 mm. of oxygen: results are here quoted for the pressure region below 0.5 mm. Under such conditions both the chlorophyll content and the oxygen pressure become simultaneously variable factors, in contrast to the oxidations at 100 mm. oxygen, when the fractional change in oxygen pressure is so small as to render the oxygen pressure sensibly constant, and without appreciable effect on the rate of oxidation (p.55).

In this low pressure region the rates obtained with copper-containing chlorophyll Sample 1. were so low as to render observation difficult and inaccurate. With chlorophyll

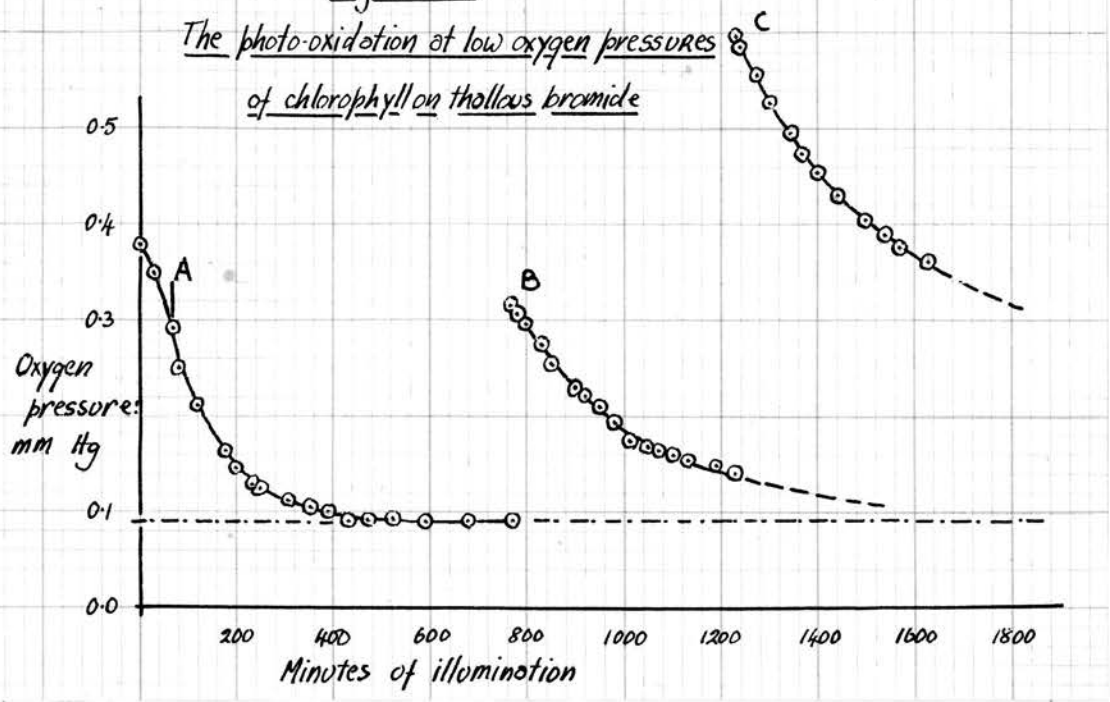
Sample 2, however, accurate measurement was possible and the following results refer to this preparation.

Experimental. 2.0 mg. of chlorophyll Sample 2. were deposited on thallous bromide crystals from acetone solution in the cylindrical vessel. Into the evacuated system was introduced oxygen sufficient to give a rise in scale reading of 16.0 divisions, equivalent to a pressure of 0.39mm. Hg. The system was exposed to 'white' light at 20°C.

At this low pressure the vibration of the gauge pointer was marked, making observation difficult. Accordingly, after an oxygen uptake corresponding to 4.0 scale divisions illumination was interrupted to allow the introduction of 50 mm. of carbon dioxide, a gas shown to be inert in this reaction (p. 75). Illumination was recommended and observations continued to very low oxygen uptake rates. Fig. 29. is the plot of pressure against time for this and following runs. (A) indicates the point at which the carbon dioxide was introduced.

Figure 29.

The photo-oxidation at low oxygen pressures of chlorophyll on thallous bromide



(B) and (C) indicate further interruptions of illumination during which the system was pumped out and a fresh small amount of oxygen and 50 mm. of inert gas introduced; (B) 13.1 scale divs. (= 0.32mm. Hg) (C) 24.8 scale divs. (= 0.60mm. Hg).

In the initial run (Curve (A) of fig. 29) the partial pressure of oxygen fell to a value of 0.9 mm. approximately. This value was reached after an exposure time of about 450 mins., and was maintained constant during a further 250 mins. of illumination. The fall in pressure in the following run was not continued to such low values, but

curve (B) is obviously asymtotic to a similar limiting oxygen pressure, although in this case an exposure time of about 15 hours would have been required to reach this pressure.

Only a representative selection of the experimental observations are plotted in Fig. 29, and the rate curves of Fig. 30. were obtained from the complete plots.

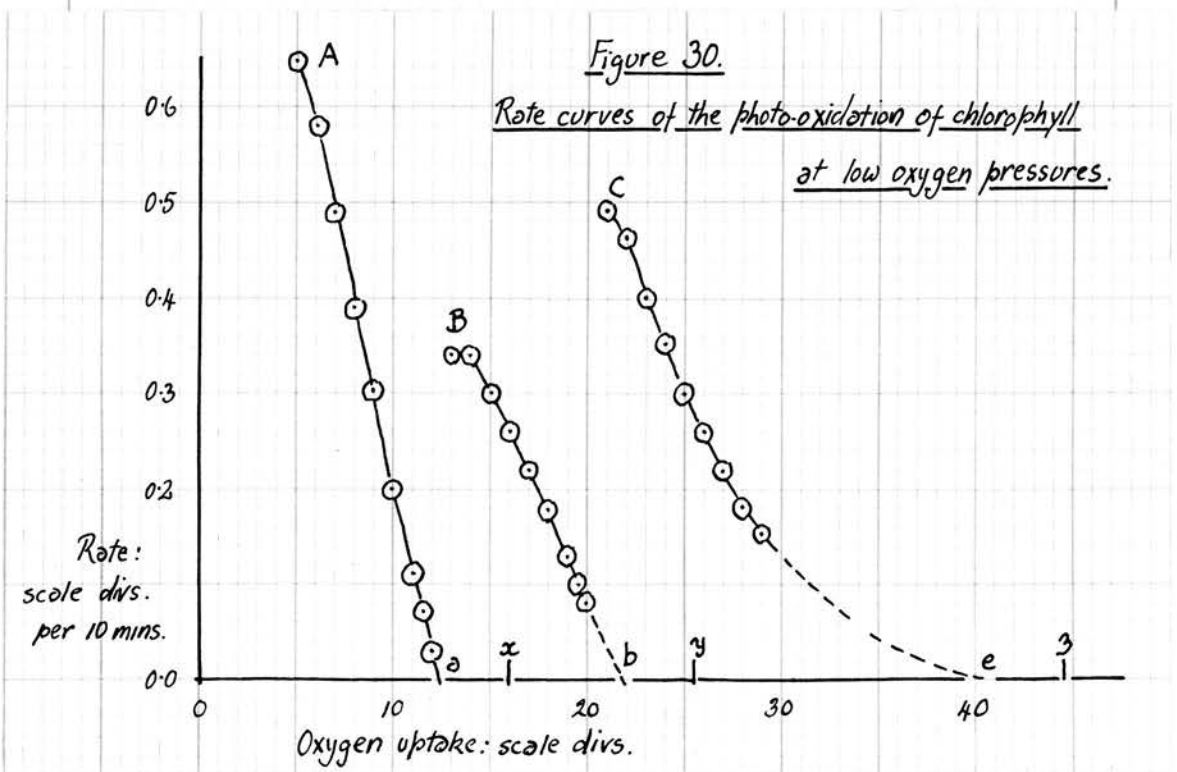


Figure 30.
Rate curves of the photo-oxidation of chlorophyll at low oxygen pressures.

In contrast to the rate curves for exposures at high oxygen pressures the rate curves of Fig. 30 are never linear, an effect arising from the simultaneous variation of both the chlorophyll and oxygen 'contents' of the system. Despite this,

extrapolated total oxygen uptake values for each run can be obtained with fair accuracy. For the runs commencing at (A), (B) and (C) respectively these values are 12.4, 21.7 and c.40 scale divisions. In each case the value falls short of consumption of all the oxygen present by several scale divisions ($(x - a)$, $(y - b)$, $(z - c)$: Fig. 30).

Under otherwise identical conditions but at an oxygen pressure of 100 mm., 2.0 mgm. of chlorophyll Sample 2. is capable of taking up oxygen corresponding to 44 scale divisions. Ignoring the possibility of reversal during the evacuations at (B) and (C), a procedure perhaps justified by the low rates to which oxidation was taken previous to the evacuations, it is possible to give a value for the chlorophyll content of the system at any stage in the experiment, in terms of the amount of oxygen that the deposit was still capable of taking up.

We can therefore determine, from the rate curves of Fig. 30, the oxygen pressure and the chlorophyll content, both in terms of scale divisions, at any stage in the experiment.

This has been done for several points on each rate curve and the results tabulated (Table 13).

Table 13.

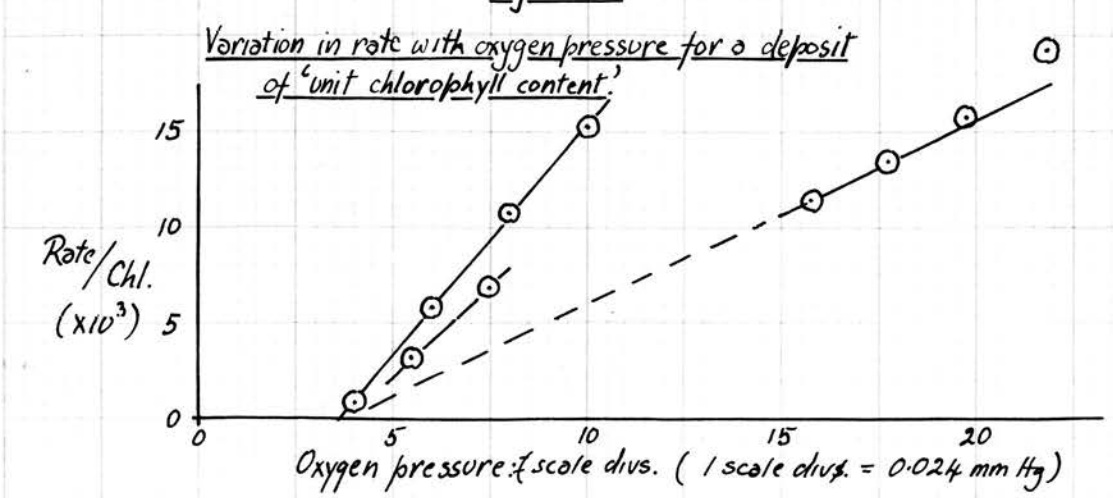
Chlorophyll content (scale divs.)	15	17	19	21	24	26	32	34	36	38
Oxygen pressure (scale divs.)	15.8	17.8	19.8	21.8	5.5	7.5	4.0	8.0	8.0	10.0
Rate (scale divs./10 mins.)	0.17	0.23	0.30	0.40	0.08	0.18	0.03	0.20	0.39	0.58
Rate/Chlorophyll Content ($\times 10^3$)	11.3	13.5	15.8	19.1	3.33	6.92	0.94	5.88	10.8	15.3

On the assumption that the rate of oxygen uptake is proportional to the chlorophyll content as defined above, we may attempt to eliminate one of the variables by dividing the rate at any point in the run by the corresponding chlorophyll content.

It has however been shown for chlorophyll Sample 2. deposits at high oxygen pressures that the relationship between the rate and the oxygen uptake capacity during a run is apparently complicated by the formation of oxygen absorbing intermediates (p.87). The presence of carotene is an added complication.

Fig. 31. is the plot of the rate of oxygen uptake per unit oxygen uptake capacity (Rate/Chlorophyll content of Table 13) against oxygen pressure, at various stages during the oxidation.

Figure 31.



Three groups of points are obtained, each derived from a different run. It is obvious that the rate of oxygen uptake at low pressures is not directly proportional to the oxygen uptake capacity, as the required coincidence of the three curves is not obtained. The effect is analogous to the observation at high pressures that the slope of the descending portion of the rate curve becomes less steep in the final stages (p. 86).

As has already been noted the extrapolated value of the oxygen uptake for each run falls short of the total oxygen available by several scale divisions. This residual pressure of oxygen may be obtained from any of the plots of Figs. 29,30, and 31, and is about 3.7 scale divisions (3.7 x 0.024 = 0.088mm.). Oxygen uptake apparently ceases at this pressure.

In view of the reversibility during dark intervals at reduced pressure noted in earlier experiments (p. 90) this limit must represent the dissociation pressure of the peroxidic products of the reaction. In view of the complex nature of the absorbing solid phase; chlorophyll, chlorophyll monoperoxide and carotene being simultaneously present; further experimental work is required

before the nature of the equilibrium established may be more fully discussed.

The ultimate formation of an irreversible peroxidic end-product would lead one to expect continued absorption of oxygen to pressures below the true dissociation pressure. However the very transient existence of a molecule of the reversible peroxide to be expected at pressures sensibly below the dissociation pressure would render the rate of transformation into the irreversible form very slow indeed, and the amount of absorbed oxygen 'stabilised' at pressures below the dissociation pressure in this way would be very small.

PRELIMINARY EXPERIMENTS IN COLOUR FADING.

Several identically prepared deposits of chlorophyll Sample 2. on thallous bromide crystals were exposed in air to Osira lamp 'white' light for varying times. The partially bleached systems were extracted with acetone and the residual 'chlorophyll equivalents' estimated in the red and violet spectral regions by means of a Spekker photometer.

Experimental

0.100 g. portions of thallous bromide crystals were weighed out into 100 ml. Pyrex conical flasks, chosen for the flatness of their bases. The crystals were separated by vigorous shaking with a few ml. of acetone. This decanted off, the walls of the flask were washed down with a further few drops of acetone, and this used to spread the crystals in a uniform layer on the bottom of the flask. The acetone was them pumped off at the filter pump leaving a fairly uniform circular layer on the bottom of the flask.

Into each of the flasks thus prepared was introduced 1.70 mgm. chlorophyll/carotene mixture Sample 2. (2 ml. 0.850 gm./l.acetone solution). The acetone solvent was allowed to evaporate off at room temperature in the dark. Final removal of

solvent was made by pumping out for 20 minutes on the oil pump, light being excluded.

Pigmentation of the crystals appeared uniform, and only very slight traces of chlorophyll were noted on the rounded portions of the bases of the flask outside the crystal boundary.

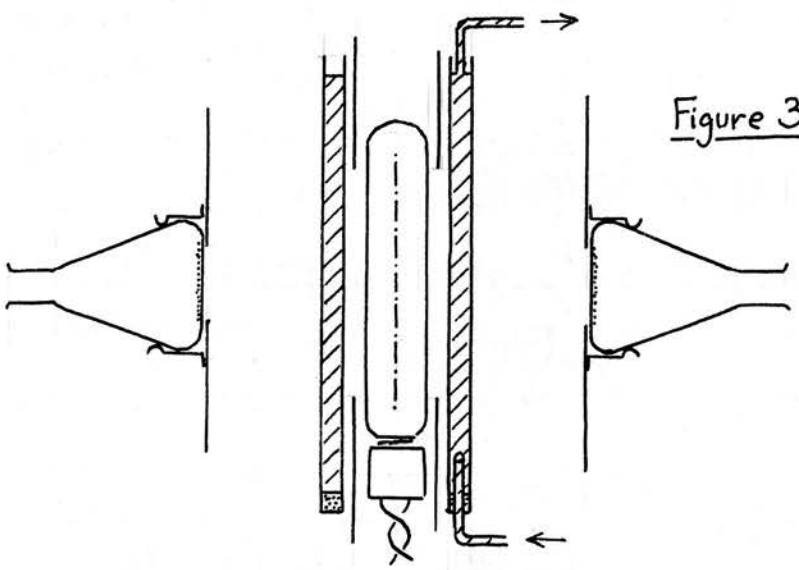


Figure 32.

The exposure apparatus illustrated in Fig. 32 consisted of a 230V/400W Osira lamp mounted vertically. Soft glass tubes formed a 2 cm. air gap and a 2 cm. water filter concentric about the lamp. During illumination the flasks were mounted radially about the lamp in clips on a housing. The housing was blackened inside. The water in the filter was changed continuously, being introduced tangentially at the bottom of the filter and sucked off by means of a water pump at the top of the filter.

The filter combination soft glass/water

eliminated ultra violet light of wavelength less than the 3650 A.U. line, and much of the infra red radiation. In operation, with a room temp. of 15°C, the temperature of the crystals during illumination was maintained at about 35°C.

The prepared flasks were exposed for the times noted in Table 14, being removed and stored in the dark when their illumination was discontinued. The partially bleached deposits were then extracted with 5 mls. of acetone for 2 minutes and the resulting solution compared with a standard solution of 2 mls. of the original chlorophyll solution in acetone, diluted to 5 mls., in a Spekker photometer, using the colour filters Spectrum Violet 601 and Spectrum Red 608. The instrument was calibrated over the necessary range using various dilutions of the chlorophyll solution.

Table 14.

Time of Illumination mins.	Chlorophyll equivalent: Violet.	mls. standard solution. Red.
Blank	1.94	1.95
Blank	2.00	2.00
2	1.46	1.70
5	1.12	1.31
10	0.96	1.12
15	1.02	1.00
30	0.87	0.77
60	0.54	0.45
60	0.54	0.45
85	0.47	0.36

Figure 33.

Colour fading during photo-oxidation for a chlorophyll deposit on thallous bromide crystals.

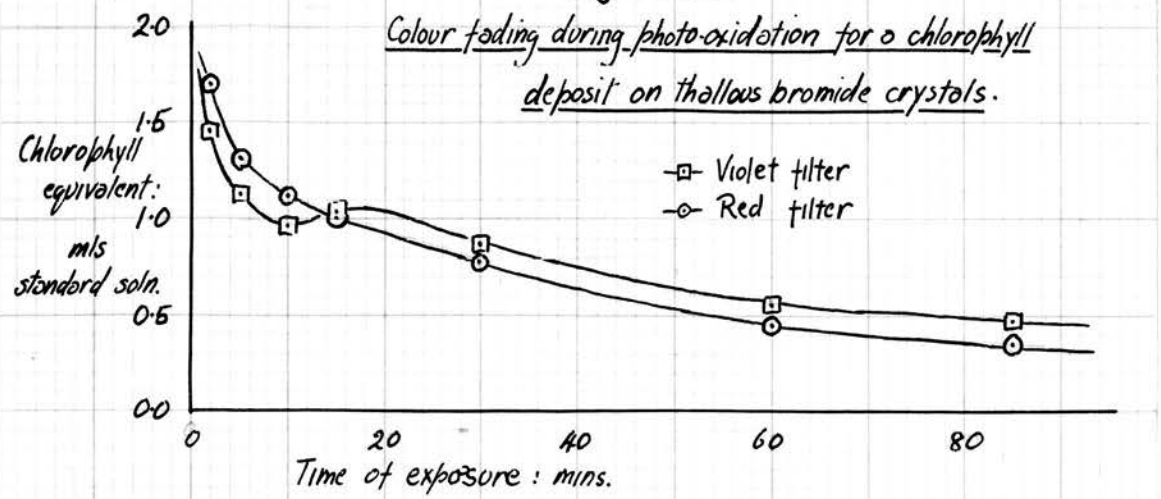


Fig. 33 is the plot of chlorophyll equivalent (Table 14) against time.

The following points may be noted from the curves of Fig. 33:-

(a) the initial more rapid fading of 'chlorophyll' as measured by 'blue absorption.

(b) The sygmoidal form of the 'blue' curve leading to a crossover of the red and blue 'chlorophyll equivalent' curves.

(c) the persistence of blue absorption in the later stages.

In explanation of (a) we may postulate a first product in which both red and blue maxima are suppressed, the blue maximum more strongly. The blue maximum may even be completely suppressed in this initial product, but there must be some red

absorption or the two curves would coincide.

The form of the blue curve and the cross-over noted in (b) seem to demand the appearance of a blue absorbing secondary product. The red absorption of this product must be less than its blue absorption (relative to chlorophyll as a standard) and may be zero. This substance would not appear to be the end product of the reaction since as the curves do not tend to diverge further, as would be expected if this substance accumulated. Both curves tend to zero and the final product may be colourless.

In the light of the deductions already made concerning the mechanism of the oxygen uptake by this chlorophyll preparation, it is attractive to equate these three stages with the reaction steps postulated.

$\overline{Chl} + O_2 \xrightarrow{h\nu} \overline{Chl}.O_2$ (red absorbing: blue maximum suppressed)

$\overline{Chl}O_2 + O_2 \xrightarrow{h\nu} \overline{Chl}.2O_2$ (blue absorbing: red maximum suppressed)

$\overline{Chl}.2O_2 \rightarrow$ irreversible di-peroxide (colourless)

The presence of both chlorophylls a and b and carotene in the system makes necessary further work on these lines on the separated components before the discussion may be pressed. In support

of the present findings and conclusions, analogous changes in the absorption spectrum have been reported for the aut-oxidative fading of illuminated solutions of chlorophyll.[35].

THE PHOTO-OXIDATION OF CHLOROPHYLL ON GLASS.

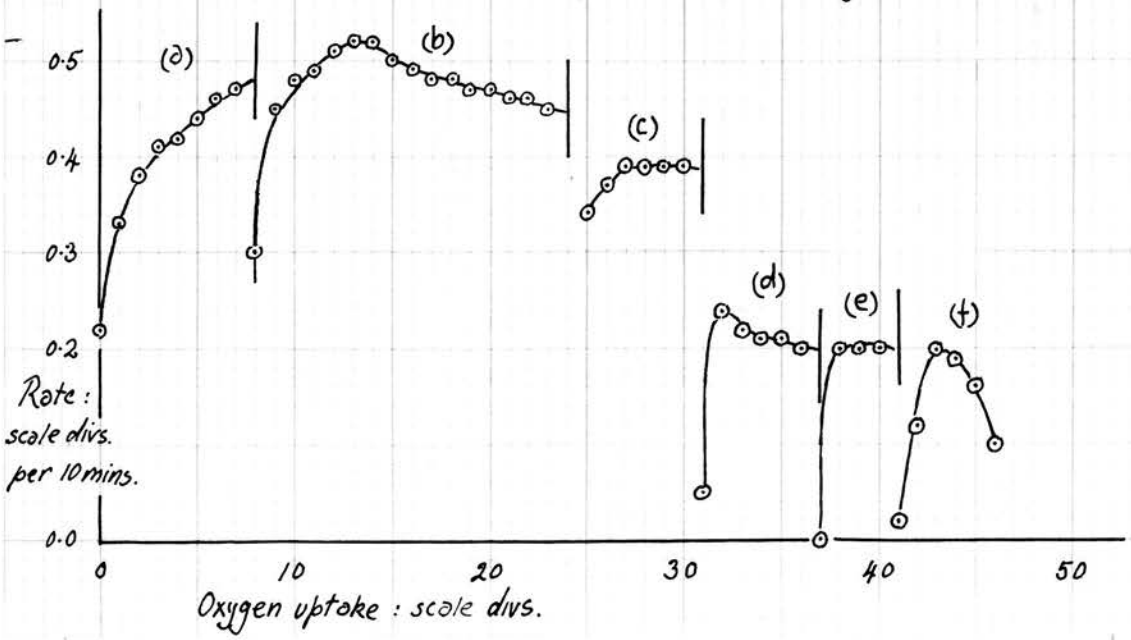
The majority of the experiments of the preceding sections were carried out with pigment deposits on thallous bromide crystals, a system giving conveniently measurable oxygen uptake rates. No changes in pressure were observed with thallous bromide in the absence of pigment for otherwise comparable conditions, and it therefore appeared that thallous bromide merely acted as an otherwise inert photosensitiser. It was, however, thought desirable to attempt to confirm the main features of the oxidation with deposits on glass itself as the simplest system free from any complications. The preliminary experiments had shown that deposits on the plane end face of the reaction vessel gave, on illumination at the highest conveniently obtainable intensity, very low oxygen uptake rates. In an attempt to obtain higher rates, it was decided to use chlorophyll spread on small glass beads in one of the cylindrical vessels.

Small glass beads of approximately 2 - 3 mm. diameter, sufficient in number to fill the chosen reaction vessel, were cleaned with chromic acid, rinsed with distilled water, washed and dried with acetone. Approximately two-thirds of these

beads were then transferred to a second vessel, and, in dim artificial light, 2.31 ml. of an acetone solution of chlorophyll Sample 1. were dried on them at the water-pump, the vessel being rotated to ensure as far as possible even distribution. The walls of the vessel were then "rinsed" with the remaining beads in two portions, by the addition of a small volume of acetone and drying as before. All the beads were then mixed and transferred to the reaction vessel. This was attached to the main apparatus, pumped out for one hour, left evacuated for 15 hours, pumped out for 2 hours, and then exposed to full 'white' light at 100.2 mm. of oxygen.

Figure 34.

Chlorophyll photo-oxidation on glass beads.



The maximum rates of oxygen uptake are still low, but in comparison with experiments in the absence of the beads, they show an increase by a factor of approximately three, after allowance has been made for the decrease in volume of the system.

All the curves show marked 'induction' periods, this being especially marked in the later stages of the illumination, e.g. in (d), (e) and (f) of Fig. 34. the initial rate on resuming illumination is almost zero. The maximum rates obtained in (b), (c), (d), (e) and (f) show a general decrease, but the factors governing the extent of the decrease between adjacent runs are obscure.

In contrast to the thallous bromide systems a dark period under vacuum does not appear to cause obvious reversion, while contact with 100 mm. of oxygen in the dark, which for thallous bromide systems gave practically no change in rate, has here caused a marked reduction.

If we project the linear portion of curve (b) to cut the oxygen uptake axis, the calculated oxygen to chlorophyll ratio (taking the molecular weight as 900 and neglecting the carotene fraction) is approximately 1.9, this to be compared with the value of 1.2 obtained with thallous bromide under

similar assumptions. The value 1.2 would correspond to an oxygen uptake of some 55 scale divisions; the rate curves of Fig. 34. do seem to be approaching in a general sense this figure.

DISCUSSION.

While it is unfortunate that the main features of the oxidation on thallous bromide crystals have not been exactly duplicated on glass, and, therefore a common mechanism of oxidation can not rigidly be taken to occur, a general mechanism can be put forward to cover the main trends of the observations. To simplify the argument the term chlorophyll is used generally for these general preparations and mixtures and only when necessary is the nature of the actual preparation indicated.

Reaction is initiated by the absorption of a quantum of the incident radiation by a ground state chlorophyll molecule, an excited chlorophyll molecule of higher electronic state being formed. We denote these molecular species $\overline{\text{Chl}}$ and $\overline{\text{Chl}}^*$ respectively and formulate the initial step as follows:-

$$\overline{\text{Chl}} + h\nu \longrightarrow \overline{\text{Chl}}^*$$

The observed quantum efficiency is low and much of the absorbed light energy is degraded without causing chemical reaction. A high proportion of the excited molecules must therefore revert to the ground state by some process of internal

degradation, and only a few enter the relatively long-lived 'reactive' state, which still possesses an energy higher than the ground state. To this long-lived reactive species, for the existence of which much evidence has been presented in the literature,[36.] we give the symbol $\overline{\text{t-Chl}}$.

$$\overline{\text{Chl}}^* \rightarrow \overline{\text{Chl}}$$

$$\overline{\text{Chl}}^* \rightarrow \overline{\text{t-Chl}}.$$

This reactive species too may revert to the ground state but has, asnnoted, a relatively long half-life, enabling it to react rapidly and reversibly with molecular oxygen to give a chlorophyll peroxide $\overline{\text{t-Chl}}.O_2$

$$\overline{\text{t-Chl}} \rightarrow \overline{\text{Chl}}$$

$$\overline{\text{t-Chl}} + O_2 \rightleftarrows \overline{\text{t-Chl}}.O_2$$

Irreversible oxidation takes place by the slow transformation of the reversible peroxide into an irreversible peroxide $\overline{\text{Chl}}.O_2$ by a process which may be photochemical.

$$\overline{\text{t-Chl}}.O_2 \xrightarrow{h\nu\ ?} \overline{\text{Chl}}O_2$$

The species $\overline{\text{t-Chl}}O_2$ and $\overline{\text{Chl}}.O_2$ strictly represent only the chlorophyll Sample 1. derivatives.

The reversible autoxidation stage

$$\overline{\text{t-Chl}} + O_2 \rightleftarrows \overline{\text{t-Chl}}.O_2$$

(solid) (gas) (solid)

requires separate consideration.

Here a gas in contact with two chemically distinct solid species forms a two component system of one gaseous and two solid phases. The familiar case of the dissociation of calcium carbonate is of identical type.

$$\underset{\text{(solid)}}{CaO} + \underset{\text{(gas)}}{CO_2} \qquad \underset{\text{(solid)}}{CaCO_3}$$

By the phase rule both systems are univariant. In the calcium carbonate system equilibrium at a given temperature is attained at a certain definite pressure of carbon dioxide; the dissociation or equilibrium pressure at that temperature. The equilibrium pressure is determined solely by the free energy changes involved in the reaction of equivalent quantities of the species concerned and is independent of the relative or absolute amounts of the solid phases and of the volume of the gaseous phase. At a constant temperature a decrease in the carbon dioxide pressure imposed on the system will cause dissociation of the carbonate until the equilibrium pressure appropriate to the temperature is re-established. If the amount of carbonate present in the system is insufficient to re-establish the equilibrium pressure then the carbonate solid phase will disappear from the system. Similarly an imposed

increase in the carbon dioxide pressure will bring about the formation of carbonate at the expense of the calcium oxide until either the equilibrium pressure is re-attained or the calcium oxide phase has disappeared. When conditions are such that only one solid phase is present the system becomes bivariant and the carbon dioxide pressure and the temperature may be chosen independently.

The transference of these arguments to the analogous chlorophyll peroxide system makes possible the following explanations of a number of the experimental observations.

The higher pressures of oxygen used are well in excess of any possible dissociation pressure of the reversible peroxide, this solid phase being stable under the prevailing conditions. When the reactive chlorophyll phase is introduced into the system by light action it will therefore react with molecular oxygen at a rate proportional to some function of the oxygen pressure in excess of the dissociation pressure and of the number of molecules of the reactive chlorophyll species which temporarily exist. This will be the experimentally measured rate of reaction.

In the dark on the cessation of illumination at an oxygen pressure higher than the

dissociation pressure of the reversible peroxide, the prevailing oxygen pressure will prevent appreciable dissociation of the peroxide. The amount of the reactive chlorophyll species which will exist under these conditions will be exceedingly small. On the resumption of illumination the system will therefore be found to be sensibly unchanged, a result in agreement with experiment.

A dark interval during which the oxygen pressure is lowered to a value less than the dissociation pressure will lead to the complete dissociation of the reversible peroxide, and the reactive chlorophyll so formed will revert, irreversibly in the absence of light, to the ground state. On the re-introduction of oxygen at a higher pressure the ground state chlorophyll will be unable to react and no dark uptake of oxygen will be observed. Subsequent illumination will reveal the autoxidation to have been reversed to some extent during the dark interval in vacuo.

The experimental work at low oxygen pressures gives more direct evidence for the existence of a dissociation pressure for the reversible peroxide. The rate of oxygen uptake falls to zero at an oxygen pressure of about 0.1 mm. and it must be presumed that below this pressure the

reversible peroxide is unstable however much of the reactive chlorophyll be formed by light action.

A kinetic expression for the observed rate of oxygen uptake may then be derived from the proposed reaction mechanism.

$$\overline{Chl} + h\nu \rightarrow \overline{Chl}^* \qquad (1) \ldots\ I_{abs}$$

$$\overline{Chl}^* \rightarrow \overline{Chl} \qquad (2) \ldots\ k_2$$

$$\overline{Chl}^* \rightarrow \overline{t\text{-}Chl} \qquad (3) \ldots\ k_3$$

$$\overline{t\text{-}Chl} \rightarrow \overline{Chl} \qquad (4) \ldots\ k_4$$

$$\overline{t\text{-}Chl} + O_2 \rightarrow \overline{t\text{-}Chl}.O_2 \qquad (5) \ldots\ k_5$$

$$\overline{t\text{-}ChlO_2} \rightarrow \overline{t\text{-}Chl} + O_2 \qquad (6) \ldots\ k_6$$

$$\overline{t\text{-}ChlO_2} \rightarrow \overline{Chl}.O_2 \qquad (7) \ldots\ k_7$$

For steady state conditions

$$\frac{d[\overline{Chl}^*]}{dt} = I_{abs} - k_2[\overline{Chl}^*] - k_3[\overline{Chl}^*] = 0 ,$$

whence $[\overline{Chl}^*] = I_{abs} / (k_2 + k_3)$.

$$\frac{d[\overline{t\text{-}Chl}.O_2]}{dt} = k_5[\overline{t\text{-}Chl}][O_2] - k_6[\overline{t\text{-}Chl}.O_2] - k_7[\overline{t\text{-}Chl}.O_2] = 0$$

whence $[\overline{t\text{-}Chl}.O_2] = k_5[\overline{t\text{-}Chl}][O_2] / (k_6 + k_7)$.

$$\frac{d[\overline{t\text{-}Chl}]}{dt} = k_3[\overline{Chl}^*] - k_4[\overline{t\text{-}Chl}] - k_5[\overline{t\text{-}Chl}][O_2] + k_6[\overline{t\text{-}Chl}.O_2]$$

$$= \frac{k_3}{k_2 + k_3} I_{abs} - k_4[\overline{t\text{-}Chl}] - k_5[\overline{t\text{-}Chl}][O_2] + \frac{k_5 k_6}{(k_6 + k_7)}[\overline{t\text{-}Chl}][O_2]$$

$$= 0 ,$$

whence $[\overline{\text{t-Chl}}] = \frac{k_3}{(k_2+k_3)} \cdot I_{abs} \cdot \frac{1}{\left(k_4 + k_5[O_2] - \frac{k_5 k_6}{(k_6+k_7)} \cdot [O_2]\right)}$.

Now when $[O_2] = [O_2]_{equilibrium}$

$$k_5[\overline{\text{t-Chl}}][O_2]_{eq} = k_6[\overline{\text{t-Chl}}.O_2] \quad .$$

In the general case

$$-\frac{d[O_2]}{dt} = k_5[\overline{\text{t-Chl}}][O_2] - k_6[\overline{\text{t-Chl}}.O_2]$$

$$= k_5[\overline{\text{t-Chl}}][O_2] - k_5[\text{t-Chl}][O_2]_{eq}$$

$$= k_5[\text{t-Chl}]([O_2] - [O_2]_{eq})$$

$$= \frac{k_5 \frac{k_3}{(k_2+k_3)} \cdot I_{abs} \cdot ([O_2] - [O_2]_{eq})}{k_4 + k_5[O_2] - \frac{k_5 k_6}{(k_6+k_7)} \cdot [O_2]}$$

$$= \frac{\left(1 + \frac{k_6}{k_7}\right)\left(\frac{k_3}{k_2+k_3}\right) I_{abs} \left([O_2] - [O_2]_{eq}\right)}{\frac{k_4}{k_5}\left(1 + \frac{k_6}{k_7}\right) + [O_2]} \quad ,$$

which may be reduced to the form

$$-\frac{d[O_2]}{dt} = \frac{a \cdot I_{abs}([O_2] - [O_2]_{eq})}{1 + b[O_2]}$$

where

The term $[O_2]$ is the oxygen pressure above the film :

This equation should read

$$\frac{d[O_2]}{dt} = \frac{a \cdot I ([O_2] - [O_{20}])}{1 + b[O_2]}$$

and $[O_2]_{ABS} = \frac{a[O_2]}{1 + b[O_2]}$

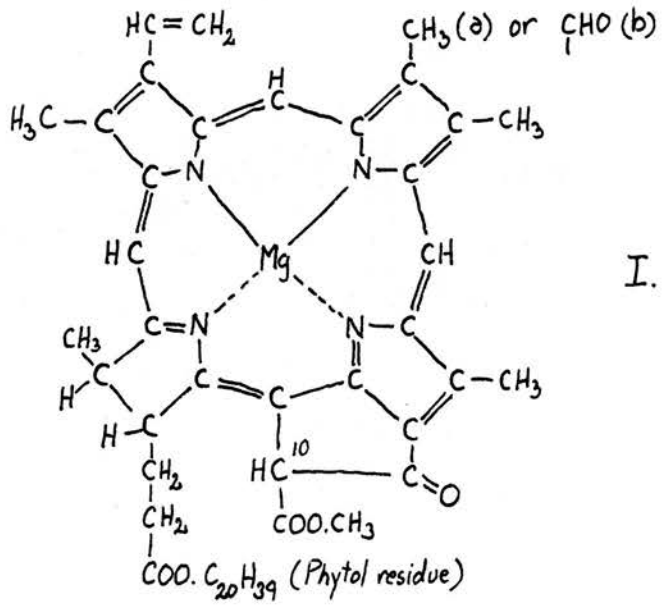
$$\boxed{\frac{d[O_2]}{dt} = a \cdot I \cdot [O_2]_{ABS}.}$$ P/O

It is the latter that should be removable from the mechanism.

a and b involve only constants.
At the equilibrium $[O_2]$ pressure, $[O_2]_{eq}$, the rate will be zero. At high oxygen pressures, where $[O_2]_{eq}$ may be neglected, this expression is of the same form as that derived empirically for the relation of rate to oxygen pressure and light intensity, although in the latter case only incident light intensity was experimentally investigated.

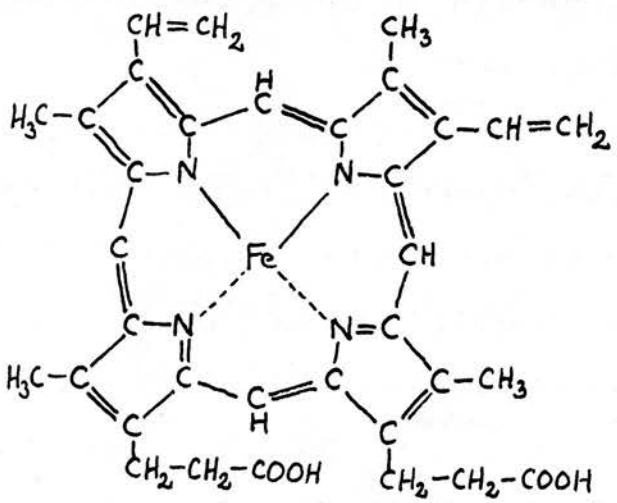
The maximum quantum efficiency $\frac{dO_2}{dt} / I_{abs}$ is mainly determined by the factor $\frac{k_3}{(k_2 + k_3)}$ the proportion of the excited molecules which will enter the reactive state.

The derivation of a detailed chemical picture to coincide with the proposed energetic and kinetic schemes cannot be undertaken without further investigation of the chemical nature of the reversible and irreversible peroxides formed, but a little speculation is perhaps justifiable.



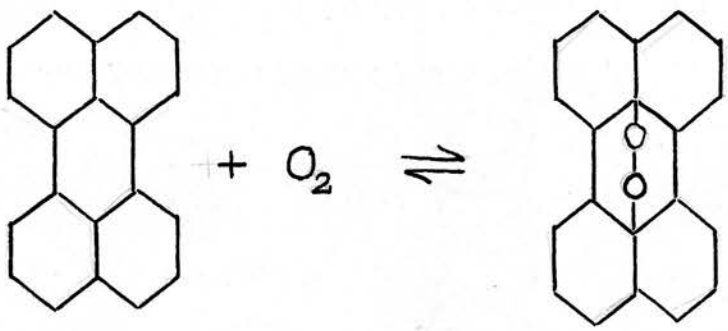
I.

The chlorophyll molecule (I) is a peculiarly delicate chemical structure possessing more than one grouping susceptible to oxidation. In the absence of any certain knowledge of the exact mode of entry of the oxygen molecule into the chlorophyll structure it is only possible to suggest similarities between this process and others about which more precise information is available. The most striking of reversible autoxidations is the reversible oxygen uptake by the blood pigment haemoglobin. The structural similarities between chlorophyll and the functional group of haemoglobin (II) make this comparison particularly interesting.



(II.)

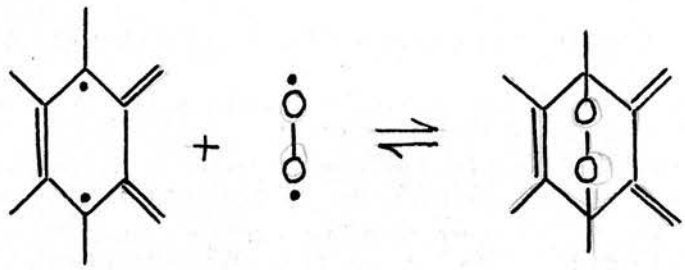
The point of attachment of the oxygen in oxyhaemoglobin is the ferrous atom. The formation of the oxygen-iron linkage does not involve any change in the valency state of the iron. It is not obvious how the magnesium atom of even photo-activated chlorophyll could behave in like manner.



(III)

Another class of reversible peroxides are those derived by the photo-autoxidation of hydrocarbons of the rubrene series (III).[37] These molecules form a peroxide on illumination at high oxygen pressure, the reverse reaction taking place in the dark only at low pressures or high temperatures. It may be suggested that an energetic scheme very similar to that derived for chlorophyll would be applicable to these hydrocarbons. Rubrene photo-autoxidation has been studied mainly from the structural aspect and the structural formula for the peroxide is well established (IV). The oxygen molecule is doubly linked to the hydrocarbon system.

The reaction may be considered as one between two biradial structures.



The biradial nature of excited rubrene has not been established as yet by the classical methods of paramagnetic susceptibility determination[38]. but little doubt exists as to the validity of the hypothesis. We have already noted in the introduction, the role played by radical species in autoxidative processes. These mono-radical forms were generally derived by the removal of a hydrogen atom, a process requiring much energy. Biradical structures may, however, be formed with a moderate requirement of energy and much recent work has been directed towards the demonstration of the biradical nature of the excited states of complex coloured molecules.[38].

The observation of phosphorescence by suitably dispersed organic pigments has been explained by the existence of a metastable high energy state of the molecule.[39]. This metastable state has been identified with a triplet energy

level of the molecule.[40] Transition between such a triplet state and the normal singlet states of the molecule by absorption or emission of light energy, is forbidden and the triplet state is accordingly relatively long-lived. Triplet states arise from the decoupling of the spins of two electrons so that they are unable to share a normal bonding orbital. In the simplest cases these electrons may become localised upon convenient atoms of the molecule, forming what may be considered to be a diradical species. This has been already postulated above in the case of rubrene, but resonance stabilisation between many canonical forms probably occurs. As the spins of these electrons are parallel and do not cancel out the molecule will display paramagnetic properties similar to those possessed by monoradical forms possessing a single odd or uncoupled electron.

Such a triplet state has already been suggested for photo-excited chlorophyll and closely related compounds in explanation of the phosphorescence and reversible bleaching which has been observed.[41] There is, however, no evidence for the localisation of the uncoupled electrons at particular points in the structure.

Chlorophyll in organic solvents exhibits photo conductivity.[42] This, and the photosensi-

tising properties of chlorophyll in AgBr photography suggest the possibility of the loss of an electron by the excited molecule. Such a process may be reconciled with the triplet state view expressed above if the non-localisation of the uncoupled electrons be such that one or other of them may leave the molecule to enter either an adjacent molecule in solution, or, in the case of the photosensitisation, the conductivity level of the supporting solid.

The catalytic effect of the thallous bromide may therefore be due to an extension of the lifetime of the metastable state by increasing what may be considered to be the resonance stabilisation of the pigment-substrate complex diradical.

Thallous bromide resembles silver bromide in forming a latent image provided a bromine atom acceptor such as gelatin is present. Chlorophyll and carotene may act in such a capacity by virtue of their unsaturated nature. If a thallium latent image were here formed, it would however, be expected to react with water vapour and carbon dioxide in the presence of oxygen, and such reaction would be accompanied by a decrease in pressure:

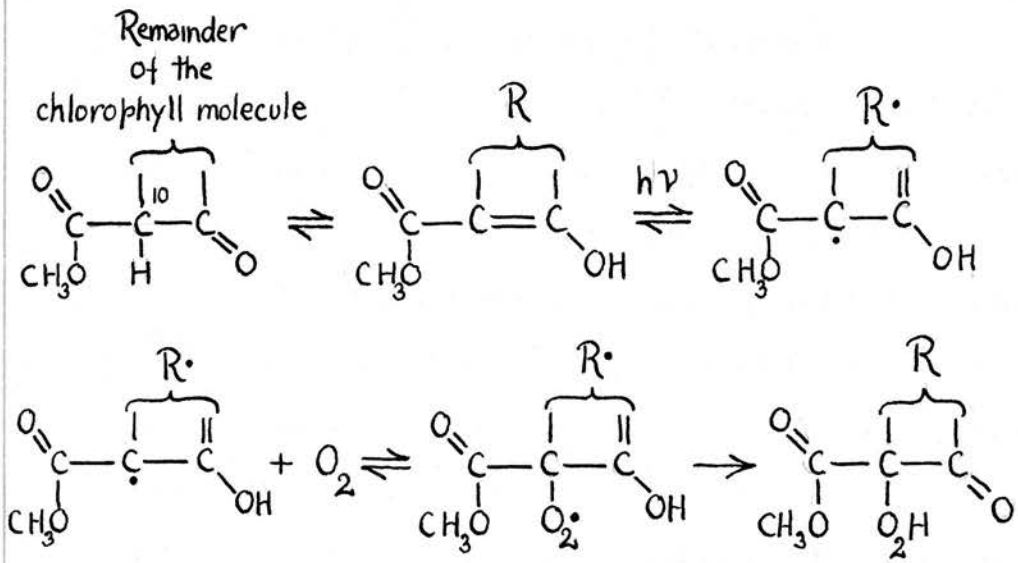
$$4Tl + 2H_2O + O_2 = 4TlOH$$

$$4Tl + 2CO_2 + O_2 = 2Tl_2CO_3$$

As already noted, (p. 75) no effect of water vapour and carbon dioxide was observed, either on the magnitude of the rate of pressure decrease or on the final gas uptake.

The position (10) of the chlorophyll molecule is particularly reactive and is a possible point of attack of oxygen. The process of allomerisation has been postulated, but not proved, to involve this position. The nature of the groupings around this position render the hydrogen atom extremely labile and enolisation readily occurs.[43].

The following processes might conceivably explain the reversible photo-oxidation reported here.



As shown enolisation is assumed to take place before the absorption of light, but could probably equally well occur after the absorption act.

The intermediate singlet excited state is not depicted in the scheme above. The diradical formed has one of its uncoupled electrons localised upon the position (10) carbon atom while the other is located somewhere in the rest of the molecule or even outside the molecule. The final irreversible product is formed on the return of the labile hydrogen atom from its enolised position and redistribution of the bonding in the molecule. This final product would probably be very stable by reason of the bonding of the hydrogen of the hydroperoxide with the adjacent carbonyl groupings.

For solid phase sensitised photoautoxidations the participation of the hydroperoxide radical HO_2 has been postulated. A very general mechanism for this process has been given.[44.] The present

$$HD \xrightarrow{h\nu} HD^*$$

$$HD^* + O_2 \rightleftharpoons HDO_2$$

$$HDO_2 \rightleftharpoons D.O_2H$$

$$DO_2H \rightleftharpoons D\cdot + HO_2\cdot$$

work may lend support to such mechanisms by demonstrating the existence of high energy peroxides retaining the power of spontaneously severing the

carbon oxygen link formed. Hydroperoxide radical formation has not been detected in the present instance, the introduction of a pressure of hydrogen gas causing no change in the observed rate of pressure decrease as might be expected from the known resctions of this radical species.[38.]

An application of the results of the present work to the problem of photosynthesis must be highly speculative. One of the problems of photosynthesis is the mechanism of the release of oxygen, which must be accomplished with minimal loss of energy.[45.] Any process involving the decomposition of hydrogen peroxide or normal organic peroxides must necessarily result in the decrease in the free energy of the system, but here we may have a system which accomplishes oxygen release while retaining the energy. Admittedly the conditions required to obtain reversal are violent, speaking from a biological point of view, but allowance of unknown extent must be made for the effect of the detachment of the chlorophyll molecule from the protein to which it is attached in the plant cell. Were natural conditions such as to raise the dissociation pressure to a figure more nearly that of atmospheric oxygen or to that in oxygenated liquid water the mechanism presented would be of great value, but would entail

an additional function of the chlorophyll molecule in photosynthesis, making it not only the centre of water oxidation and carbon dioxide reduction but also the point of oxygen release.

The present work cannot claim to have exhausted the problems even of the limited field chosen, and the results obtained only indicate the many further lines of enquiry that must be pursued before full knowledge of the system is realised and before the velocity coefficients of the kinetic expressions derived can be determined. The experimental methods used appear to be applicable to a wide range of both fundamental and applied problems and may be expected to yield much new knowledge of the photo-chemistry of pigments in the solid state.

S U M M A R Y.

Examination has been made of the photo-oxidation, in visible light, of chlorophyll preparations, deposited on various solids, by means of the direct measurement of oxygen pressure decrease.

Rates of oxidation were very low on glass, zinc oxide, aluminium oxide, ferric oxide, and on talc, but were some five to ten times more rapid on thallous bromide and thallous iodide. The maximum quantum efficiency on glass in light of wave length 4358 A.U. was of the order 10^{-2}, increasing slightly with decreasing film thickness.

Chromatographic and solvent partition separation methods applied to two chlorophyll preparations enabled two main fractions to be examined, a yellow carotene fraction and the green chlorophyll fraction. On thallous bromide, the oxygen to carotene and the oxygen to chlorophyll molar ratios in oxidation were unity. The chlorophyll fraction of a second sample showed an apparent oxygen to chlorophyll molar ratio of approximately two. Oxidations were partly reversible by simple pressure reduction. For one chlorophyll preparation, a limit of oxygen pressure of approximately 0.1mm. was established, below which no oxidation occurred.

A possible mechanism has been discussed in relation to the experimental results and to the general nature of such preparations.

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