THE PHOTO-OXIDATION OF CHLOROPHYLL

Thesis for the degree of Doctor of Philosophy

Presented by

Janet M. Norbury, B.Sc.

May, 1961

University of Edinburgh

ABSTRACT OF THESIS

Name of Candidate JANET M. NORBURY.

Address

Degree Doctor of Philosophy. *Date* May, 1961.

Title of Thesis "The Photo-oxidation of Chlorophyll."

The present work showed that on illumination of films of freshly extracted chlorophyll deposited on a thallous bromide substrate in the presence of 50 mm oxygen, the total oxygen uptake was approximately 2 moles per mole of chlorophyll present, while quantities of water vapour varying from 0.3 to 1.0 moles under different conditions were released during oxidation. Between 80 per cent and 100 per cent of the total oxygen taken up within one day was estimated as hydroperoxide in the oxidised film. On prolonged oxidation, however, this unstable hydroperoxide decomposed to give water vapour as one decomposition product.

Two positions in the chlorophyll molecule were found where hydroperoxide formation could occur, namely, the phytol chain and the labile hydrogen atom on the carbon atom C_{10} of the cyclopentanone ring. Hydroperoxide formation also occurred on oxidation of compounds related to chlorophyll containing a cyclopentanone ring, e.g. copper chlorophyll, pheophytin, pheophorbide and ethyl chlorophyllide, but not in chlorin e which contains no isocyclic ring.

It was thought that bleaching of the film on prolonged oxidation was associated with pheophytinisation and the final rupture of the conjugated bond system of the molecule.

Experiments were also carried out to investigate the dark and photoconductivity of thin films of thallous bromide and chlorophyll placed between platinum electrodes under the influence of an applied voltage in vacuo and in 50 mm oxygen. In all cases, the dark and photocurrents were proportional to the applied voltage, and the maximum photocurrent was proportional to the intensity of the incident light. While films of thallous bromide reached a maximum photocurrent within one minute after the commencement of illumination, for films of chlorophyll the photocurrent increased slowly with time to reach a saturation value after approximately 25 minutes illumination. Decay back to the original dark current value also occurred within 25 minutes.

The maximum photocurrent was always caused by illuminating chlorophyll films with light of wavelengths between 5500 and 6000°A, which did not coincide with the absorption maxima at 4300 and 6600°A for ether solutions of the pigment. A large decrease in photocurrent occurred on admission of 50 mm oxygen to the system, and after illumination the film extracted in methanol gave a peroxidic reaction with ferrous thiocyanate.

The energy gap $\Delta\epsilon$ representing the energy required to transport an electron from the highest filled band to the lowest unfilled band of the chlorophyll molecule was determined by temperature variation of conductivity. For dark conductivity, an energy gap of the order 2.6 ± 0.1 e.v. was obtained, increasing as the sample aged. For photoconductivity, the energy gap was considerably less, a value of 1.6 ± 0.7 e.v. being determined.

Use other side if necessary.

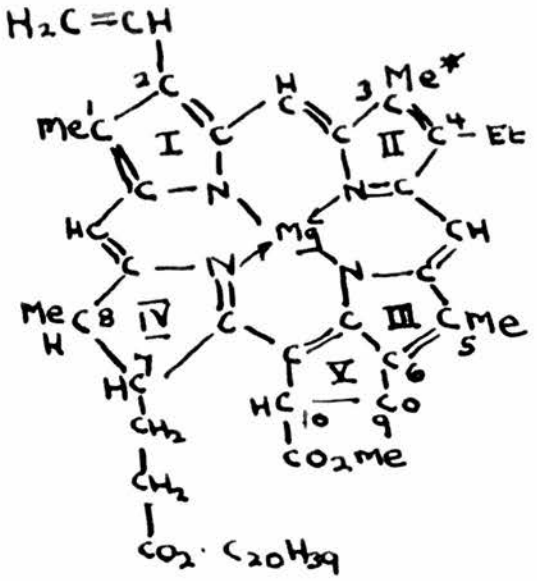
C O N T E N T S

PART I

INTRODUCTION

In spite of the attention attracted by the pigment system of plants due to their striking colour and their relationship to the primary process in photosynthesis, the many problems their biological function presents have only been partially solved. Difficulty is at once encountered in extracting the pigments in a pure state from their natural environment, since contact with oxygen, solvents or adsorbents tends to decompose them chemically. Complete separation of the various components of the pigment system is made more difficult by the fact that each constituent contains isomers or other substances of very similar chemical composition whose chemical properties and solubility differ very little from those of the parent compound.

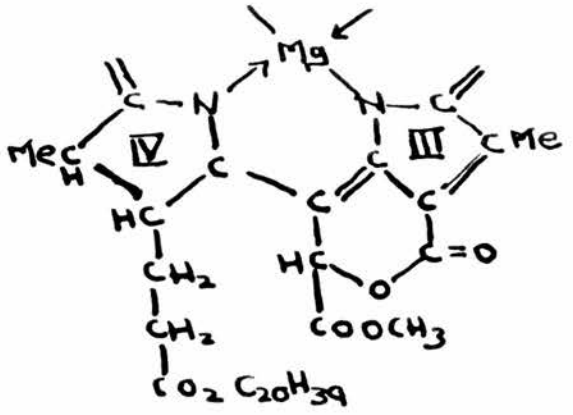
The formula of chlorophyll (fig. 1) was eventually established as a result of the arduous researches of Emil Fischer (1), and has recently been confirmed beyond all possible doubt by the brilliant synthesis of the compound by Woodward (2). Chlorophyll is a dihydroporphyrin occurring in nature as chlorophyll a. and chlorophyll b. in which an aldehydic group is substituted for a methyl group as shown in fig. 1, and also as various isomers of these substances. The first reliable absorption spectra of chlorophylls a. and b. were published by Zscheile and Comar, and show maxima for chlorophyll a. at 4300°A and 6600°A, with very low absorption in the green (3). Chlorophyll contains a centrally complexed magnesium atom which



CHLOROPHYLL A

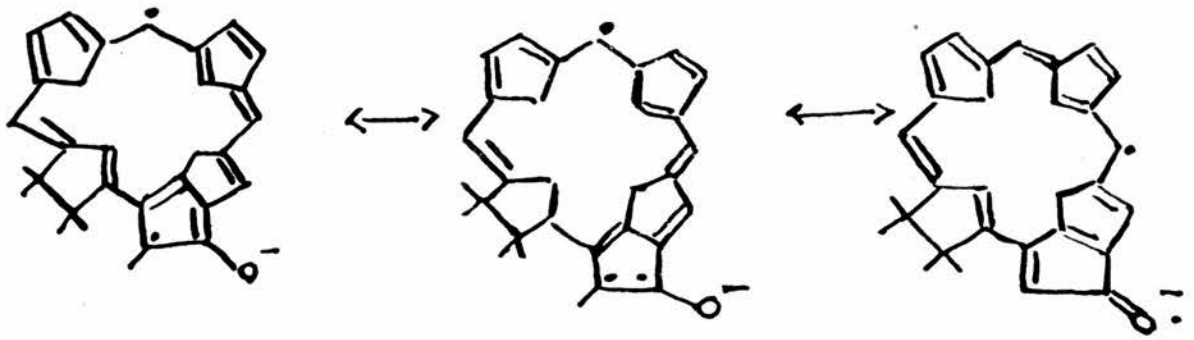
* POSITION OF -CHO GROUP IN CHLOROPHYLL B

FIG. 1



ALLOMERISED CHLOROPHYLL

FIG. 2



RESONATING BIRADICAL STRUCTURES

FIG. 3

in acid solution is replaced by two hydrogen atoms, yielding pheophytin. The absorption spectrum of this compound shows diminished maxima in the blue and the red by comparison with that of chlorophyll, and increased absorption in the green, imparting to pheophytin a dull olive green colour which contrasts sharply with the pure green of chlorophyll itself.

If the pigments are extracted in alcoholic solution, action of the enzyme chlorophyllase leads to the formation of chlorophyllides in which the phytyl ester grouping is replaced by a methyl group. These are difficult to identify spectroscopically since their absorption spectra differ hardly at all from that of the parent compound. Strong acids perform the double function of hydrolysing off the phytol chain and removing magnesium, yielding pheophorbides, while the action of hot alcoholic alkali leads to the elimination of magnesium and the rupture of the isocyclic ring with the formation of chlorins.

Certain reactions undergone by chlorophyll which had hitherto been considered to involve one simple reaction have since been shown to be the result of a more complex series of changes. Under various chemical conditions, chlorophyll undergoes different types of oxidation. One of these reactions, the "allomerization" of chlorophyll, has puzzled chemists for many years. This transformation occurs when alcoholic solutions of chlorophyll are exposed to air or oxygen in the dark. The solution remains green in colour and the absorption spectrum is virtually unchanged, but after allomerization chlorophyll fails to give the "Molisch phase test". This

consists of the transient appearance of a brown coloration upon the addition of alcoholic potassium hydroxide to ethereal solutions of chlorophyll and is undergone by all freshly extracted samples of the substance. Conant and his co-workers interpreted this allomerization as an oxidation and attributed it to the uptake of one mole of oxygen per mole of chlorophyll (4). Later, Stoll and Fischer suggested that the reaction was due to oxidation of the hydrogen atom on the tertiary carbon atom C_{10} (5). This hydrogen atom must be characterised by extreme lability since it is situated between a carbonyl and a carbmethoxy group, and Fischer suggested the formation of a chlorophyll peroxide at this position:

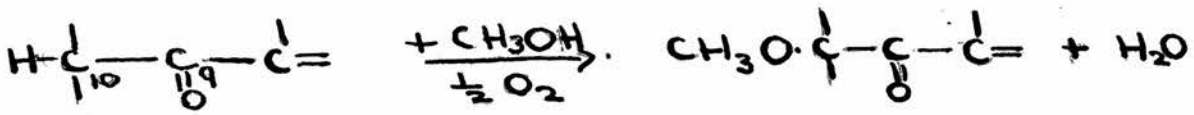
$$-\overset{|}{\underset{|}{C}}_{10}-H \;+\; O_2 \;\rightarrow\; -\overset{|}{\underset{|}{C}}_{10}-O-OH$$

However, he was unable to isolate any such peroxide.

The above interpretation was subsequently shown to be an over-simplification. In 1954, Holt and Jacobs found that allomerized chlorophyll a. could be separated chromatographically on a sugar column into three components (6). In addition to a main component, there was one more readily adsorbed fraction with a spectrum almost identical with that of chlorophyll a. and a smaller less readily adsorbed fraction with a red peak towards longer wavelengths. All three fractions gave no "brown phase" test with alkali.

It is suggested by Holt and Jacobs that the main product involves disruption of ring V with the formation of a chlorin lactone (fig. 2), an idea which had previously been put forward by Fischer and Pfeiffer in 1944 (7). Failure to give the phase test in the more readily adsorbed fraction was attributed to substitution of the hydrogen atom

on C_{10} by a methoxy group:



The third fraction still remains to be identified. Thus allomerization is shown to be the result of a complex set of reactions rather than a simple one-stage oxidation and is one example of the complicated nature of chlorophyll chemistry.

The "phase test" itself is not yet completely understood. In 1951, Dunicz, Thomas, Van Pee and Livingston studied the brown intermediates resulting from the addition of alcoholic alkali to fresh chlorophyll solutions (8). They found that these were formed without the participation of oxygen, but were subsequently converted to green chlorins by reaction with atmospheric oxygen. Weller (9) suggested that the brown intermediate was a diradical form of the anion formed by enolization and acid dissociation in position 9 (fig. 3). This radical is considerably stabilized by resonance with free valencies in different positions in the normally conjugated ring system. Many of the oxidation and reduction reactions undergone by chlorophyll can be explained in terms of a biradical structure interrupting the conjugation of the ring system.

A further example of the diverse reactions of this versatile compound is the instantaneous colour change from green to yellow occurring when ferric salts are added to methanolic solutions of chlorophyll. The reverse reaction can be brought about by the addition of ferrous chloride and other methanol-soluble salts to the solution. This was explained as a reversible oxidation by Rabinowitch

and Weiss (10):

$$Chl + Fe^{+++} \longrightarrow o\text{-}Chl + Fe^{++}$$

Objections to this oxidation theory were raised by Strain in 1949 (11) and Ashkinazi, Glikman and Dain in 1950 (12). They found that bleaching could also be caused by Al^{3+} and Sn^{2+}, and proposed that this was due to the essential acid nature of these salts, causing pheophytinisation of the chlorophyll. They explained the reverse reaction by the formation of metal complexes of pheophytin with Fe^{2+}, Cu^{2+} and Zn^{2+} ions by replacement of hydrogen leading to restoration of the green colour of the solution and of the original absorption spectrum. However, Rabinowitch and Weiss refute this theory on the grounds that pheophytinisation of chlorophyll in acid solution is a slow process and could never happen instantaneously to give a yellow solution: also the absorption spectra of metal-pheophytin complexes differ considerably from each other and from that of the original pigment. The absorption spectrum of the oxidised form shows a striking similarity to that of the phase-test intermediate and possibly has the same biradical structure. If this is the case, the original explanation of Rabinowitch and Weiss is probably nearer the truth, the reversible oxidation-reduction being the cause of interrupted conjugation with consequent colour change.

These are but three examples from the interesting but far from completely understood chemistry of this delicate system. The solution to the problem rests with the basic electronic structure of chlorophyll and the varied transitions allowable between its many electronic states as a result of absorption of radiation in the visible and near ultra

violet regions of the spectrum.

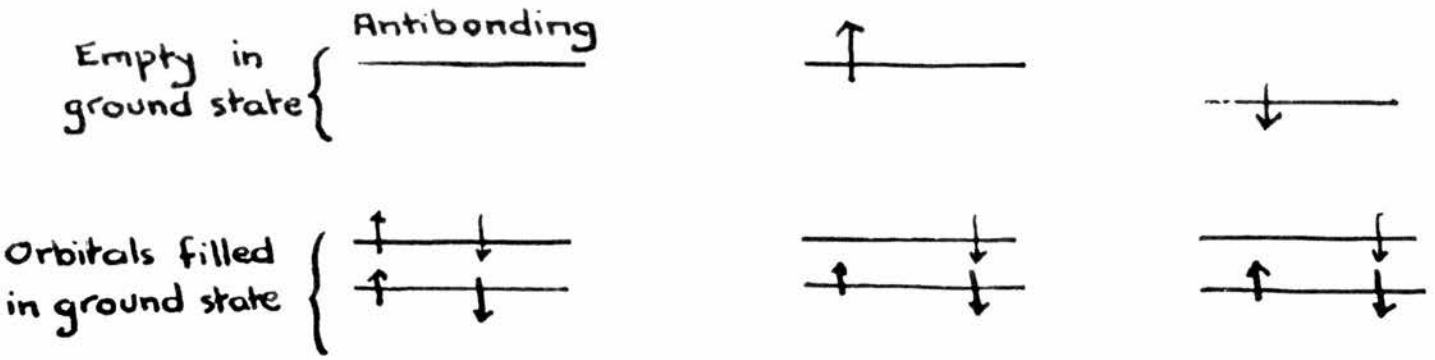
The primary process in photochemical reactions is the absorption of a quantum of light energy $h\nu$ which supplies the activation energy necessary for the reaction to proceed. Absorption of a quantum $h\nu$ will occur when there is an energy difference equal to $h\nu$ between two energy levels where transition is allowable according to symmetry and momentum conservation rules. A photon of light in the visible or the ultra violet region of the spectrum has sufficient energy to bring about electronic transitions in molecules containing π-electrons which absorb throughout this range.

Such molecules have an even number of π-electrons, and in the ground state all the electrons are paired. According to the Pauli exclusion principle, since the quantum numbers of both electrons in each pair are identical, the spins of the electrons must be opposed, so that in its singlet state the molecule is diamagnetic. If one of the paired electrons is excited by radiation to a higher energy level, there are two possibilities. Either the spins may again be opposed giving a diamagnetic "singlet", or the spins may be parallel, in which case the effects of the spinning electrons are added and contribute to the angular momentum of the system, resulting in paramagnetism. The multiplicity of a state is given by the rule:

Multiplicity = $2s + 1$ where s is the number of unpaired electrons.

When $s = 0$ there is only one state, the singlet state, but when $s = 1$ there are three states known as the triplet state. According to Hundt's rule, a system in which electron spins are parallel has lower energy than one in which the spins are antiparallel, so that the first

ORBITAL STRUCTURE OF MOLECULE



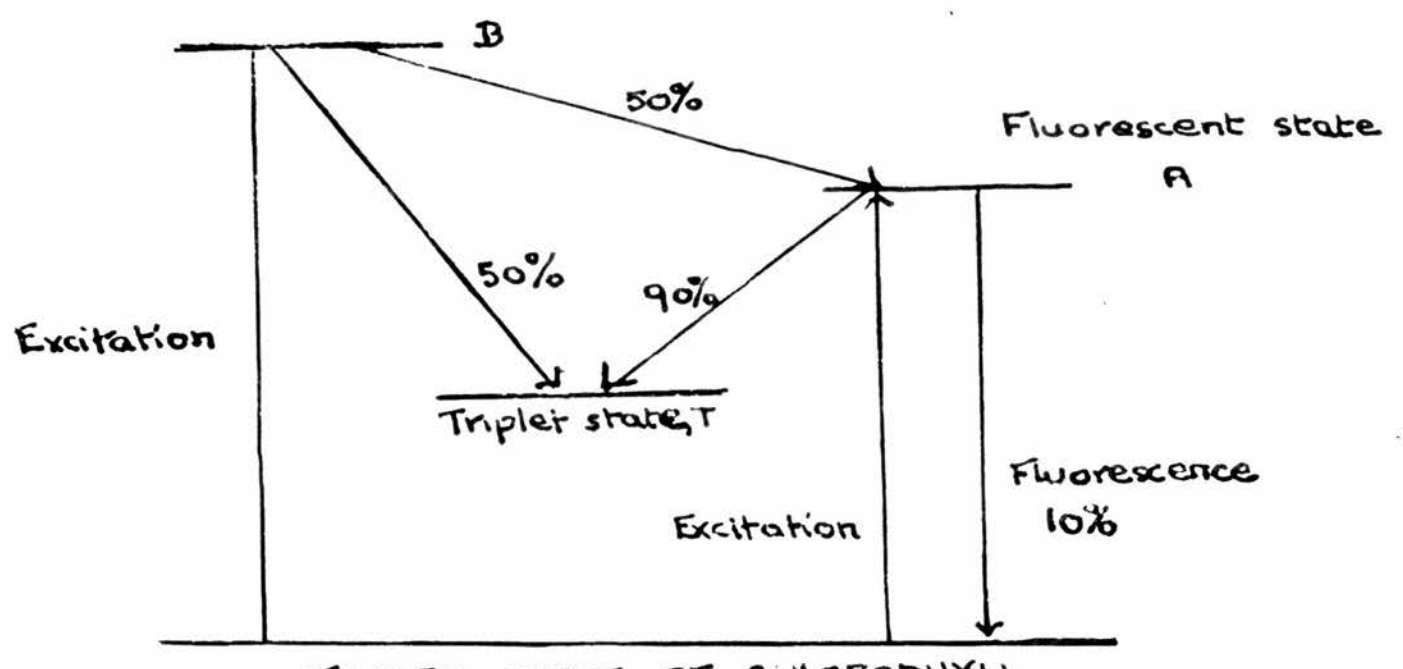
(a) Ground singlet state S=0. No unpaired electrons.

(b) First excited singlet state S=0 (No unpaired electrons)

(c) S=1

In case (c), S=1. This results from inversion of the spin of an excited electron to give 2 unpaired electrons. ∴ 2S+1 = 3. This is a triplet state

Fig. 4



TRIPLET STATE OF CHLOROPHYLL

Fig. 5

triplet level lies below the first excited singlet level (fig. 4).

In small molecules, electronic transitions involving changes in the multiplicity of the molecule are forbidden, but in large molecules these transitions can occur due to perturbation of the energy levels. Many aromatic molecules and dyestuffs exhibit phosphorescence with a decay time of the order of seconds when illuminated in the solid state. Jablonski (13) interpreted this property by postulating a metastable state of energy between the ground state and the first excited singlet state. In 1944, Lewis and Kasha (14) identified the phosphorescence of a large range of aromatic molecules with a slow transition from a triplet to a ground state. This was experimentally confirmed in 1945 by Lewis and Calvin (15) who showed through paramagnetic susceptibility measurements the occurrence of paramagnetism in the phosphorescent state which was identified with the presence of unpaired electrons.

More recently, Kasha and Becker have proved that these singlet-triplet transitions occur in the porphyrin series (16), as a result of examination of their fluorescent and phosphorescent properties. Fluorescence occurs from the first excited singlet state; phosphorescence from the lowest triplet state. Livingston, Porter and Windsor (17) have photographed transient triplet state spectra for chlorophylls a. and b. and pheophytin a. by the flash photolytic technique after exposure to a flash of intense light.

Chlorophyll molecules excited by light in the blue or the near ultra violet to a higher state B have the choice of going directly to the triplet state T, or first to state A and from there to T (fig. 5). Molecules reaching state A, however, either from B or by direct

excitation by red light, have a 90 per cent probability of reaching state T and a 10 per cent probability of reaching the ground state again by fluorescence. Of those molecules reaching state B, 50 per cent pass directly to state T, and 50 per cent to the fluorescent state A. Of these 50 per cent, 10 per cent reach the ground state again by fluorescence and 90 per cent are transferred to the triplet state; thus it can be seen from the diagram that the total probability of conversion to the triplet state is 95 per cent for molecules excited in the blue and 90 per cent for those excited in the red.

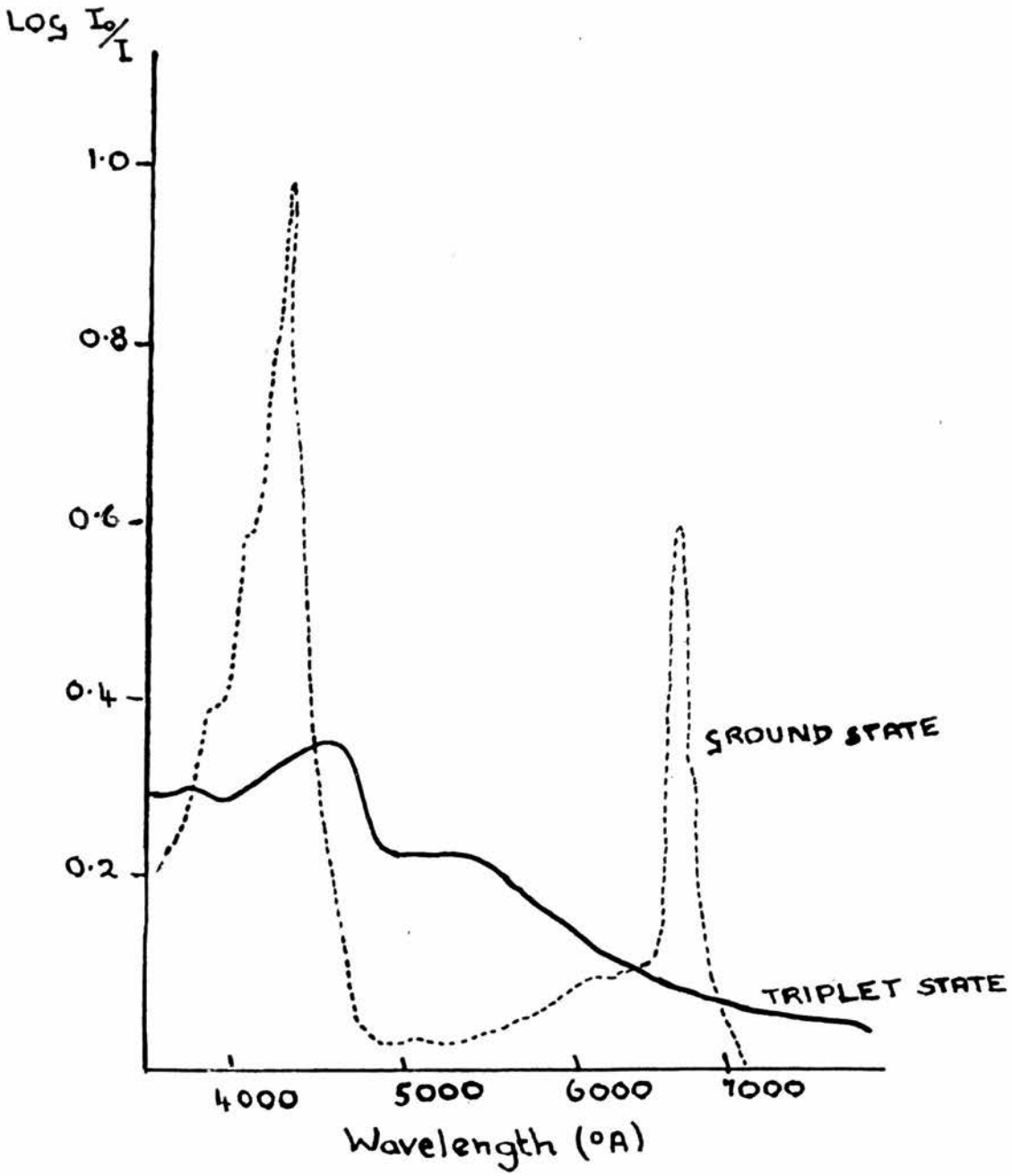
After the initial excitation to the lowest singlet state, the molecule can lose its excitation energy in several ways, of which the following three will be discussed in relation to chlorophyll chemistry:

(1) Photochemical reaction. Reaction with oxygen is of primary importance and can occur with the triplet state.

(2) Energy transfer non-radiatively to a neighbouring molecule. This is the basis of the function of chlorophyll as a sensitiser.

(3) By fluorescence, which is a radiative conversion from the first excited singlet state to the ground state.

Many chemical reactions of electronically excited molecules appear to involve the triplet rather than the excited singlet state. The absorption spectrum of the triplet state has been determined using flash photolytic apparatus (fig. 6). These spectra are broad and diffuse in character, in contrast to the sharpness of the bands for the singlet state. Several overlapping electronic bands are probably involved. Since molecules in this triplet state can enter into reactions of the free radical type, it was suggested by chemists that



The absorption spectra of chlorophyll in its triplet and in its ground state

Fig. 6

in diatomic molecules the triplet state could be considered as a diradical and that there must be a localisation of electrons which did not occur in the singlet state. The addition of oxygen to polynuclear aromatic hydrocarbons, e.g. anthracene, rubrene, pentacene, with the formation of transannular peroxides is believed to be a radical reaction resulting in the formation of an oxygen bridge joining the parts of the molecule where electrons are localised.

Livingston and Ryan have explained the reversible photobleaching of chlorophyll in terms of a similar metastable state (18). This reaction can, however, occur in complete absence of oxygen and is therefore attributed to reversible oxidation of the triplet state by the solvent (19). Oxygen itself has actually an inhibiting effect on reversible bleaching due to deactivation of the triplet state by the paramagnetic oxygen molecules.

Krasnovsky and Vojnovskaja (20) noted that the tetrahydroporphyrin bacteriochlorophyll dissolved in alcoholic solution oxidised in air to give a strong peroxidic reaction with ferrous thiocyanate. The pigment could be regenerated with ascorbic acid or hydrogen sulphide even after standing for 10 hours. This autoxidation was interpreted (20) by Krasnovsky as addition of oxygen to a photochemically produced biradical.

The classic experiments on the photo-oxidation of porphyrins were carried out by Calvin and Dorough in 1948 (21). They found that solutions of zinc tetraphenyl chlorin changed from green to yellow on standing in air due to a light induced oxidation causing dehydrogenation of the chlorin to the corresponding phorbin. The same result was obtained with o- and p-quinones.

The light required for the reaction was that absorbed by the chlorin at its maximum absorption at 6212°A. The spectrum of the final product showed that this maximum had disappeared completely with the appearance of a new peak at 5200°A showing the presence of the dehydrogenated porphin structure. The significant feature of these reactions was that the rate of oxidation was found to be independent of the concentrations of oxygen or quinone present. Calvin concluded that the primary step involved the passage of the excited molecule into the metastable state by a radiationless transition. Once a molecule had reached this state, oxidation always took place. Had the oxidants reacted with the excited singlet state, the rate of reaction would have depended on their concentration because they would have had to compete for the excited chlorin which could be deactivated by fluorescence or solvent and wall deactivation. After the primary oxidation a secondary reaction occurs where hydrogen peroxide formed as a biproduct further attacks the porphin, probably at one of the methine bridges, causing rupture of the ring to give a tetrapyrrole.

Weiss (22) has studied oxidation-reduction processes sensitised by chlorophyll and has shown that the sensitiser must exist in a long-lived excited state. Chlorophyll efficiently sensitises the autoxidation of a wide variety of reducing agents and this function may be of importance in explaining the primary photochemical process in photosynthesis.

Krasnovskii studied the reversible photoreduction of chlorophyll by a number of reducing agents in 1948 (23). The reduced form of chlorophyll was found to be a pink substance which rapidly reverted to the original green compound on admission of air or oxidising agents.

Complete regeneration of chlorophyll does not occur, there being also a certain amount of pheophytinisation taking place. It has since been shown that this photoreduction encompasses a complex series of reactions involving the metastable state (24). Several intermediates are formed including the pink pigments originally observed by Krasnovskii.

Chlorophyll fluoresces in all polar solvents but only slightly or not at all in dry non-polar solvents. Livingston (25) suggested that fluorescence in polar solvents was due to an addition reaction between the labile hydrogen of the nucleophilic solvent and the keto-oxygen in ring V of chlorophyll, causing stabilisation of the keto form which was fluorescent. In absence of the stabiliser, it was supposed that the substance existed in a chelated enol form which was non-fluorescent. However Evstigneev and his co-workers (26) later proved that the fluorescence activator was bound to the central magnesium atom of the pigment, on the grounds that polar solvents do not affect the fluorescence spectrum of pheophytin. An even more convincing argument is that polar solvents enhance the fluorescence of magnesium phthalocyanine but not of the metal-free phthalocyanine itself. Since this substance contains no cyclopentanone ring, Livingston's theory is disproved.

It has been established that chlorophylls and metal complexed chlorins form stable 1:1 addition compounds with nucleophilic reagents such as hydroxylic solvents, amines, ethers and ketones (27). Exhaustive drying of non-polar solvents causes broadening of the absorption bands of chlorophyll - the normal published spectra are those of the solvated form.

The fluorescence of certain dyestuffs adsorbed on silica gel is considerably weakened by oxygen at a pressure of several hundred millimetres. The same effect is observed for solutions of chlorophyll in acetone. Since effective quenchers are oxidising rather than reducing agents, it appears that quenching is a result of an electron-transfer process.

Although most aromatic hydrocarbons exhibit phosphorescence at low temperatures in glassy solvents, chlorophyll a. surprisingly shows no phosphorescence, while chlorophyll b. phosphoresces only weakly at liquid nitrogen temperatures (28). Copper pheophorbide, however, which is non-fluorescent, phosphoresces strongly in rigid solvents at low temperatures.

The complexity and diversity of the photochemical reactions undergone by chlorophyll are apparent from the above evidence. It was the object of the present work to investigate the nature of the oxidation which occurred when solid films of chlorophyll were illuminated in the presence of oxygen. Oxidation reactions occurring in solution are always complicated by the presence of the solvent, and therefore previous workers (29) decided to eliminate this factor by using only solid films of chlorophyll.

The earliest work on solid films of chlorophyll was undertaken by Wager in 1913 (30). He established that visible light destroyed the colour of pigments extracted from plants, and that the photo-product dissolved in petroleum ether solution released iodine from potassium iodide solution. In 1949, Lonie (29) established that oxidation proceeded at a far greater rate if the solid chlorophyll

film was deposited on finely ground thallous bromide crystals rather than on the walls of the reaction vessel or on powdered glass; the catalytic effect of the photoconducting thallous bromide was probably due to an extension of the lifetime of the metastable state of chlorophyll by energy transfer from the thallous bromide.

The films were illuminated with a mercury vapour lamp and pressure changes due to uptake of oxygen by the film were followed on a Bourdon gauge. Initially, commercial copper stabilised chlorophyll was used and it was estimated that oxygen was taken up in unit ratio to the chlorophyll present. The oxidised film extracted in methanolic solution was shown to give a peroxide reaction with ferrous thiocyanate reagent (29), and there was considerable confusion as to whether this peroxide could revert to the original chlorophyll. The grounds for the supposed reversibility of the reaction were that evacuation of the reaction space after oxidation followed by subsequent readmission of oxygen and illumination resulted in an accelerated rate.

Oxidations carried out in the presence of various absorbents showed that the actual uptake of oxygen was, in fact, approximately two moles with the release of one mole of water vapour, giving an apparent oxygen uptake of one mole per mole of chlorophyll present.

The measurements of the quantity of water vapour released carried out by Sunmers (31) were never reproducible, lack of reproducibility being attributed mainly to adsorption of water vapour on the film and on the glass walls of the vessel. Difficulty was also encountered in extending the work to natural chlorophyll extracted from spinach and stinging nettles, since this constantly tended to degrade to

pheophytin.

It was the object of the present work to investigate more fully the oxidation of natural chlorophyll films deposited both on powdered glass and thallous bromide substrates. An attempt has been made to correlate the three variables in this reaction, i.e. the oxygen uptake, the weight of peroxide formed and the water vapour produced. No previous worker has analysed all three factors for the same film, so that no overall picture of the process has been constructed. There has also been an endeavour to explain some of the anomalies encountered by previous workers.

Experimental methods

The photo-oxidation of chlorophyll was carried out in the apparatus shown in fig. 7. The reaction vessel was of capacity about 30 ml and was attached to a Bourdon gauge by an A10 joint. The gauge and its jacket were attached to the main vacuum line along with manometers, gas storage bulbs and various traps, as shown in the diagram. The line could be evacuated through a cooled trap A to 10^{-3} mm using a 'Hyvac' oil pump. The apparatus was built entirely of soda glass and all traps were lubricated with Apiezon 'L' grease. Oxygen was admitted to the system from the storage bulbs, and dry CO_2-free air through the air leak.

The system was accurately thermostated by circulating water from a tank kept at a constant temperature of 25 ± 0.1°C round the reaction vessel and the gauge, which was jacketed. The water in the tank was kept at constant temperature by means of a lamp heater controlled by a chloroform-mercury regulator and a valve relay control. A Stuart Turner pump was used to circulate water from the tank to the vessel and the gauge.

Pressure changes in the reaction space were followed on a telescope containing a graduated scale focused on the illuminated platinum tip of the gauge pointer. The telescope scale was graduated in terms of millimetres of mercury as follows:

The number of scale divisions travelled by the pointer in one complete sweep of the scale was equivalent to a pressure change too small to be detected on the manometer, so the total number of

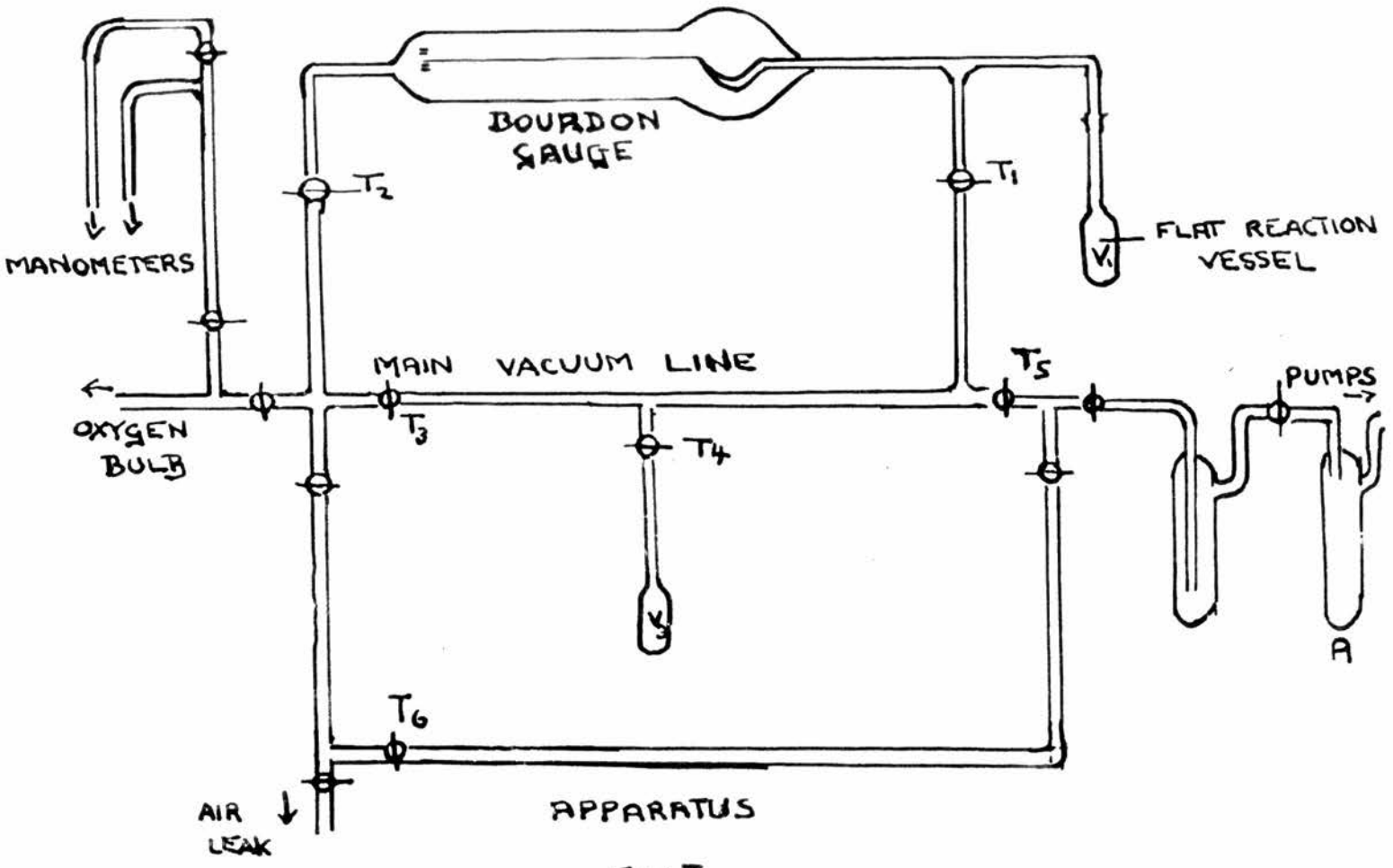
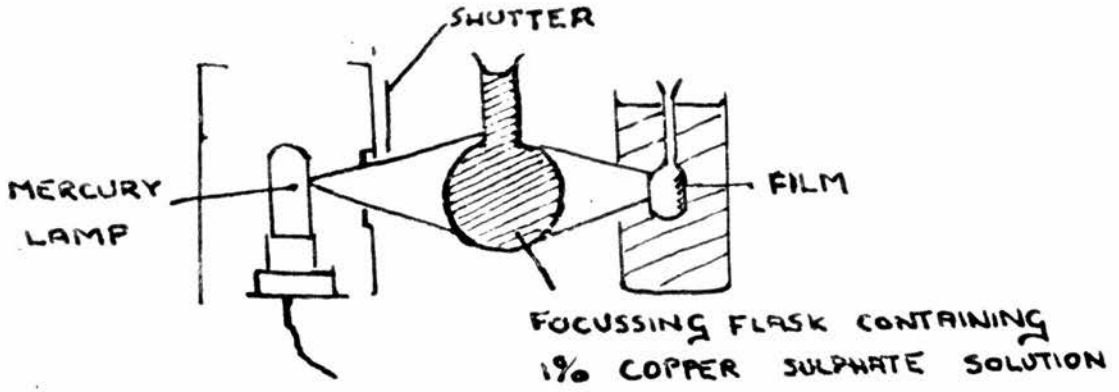


FIG. 7



LAMP AND FOCUSSING SYSTEM

FIG. 8

divisions travelled in several sweeps was recorded as small quantities of gas were removed from the gauge through T_1. At the end of each sweep the pointer was brought back to its starting point by removing the same pressure of gas from the jacket through T_6, thus producing an appreciable reading on the manometer.

Total No. of divisions	Pressure change	Sensitivity (mm./div.)
462.5	23.0 mm.	0.050
611.9	30.4 mm.	0.049

The sensitivity was found to be the same when determined at 50, 100 and 150 mm pressure. Each division was estimated to the nearest tenth of a division, readings being recorded to 0.005 mm.

In order to determine the volume of the reaction space, the apparatus was filled with dry air to a pressure of approximately 100 mm, and taps T_1 and T_3 were then closed. A bulb of known volume V_3 was then attached to the apparatus at T_4 and the bulb and vacuum line between taps T_1, T_3, T_4 and T_5 were then evacuated. With T_4 closed, the air in the reaction space was expanded through T_1 into the space T_1, T_3, T_4, T_5, the pressure in the jacket being decreased simultaneously through T_6 to balance the gauge. The equilibrium pressure was read on the manometer. Air from the reaction space was next expanded into V_3 (T_4 open) and the above procedure repeated until equilibrium was again attained.

Let V_1 = Volume of reaction space.

V_2 = Volume of connecting lines T_1, T_3, T_4, T_5.

V_3 = Volume of standard vessel.

P_1 = Initial pressure.

P_2 = Equilibrium pressure in V_1 and V_2.

P_3 = Equilibrium pressure in V_1, V_2 and V_3.

Now $P_1V_1 = P_2(V_1 + V_2) = P_3(V_1 + V_2 + V_3)$

Substituting for $V_2 = V_1 \frac{P_1 - P_2}{P_2}$ in $V_1P_1 = P_3(V_1 + V_2 + V_3)$

$$\therefore V_1P_1 = P_3\left(V_1 + V_3 + V_1 \frac{P_1 - P_2}{P_2}\right)$$

$$= P_3V_1 + P_3V_3 + V_1(P_1 - P_2) \cdot \frac{P_3}{P_2}$$

$$\therefore P_3V_3 = V_1\left(P_1 - P_3 - \frac{(P_1 - P_2) \cdot P_3}{P_2}\right)$$

$$\therefore V_1 = \frac{P_3V_3}{P_1 - \frac{(P_1 - P_2) \cdot P_3}{P_2} - P_3}$$

V_3 was found by weighing the standard vessel first empty then filled with water. One set of results was as follows:

$P_1 = 10.18$ cm. $P_2 = 6.31$ cm. $P_3 = 3.00$ cm. $V_3 = 56.52$ ml.

$$\therefore V_1 = \frac{3.00 \times 56.52}{10.18 - \frac{(10.18 - 6.31) \times 3.00}{6.31} - 3.00}$$

$$= 31.8 \text{ ml.}$$

The above experiment was repeated several times and the maximum error was estimated at 0.5 per cent.

The substances examined were illuminated with a Mazda 250 watt mercury vapour lamp contained in a metal box situated behind the

reaction vessel (fig. 8). The beam of light was admitted by a shutter and focussed by a 500 ml flask filled with a 1 per cent copper sulphate solution which also acted as a heat filter. The soda glass of the system removed all ultra violet light from the beam, which therefore contained no light of wavelength less than 3650°A.

The chlorophyll samples used were freshly extracted from spinach or stinging nettles by a modification of the method of Zscheile and Comar (32) using an apparatus designed by Griffiths and Jeffrey and Le Rosen (33).

250 g of freshly gathered spinach were shredded and extracted with 400 ml A.R. acetone in a Townsend and Mercer macerator for 5 minutes. A few pieces of crushed ice were added to keep the temperature down. The extract was filtered and washed with acetone, and the solution was added to 250 ml diethyl ether in a large separating funnel. Distilled water was added and the funnel was rotated to prevent emulsion formation between the ether and the water. Two layers formed, the pigments being transferred to the ether layer. The mixed pigments were scrubbed through distilled water using the apparatus shown in fig. 9. The solution of chlorophyll flows by gravity down the capillary tube 1 and up the washing tube 2. It eventually passes into a large coil cooled with an ice and salt mixture which freezes the water out of the ethereal solution, which is finally collected in the separating funnel 'S'. Any water frozen out of the washed ether solution is then removed. The solution was dried overnight over anhydrous sodium sulphate. The average batch

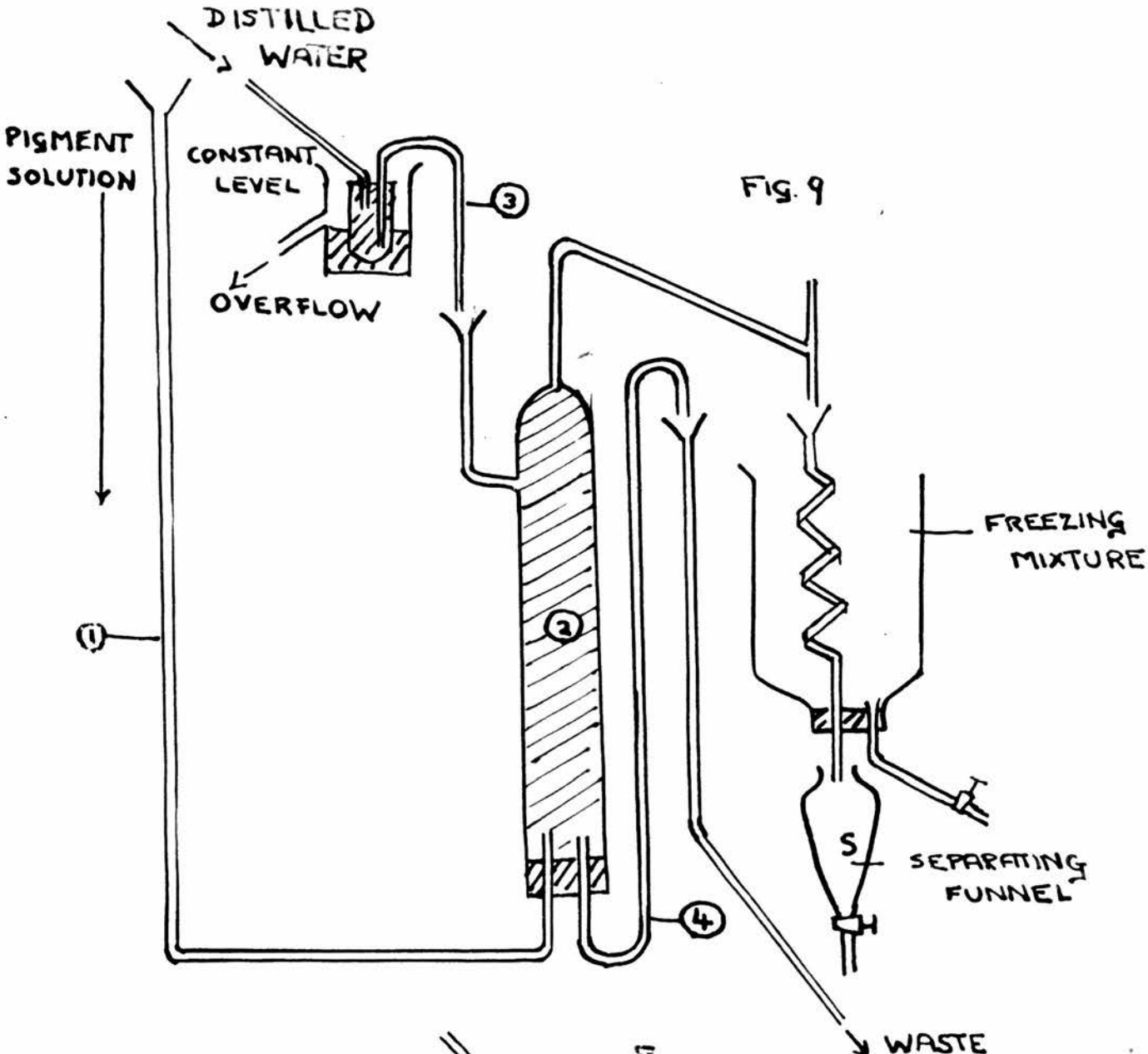


FIG. 9

WASHING APPARATUS

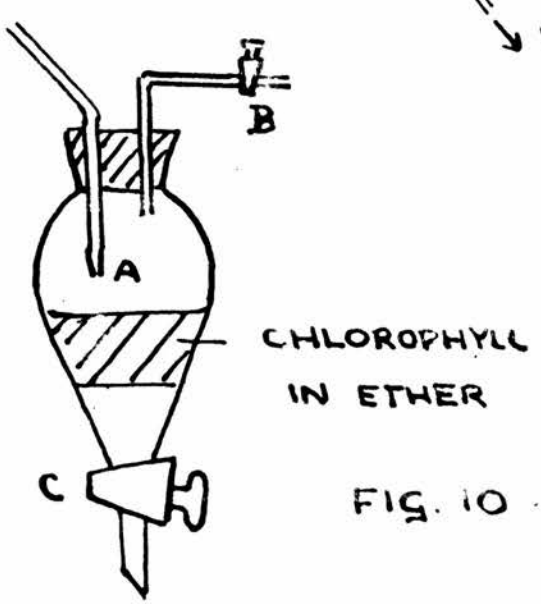


FIG. 10

of 250 ml solution could be washed in less than an hour by this method. All operations were performed in dim red light.

The pigments were separated on columns of dimensions about 5 x 50 cm. containing dried confectioner's sugar in petroleum ether, B.P. 35-40°C. About 20 ml of mixed pigment solution was added to the top of the column and the carotenes were eluted with about 200 ml petroleum ether. When all traces of carotene had been removed, the chlorophylls themselves were eluted with diethyl ether, components a. and b. not generally being separated since previous work has shown that the reaction under investigation is common to both.

After elution the solution was washed free of dissolved sugar using the device described by Le Rosen, fig. 10. Distilled water was run from the fine jet A onto the surface of the chlorophyll solution until the separating funnel was half full. Tap B was then closed and C opened so that the washing water ran through the solution continuously, the liquid levels remaining constant. 100 ml of ether solution could be washed with 1 litre of water in 30 min. The solution was dried overnight over anhydrous sodium sulphate before determining its concentration and making it up to a standard volume with ether.

All operations were performed in dim red light and the solutions were stored at -5°C in the dark. The absorption spectra of the chlorophyll was taken on a Unicam S.P. 600 spectrophotometer from 3500°A to 7000°A. The purity of each preparation was determined largely by its spectrum, using as a reference the spectra of Zscheile

and Comar (32).

The concentrations of the solutions were found by evaporating the solvent off 10 ml samples under vacuum, and weighing the residue.

Preparation of pheophytin

Pheophytin was prepared from chlorophyll by an adaptation of the method of Willstatter (34). 25 ml of a 1 mg/ml concentration solution of chlorophyll were shaken with 5 mg oxalic acid in the dark for 24 hours. The pheophytin formed was extracted into the chloroform layer of a 10 ml 1:1 chloroform/water solution. The aqueous layer was discarded and the chloroform layer washed with distilled water until free from oxalic acid, shown by testing the washings with dilute potassium permanganate solution. The chloroform solution was dried over sodium sulphate overnight, and the concentration of pheophytin found by evacuation to constant weight at the oil pump. The residue was then made up to 10 ml with ether and the spectrum determined.

Preparation of ethyl chlorophyllide

This was prepared by enzymic hydrolysis of chlorophyll according to the method of Willstatter and Stoll (35). Plants recommended are those of the Hogwort family to which hedge parsley belongs. Fresh hedge parsley leaves were extracted in 80 per cent alcoholic solution in a macerator for 5 minutes. A little chalk was added to counteract

the effect of any acidity in the plant. The extract was filtered and the filtrate allowed to stand in the dark for several days while a fine precipitate separated out. The very small crystals formed were filtered off and washed with ether. The poor yield obtained could be increased by adding a little water to the filtrate. The crystals were dark and powdery in appearance, and could be precipitated from acetone solution by the addition of water to form a suspension of microcrystals (chlorophyll forms a colloidal suspension under the same conditions).

The crystals were dried, weighed, and made up to 10 ml solution with ether. The absorption spectrum was found to be very similar to that of chlorophyll.

The phytol used in these experiments was supplied by B.D.H. A solution of known concentration of the very pale yellow alcohol was prepared in ether.

Preparation of pheophorbide

An ether solution of chlorophyll was evaporated and the solid residue treated with concentrated hydrochloric acid. A little acetone was added to facilitate the mixing of acid and wax and the solution was allowed to stand overnight. The solid was then extracted with ether and washed with distilled water until free from acid. The ether solution was dried over sodium sulphate, filtered, and the ether evaporated off. The residue was dissolved in

petroleum ether, B.P. 40°C - 60°C, and the pheophorbide separated from phytol on a sugar column which was washed exhaustively with petroleum ether to ensure removal of all the phytol, according to the method of Willstatter (36).

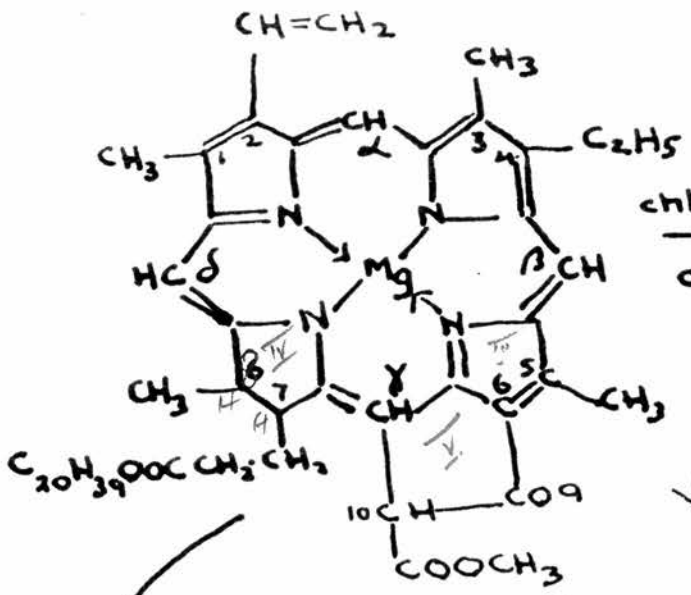
The pheophorbide band was removed from the column and stirred into 20 ml ether. The sugar was filtered off through sintered glass, and the ether extract was washed with distilled water and then dried over sodium sulphate. The concentration of the solution and its spectrum were determined as before.

Preparation of chlorin e

Treatment of chlorophyll with concentrated alcoholic alkali in the cold detaches the ester groupings giving water soluble alkali metal salts of the chlorophyllins along with phytol and methanol. However, this treatment also leads to disruption of the isocyclic ring as in the phase test with the formation of chlorins (37).

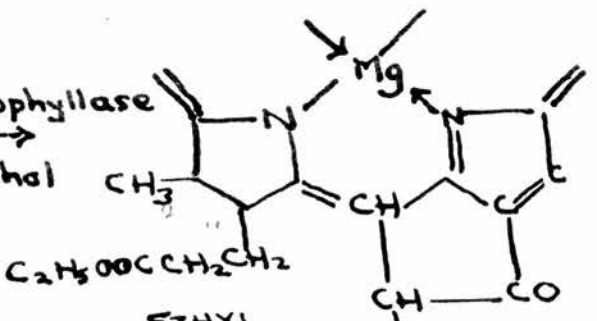
100 ml of a 2 mg/ml concentration solution of chlorophyll in ether was evaporated down to 20 ml. Concentrated alcoholic potassium hydroxide was then added until the phase change was complete, i.e. until the solution remained green on further addition of alkali with no subsequent formation of the brown phase. Distilled water was added to the solution which was transferred to a separating funnel. Addition of petroleum ether removed the free phytol from the solution - the chlorins remained dissolved in the aqueous/methanol phase.

CHLOROPHYLL AND RELATED COMPOUNDS



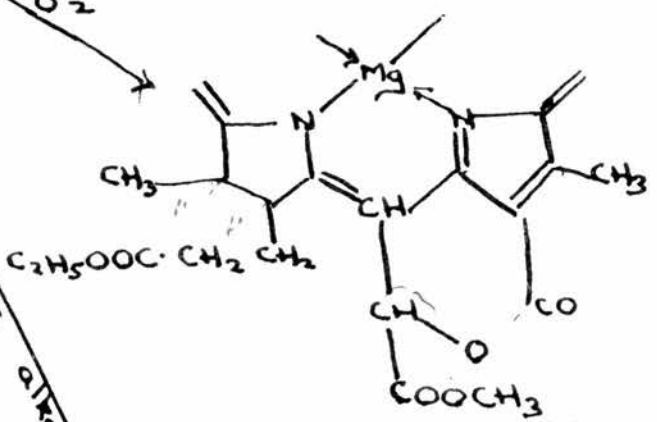
CHLOROPHYLL A

chlorophyllase → alcohol



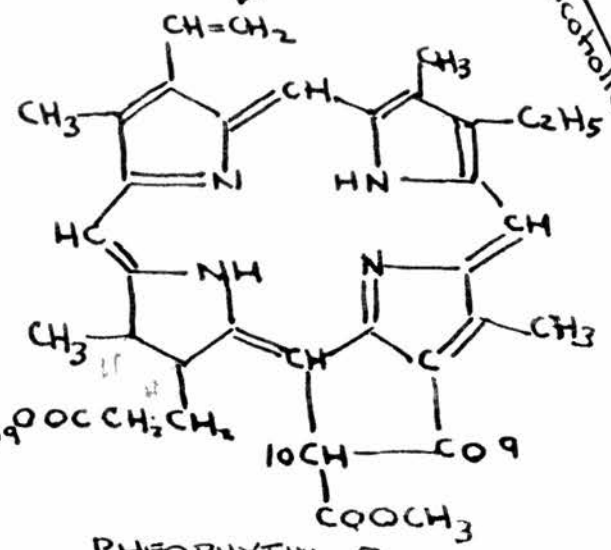
ETHYL CHLOROPHYLLIDE

alcohol + O_2



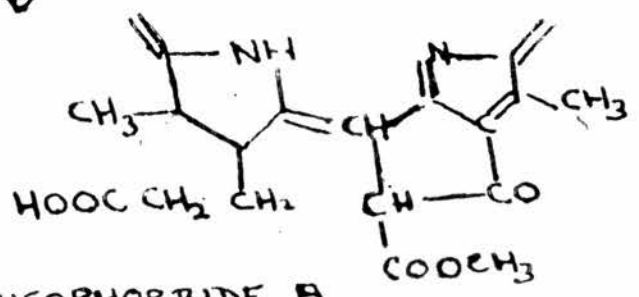
ALLOMERISED CHLOROPHYLL (chlorin lactone)

dilute acid



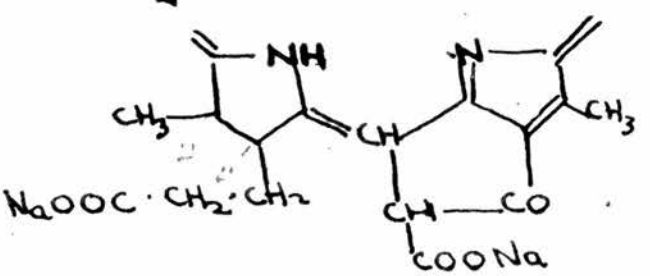
PHEOPHYTIN A

conc. acid

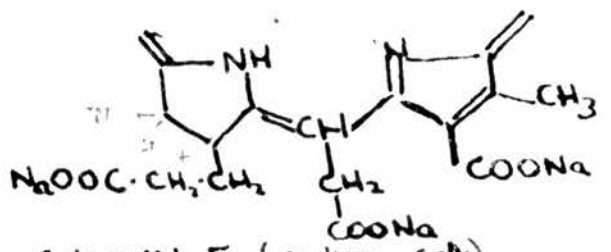


PHEOPHORBIDE A

alcoholic alkali



CHLOROPHYLLIN (sodium salt)



CHLORIN E (sodium salt)

FIG. 11

The aqueous solution was then dialysed through cellophane for several days until neutral to litmus, in order to remove alkali and methanol. Water was removed from the solution by freeze-drying for 24 hours, after which the chlorins remained behind as a dark green powder. The powder was weighed, made up to 10 ml solution in methanol and the spectrum determined.

Preparation of allomerised chlorophyll

Alcoholic solutions of chlorophyll, when left exposed to air in the dark, take up one molar proportion of oxygen per mole of chlorophyll present (4), with the formation of a chlorin lactone (7) (fig. 11).

10 ml of an ether solution of chlorophyll were evaporated to dryness and the residue was dissolved in absolute ethanol. The alcoholic solution was aerated for four to five days until the 'phase test' was negative, i.e. no transient brown phase appeared because the solution was completely allomerised. The solution was again evaporated to dryness and the concentration of allomerised chlorophyll present and its absorption spectrum in ether solution were determined.

Preparation of partially substituted copper chlorophyll

90 per cent acetone/10 per cent aqueous solutions of chlorophyll were shaken with 2 mg copper sulphate until the solution showed no red fluorescence in ultra violet light. The copper chlorophyll was extracted with 10 ml ether and washed thoroughly to get rid of excess

copper sulphate. The ether extract was then dried and its concentration and absorption spectrum found as before.

Method of photo-oxidation

Two substrates were used to support the chlorophyll films: thallous bromide and Jena glass 'J'.

Thallous bromide was prepared by adding 100 ml of thallous nitrate solution containing 5.32 g. thallous nitrate slowly to 50 ml of an aqueous solution containing 2.50 g potassium bromide with continuous shaking. The precipitated thallous bromide was washed by decantation, filtered, then vacuum dried over phosphorus pentoxide. The powder was stored in the dark.

0.100 to 0.150 g substrate were weighed out and transferred to the reaction vessel. 1 to 2 ml pigment solution (concentration between 1 and 2 mg per ml) were then added by pipette and the bulk of the solvent was removed under water pump vacuum, the vessel being gently swirled during evacuation so that a smooth film formed on one face of the flat-sided vessel. The film was prepared in dim red light, then transferred to the reaction position and evacuated for predetermined periods varying from 2 to 24 hours. Any water vapour or solvent remaining on the film was condensed out by surrounding trap B with liquid oxygen. 50 mm oxygen were next admitted to the system, the gauge and jacket taps were closed and the film was left in the dark for a short time to allow the oxygen to come to thermal equilibrium. When the gauge pointer had remained steady for about

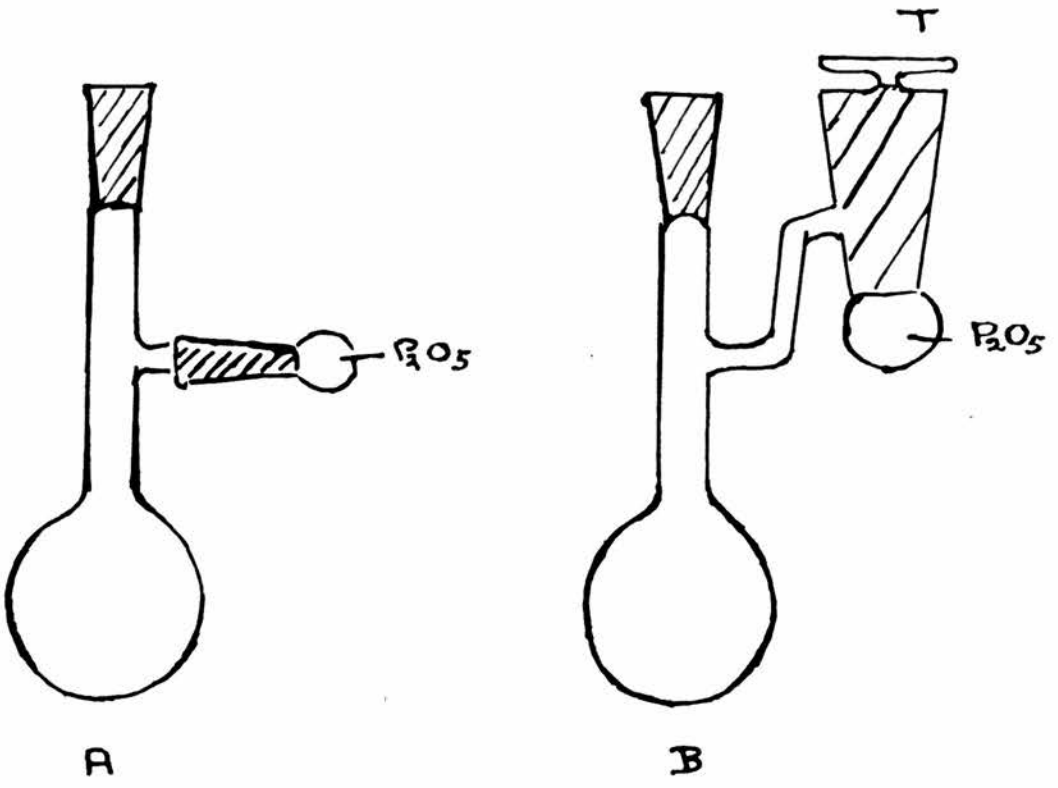
20 minutes, the shutter was raised and the film illuminated, gauge readings showing change in pressure being plotted against time. From this curve a 'rate' curve was derived by plotting the change in pressure during a fixed interval of time against the total pressure change achieved in the middle of this period of time. The final portion of this rate curve was found to be linear, and extrapolation of this linear portion to zero rate gave an estimation of the pressure change that would have occurred for 100 per cent reaction.

The water vapour produced in the reaction was estimated by using a reaction vessel containing a side-arm carrying phosphorus pentoxide. Two such types of vessel were used (fig. 12). In vessel A, the P_2O_5 was in contact with any water vapour given off during the course of the run, whereas in B water vapour was shut off from the absorbent by tap T during the run. At the end of the run, tap T was opened and any water vapour produced during the oxidation was absorbed by the P_2O_5. Any subsequent pressure decrease was recorded.

For preliminary investigations an ordinary flat-sided reaction vessel with no side-arm was used. All vessels were flat-sided rather than spherical in order to keep the volume of the reaction space small.

Analysis of hydroperoxide in the oxidised films

The oxidised film had previously been shown to give a peroxide reaction to both ferrous thiocyanate (38) and potassium iodide (31). It was the object of the present work to investigate the relationship



REACTION VESSELS

FIG. 12

between the oxygen uptake and the amount of peroxide formed, and to try to determine the position in the chlorophyll molecule at which this peroxide formed. Various methods of peroxide analysis were tried out to see which was most suitable for further development.

It was found that the peroxide reaction only occurred if the film was extracted with 100 per cent methanolic or isopropanol solutions. The reactive peroxide group was water insoluble and it was therefore not possible to use methods of analysis which involved aqueous solutions of the reagent. The stannous chloride (39) method was therefore discarded, since this also had the added disadvantage of requiring highly acid solutions, which would degrade the chlorophyll molecule and introduce complications. The titanous sulphate method also involved acid solutions and depended on the formation of a yellow-coloured complex with maximum absorption at 4100°A (39). This yellow colour would have been largely masked by the green colour of chlorophyll itself, which also has a maximum absorption at this point.

Thus the two most suitable methods available were the potassium iodide and ferrous thiocyanate reactions, since these two reagents could be dissolved in organic solvents and only very weakly acid solutions were required. The method of Kotanur and Jelling (40) utilised the reaction of peroxides with potassium iodide. The oxidised film was extracted in isopropanol, filtered, and made up to 10 ml solution. A 2 ml sample was heated with one drop of saturated aqueous potassium iodide and one drop of acetic acid for 5 minutes in an atmosphere of nitrogen, then made up to 10 ml solution with isopropanol in a graduated flask. After allowing any undissolved

potassium iodide to settle, the optical density was measured at 3800°A using 2 cm cells in a Unicam S.P. 600 spectrophotometer, the absorption due to unreacted chlorophyll being compensated for by measuring that of an untreated sample with the same absorption and subtracting. Calibration was achieved by means of standard iodine solutions prepared by dissolving 0.06 g resublimed iodine crystals in 100 ml isopropanol and diluting 5 times. Starch indicator could not be used in this case unless the isopropanol solutions were considerably diluted with water. This is unsatisfactory because it forms a heterogeneous system.

Owing to the high absorption due to unreacted chlorophyll at 3800°A, the above method was never very satisfactory. A further serious disadvantage is that chlorophyll is known to react with hydrogen iodide in glacial acetic acid solution to give a colourless leuco-compound which is oxidised in air to pheoporphyrin a_5 (37). Also many chlorophyll porphoryins can be oxidised by iodine at position 10 to give an OH group, so that this method also is subject to many complicating factors.

It was thus found that the most satisfactory method for this particular peroxide analysis involved the use of the reagent ferrous thiocyanate in methanol according to the method of Young, Vogt and Nieuwland (41). This had many advantages:

(1) The reagent could be made up in methanol.

(2) The reaction occurred quickly in the cold.

(3) The maximum absorption of the red colour complex was at 5100°A, which was a wavelength of minimum absorption for

chlorophyll itself, so that the correction factor allowing for the absorption due to unreacted pigment was small.

Using this reagent, the optical density has been shown to be proportional to the concentration of peroxide present for several peroxides, e.g. hydrogen, ethyl tertiary butyl, diethyl (39) or decalin (38) hydroperoxides. Furthermore, it can be shown that if the optical density for these organic peroxides with the reagent is plotted against the weight of hydroperoxide present per mole as peroxidic oxygen rather than against the total weight of the peroxidic compound, the same straight line is obtained as for hydrogen peroxide for concentrations of hydroperoxide below 20 µg. Thus it was justifiable to use a hydrogen peroxide/ferrous thiocyanate calibration graph in order to estimate the amount of peroxide or hydroperoxide present in the oxidised chlorophyll films.

The oxidised film was therefore finally extracted in methanol, filtered and made up to 10 ml solution, and analysed for hydroperoxide according to the method of Young, Vogt and Niewland (41). The reagent was prepared by dissolving 5 g ammonium thiocyanate in 500 ml methanol, adding a few drops of concentrated sulphuric acid, and making the volume up to 1 litre with methanol. Before each determination sufficient ferrous ammonium sulphate was added to the required volume of this solution to saturate it, and after shaking, the resulting solution of ferrous thiocyanate was decanted and used immediately. A calibration graph was obtained by measuring the absorption of solutions of hydrogen peroxide plus reagent on a Unicam S.P. 600 spectrophotometer at 5100°A, which is the maximum absorption for

the complex.

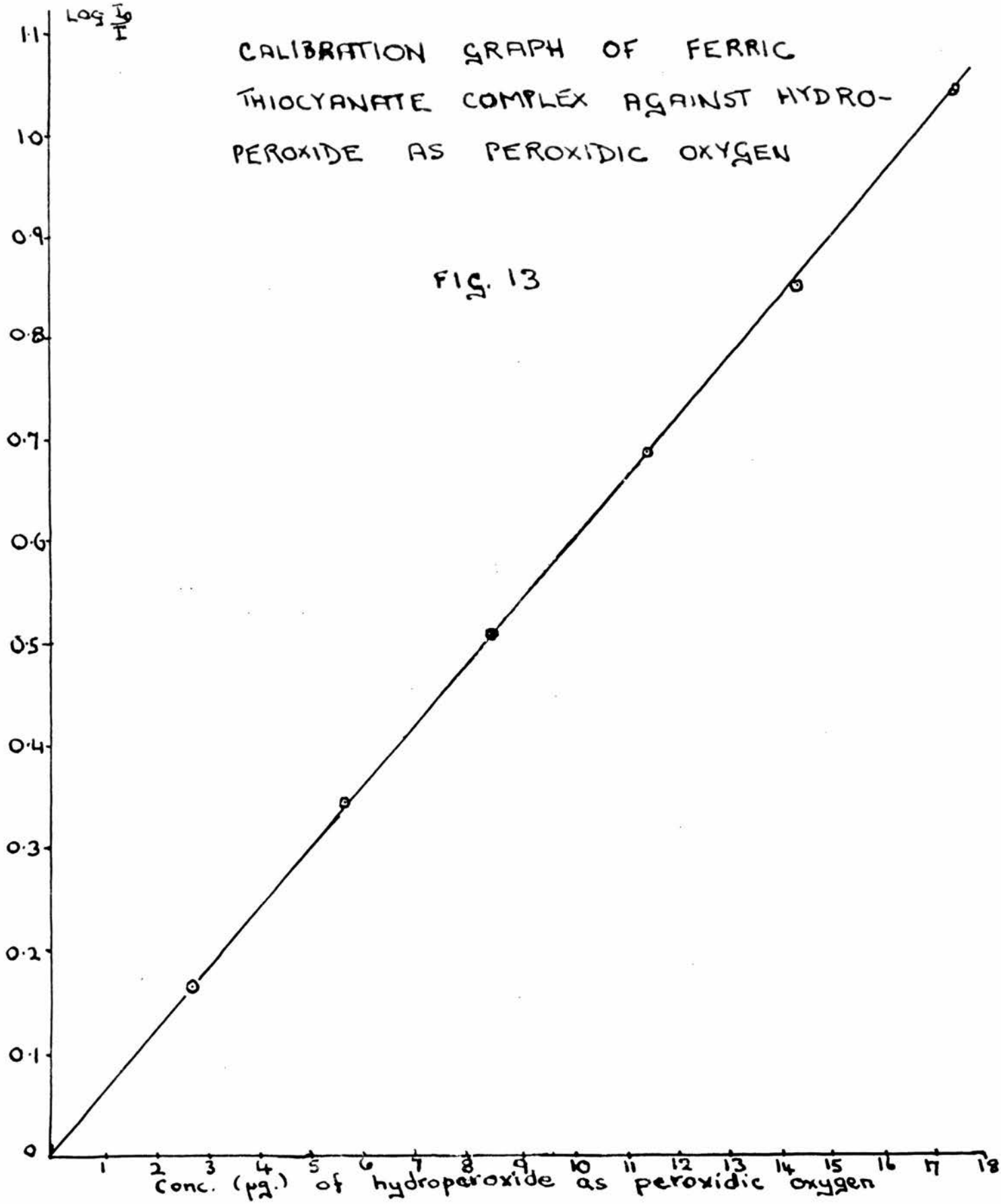
An approximately decinormal solution of commercial hydrogen peroxide in methanol was prepared and its concentration found by direct titration in acid solution with decinormal potassium permanganate, previously standardised by the oxalic acid method. The hydrogen peroxide solution was then diluted 50 times with methanol and to known volumes of this solution (< 1 ml), 5 ml ferrous thiocyanate was added and the solution was made up to 10 ml with methanol. The colour developed in each was compared with that of 5 ml reagent made up to 10 ml with methanol at 5100°A, using 2 cm cells. A 10 minute time interval was allowed after making up the solutions before measuring the absorption to ensure maximum development of the colour. The curve obtained by plotting log $^{Io}/_{I}$ against the number of micrograms of peroxide present was found to be linear up to 20 ug peroxidic oxygen. The calibration was repeated several times (fig. 13).

Concentration of hydroperoxide in H_2O_2	log $^{Io}/_{I}$
2.92	0.165
5.84	0.342
8.76	0.504
11.68	0.681
14.60	0.850
17.52	1.040

10^{-6} moles × 32 µg.

See p 77

To suitable aliquot portions of the methanolic extract of the oxidised film (1, 2 or 4 ml samples, depending on the extent of


CALIBRATION GRAPH OF FERRIC THIOCYANATE COMPLEX AGAINST HYDRO-PEROXIDE AS PEROXIDIC OXYGEN
FIG. 13
Log I₀/I
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Conc. (μg.) of hydroperoxide as peroxidic oxygen

oxidation), 5 ml reagent were added and the solution was made up to 10 ml with methanol. Since the absorption due to unoxidised chlorophyll, although small at 5100°A, was not negligible, before each determination a solution of unoxidised chlorophyll with the same absorption as that of the oxidised sample was made up, by adding chlorophyll to the solvent until the absorptions were equal. The colour developed with the reagent and oxidised chlorophyll was compared against that of the reagent together with the same concentration of unoxidised chlorophyll made up to 10 ml solution with methanol.

One further preliminary experiment was carried out. 1 ml, 2 ml, 3 ml and 4 ml samples of a solution of the oxidised film in methanol were reacted with 5 ml reagent and made up to 10 ml solution as before. The absorption of these samples was found to be proportional to the original volume of solution, showing that the absorption of chlorophyll peroxide plus reagent varies linearly with peroxide concentration. Thus in all estimations of peroxide in oxidised films, a convenient check on all readings was to measure the absorption for, say, 1 ml and 2 ml samples of the 10 ml methanolic solution of the oxidised film.

Paper chromatography of oxidised films

Cartlidge and Tipper (42) have developed methods for the analysis of peroxides by paper chromatography. Whatman No. 1 paper in strips of dimensions 3 x 25 cm was used, both untreated and soaked in a 5 per cent or 20 per cent solution of ethylene glycol in acetone, and subsequently dried in air.

The solvents used were:

(1) For untreated paper, ether and water : ether : n butanol (1:10:10).

(2) For paper soaked in 5 per cent ethylene glycol solution: 10 vol. per cent butanol in petroleum ether, B.P. 80-100°C.

(3) For paper soaked in 20 per cent glycol solution: 5 vol. per cent ether in 80-100°C petroleum ether, and chloroform : 80-100°C petroleum ether (50:50).

The films were extracted in concentrated form with a few drops of ether and deposited as a spot about ½ cm in diameter on the paper, the procedure being carried out in dim red light. A spot of unoxidised chlorophyll of similar concentration was placed alongside the oxidised spot.

A large glass tank made air-tight at the top by a glass plate sealed with vaseline contained the trough for the moving phase. This was also present in the bottom of the tank so that the atmosphere was saturated with solvent during the run. All air was initially driven out of the tank by a stream of nitrogen to prevent further oxidation of the chlorophyll. The tank was also kept in the dark. The papers were suspended with their ends dipping about 1 cm into the solvent in the trough and left for 2 to 4 hours, by which time the solvent had almost reached the bottom of the paper. The paper was then removed and allowed to dry before being sprayed with a concentrated solution of ferrous thiocyanate in methanol.

Estimation of the amount of copper present in copper chlorophyll

The reagent used was 0.1 per cent aqueous disodium diethyl dithiocarbanate, which reacts with copper to form a yellow complex. The intensities of the colour developed in standards and in the copper chlorophyll were measured against that of the reagent on the Unicam at 4600°A (43).

5 ml of copper chlorophyll solution (concentration 1 mg/ml) were evaporated to dryness. 2 drops of aqua regia were then added and the solution was again evaporated to dryness on a hot plate. The residue was dissolved in a minimum quantity of copper-free distilled water and made up to 10 ml solution.

For calibration, 2, 4, 6 and 8 ml portions of a standard solution of copper sulphate (concentration 0.01 mg copper/ml) were added to 10 ml 4 N ammonia solution and 5 ml reagent, and the solution was made up to 25 ml. The absorption was compared immediately with that of the reagent at 4600°A. Beer's law was obeyed and the intensity of colour developed in the solutions was proportional to the concentration of copper in each. 5 ml samples of the copper chlorophyll solutions were treated in exactly the same way, and the concentration of copper in each was determined from the calibration graph.

Infra red spectra of chlorophyll and its oxidation product

The infra red spectra were measured by J.L. Duncan on a Hilger H800 double beam spectrometer fitted with rock salt prisms. Films

of solid chlorophyll and oxidised chlorophyll deposited from ether solution were used.

Experimental Results

Previous workers (29) have established that the illumination in visible light of partially substituted copper chlorophyll in oxygen results in a pressure decrease in unit ratio to the amount of chlorophyll present. Attempts to confirm these results with samples of chlorophyll extracted from spinach and stinging nettles showed that although ratios of one were sometimes obtained, many such ratios tended to be greater than one, particularly when the chlorophyll had been extracted for some time. This effect was attributed to either one or both of two causes.

(a) Degradation of the chlorophyll leading to formation of pheophytin by loss of magnesium. Oxidation of pheophytin leads to a pressure decrease ratio of approximately three, and thus partial degradation of chlorophyll to pheophytin would lead to ratios between one and three, depending on the amount of pheophytin present. Sunners (31) analysed samples of chlorophyll showing high pressure ratios for magnesium, and invariably found that the amount of magnesium present was less than that required for pure chlorophyll. He also found a correlation between the lowering in the magnesium concentration and the increased pressure ratio. The amount of pheophytin in the sample could be calculated from the magnesium loss, and assuming that the pressure ratio for pheophytin is three, the increased ratio to be expected for the degraded chlorophyll preparations could be estimated. The calculated values agreed very well with the experimental results.

(b) Sunners also showed that the apparent unit molar oxidation ratio was actually the result of an uptake of two moles of oxygen with the release of one mole of water vapour, although it proved very difficult to obtain reproducible estimates of the amount of water vapour given off. Thus increased pressure ratios could be due to adsorption of water vapour on the surfaces of the substrate and the glass walls of the apparatus. For the present the observed pressure decrease is referred to as the apparent oxygen uptake.

It has also been shown by Lawrie (38) that the oxidised film contains a peroxidic group, and preliminary experiments were here carried out to investigate the relationship between the amount of peroxide formed and the apparent oxygen uptake, equivalent to the pressure decrease, using both thallous bromide and powdered glass as substrates for the chlorophyll film.

The method adopted is described in relation to a sample of 2.4 mg copper chlorophyll on 0.100 g thallous bromide. Copper chlorophyll is chosen as an example because this gave more reproducible ratios than spinach chlorophyll due to stabilisation of the molecule by copper which is a better co-ordinating element than magnesium.

A film was prepared as described previously and the reaction vessel attached to the Bourdon gauge and evacuated overnight.

Next morning 50 mm of oxygen were admitted to the system, taps T_2 and T_6 were closed, and the gauge pointer was allowed to come to a steady equilibrium value. The shutter was then raised and illumination commenced. The gauge pointer position was read from

TABLE 1.

Time (Min.)	Gauge Reading	Δp	Time	Gauge Reading	Δp
0	4.60	0	180	7.35	27.5
10	5.20	6.0	190	7.42	28.2
20	5.60	10.0	200	7.50	29.0
30	5.86	12.6	210	7.56	29.6
40	6.05	14.5	220	7.62	30.2
50	6.20	16.0	230	7.68	30.8
60	6.30	17.0	240	7.75	31.5
70	6.43	18.3	250	7.81	32.1
80	6.54	19.4	260	7.88	32.8
90	6.64	20.4	270	7.94	33.4
100	6.73	21.3	280	7.98	33.8
110	6.83	22.3	290	8.02	34.2
120	6.90	23.0	300	8.08	34.8
130	6.99	23.9	310	8.12	35.2
140	7.06	24.6	320	8.16	35.6
150	7.13	25.3	330	8.20	36.0
160	7.20	26.0			
170	7.28	26.8			

TABLE 2.

R_{20}	Δp	R_{20}	Δp
10	5	1.3	30.4
4.2	14.2	1.2	31.6
3.0	17.2	1.2	32.8
2.3	19.5	1.1	33.9
1.9	21.4	0.9	34.4
1.8	23.2	0.8	35.2
1.6	24.8		
1.5	26.3		
1.4	27.7		
1.4	29.1		

GRAPH OF PRESSURE DECREASE Δp/TIME

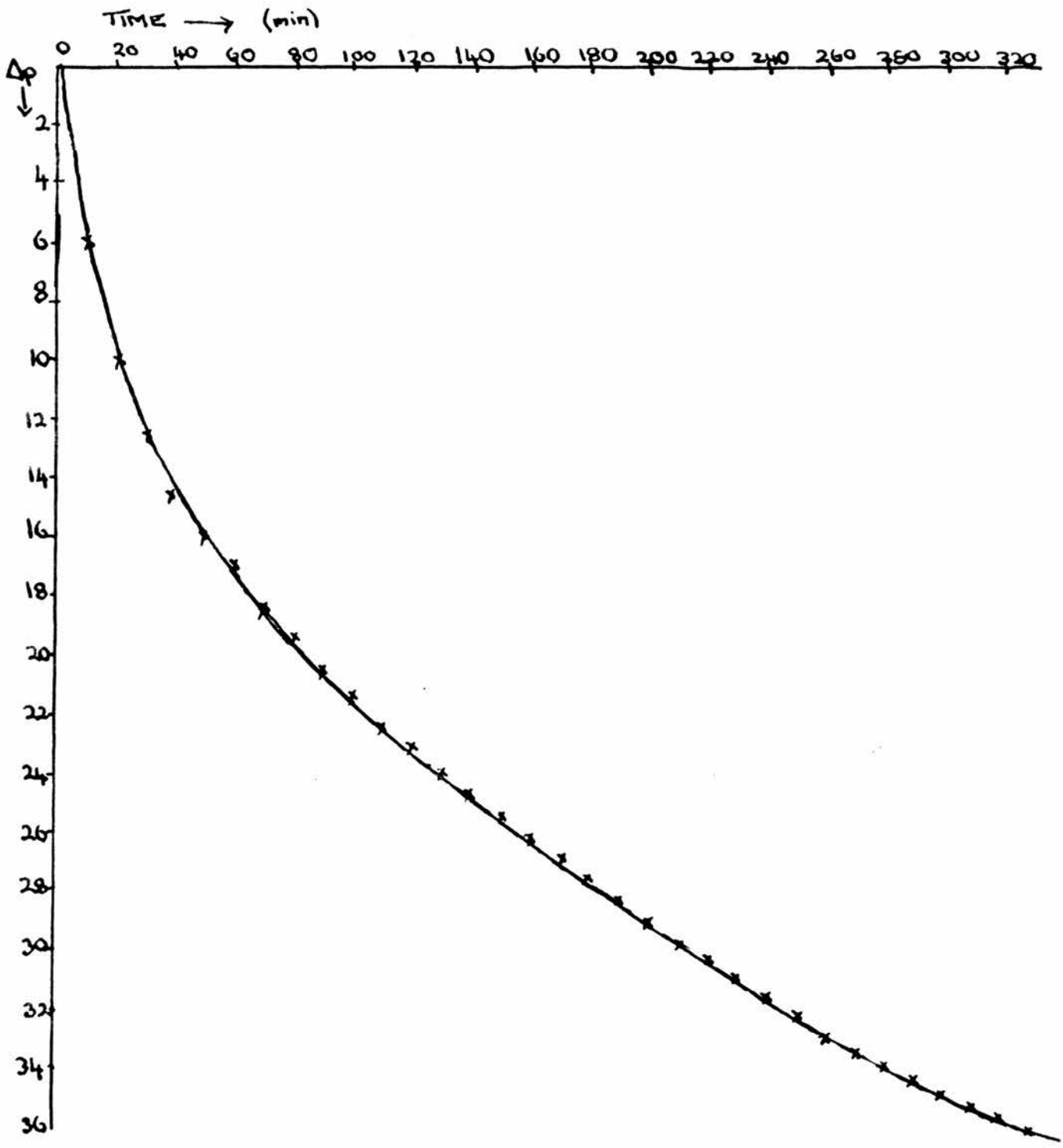


Fig. 14

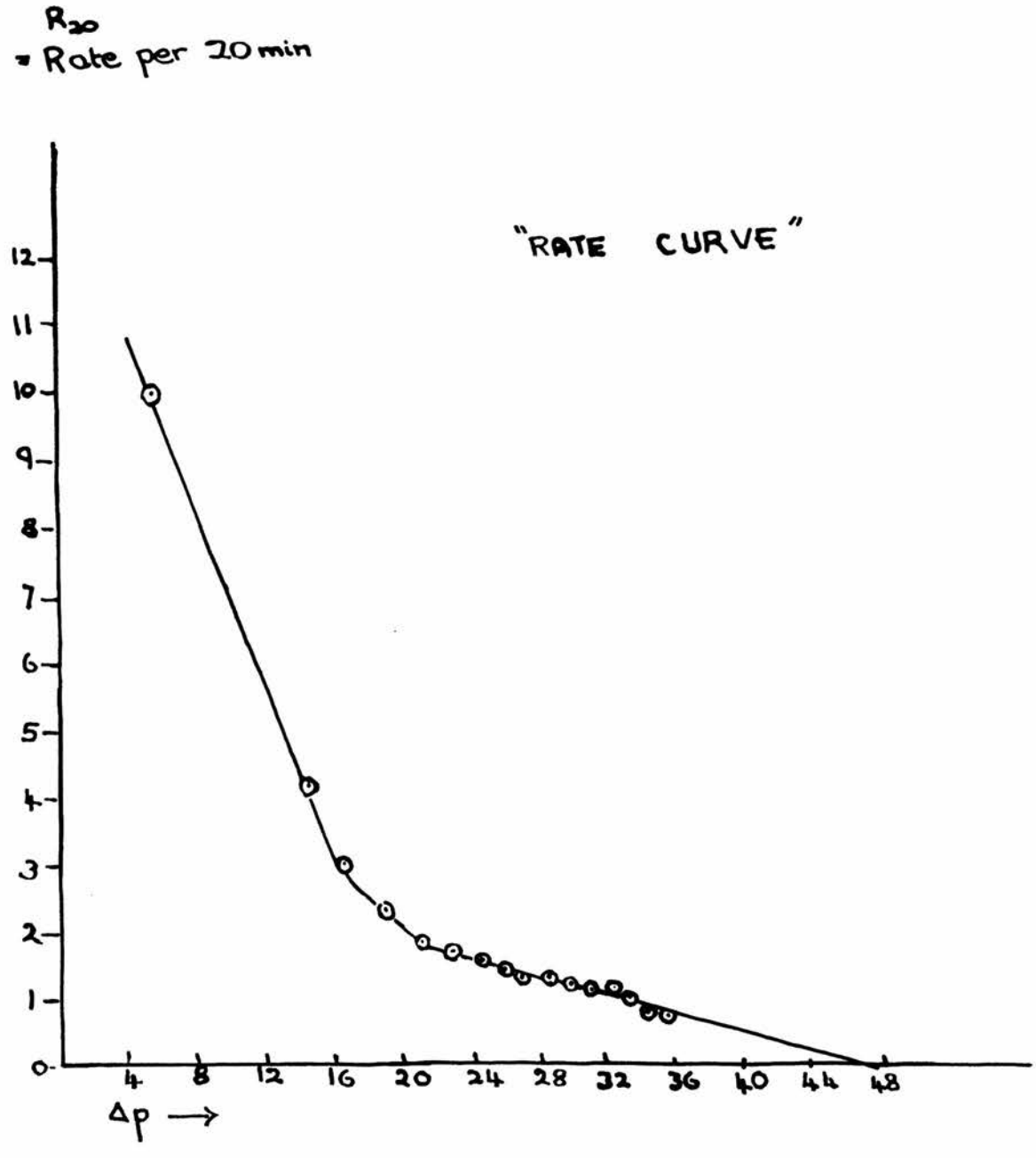

R_{20}
= Rate per 20 min
"RATE CURVE"
12
11
10
9
8
7
6
5
4
3
2
1
0
4
8
12
16
20
24
28
32
36
40
44
48
Δp →

FIG. 15

the telescope scale every ten minutes, and the gauge readings were recorded against the time (t). From the gauge readings the change in pressure Δp from the beginning of the reaction was calculated, and a graph of Δp against t was plotted (fig. 14).

From the curve obtained the rate of change of Δp per 20 seconds (R_{20}) was plotted against the change in pressure Δp at the mid-point of the time interval over which the rate was measured. The rate curve was linear in its final stages, and could therefore be extrapolated linearly to give an estimate of the total pressure decrease at zero rate (Δp_o) (fig. 15).

Calculation

Temperature of thermostat = 26.3°C

Volume of reaction space = 29.82 ml

Sensitivity of gauge = 0.035 mm/scale division

Δp_o = 47 scale divisions (S.D.)

Total pressure decrease at 100 per cent reaction, corrected to N.T.P.:

$$\frac{47.0 \times 0.035 \times 29.82}{760 \times 22400} \times \frac{273}{298} = 2.63 \times 10^{-6} \text{ moles}$$

Amount of chlorophyll present = 2.41 mg = $\frac{2.41 \times 10^{-3}}{900}$

$= 2.68 \times 10^{-6}$ moles

$$\therefore \text{Pressure ratio} = \frac{\text{No. of moles of oxygen taken up at zero rate}}{\text{No. of moles of copper chlorophyll present}}$$

$$= \frac{2.68}{2.63} = \underline{1.0(2)}$$

From the pressure decrease at the point at which illumination

was ceased, the weight of oxygen taken up by the film was calculated. After extracting the oxidised film in methanol, the weight of hydroperoxide in the oxidised film was determined as previously described, and the ratio $\frac{\text{hydroperoxide}}{\text{apparent weight of oxygen taken up}}$ was calculated. This was found to be of the order 2:1.

The form of the rate curve of figure 15 may be briefly considered at this point. Since the total decrease in pressure recorded during the course of the run could be regarded as constant, the linear nature of the terminal section of the rate curve indicated that the rate of oxygen uptake was directly proportional to the amount of chlorophyll remaining in the system. The mathematical expression for this is that of a first order reaction:

$R = k[Chl]$ where R = rate of oxygen uptake = rate of disappearance of chlorophyll and [Chl] is the concentration of unoxidised chlorophyll at the time when the rate is measured. It follows from this expression that the unoxidised chlorophyll content is zero when R = 0, i.e. extrapolation of the rate curve to zero rate should give the amount of oxygen that would be taken up for complete oxidation.

The following runs were carried out on a batch of freshly extracted chlorophyll.

Photo-oxidation of 2 mg samples of chlorophyll on 0.175 g thallous bromide.

Two ratios were calculated from the results:

(a) The ratio $\frac{\text{"oxygen" uptake at zero rate}}{\text{No. of moles of chlorophyll present}}$, defined as the 'pressure ratio'.

(b) The ratio $\frac{\text{hydroperoxide present}}{\text{apparent weight oxygen taken up}}$ which had not previously been estimated for films of chlorophyll on thallous bromide. Runs were of length between 5 and 10 hours.

TABLE 3.

Pressure ratio	Weight of Oxygen taken up (µg.)	Hydroperoxide (µg.)	$\frac{\text{Hydroperoxide}}{\text{apparent oxygen}}$
0.98	44.3	41.7	0.94
0.76	35.9	32.5	0.91
1.02	125.3	85.8	1.46
0.93	40.7	65.0	1.59
0.91	71.1	115.0	1.62

Although the pressure ratios for these five runs were approximately unity, the $\frac{\text{hydroperoxide}}{\text{apparent oxygen}}$ ratios varied from 0.9 to 1.6, showing distinct lack of reproducibility.

A second set of runs was carried out on powdered glass. Difficulty was at once encountered in reproducing the original form of the Δp/time curve. There was an initial acceleration period until a constant rate was maintained for almost the whole of the first day, there being a slight deceleration latterly. If the illumination was discontinued overnight but recommenced on a second day, there was an initial increase in rate followed by a steady decrease (fig. 16).

A linear extrapolation of the $R_{10}/\Delta p$ curve for the second day was parallel to the beginning of a linear portion obtained at the end

OXIDATION OF CHLOROPHYLL ON JENA GLASS

Δp / TIME CURVE

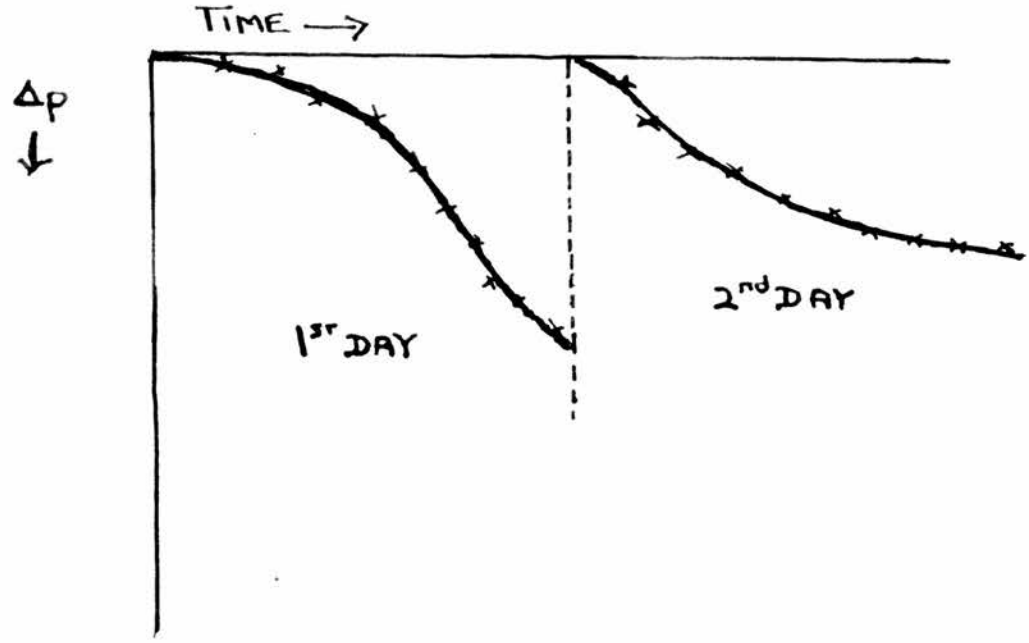


FIG. 16

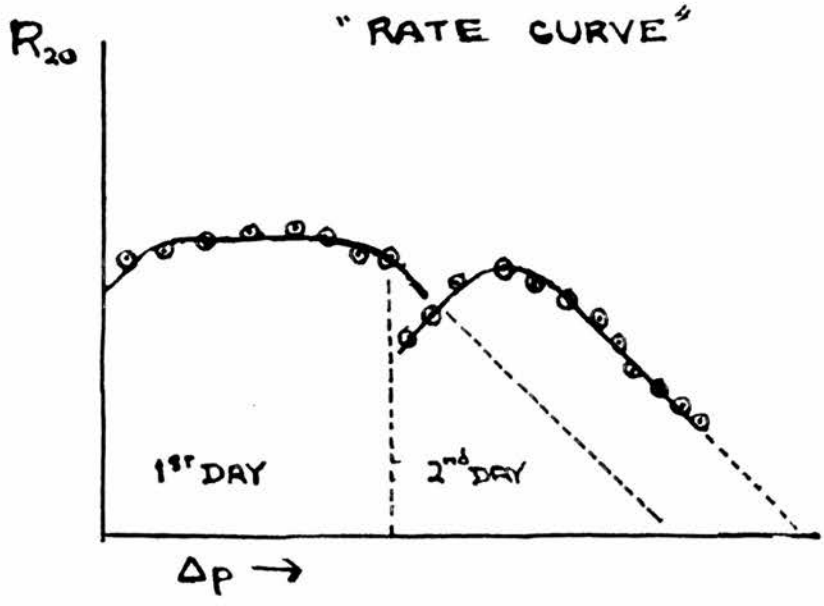


FIG. 17

of the second day. Thus a rough estimate of the value of Δp_o at zero rate for an undisturbed reaction could be made by extrapolating the first day rate curve parallel to the slope of the linear portion obtained for the second day (fig. 17).

1.5 mg chlorophyll on 0.05 g powdered glass.

TABLE 4.

Pressure ratio	Oxygen uptake (µg.)	Hydroperoxide (µg.)	$\frac{\text{Hydroperoxide}}{\text{apparent oxygen}}$
1.26	90.6	130.0	1.44
1.20	89.5	87.5	0.98
1.20	129.3	55.0	0.43
1.21	149.0	52.5	0.35
1.67	91.3	87.5	0.99
3.00	178.4	132.0	0.74

The ratios obtained on powdered glass were even less satisfactory than those for thallous bromide. The increase in pressure ratios suggests either degradation of the chlorophyll or adsorption of water vapour on the glass substrate. A high value for the apparent oxygen uptake would automatically lead to a lower $\frac{\text{hydroperoxide}}{\text{oxygen}}$ ratio than was obtained for thallous bromide.

The absorption spectrum of the chlorophyll solution was re-determined at this stage. The peak heights at 4300°A and 6600°A were found to be considerably diminished, while a small peak was beginning to appear at 5050°A, indicating the presence of pheophytin

in the preparation. The following runs were therefore carried out on freshly extracted chlorophyll. The reaction was terminated at the end of one day and the hydroperoxide estimated immediately so that no decomposition had occurred. There was always this possibility in runs which were discontinued overnight then recommenced the next day.

1.67 mg chlorophyll on 0.05 g powdered glass.

TABLE 5.

Apparent oxygen uptake (μg.)	Hydroperoxide (μg.)	$\frac{\text{Hydroperoxide}}{\text{apparent oxygen}}$
44.9	55.0	1.21
48.9	77.5	1.59
46.0	84.6	1.87
52.6	57.5	1.09
36.6	62.5	1.71
41.1	46.0	1.12
31.5	52.5	1.67

The $\frac{\text{hydroperoxide}}{\text{oxygen}}$ ratios in this case were higher and agreed more closely with those obtained on thallous bromide. If the hydroperoxide formed initially is unstable, and does decompose during longer runs, then the $\frac{\text{hydroperoxide}}{\text{oxygen}}$ ratios would be larger for short than for long runs.

The runs on glass were repeated for chlorophyll extracted from stinging nettles. The method of extraction was similar to that for spinach chlorophyll, except that a small quantity of calcium carbonate

was introduced into the macerator to counteract acidity in the plant. The spectrum corresponded closely with that for spinach chlorophyll. Pumping was continued for only 6 hours instead of overnight, to see how this affected the ratios obtained.

1.75 mg nettle chlorophyll on 0.05 g powdered glass.

TABLE 6.

Apparent oxygen uptake (µg.)	Hydroperoxide (µg.)	Hydroperoxide / apparent oxygen
49.2	70.5	1.43
70.4	130.0	1.87
52.5	130.0	2.48
58.5	122.0	2.08
51.0	120.0	2.35
47.5	100.0	2.10

Since the pressure ratios for this preparation were approximately unity, and the chlorophyll was spectroscopically pure, the most likely reason for the wide spread in these ratios is that the water vapour given off during the course of the reaction is adsorbed to a greater or lesser extent on the powdered glass, depending on the degree of dryness of the film prior to illumination. High $\frac{\text{hydroperoxide}}{\text{apparent oxygen}}$ ratios for nettle chlorophyll could therefore be explained on the grounds that pumping had only been continued for 6 hours instead of overnight, leading to the adsorption of a smaller quantity of water vapour and consequently a lower apparent oxygen uptake.

In order to ensure that only a negligible quantity of water vapour produced during the reaction could be adsorbed, three runs were commenced after pumping for only one hour. These runs proceeded at a very slow rate, Δp being only of the order 5 to 6 S.D. at the end of one day, as opposed to 15 to 20 S.D. in previous runs. The following results were obtained:

TABLE 7.

Apparent oxygen uptake (μg.)	Hydroperoxide (μg.)	Hydroperoxide / apparent oxygen
21.1	55.0	2.7
18.2	51.2	2.8
14.2	56.0	3.96

The excessively high ratios obtained here suggest that the values of Δp obtained were far too low, probably because considerable quantities of water vapour were given off from the film during illumination as a consequence of insufficient pumping. This was, in fact, proved to be the case. One film was illuminated in vacuo for 6 hours after only one hour's pumping, and the gauge pointer rose by 7 S.D. On the other hand, a film which had previously been pumped overnight was treated in the same manner, and no movement of the pointer was observed.

A vessel containing P_2O_5 in a side-arm was used for one run so that any water vapour produced could be absorbed during the reaction. Even after two days continuous illumination, oxygen was still being

taken up at a reasonable rate, so that the pressure ratio obtained was very high (4.2). The $\frac{\text{hydroperoxide}}{\text{oxygen}}$ ratio, however, was only 1.0. These results seem to indicate that removal of water vapour during the course of the run tends to decompose the hydroperoxide, while oxygen uptake continues. This also provides some evidence that the peroxidic group present is in fact a hydroperoxide, since hydroperoxides are known to decompose with the evolution of water vapour.

Oxidation of phytol

Sunners (31) has shown that oxidation of phytol, the esterifying alcohol occurring in the side-chain of the chlorophyll molecule attached to C_7, does occur on illumination in presence of oxygen. A comparison was therefore made between the photo-oxidation of chlorophyll and that of phytol under the same conditions, to see what role the phytol side-chain plays in this reaction.

A solution of phytol in acetone was accurately made up and the oxidation procedure was carried out using 1.6 mg phytol on 0.05 g powdered glass. A methanolic extract of the oxidised film gave a peroxide reaction with ferrous thiocyanate, but no reaction with Schiff's solution. The concentration of hydroperoxide in each oxidised film was estimated after the run, as for chlorophyll. For runs lasting one day, the following results were obtained:

TABLE 8.

Apparent oxygen (µg.)	Hydroperoxide (µg.)	Hydroperoxide / apparent oxygen
21.0	24.0	1.15
66.8	48.0	0.72
27.8	20.0	0.72
45.8	30.0	0.66

The Δp/time curve and rate curves obtained were of exactly the same form as for chlorophyll, so that it was possible to extrapolate the rate curve to give an estimate of the total apparent oxygen uptake at zero rate.

In one run the oxidised phytol film was left under vacuo for 2 days before estimating the hydroperoxide formed, and it was found that the ratio had diminished to 0.27. Two runs were therefore continued overnight and the hydroperoxide content was estimated the next day.

TABLE 9.

Pressure ratio	Oxygen uptake (µg.)	Hydroperoxide (µg.)	Hydroperoxide / oxygen uptake
0.47	27.7	19.5	0.24
0.36	48.8	11.0	0.23

These results are in accordance with the theory that the hydro-

peroxide does decompose to water vapour and another product during long runs. A further run using phosphorus pentoxide in the side-arm of the reaction vessel gave a pressure ratio of 0.72, but a $\frac{\text{hydroperoxide}}{\text{oxygen uptake}}$ ratio of only 0.04 indicating almost complete decomposition of the hydroperoxide. The pressure ratio of 0.72 is approximately double that obtained when no phosphorus pentoxide is present, so that the number of moles of oxygen taken up is approximately double the number of moles of water vapour given off.

All the above experiments have shown that it is difficult to obtain reproducible results for runs carried out on powdered glass, due to its readiness to adsorb water vapour produced during the reaction. In a control experiment, 30 S.D. of water vapour were admitted to an evacuated reaction vessel containing a chlorophyll film on powdered glass which had previously been pumped out overnight. It was found that about 20 per cent of the water vapour was taken up by the film within 15 minutes. On a second admittance of water vapour, however, less than 5 per cent was adsorbed. There seemed to be no easy way of eliminating this difficulty, since if a film was insufficiently pumped prior to illumination, it actually gave off water vapour when illuminated in vacuo.

Also, films oxidised on glass were found to be olive green in colour after the reaction showing that degradation to pheophytin was occurring on the glass surface. Because of these two disadvantages, glass was abandoned as an unsuitable substrate and thallous bromide was used in all subsequent runs. This had the advantage that very little water vapour was adsorbed when admitted to an evacuated film

(about 5 per cent), and also that the chlorophyll seemed to retain its pure green colour much more readily during oxidation. An added asset was that because oxidation proceeded at a much quicker rate, a run had virtually reached completion by the end of the first day. A thallous bromide film illuminated in oxygen by Sunners showed no pressure change.

A second improvement was introduced. Previously the gaseous products of the reaction have been reported to be CO_2, H_2O and an intermediate fraction which could be the solvent of introduction in the ratio 4:90:6 (44). However, in the present work, only water vapour could be detected as a gaseous product - no CO_2 or acetone were detected either by infra red or vapour phase chomatography for runs of normal length. After very long runs, where considerable degradation of the chlorophyll film had occurred, very small quantities of a gas which could be CO_2 were isolated. In any case, for a normal run in which the water vapour is, say, 20 S.D., only 1 S.D. of CO_2 or solvent could be present on this analysis, which is hardly a significant amount, and could be due to a small leak in the system. It is possible that CO_2 and acetone were produced from small quantities of acetone used for binding the films and retained by the chlorophyll. For this reason, ether was always used as a solvent for chlorophyll in these experiments. McLean (45) has reported that CO_2 was given off in large quantity from acetone-bound films of TiO_2, illuminated in oxygen, whereas no CO_2 was detected when water-bound films were used.

Water vapour was therefore analysed for by introducing a special

reaction vessel having a side-arm containing P_2O_5 which could be closed off during the course of the oxidation by a tap. After illumination was terminated, the tap was opened connecting the reaction space and the absorbent, and the resulting dark pressure decrease was recorded with time until a steady pressure was attained. All the water vapour was absorbed within between one and two hours (fig. 18). The main advantage of this method was that removal of water vapour did not take place during illumination so that the equilibrium between the gaseous and solid phases was not continually disturbed. It also enabled a comparison to be drawn between the total rather than the apparent oxygen uptake and the hydroperoxide formed. The side-arm was given the same pre-reaction treatment as the film, and after admitting oxygen to the reaction vessel, the side-arm was isolated from the reaction space and the run allowed to proceed as usual. A volume correction was necessary when comparing the pressure decrease under illumination and that produced on introducing the absorbent,

e.g. Volume of reaction space = 37.5 ml

Volume of side-arm = 3.9 ml

$\therefore$ 11.4 S.D. H_2O in (37.5 + 3.9) = 41.4 ml $\equiv$ 12.6 S.D. in 37.5 ml.

1.5 mg samples of freshly extracted spinach chlorophyll on 0.1 g TClBr were used in the following runs. Ratios were calculated for both $\frac{\text{hydroperoxide}}{\text{total oxygen uptake}}$ and $\frac{\text{hydroperoxide}}{\text{apparent oxygen}}$, together with $\frac{H_2O}{\Delta p}$ where Δp is the observed deflection of the gauge pointer before absorption of water vapour. All runs were discontinued at the end of one day.

TABLE 10.

$\equiv \Delta p + H_2O$ (μg)	Hydroperoxide (μg)	Hydroperoxide / Total oxygen	Hydroperoxide / Apparent oxygen	$\frac{H_2O}{\Delta p}$	
86.9	76.0	0.88	1.30	0.49	
57.6	45.5	0.79	1.20	0.52	
143.3	132.0	0.92	1.47	1.1	Pumped for 2 hours
43.4	34.5	0.79	1.43	0.80	
132.5	93.5	0.71	0.96	0.36	Pumped overnight
30.3	25.0	0.83	1.40	0.69	Pumped for 7 hours
92.4	75.0	0.81	1.42	0.75	

This new method gave greatly improved results. The $\frac{\text{hydroperoxide}}{\text{total oxygen}}$ results are reasonably reproducible, the values ranging between 0.7 and 0.9, due to the fact that discrepancies caused by adsorption of water vapour are eliminated by considering the total rather than the apparent oxygen uptake. From fig. 19, it is evident that if an extra quantity of water vapour δx is adsorbed by the film and the glass walls of the apparatus, then the observed pressure decrease will be $(\Delta p + \delta x)$ S.D., while the water vapour observed at the end of the run will be the actual water vapour given off by the film minus δx = $(H_2O - \delta x)$ S.D. However, the <u>total</u> oxygen uptake will still be $\Delta p + \delta x + H_2O - \delta x = \Delta p + H_2O$. It may also be noted that the $\frac{\text{hydroperoxide}}{\text{apparent oxygen}}$ ratios agreed well with previous values and that removal of water vapour at the end of the reaction had no effect on

PRESSURE DECREASE ON ABSORPTION OF WATER VAPOUR BY P_2O_5

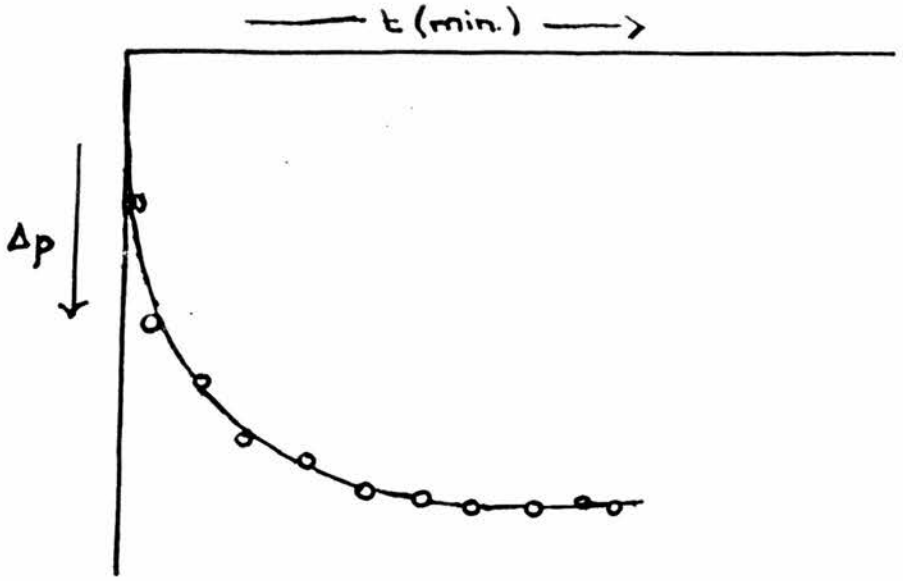
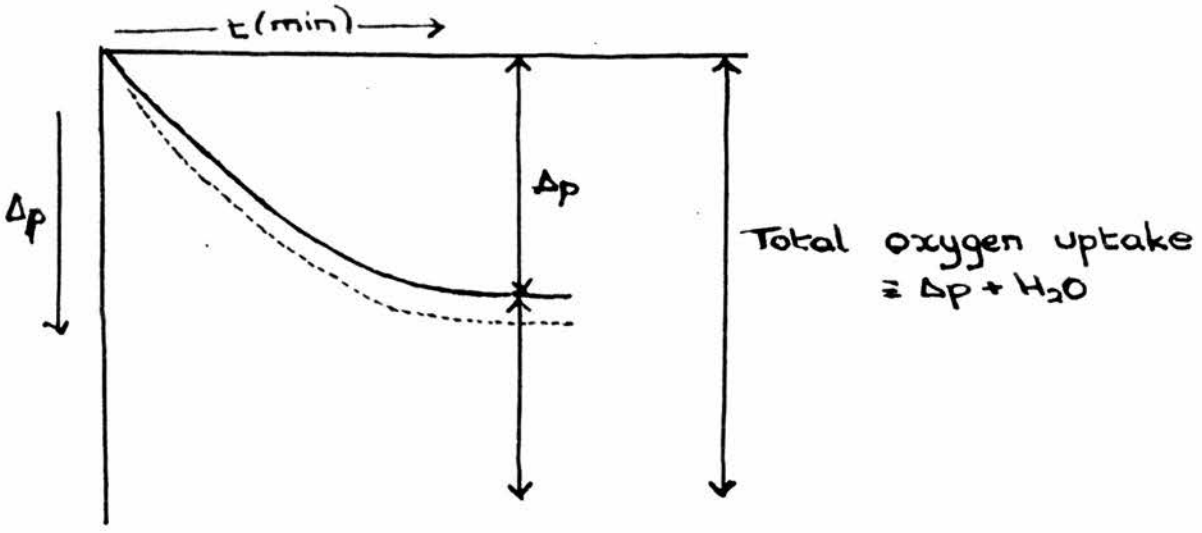


FIG. 18

TOTAL OXYGEN UPTAKE



If an extra quantity of water vapour δx is adsorbed by the film or the glass walls of the apparatus, then the observed pressure decrease will be $\Delta p + \delta x$, and the water vapour $H_2O - \delta x$, but the <u>total</u> oxygen will still be $\Delta p + H_2O$

FIG. 19

the final concentration of hydroperoxide.

The $\frac{H_2O}{\Delta p}$ ratios are high ($\simeq$1) for runs where the film had been pumped for only 2 hours, and very much lower when pumping had been continued all night, with intermediate values for 5 to 6 hours evacuation.

Oxidation of copper chlorophyll

These runs were repeated on partially substituted copper chlorophyll prepared from the freshly extracted spinach chlorophyll. Copper chlorophyll was found by analysis to contain 0.7 per cent copper which is equivalent to the replacement of one magnesium atom in ten by one atom of copper. Now partial replacement of magnesium by copper results in quenching of fluorescence, a negative phase test and greater resistance to bleaching. The overall effect is one of stabilisation because copper is a better co-ordinating atom than magnesium, so that it was expected that the most satisfactory results would be obtained on samples of copper stabilised chlorophyll. This was indeed the case for runs lasting only one day.

TABLE 11.

Pressure ratio	Total oxygen $\equiv \Delta p + H_2O$ (μg)	Hydroperoxide (μg)	$\frac{\text{Hydroperoxide}}{\text{Total oxygen}}$	$\frac{H_2O}{\Delta p}$
0.8	89.7	82.0	0.91	1.17
1.2	87.0	89.0	1.0	0.29
1.1	112.2	113.0	1.0	0.15
0.95	114.0	100.0	0.88	0.33
1.1	60.0	60.0	1.0	0.26

These results agree very well with those obtained for spinach chlorophyll. It appears that for this very stable preparation the $\frac{\text{hydroperoxide}}{\text{total oxygen}}$ ratio is unity, at least for short runs. Low $\frac{H_2O}{\Delta p}$ values occurred where pumping had been continued overnight prior to illumination.

Oxidation of pheophytin

The next stage in the investigation was to carry out the same procedure on pheophytin, the degradation product of chlorophyll produced by the action of dilute acids which replace the magnesium atom by two hydrogen atoms. It had previously been established that oxidation of solid films of pheophytin resulted in a pressure ratio of approximately three caused by an uptake of four moles of oxygen with the release of one mole of water vapour (31). It was found that in certain runs pressure ratios greater than three were obtained, but this was always associated with a $\frac{H_2O}{\Delta p}$ ratio of less than $\frac{1}{4}$, and so could be accounted for by adsorption of water vapour during the reaction. This effect was particularly noticeable on long runs.

The following results were obtained for runs allowed to go nearly to completion, i.e. where the rate of oxygen uptake had diminished almost to zero.

TABLE 12.

	Pressure ratio	$\Delta p + H_2O \equiv$ Total oxygen (μg)	Hydroperoxide (μg)	$\frac{\text{Hydroperoxide}}{\text{Total oxygen}}$	$\frac{H_2O}{\Delta p}$
0.96 mg pheophytin on 0.1 g TlBr.	3.8	117.2	43.8	0.37	0.14
	2.5	111.0	35.0	0.32	0.36
	2.4	104.8	35.5	0.32	0.89
1.68 mg pheophytin on 0.1 g TlBr.	2.4	255.6	95.0	0.37	0.90
	4.0	259.7	105.1	0.40	0.40
	3.4	299.7	119.0	0.40	0.33

The $\frac{\text{hydroperoxide}}{\text{total oxygen}}$ ratio is between 0.3 and 0.4, i.e. almost half to one third of that obtained for chlorophyll; the actual weight of hydroperoxide formed in the film is, however, of the same order per milligram of pheophytin as was found per milligram of chlorophyll. It therefore is possible that the hydroperoxide is formed initially in the same position as in chlorophyll, but that the excess oxygen taken up after this stage no longer leads to the formation of a hydroperoxide. This possibility was examined by stopping several runs after only half the calculated total weight of oxygen had been taken up, i.e. after an uptake of approximately 2 moles of oxygen instead of 4.

Sunners' observation that the pressure ratio for pheophytin was approximately three due to an uptake of four moles of oxygen and the release of one mole of water vapour was confirmed by these results.

1.15 mg pheophytin on 0.1 g TlBr.

TABLE 13.

	$\Delta p + H_2O \equiv$ total oxygen (µg)	Hydroperoxide (µg)	$\frac{\text{Hydroperoxide}}{\text{Total oxygen}}$	$\frac{H_2O}{\Delta p}$	Extent of Oxidation
Runs of length 3-4 hours	85.5	81.0	0.95	0.27	half complete
	89.4	87.5	0.98	0.24	" "
	119.5	107.5	0.90	0.14	slightly more than half complete
	108.2	86.5	0.80	0.30	" "
Run of length 24 hours	203.2	70.0	0.35	0.26	complete oxidation

These results seem to bear out the above theory, i.e. the first two moles of oxygen taken up by pheophytin are involved in hydroperoxide formation, whereas the second two moles are associated with some further degradative attack on the molecule.

Oxidation of chlorophyll treated with phosphoric acid

Phosphoric acid solution was added to the chlorophyll film in the ratio of one mole of acid per mole of pigment for the first run, and $\frac{1}{50}$ mole acid per mole of chlorophyll in the second run.

2.72 mg chlorophyll + 1 ml phosphoric acid solution (conc. 0.294 g/litre).

TABLE 14.

	Pressure ratio	Total oxygen (µg)	Hydroperoxide (µg)	$\frac{\text{Hydroperoxide}}{\text{total oxygen}}$	$\frac{H_2O}{\Delta p}$
Run 1	2.32	172.2	147.5	0.86	0.23
Run 2	1.72	159.0	132.0	0.83	0.10

The films were pumped for 24 hours over liquid oxygen to eliminate water from the phosphoric acid solution.

Both runs gave substantially the same ratios, which eliminates the possibility that any oxidation of the acid is occurring. This indicates that pretreatment of the film with phosphoric acid caused pheophytinisation, borne out by the olive green colour of the film, although the pressure ratios were rather low. It was unexpected that $\frac{1}{50}$ mole of phosphoric acid would have the same effect as 1 mole. Apparently a very small quantity of the acid is sufficient to cause pheophytinisation of the film, and it is not necessary to have a 1:1 molar ratio of acid to chlorophyll.

Spectra of oxidised pheophytin films

By comparison with the spectrum of unoxidised pheophytin, the maxima at 4300 and 6600°A had both diminished to approximately half their previous values. This spectrum remained the same for several

days, which suggested that the final product of oxidation was stable.

Oxidation of phytol

The oxidation of phytol was repeated on thallous bromide to make sure the results were substantially the same as for glass.

TABLE 15.

	Length of run	Total oxygen uptake (μg)	Hydroperoxide (μg)	$\frac{\text{Hydroperoxide}}{\text{Total oxygen}}$	$\frac{H_2O}{\Delta p}$
1.96 mg phytol on 0.1 g TlBr.	3 hours	246.0	116.0	0.47	0.9
		129.6	51.0	0.39	1.4
		334.0	122.0	0.37	0.7
0.95 mg phytol on 0.1 g TlBr.	7 hours	208.8	53.0	0.25	1.2
		107.2	26.5	0.25	0.6
	36 hours	280.0	Almost zero	0	0.8

Pressure ratios were approximately 0.6, and since the $\frac{H_2O}{\Delta p}$ ratio by phosphorus pentoxide treatment had an average value of approximately 1.0, the total oxygen uptake was double the quantity of water vapour given off.

For runs of length one day, the $\frac{\text{hydroperoxide}}{\text{total oxygen}}$ ratios were between 0.35 and 0.45, which is half the $\frac{\text{hydroperoxide}}{\text{apparent oxygen uptake}}$ ratio obtained originally on glass. This is to be expected, since the apparent oxygen uptake for phytol is approximately half the total

oxygen uptake. For long runs, however, this ratio decreases progressively with the length of the run, indicating that decomposition of the unstable hydroperoxide is constantly occurring.

Variation of $\frac{\text{hydroperoxide}}{\text{total oxygen}}$ ratio with length of run using films of copper chlorophyll on thallous bromide

Runs of length from 3 hours to 5 days were carried out on copper chlorophyll. This was chosen because due to its greater stability it was less likely to degrade to pheophytin during these long runs than natural chlorophyll.

TABLE 16.

Length of run (hours)	Total oxygen (µg)	Hydroperoxide (µg)	$\frac{\text{Hydroperoxide}}{\text{Total oxygen}}$	$\frac{H_2O}{\Delta p}$
3	63.0	43.5	1.0	0.27
20	108.2	32.5	0.30	1.30
22	186.4	44.0	0.24	1.20
5 days	191.0	28.0	0.13	0.90

Similar results were obtained for spinach chlorophyll, and confirm that the hydroperoxide formed in chlorophyll oxidations does decompose under the prevailing conditions.

All the above experiments have shown that a hydroperoxide is formed in chlorophyll oxidations, and that a probable position of attack is the phytol chain. However, if oxidation occurs only in

the phytol chain, it is difficult to see why two moles of oxygen are taken up per mole of chlorophyll, since approximately one mole of oxygen is taken up per mole of phytol.

The solution of this problem hinges on the photo-oxidation of ethyl chlorophide which is formed when phytol is hydrolysed off the chlorophyll molecule by the enzyme chlorophyllase in alcoholic solution. Sunners (31) reports a very small uptake of oxygen for films of this compound, which he states may be due to a slight uptake of oxygen by thallous bromide. However, the present worker found absolutely no oxygen uptake on illuminating films of thallous bromide in presence of oxygen. Sunners did not analyse for either water vapour or hydroperoxide. Now the preparation of ethyl chlorophyllide involves aqueous solutions, and since he only pumped the films for 2 hours using an oil rotary pump, it is more than probable that the film was so wet that it gave off considerable quantities of water vapour during illumination, making the apparent oxygen uptake very small.

Oxidation of ethyl chlorophyllide

The following runs were therefore performed on 1.62 mg ethyl chlorophyllide on 0.1 g thallous bromide, the film having been pumped overnight over liquid oxygen to make sure that it was thoroughly dry.

TABLE 17.

Length of run	Total oxygen (μg)	Hydroperoxide (μg)	$\frac{\text{Hydroperoxide}}{\text{Total oxygen}}$	$\frac{H_2O}{\Delta p}$
3-4 hours	133.0	120.0	0.90	0.23
	114.0	85.0	0.75	0.31
	98.6	78.0	0.79	0.46
	100.9	77.0	0.77	0.23
24 hours	263.7	56.0	0.21	0.51
36 hours	374.2	60.0	0.16	0.11

These results show that oxidation of ethyl chlorophyllide definitely does take place with the formation of a hydroperoxide. The ratio 0.8 for short runs is reasonable, and indicates that for each mole of oxygen taken up, one mole of hydroperoxide is formed in this part of the molecule. The ratios $\frac{\text{total oxygen}}{\text{No. of moles of chlorophyllide}}$ were slightly greater than one in most cases, probably due to slight degradation to pheophorbide. (The $\frac{\text{apparent oxygen}}{\text{chlorophyllide}}$ ratios were not calculated because due to the dryness of the film the $\frac{H_2O}{\Delta p}$ ratios were very low, indicating that considerable adsorption of water vapour by the film had occurred.)

In order to confirm that hydroperoxide formation does in fact occur in the main bulk of the chlorophyll molecule as well as in the phytol chain, pheophorbide was oxidised in the same way. This is formed either by the action of dilute acids on ethyl chlorophyllide or strong acids on chlorophyll itself with the elimination of magnesium

and removal of the phytol chain.

1.65 mg pheophorbide on 0.1 g thallous bromide.

TABLE 18.

Length of run	Total oxygen (μg)	Hydroperoxide (μg)	$\frac{\text{Hydroperoxide}}{\text{Total oxygen}}$	$\frac{H_2O}{\Delta p}$
6 hours	179.2	82	0.46	0.28
	171.7	92	0.54	0.11
24 hours	159.6	39	0.24	0.16
36 hours	131.7	15	0.11	0.58

These values fit in very well with those obtained for pheophytin when the run was allowed to go almost to completion. The pressure ratio obtained for pheophorbide was approximately 2.0, as would be predicted from the fact that the same ratio for pheophytin is 3.0. The presence of a hydroperoxide in both ethyl chlorophyllide and pheophorbide oxidation confirms that there is a point of attack in the main bulk of the chlorophyll molecule as well as in the phytol chain.

It remained unknown which was the actual position in the molecule where oxidation occurred. There are two particularly susceptible points: the two "extra" hydrogen atoms in ring IV, and the lone hydrogen atom on C_{10}. The hydrogenated bond in ring IV is responsible for the characteristic absorption peak at 6600°A, so its behaviour can be followed by comparing the spectra of the oxidised product with that of pure chlorophyll. Now the spectrum of a film which had been

oxidised almost to completion showed that the peak height at 4300°A had been reduced to 0.17 times its previous value, while that at 6600°A was diminished 0.19 times. Other measurements on the spectra of films bleached to varying degrees showed that the maxima in the blue and the red were always reduced by approximately the same amount, and in no case did the red maximum disappear completely (fig. 20). Now in the photo-oxidation of zinc tetraphenyl chlorin to the corresponding phorbin where the hydrogen atoms on ring IV are known to be removed (21), there is a complete disappearance of the peak at 6212°A, and the appearance of a new peak in the blue indicating the appearance of a phorbin stricture. It therefore seems likely that in this type of chlorophyll oxidation the diminished maxima are due to the rupture of the conjugated ring system rather than to the removal of hydrogen atoms at positions 7 and 8. In any case, oxidation in positions 7 and 8 would not explain hydroperoxide formation.

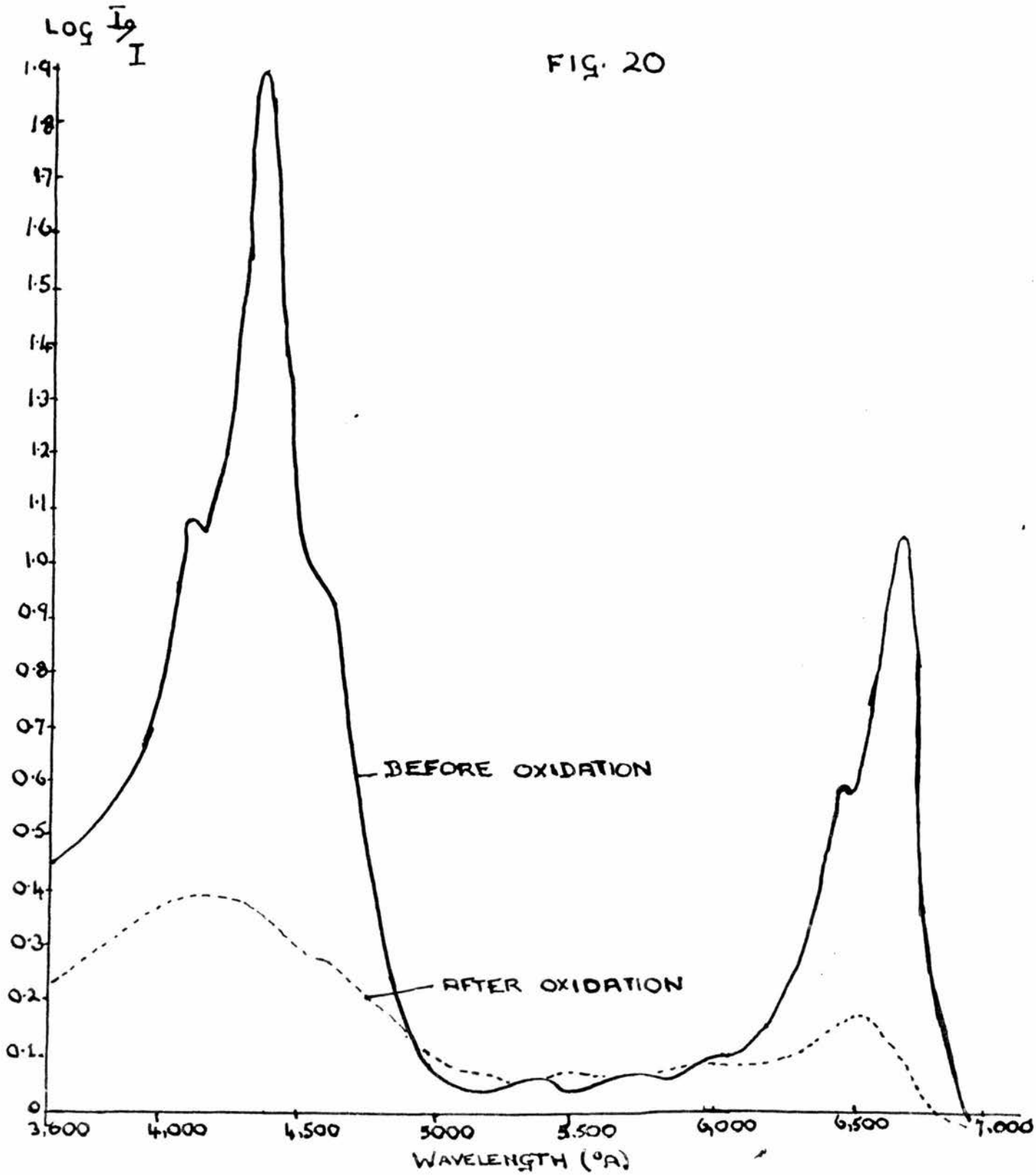
The more likely possibility is the hydrogen atom on the tertiary carbon atom C_{10}. This must be characterised by great lability since a carbonyl and a carbmethoxyl group are located on either side of it, and it is this property that is responsible for allomerisation and the phase test. Now if the hydroperoxide is formed in the isocyclic ring, then oxidation of chlorophyll derivatives containing no isocyclic ring should give no hydroperoxide.

By treating chlorophyll with concentrated alcoholic alkali magnesium was eliminated, the esterifying phytol group was split off and the isocyclic ring was ruptured, giving chlorin e. A film of 1.74 mg chlorin e on 0.1 g thallous bromide was illuminated with

SPECTRA OF CHLOROPHYLL IN ETHER SOLUTION

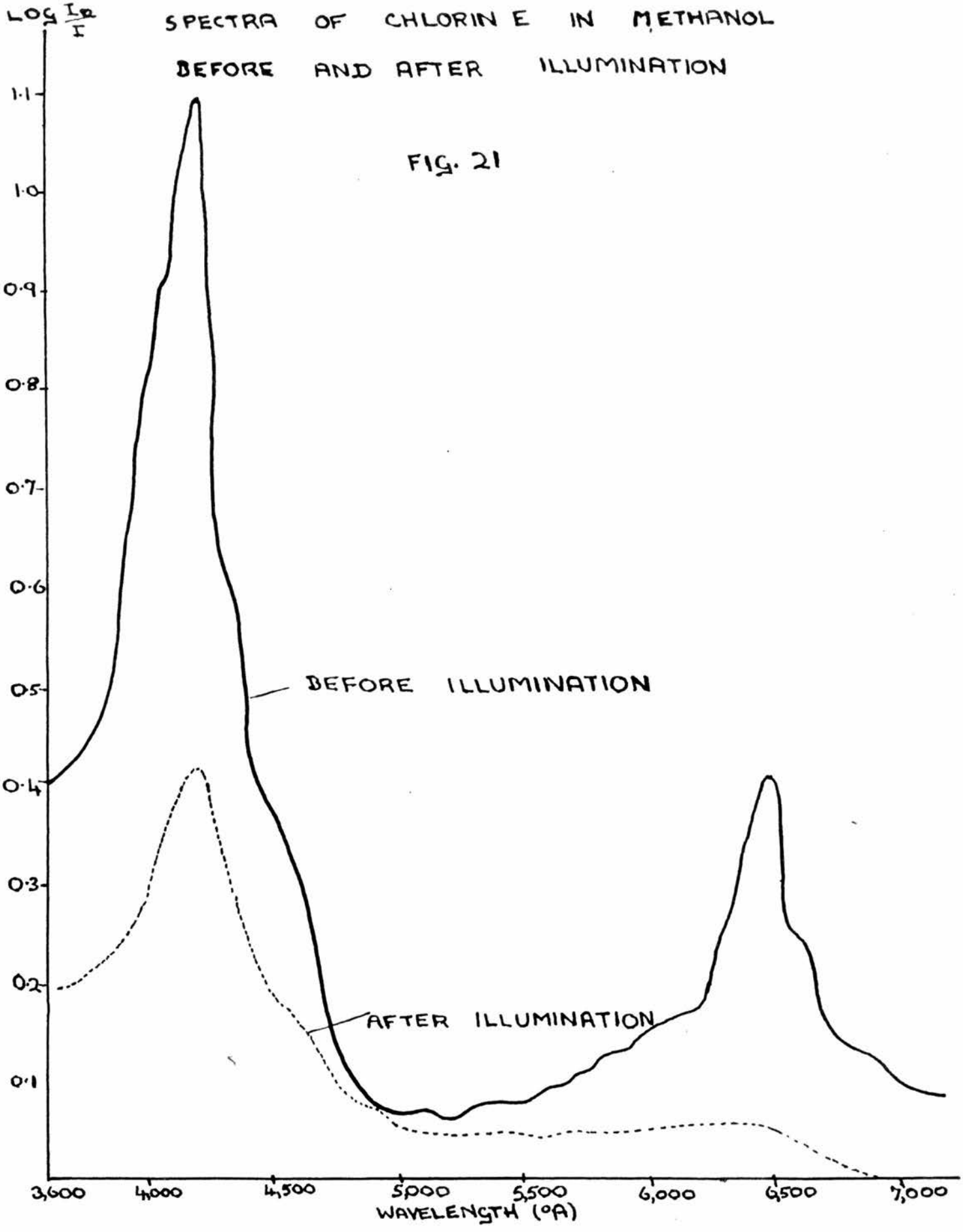
BEFORE AND AFTER OXIDATION

FIG. 20



50 mm oxygen for 24 hours. No movement whatsoever of the gauge pointer was observed, and a methanolic extracted of the film after illumination gave no hydroperoxide reaction whatsoever. However, the film was considerably bleached, and the thallous bromide had become grey in colour indicating that free thallium had been liberated. (This had never happened in any previous oxidation - the thallous bromide had never changed visibly before.) Comparison of the spectra of the oxidised and unoxidised products showed that the peak at 6400°A had completely disappeared whereas that at 4200°A, although diminished, still remained (fig. 21).

Repetition of the above experiment gave the same results. In neither case were any condensable gases isolated. Since there is no uptake of oxygen and no water vapour is liberated it seems likely that the thallous bromide itself was responsible for bleaching the chlorin. Illumination of films of chlorin e in absence of thallous bromide both in vacuo and in 50 mm oxygen for 6 hours produced no bleaching of the film, its spectrum remaining unchanged before and after illumination. Nor was there any oxygen uptake, hydroperoxide formation or production of water vapour. On the other hand, illumination of a film of chlorin e on 0.1 g thallous bromide for 6 hours in vacuo produced marked bleaching of the film together with a reduction in the maximum at 6400°A by half. It therefore seems likely that the bromide ion from the thallous bromide has removed the labile hydrogen, liberating free thallium with consequent greying of the substrate. This is an analagous reaction to the latent image effect exhibited by silver bromide.


LOG $\frac{I_0}{I}$
SPECTRA OF CHLORIN E IN METHANOL
BEFORE AND AFTER ILLUMINATION
FIG. 21
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
BEFORE ILLUMINATION
AFTER ILLUMINATION
3,600
4,000
4,500
5,000
5,500
6,000
6,500
7,000
WAVELENGTH (°A)

The important point, however, is that when there is no isocyclic ring in the molecule, there is no uptake of oxygen and no hydroperoxide formation. This proves that photo-oxidation is definitely a function of the isocyclic ring of the chlorophyll molecule. It may also be noted that the extra two moles of oxygen taken up by pheophytin cannot be associated with the -NH groups since chlorin e would have taken up two moles of oxygen if this had been the case.

There was a possibility that the product of photo-oxidation was identical with that obtained as a result of allomerisation. 10 ml ethanolic solution of chlorophyll, concentration 1 mg/ml, were allomerised by bubbling air through the solution until the phase test was negative. The allomerised chlorophyll was still green in colour and its spectrum closely resembled that of the original chlorophyll. There was no hydroperoxide reaction, so that the product of photo-oxidation cannot be a chlorin lactone, which is known to be the final product of allomerisation (7). The allomerised chlorophyll could be oxidised in a similar manner to normal chlorophyll, and this time the final product did give a hydroperoxide reaction. These experiments therefore proved that allomerisation and photo-oxidation are not identical types of reaction and give different final products, even though they do appear to occur at the same position in the molecule.

Decomposition of the hydroperoxide

It still remained necessary to establish conclusively that at least some of the water vapour produced during the reaction was a

result of decomposition of the hydroperoxide. After illumination was ceased all the water vapour was removed from the reaction space and the side-arm containing P_2O_5 was shut off. The oxidised film was left overnight, taps T_2 and T_6 remaining closed. The following morning the gauge pointer had risen 6 S.D. from the steady position maintained the previous night. On admitting the absorbant P_2O_5 into contact with the reaction space, the pointer returned to its position of the night before. The low $\frac{\text{hydroperoxide}}{\text{total oxygen}}$ ratios obtained (0.3 - 0.4) were additional evidence that the hydroperoxide had decomposed during the night giving off water vapour. Removal of water vapour from the reaction space appears to disturb the equilibrium

$$\text{Chl. OOH} \rightleftharpoons H_2O + \text{decomposed hydroperoxide},$$

so that more water vapour must be formed by decomposition of the hydroperoxide.

However, if the film was left overnight in equilibrium with its water vapour so that the reaction products were undisturbed, the gauge pointer remained steady and the $\frac{\text{hydroperoxide}}{\text{total oxygen}}$ ratios were the same as for previous runs. Exactly the same results were obtained for pheophytin and phytol.

This experiment also explains why the $\frac{\text{hydroperoxide}}{\text{total oxygen}}$ ratios were always low in runs where P_2O_5 removed water vapour from the reaction space continually during the reaction so that the hydroperoxide must have been decomposed almost as it was formed. There may also be some decomposition merely on estimating the water vapour present at the end of a run. However, as this was always done quickly (within 1 hour) and the hydroperoxide was always estimated immediately afterwards, this should not be serious.

Experiments on oxidised films

The collected methanolic extracts of many runs were evaporated down until the total volume was between 10 and 20 ml and poured onto a sucrose column. Petroleum ether, B.P. below 40°C, was poured down the column and eventually a pale yellow solution was eluted. This was evaporated to dryness at the water pump, dissolved in methanol and found to give a faint hydroperoxide reaction with ferrous thiocyanate. Further elution of the column with ether carried down the main green portion of the pigment. This was also evaporated to dryness, dissolved in methanol, and found to give a positive hydroperoxide reaction. The fact that two main fractions came off the column suggested that the phytol chain had split off the molecule either during the reaction, or on the column or while the oxidised samples had been stored in methanolic solution.

A second set of collected methanolic extracts were evaporated down almost to dryness at the water pump. A yellow oil and a green waxy microcrystalline deposit separated out. The two were separated by filtration and the filtrate was washed cautiously with methanol. Both the green product and the yellow oil gave a strong peroxidic reaction. However, after leaving these two products for two or three days, neither gave any reaction with the reagent, showing the instability of the hydroperoxide group, particularly when no longer stored in solution. The yellow oil decolourised a dilute solution of bromine water, showing the presence of an unsaturated grouping as found in phytol. It can therefore be concluded that the methanolic

solution contained phytol hydroperoxide and methyl chlorophyllide hydroperoxide (probably along with pheophorbide hydroperoxide and other degradation products). It was uncertain whether the split occurred during oxidation or on storing in solution.

After one long run the oxidised film was extracted in quinoline and transferred to a corked tube from which a delivery tube dipped into a solution of lime water. The quinoline solution was boiled for several minutes and the issuing gases were allowed to bubble through the lime water. This did not turn milky, indicating that no carbon dioxide was being given off. It was therefore concluded that no free carboxyl group was present in the actual oxidised film (48). Thus the phytol group must split off on prolonged standing in methanolic solution.

Bleaching of chlorophyll in vacuo

2.32 mg chlorophyll on 0.1 g thallous bromide were illuminated in vacuo for seven hours. There was no movement of the gauge pointer and no water vapour given off. The spectra of equal concentrations of the chlorophyll extracted in ether before and after illumination were determined. There was a marked change in the spectrum of the illuminated sample. The maxima at 4300 and 6600°A had been reduced to $\frac{2}{3}$ of their original value, and there was a shift of the peak at 4300 to 4100°A, along with new small maxima at 5050, 5300, 5650 and 6200°A, all indicating that pheophytinisation of the film had occurred. The fact that considerable changes in the spectrum of chlorophyll can

occur on illumination in vacuo are an indication that bleaching is as likely to be due to pheophytinisation as to oxidation.

It was thought that thallous bromide might have a tendency to precipitate this degradative process, so several runs were carried out with chlorophyll deposited as a film directly on the walls of the glass vessel. However, such runs were so slow (only 4-5 S.D. being taken up in seven hours) that the idea was abandoned.

Oxidation of chlorophyll at different wavelengths

In order to find out to what extent each wavelength of light in the mercury spectrum was responsible for catalysing the oxidation, the 3650 line was isolated using a chance OX1 filter and the 4360 and 4050 lines together using a chance OB10 filter.

1.75 mg chlorophyll on 0.1 g thallous bromide.

TABLE 19.

Filter	Total oxygen (µg)	Hydroperoxide (µg)	$\frac{\text{Hydroperoxide}}{\text{Total oxygen}}$	Rate of oxidation
OB10	42	37.5	0.88	1 S.D./hour
OX1	89	34.0	0.38	2 S.D./hour

It can be seen from these results that whereas oxidation proceeds at twice the speed using the OX1 filter, the $\frac{\text{hydroperoxide}}{\text{total oxygen}}$ ratio is less than half that obtained using the OB10 filter. This suggests that it is the near ultra violet light in the mercury vapour spectrum

that is responsible for decomposition of the hydroperoxide. Since in both cases the rate of oxidation was very slow due to the diminished light intensity, there would have been little value in carrying out a series of runs using the OB10 filter.

The same experiment was repeated on phytol.

1.67 mg phytol on 0.1 g thallous bromide.

TABLE 20.

Filter	Total oxygen (μg)	Hydroperoxide (μg)	Hydroperoxide / Total oxygen	Rate of oxidation
OB10	78.9	1.8	0.23	2 S.D./hour
OX1	53.5	2.5	0.05	1.5 S.D./hour

This experiment provided further evidence that light of wavelength 3650°A decomposes the hydroperoxide formed during oxidation.

It is at first surprising that although chlorophyll absorbs more strongly in the blue (i.e. in the region 4050 - 4360°A) than in the near ultra violet, the reaction actually proceeded at double the rate when light of wavelength 3650°A was used. This could be due either to the fact that decomposition of the hydroperoxide caused an increased rate of oxidation, or may be attributed to the function played by the sensitiser in these oxidations. Thallous bromide absorbs more strongly at 3650°A than 4050 - 4360°A and would therefore be expected to sensitise more effectively at this wavelength. The necessity for a sensitiser was proved by the very slow rate of reaction for films deposited directly on the glass walls of the reaction vessel.

Analysis of peroxides in oxidised films by paper chromatography (42).

Taylor (46) has shown that hydrogen peroxide can be separated from other peroxides on untreated paper, using ether as the moving phase. The R.F. value for hydrogen peroxide is 0.14, and for other hydroperoxides is of the order 1.0. No hydrogen peroxide was detected on spraying the paper with ferrous thiocyanate solution - there was only a faint peroxide in the main green bulk of the material which had moved down the paper with the solvent front.

Paper treated with ethylene glycol in acetone solution along with butanol and petroleum ether as eluting solvents is more suitable for separating higher peroxides. Only very faint peroxide reactions could be detected, and these always occurred in the green component of the material. No colourless peroxide separated out. Cartlidge and Tipper (42) found that some peroxides decomposed on the paper, while other very stable peroxides were not detected. These experiments do show the presence of a hydroperoxide in the nucleus of the chlorophyll molecule although it seems likely that this underwent considerable decomposition on the paper. Phytol peroxide was not isolated, either because the phytol chain remained attached to the chlorophyll molecule or because it had decomposed on the paper.

Further proof that no low molecular weight hydroperoxide was formed is that no peroxide reaction could be detected in aqueous extracts of the film, or even in 50:50 H_2O : methanol and 25:75 H_2O : methanol solvents. The hydroperoxide could only be extracted in solvents containing more than 80 per cent methanol which points to the presence of a high molecular weight hydroperoxide.

Infra-red spectra of chlorophyll and its oxidation product

The characteristic bands shown by unoxidised chlorophyll were as follows:

(1) An intense absorption band in the region of characteristic frequency of -OH bonds at 3400 cm^{-1}.

(2) Bands at 1740 cm^{-1} and 1701 cm^{-1}. These are interpreted by Sidorovand Terenin (47) as due to the vibrations of the ether groups C = O at atoms C_7 and C_{10}, and to the vibrations of the keto group of the cyclopentanone ring respectively.

(3) A band at 1611 cm^{-1} which is the region of characteristic frequency of C=C bonds and interpreted by the same authors as caused by the C=C double bond present in the enolic form of the cyclopentanone ring.

(4) The band at 1665 cm^{-1}, according to Holt and Jacobs (49) corresponds to the vibrations of the carbonyl C=O group at C_{10} being disturbed by the intermolecular hydrogen bonds formed with the OH group found in the enol form of the cyclopentanone ring.

The following changes were observed in the spectrum of oxidised chlorophyll:

(1) Disappearance of the band at 1665 cm^{-1} and a much diminished band at 1611 cm^{-1}, together with a slight enhancement of the band at 1701 cm^{-1}.

(2) A very weak band at 992 cm^{-1} could be interpreted as due to an aryl peroxide (50) which show characteristic absorptions near

1000 cm^{-1}. This weak band disappeared completely in films which had been subjected to prolonged oxidation.

(3) The spectrum of a film oxidised for 24 hours became extremely diffuse in the region 1500 to 1750°A indicating profound changes in the structure of the molecule in the region of the cyclopentanone ring. Complete absence of bands at 1611 and 1665 cm^{-1} showed that none of the chlorophyll was present in the enol form. Broadening of the bands at 1701 and 1740 was due to changes in both the cyclopentanone ring and the side-chain attached at C_7.

The absence of an absorption band at 1665 cm^{-1} was regarded as due to disappearance of the enol form of chlorophyll on oxidation. If oxidation occurs at C_{10} to form a HOOC group, then the H atom at C_{10} will no longer be available to form intramolecular hydrogen bonds with the C=O group of the cyclopentanone ring, so that the enol form will gradually disappear. Slight enhancement of the peak at 1701 cm^{-1} corresponds to vibration of the carbonyl group in the cyclopentanone ring freed from bonding with the hydrogen at C_{10}.

Sheppard (51) has pointed out that peroxides of the type R - O - O - R' will have no characteristic bands for the - O - O- vibrations, as this mode would be relatively symmetrical and therefore not associated with much change in dipole moment so that the corresponding frequency would be expected to be weak in the infra red. Furthermore, the masses and force constants of the - O - O-group are so similar to those of the C - O and C - C groups that it is unlikely that any very characteristic frequency will result if these groups are present together in the same molecule. It is therefore not

surprising that the hydroperoxide group only shows up weakly in the spectrum of oxidised chlorophyll.

Oxidation of chlorophyll in an atmosphere of nitric oxide

One further run was performed using 50 mm nitric oxide in the reaction space instead of 50 mm oxygen. The chlorophyll film was illuminated for 6 hours, but no uptake of nitric oxide was observed, nor any release of water vapour or peroxide formation. It was therefore concluded that absolutely no reaction had taken place.

Summary of results for films deposited on thallous bromide

TABLE 21.

Compound used	Approximate pressure ratio O_2/Me	Average $\frac{\text{hydroperoxide}}{\text{total oxygen}}$ ratio for short runs (1 day)
Chlorophyll	1.0 - 1.2	0.8
Copper chlorophyll	1.0	0.95
Pheophytin	3.0 - 3.5	0.35
Phytol	0.6	0.4
Ethyl chlorophyllide	Not calculated (see results)	0.8
Pheophorbide	2.0	0.5
Chlorin e	0	0

Discussion

The present work supports the conclusion reached by Sunners (31) that the total uptake of oxygen for fresh chlorophyll preparations is approximately 2 moles per mole of chlorophyll, and that the amount of water vapour given off during oxidation is approximately 1 mole, although this measurement is very difficult to assess accurately and is dependent on such factors as the extent of evacuation of the film prior to illumination and the length of the run. Because this measurement is so variable, the actual Δp readings recorded do not have any great significance, and the slope of the Δp/time curve can only be used as a rough guide to give a rate curve and an estimate of the total apparent oxygen uptake at zero rate. This being the case, it is difficult to derive a kinetic expression for the reaction.

The difficulties which beset work of this type are almost insuperable, since chlorophyll itself begins to degrade immediately after it is extracted from the plant, even if the most careful precautions are taken and it is stored in solution in the dark at $-20^{\circ}C$. It has been reported (52) that purified preparations kept at this temperature are completely stable for only about a week before deterioration is evident in their spectra. A further complication is that so-called "dry" preparations of chlorophyll still contain 5 per cent moisture, and that 5 per cent of the chlorophyll deteriorates to pheophytin during drying (53).

That chlorophyll forms monosolvates with nucleophilic reagents has recently been confirmed by infra red spectroscopy of solid films

of the substance (47). In the spectra of these solid films there is a broad absorption band at 3400 cm^{-1} in the region of characteristic frequency of vibration of -OH bonds. This band is asymmetrical and similar in structure to the absorption band of liquid water, and can therefore be interpreted as due to the presence of water molecules linked to the chlorophyll molecule. This band was in fact observed in the spectra of both unoxidised and oxidised films in the present work(R69). The intensity of the band increases on lowering the temperature and has been observed in the spectra of pheophytin solutions below 8°C, although it diminishes rapidly on raising the temperature. It has been established that, in general, the water molecules are linked to chlorophyll at the central magnesium atom (26), although attachment can also occur to the two nitrogen atoms on the pyrrole nucleus. The metal atoms appear to activate the pigment with respect to the formation of complexes with water, and increases the stability of these complexes.

Thus one probable source of water vapour during illumination is the release of this complexed water as the reaction proceeds. If the film was pumped thoroughly (i.e. overnight), no release of water vapour was observed on illumination in vacuo, but this does not mean that it would not be driven off during the more drastic oxidation process resulting in the final destruction of the chlorophyll molecule. It is impossible to say what extent of pumping would be necessary to ensure complete removal of complexed water: in all probability such treatment would be too drastic and would lead to deterioration of the film prior to oxidation.

That the water-metal complex is not the sole source of water vapour is shown by the fact that water is released in both phytol and pheophytin oxidations, and neither of these substances contains a co-ordinating metal atom. Water vapour is certainly produced by decomposition of the unstable hydroperoxide, and possibly also from the metal-pigment complex.

By correlating the total rather than the apparent oxygen uptake and the hydroperoxide produced in each run, this work claims to have diminished the errors incurred due to the uncertainty in estimating water vapour quantitatively. As has been previously explained, a large apparent oxygen uptake will be compensated for by a low water vapour estimation, and the measurement of total oxygen taken up will not be altered. Previous workers have laid too much stress on the apparent oxygen uptake and on the importance of the 'pressure ratio' derived from extrapolation of the rate curve to zero rate. For freshly extracted samples, ratios greater than unity point to high water vapour adsorption rather than to degradation of the material. It is far better to consider the total oxygen uptake.

It is unfortunate that no suitable absolute method could be found for determining the amount of peroxidic oxygen present. However, since the same calibration graph and an identical procedure were used for every run, this is an excellent comparative method of determining the relative amounts of assumed hydroperoxide present in different compounds in the chlorophyll family after runs of varying length. From time to time, the analysis was checked using the potassium iodide method, and the results obtained were found to agree with those

obtained by the ferrous thiocyanate method, although the high absorption due to unreacted chlorophyll made the method rather inaccurate.

The function of thallous bromide in these oxidations, with the one exception of its use with chlorin e, is almost certainly that of a sensitiser where the absorbing substance acts as a catalyst and does not enter into chemical reaction. It might have been thought that because chlorophyll itself absorbs strongly in the visible and the near ultra violet, no sensitiser should be necessary. However, interaction of excited pigment molecules with certain substances of which oxygen is one can lead to "quenching" of the triplet state by the formation of certain addition compounds (54). The triplet state can be said to be quenched when its mean life-time is shortened by the addition of an added substance. Thus the function of the sensitiser is to transfer energy to the metastable state in order to prolong its mean life and speed up the kinetics of the addition reaction with the quencher, in this case oxygen.

Thallous bromide is a photoconductor, and absorption of light in the blue and near ultra violet leads to the transfer of an electron into the conduction band of the crystal.

$$Br^{-} + h\nu \rightarrow Br + \epsilon$$

Thus the catalytic process involves either electron or energy transfer from the conducting band of thallous bromide to the long-lived metastable state of chlorophyll, at a slightly lower energy level (fig. 22). Due to its sensitising action, runs carried out on thallous bromide proceeded at a rate four or five times faster than

ELECTRON TRANSFER FROM THE CONDUCTIVITY LEVEL OF THALLOUS BROMIDE TO THE TRIPLET STATE OF CHLOROPHYLL

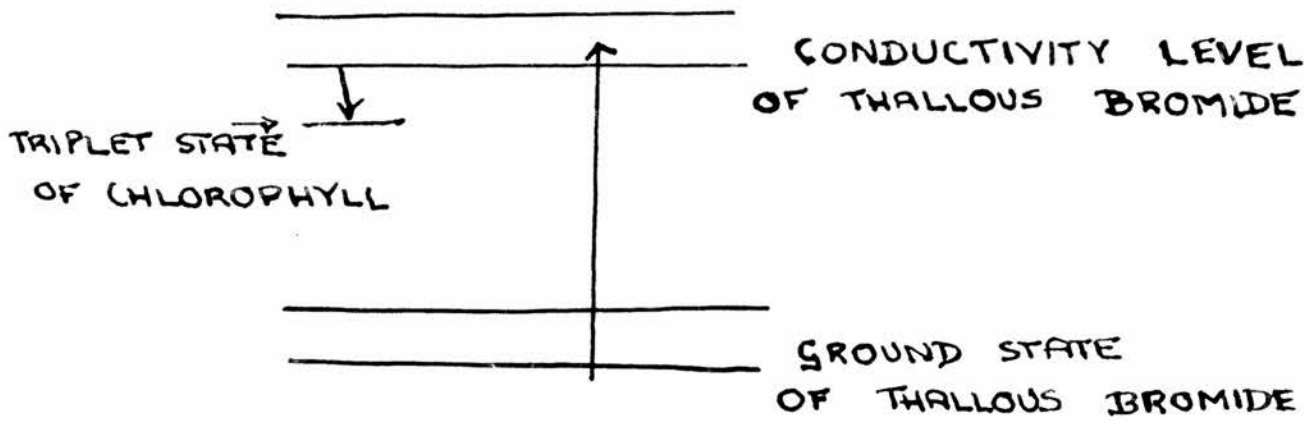


Fig. 22

those on powdered glass, which could only serve to increase the surface area of pigment exposed to oxygen.

It has been established that the main product of photo-oxidation is an unstable intermediate, probably a hydroperoxide, which decomposes on prolonged oxidation to give water vapour as one product. It has also been shown that there are two positions in the molecule where hydroperoxide formation occurs, namely, the phytol chain and the cyclopentanone ring. Although Sunners claimed that ethyl chlorophyllide only oxidised to a very small extent if at all, his paper chromatograms comparing the substance before and after illumination showed considerable change in the molecule after illumination. That both ethyl chlorophyllide and pheophorbide do form hydroperoxides has been conclusively proved in the present work.

A further proof that oxidation occurs in two positions can be deduced from consideration of the quantities of hydroperoxide produced in the various related compounds.

The pressure ratio for phytol was found to be approximately 0.6, and the total oxygen uptake was double the apparent uptake recorded by the pressure decrease Δp. The $\frac{\text{hydroperoxide}}{\text{total oxygen uptake}}$ ratio by weight was calculated to be approximately 0.4, but this also applies on a molecular basis since the hydroperoxide present was estimated as peroxidic oxygen, and 1 mole of oxygen forms 1 mole of hydroperoxide

$\therefore$ 1 mole of phytol takes up 1.2 moles of oxygen

to give $0.4 \times 1.2 = 0.5$ moles hydroperoxide.

For chlorophyll itself, the pressure ratio was approximately 1.0, the total oxygen uptake was double the apparent oxygen uptake, and the

$\frac{\text{hydroperoxide}}{\text{total oxygen ratio}}$ was approximately 0.8

$\therefore$ 1 mole of chlorophyll takes up 2 moles of oxygen

to give 0.8 x 2.0 = 1.6 moles hydroperoxide.

Since 1 mole of chlorophyll contains 1 mole of phytol, the quantities of oxygen taken up and hydroperoxide formed by chlorophyll indicate that oxidation must also be occurring in some position in the molecule other than the phytol chain.

One can therefore conclude that oxidation must occur in the main conjugated system of the molecule in approximately the same ratio as was found for chlorophyll itself. This has in fact been proved to be true - ethyl chlorophyllide gives $\frac{\text{hydroperoxide}}{\text{total oxygen}}$ ratios of approximately 0.8 as predicted from the above argument.

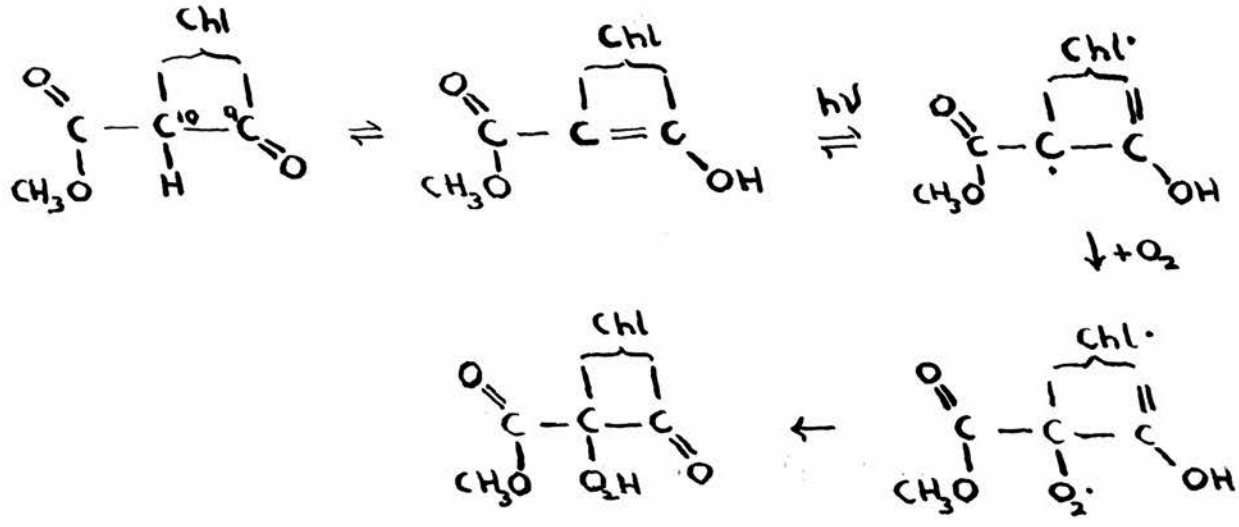
The point of attack in ethyl chlorophyllide has been shown to be the labile hydrogen atom on C_{10}. Had any attack occurred at the hydrogen atoms on ring IV, the spectrum of the oxidised product would have resembled that of protochlorophyll, which shows strong absorption in the blue and practically none in the red (55). It has already been emphasised that in the spectra of oxidised films the maxima at 4300 and 6600^{o}A were diminished in equal ratio, so this position is eliminated.

The keto-enol couple formed by the hydrogen at C_{10} and the neighbouring carbonyl group has been shown to be responsible for many of the characteristic reactions of chlorophyll, particularly oxidations. Franck and Livingston have suggested that electronic excitation energy in a dye molecule could go over, by a process of internal conversion, to a reactive energy-rich tautomer, and that the keto-enol transformation is a possible example of such tautomeric change (56). As a consequence

the system of conjugated double bonds is interrupted affecting the resonating system. The properties of this tautomer are similar to those of the metastable electronic state, so that the tautomer may have a longer life than the original structure. Internal conversion leading to tautomer formation may profoundly influence chemical behaviour, and since the tautomeric shift involves a shift of a hydrogen atom, the tautomer will in some cases, such as chlorophyll, contain a very labile hydrogen atom. Thus collision with an oxygen molecule will result in the formation of an oxidised dye radical.

Further evidence of this type can be seen in the two diradical structures proposed by Weller (9) (fig. 3) where an electron is localised on C_{10} making this position one of potential high reactivity towards oxygen. Proof that this position was in fact essential for hydroperoxide formation was provided by failure to detect any such group after illuminating chlorin e in oxygen, this porphyrin containing no isocyclic ring.

Enolisation occurs readily at C_9 and C_{10}, and 20-30 per cent of chlorophyll molecules are estimated to exist in the enol form (47). On illumination the diradical formed has one of its uncoupled electrons localised at C_{10} while the other is located somewhere in the conjugated system of the molecule. Oxygen attack occurs at this position, and the final irreversible product is formed when the labile hydrogen atom returns from its enolized position to form a hydro-peroxide:



This mechanism is supported by the infra red spectrum of the oxidised product which shows disappearance of bands due to - C = O...H - bonds and - C = C - bonds, the inference being that some of the enol form has reacted. It is interesting that this was the original mechanism proposed by Fischer (5) to account for the allomerisation reaction, but was subsequently rejected by him in favour of the chlorin lactone theory (7). It is certainly the most likely explanation of the oxidation under discussion, particularly as biradical formation is likely to occur in a photosensitised reaction.

The oxidation of phytol can also be considered to occur at the unsaturated linkage. The behaviour of both chlorophyll and phytol on photo-oxidation display many of the features of the low temperature liquid phase oxidation of olefins summarised by Bolland (57) and Bateman (58). It has been found on investigating the primary products obtained by reacting oxygen with various types of unsaturated hydrocarbon that the oxygen combines in unseparated pairs of atoms to form hydroperoxides in the case of mono-olefines. Small quantities of

non-peroxidic products are also produced, particularly in the advanced stages of the reaction. These may be aldehydes, ketones, epoxides, acids, water and carbon dioxide, and are associated with the final destruction of the molecule. The decomposition of the primary oxidation product, i.e. the hydroperoxide, serves to complicate the kinetics of the reaction. However, under mild conditions the fraction of hydroperoxide decomposing is small, and thus the overall yield of hydroperoxide is nearly quantitative in the early stages of the reaction. A feature of these oxidations is that they can be photochemically initiated.

These liquid phase oxidations are believed to occur by a chain mechanism occurring in three stages: initiation, propagation and termination. The essential role of the initiation stage is to provide reactive intermediates which are capable of setting in motion a whole series of reactions. Termination of the chain occurs when the carriers are converted into inactive species. The following reaction scheme represents this chain mechanism. RH is an olefin with an α-methylenic hydrogen atom.

<u>Initiation</u>: Production of $R\cdot$ or $RO_2\cdot$ radicals.

<u>Propagation</u>:

$$R\cdot + O_2 \rightarrow RO_2\cdot$$

$$RO_2\cdot + RH \rightarrow RO_2H + R\cdot$$

<u>Termination</u>:

$$2R\cdot \rightarrow$$
$$R\cdot + RO_2\cdot \rightarrow \quad \text{Non-propagating products}$$
$$2RO_2\cdot \rightarrow$$

Such a scheme is characterised by:

(i) High yields of hydroperoxide.

(ii) Catalysis by light.

(iii) Ease of rupture of the C - H bond in RH.

(iv) Quantum yields greater than unity.

Many of these characteristics are exhibited by the photo-oxidation of chlorophyll, e.g. high yields of hydroperoxide, photochemical initiation, and the known lability of the tertiary hydrogen atom on C_{10}. The quantum yield is rather low, a value of the order 0.6 having been determined by Lonie (59), which indicates that the reaction is not very efficient. The important points of similarity with the liquid phase autoxidation of olefins are that both types of oxidation are light catalysed and produce nearly quantitative yields of hydroperoxide in their early stages.

The parallel initiation stage for chlorophyll can be represented as follows:

$$Chl + h\nu \rightarrow Chl^{*} \quad \text{(Excited singlet state)}$$

$$Chl^{*} \xrightarrow[90\%]{} Chl^{***} \quad \text{triplet state}$$

The biradical formed in the metastable state can then react with oxygen to give a chlorophyll peroxy radical:

Propagation: $Chl\cdot + O_2 \rightarrow ChlOO\cdot$

$$ChlOO\cdot + ChlH \rightarrow ChlOOH + Chl\cdot$$

The biradical is thus regenerated.

The quantum efficiency is low because much of the light energy is wasted without causing reaction, losses being due to fluorescence,

or the quenching of the triplet state by oxygen, which would terminate the chain by formation of an unreactive species.

It is known that exchange of energy between a large number of chlorophyll molecules is physically possible (60) when the molecules are present in close proximity in concentrated solution or in the crystal. The energy quantum may change its location several times before being utilised in chemical reaction, i.e. the electronic energy of the metastable state may be exchanged between adjacent molecules. If contact with another chlorophyll molecule in the film is possible, the solid state oxidation of chlorophyll would constitute an analagous case to the chain reactions discussed in the liquid phase autoxidation of olefins. Such a mechanism would also account for the rapid rate at the beginning of runs. It was actually recorded by Bateman, Hughes and Morris (61) that autoxidation of phytene gave between 46 per cent and 50 per cent of the total oxygen taken up as peroxidic oxygen in 17 hour runs. This is in good agreement with the values quoted above for the photo-oxidation of phytol.

Another feature of the kinetics of olefin oxidation which is of interest is the mechanism proposed by these authors for the decomposition of the hydroperoxide. They consider that the hydroperoxide formed in the initial stages of the reaction subsequently decomposes bimolecularly giving off water and further chain propagating species:

$$RO\cdot\overset{H}{O}\cdots\cdots HO_2R \longrightarrow RO\cdot + H_2O + RO_2\cdot$$

Propagation would then yield products of an alcoholic type:

$$RO\cdot + RH \longrightarrow ROH + R\cdot$$

It was shown that the chief decomposition product of cyclohexanyl hydroperoxide were cyclohexanol and water. That water is a decomposition product of chlorophyll and phytol hydroperoxides has been proved, so that this particular photo-oxidation can be seen to fit into the much more general picture of the interaction of olefins with molecular oxygen. In both types of reaction, the concentration of hydroperoxide in the early stages is proportional to oxygen uptake, but as the rate of uptake of oxygen falls off, there is a decrease in the overall hydroperoxide yield becoming increasingly appreciable in the later stages. The chief decomposition products for olefins are an alcohol and water in olefins, so it can be expected that a C_{10}-OH grouping is eventually formed at C_{10} in the cyclopentanone ring.

One significant feature of the oxidised film was that for short runs where the $\frac{\text{hydroperoxide}}{\text{total oxygen}}$ ratios were near unity, the film still remained green in colour. As the film bleached, this ratio fell, so that for almost complete bleaching the ratio had fallen to approximately 0.25. A further important point was that for freshly extracted chlorophyll, the film still remained green even when the rate of oxygen uptake had fallen almost to zero. This indicates that hydroperoxide formation does not of itself cause bleaching, and it is indeed unlikely that the addition of a peroxide group to C_{10} would cause such change in the conjugation of the ring system that a drastic colour change should result.

The most likely explanation for the eventual bleaching of the film is that as the hydroperoxide decomposes in the later stages of reaction, oxygen uptake proceeds gradually beyond 2 moles due to

disruption of the conjugated system together with progressive pheophytinisation of the film due to prolonged exposure to light and oxygen. In fresh chlorophyll preparations, this rupture and deterioration is slow due to the presence of the co-ordinating magnesium atom. Copper chlorophyll retains its intact structure even more readily. However, although in these cases the reaction appears to reach almost zero rate after the uptake of approximately 2 moles of oxygen, it was always found that if the film was left under illumination for several days, a continual slow uptake of oxygen did occur, while the film changed progressively to olive green, pale yellow and finally almost colourless. The absorption spectrum of the oxidised film showed progressively decreased absorption in the red and the blue and relative increased absorption in the green, resembling the spectrum of pheophytin.

In pheophytin itself where no co-ordinating atom is present, it is likely that rupture of the conjugated ring system can occur much more easily, so that the rate of oxygen uptake does not fall almost to zero when the hydroperoxide is formed. Instead, there is a continual break-down of the molecule followed by oxidation of the degradation products.

As the hydroperoxide at C_{10} decomposed, it is likely that attack by oxygen at the methine bridge linked to C_{10} would occur. This would account for the small quantities of carbon dioxide found after long runs on chlorophyll and pheophytin. Once the ring structure was broken, further oxidation could easily occur. It is unlikely that the uptake of two extra moles of oxygen by pheophytin is

associated with the pyrrole N-H groups, because no oxidation of chlorin e was observed.

Pheophytinisation of chlorophyll films has been shown to occur after illumination in vacuo, with a decrease of two thirds in the peak at 6600°A. It therefore very probably is that pheophytinisation is an essential stage in complete bleaching. The spectrum of the final oxidised product of pheophytin bore a close resemblance to that obtained when chlorophyll was oxidised over a period of several days, which is further evidence that the final bleached material is the same in both cases, the most important feature being that the red and blue maxima were always diminished by approximately the same amount. Krasnovskii has also reported that the photo-oxidation of chlorophyll proceeds via a primary labile substance to a final more stable product with destruction of the conjugated bond system (62). It is impossible to say what this substance is without undergoing an exhaustive scheme of analysis, possibly by paper chromatography since the quantities of material involved are so small. It is, however, questionable whether there would be any great value in carrying out this work, since the degradation products of this reaction probably vary considerably with the prevailing conditions, and if illumination were sufficiently prolonged a large variety of products would probably result. The bile pigments have an open-chain linked tetra-pyrrole structure, and are possibly oxidation products of the porphyrins.

It seemed rather strange that although films of chlorin e on thallous bromide took up no oxygen, there was marked bleaching of the film after illumination and a considerable depression of the maximum

at 6400°A. Since no bleaching or change in the spectrum was observed when the film was deposited on the glass walls of the reaction vessel, while illumination on thallous bromide in vacuo did cause bleaching, it was apparent that thallous bromide rather than oxygen had reacted with the chlorin e. Disappearance of the red maximum indicated transformation from the chlorin to the porphin structure by removal of the hydrogen atoms on C_7 and C_8 in nucleus IV to give a completely unsaturated structure. This could be achieved by free bromine released by the reaction:

$$Br' + h\nu \rightarrow Br + \epsilon$$

Hydrogen bromide would be formed with the release of thallium. The thallous bromide was in fact grey in colour after illumination showing the presence of free thallium.

One point that caused considerable confusion among earlier workers was the increased rate of oxygen uptake observed when the system was evacuated in the middle of a run, and then oxygen was readmitted and illumination recommenced (29). In particular, the total oxygen uptake in such interrupted runs rose to values considerably greater than those predicted on the unit molar ratio basis. The same effect was observed when a period of darkness intervened during a run, as was shown in the present work on glass when one run was continued over two consecutive days without, however, illuminating the sample overnight. It was originally thought that these effects were due to the reversibility of the oxidation so that during a period of darkness or on evacuation the original chlorophyll was regenerated (29). However, the starting material was never in fact regained after these attempts

at reversing the reaction and the above experiments have shown that it was water vapour and not oxygen that left the oxidised film on standing overnight.

The obvious explanation is that removal of water vapour, either by evacuation or admission into contact with an absorbent, causes a displacement to the right in the equilibrium:

$$Chl{\cdot}OOH \rightleftharpoons \tfrac{1}{2}H_2O + \text{decomposed hydroperoxide}$$

The result is that further decomposition of the hydroperoxide occurs to replace the water vapour removed causing degradation of the hydroperoxide to a decomposition product which oxidises more readily than chlorophyll itself. The net result is an increased rate of reaction on readmission of oxygen to the system or on recommencement of illumination after a period of darkness. This explanation also accounts for the very large uptake of oxygen in runs where the reactants were in constant contact with phosphorus pentoxide so that constant removal of water vapour during the course of the reaction caused continuous decomposition of the hydroperoxide resulting in a very low final yield of the substance. This process promoted degradation of the film with a consequent much increased uptake of oxygen.

The present work claims to have determined the two points where oxygen attacks the chlorophyll molecule and to have accounted to some extent for the observations made by other workers on the substance. It is also hoped that this work has cleared up the confusion resulting from always considering the apparent rather than the actual oxygen uptake. The theory that this reaction proceeds through the formation

of a triplet state diradical stabilised by resonance between several structures is in agreement with the mechanisms discussed in the introduction explaining the phase test, allomerisation, anaeorobic bleaching and reversible oxidation. In all these cases, and in the present irreversible oxidation, the position of attack appears to be the cyclopentanone ring which is capable of undergoing tautomeric transformation at C_9 and C_{10} between an enol and a keto structure. The study of this particular oxidation has therefore been of value because it has contributed one further example to a set of reactions undergone by chlorophyll which at first sight appear to be unrelated. It is difficult to see how this project can be continued any further without the use of new techniques and a new approach to the problem.

PART II

THE PHOTOCONDUCTIVITY OF CHLOROPHYLL AND THALLOUS BROMIDE

Introduction

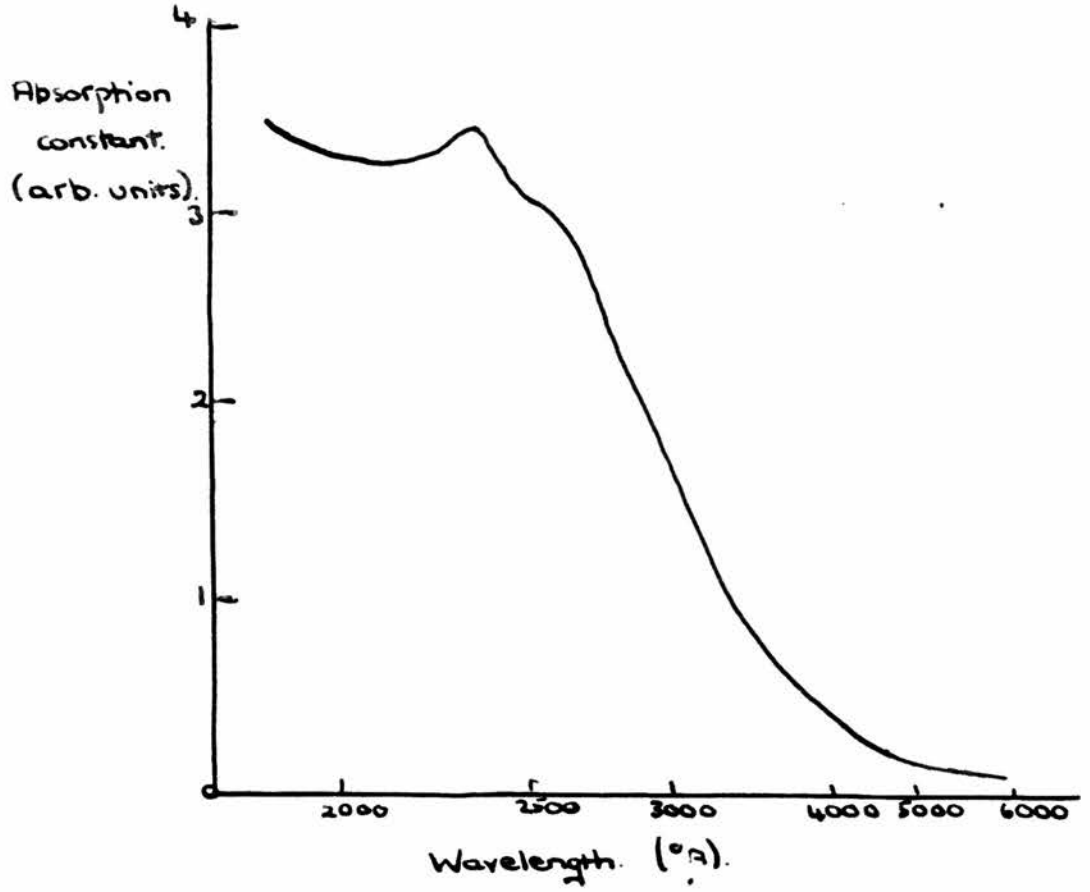
In the theoretical interpretation of the conducting properties of ionic solids, the conducting electron is considered to move in a potential field which is the average field of all the other ions and electrons. The electron can have only certain discrete energies falling into a series of allowed bands which are separated from each other by bands of forbidden energy. By the Pauli exclusion principle, no two electrons can occupy one energy state. Thus if all the energy states in a band are occupied so that the band is full, electronic conductivity is impossible because every state contains its maximum number of electrons. Substances in which these conditions exist are therefore insulators. However, if such a substance is irradiated so that an electron in the full band receives sufficient energy to lift it into an empty band, where the electron is free to move, the solid becomes a photoconductor.

When an electron leaves the lower filled band, a gap remains behind which is termed a positive hole, e.g. in an ionic halide crystal, an electron leaves a halogen ion and the ion becomes a halogen atom with an effective positive charge. An electron from a neighbouring ion could transfer itself to this atom, so the positive hole would travel through the lattice. Electronic conduction may therefore be due to both the movement of electrons in the conduction band of the crystal and positive holes in the full band. If, however, the electron remains localised near a positive hole, the two may move together through the crystal as a single unit known as an exciton.

The absorption spectrum of an ionic crystal consists of a series of sharp lines leading up to a series limit beyond which absorption occurs continuously. The spectra of the thallous halides (fig. 23) (63) show a pronounced tail on the long wave-length side of the spectrum extending into the visible region, and it is this part of the spectrum that is normally responsible for the photoconducting properties of the solid.

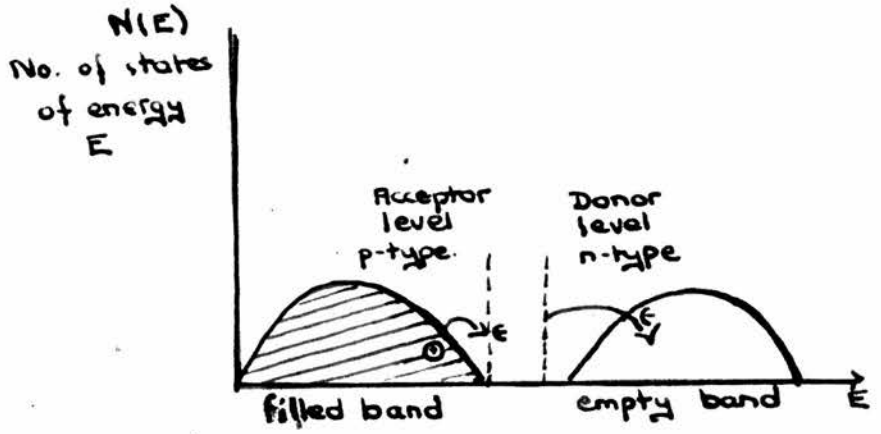
Absorption of a quantum of radiation of frequency greater than the series limit of the absorption spectrum of a crystal will give rise to free electrons and positive holes or to excitons. If a crystal irradiated with light of the required frequency is placed in contact with two metallic electrodes, then a current will flow through the circuit. This is due to the passage of electrons raised to the conduction band flowing towards the anode, and possibly also to the motion of positive holes in the full band towards the cathode.

It is an interesting fact that where the absorption maxima of the crystal occur, bulk, as opposed to surface photoconductivity, may be very small or non-existent. This is due to the high absorption coefficient in this region, which prevents the absorbed radiation from penetrating more than the surface of the film, so that conductivity can only occur at the surface of the crystal. Also, high absorption tends to favour recombination of electrons and positive holes, so that little or no current flows. However, where the absorption coefficient is much smaller, e.g. in the "tail-end" region of the spectrum of thallous bromide, the radiation can penetrate far further into the crystal and a much larger photo-



ABSORPTION SPECTRUM OF THALLOUS BROMIDE

FIG. 23



DENSITY OF STATES CURVE FOR AN IONIC SOLID.

FIG. 24

conductivity is observed. This effect is termed primary photo-conductance and is proportional to the applied voltage.

The primary photocurrent is caused directly by the exciting radiation and is due to the motion of electrons towards the anode. For n quanta of absorbed light, the maximum charge that can pass should be ne, where e is the charge on an electron. However, the conductivity of the crystal does tend to increase beyond its theoretical maximum with time due to a progressive lowering of the resistance of the crystal. This has been explained by supposing that a positive space charge accumulates near the electrodes causing a negative charge to be induced on their surface. This results in a lowering of the energy of the conduction band for electrons in the crystal relative to those in the metallic electrodes, so that some electrons can pass from the metal electrodes in the presence of an applied field. This effect is termed secondary photoconductance.

The absorption of radiation is not the only way in which substances which are normally insulators can be made to exhibit conductivity. Electrons can also be raised into the conduction band by elevating the temperature, and such solids showing conductivity at comparatively high temperatures are termed semiconductors. Most semiconductors contain a stoichiometric excess or deficit of one constituent giving rise to interstitial ions or vacancies in the lattice known as "impurity centres". Since it requires less energy to elevate an electron from such a centre into the conduction band than to bring an electron from the full valency band, semiconductivity generally arises from these impurity centres. Where stoichiometric

excess is due to interstitial cations, so that free electrons must be available to preserve neutrality, the substance is known as an n-type semiconductor. If there is a deficit of positive ions, positive holes must result, and the semiconductor is p-type (fig. 24).

If E is the work required to move an electron from an impurity centre and transfer it to the conduction band, the variation of the conductivity of the specimen with temperature can be expressed as $\sigma = \sigma_0 e^{-\epsilon/kT}$ where $\epsilon = \frac{1}{2} E$ (64).

It has been found that the adsorption of certain dyes on the surface of silver halide films can extend the spectral sensitivity of the halide in the visible region (65). Gurney and Mott suggest that light to which silver halides are insensitive is absorbed by the dye, and electrons are transferred from the dye into the conduction band of the halide. For this to occur it is essential that the excited level of the dye should be above the lowest conduction level of the halide.

In 1941, Szent Györgyi had the idea that the concept of valency electrons common to the whole system rather than to any one particular atom could be extended to biological systems (66). If an electron is raised to an excited state, it is impossible to say to which atom it belongs, and therefore the whole system can be regarded as activated. Where the molecules constituting living matter exist in a closely packed ordered arrangement, the existence of common energy levels is a feasible postulate. As early as 1930, Emerson and Arnold proposed that 2,500 molecules of chlorophyll compose one functional unit in photosynthesis, so that absorbed energy could be transported throughout

the whole system of chlorophyll molecules (67). It can be seen that such a system, where an electron excited by a quantum of light could travel to a position distant from where the energy was absorbed, would exhibit photoconductivity.

Rabinowitch has also considered whether the transfer of excitation energy between a large number of chlorophyll molecules is physically possible (68). Where similar molecules are situated in close proximity in either the liquid or the solid states, a quantum of energy will change its position several times before being emitted as fluorescent energy or partaking in chemical reaction. If the excitation energy migrates very quickly through the system, then the excited state belongs to the system as a whole, as in the case of an electron in the conductivity band of a metal. However, there is a second possibility. Excitation energy may be transferred to a long-lived metastable state so that it lingers with each molecule for a given length of time. Transfer of energy from the metastable state of one molecule to that of an adjacent molecule could then occur after a time interval, the life-time of the metastable state being of the order 10^{-8} sec., i.e. a million times longer than that of the first excited singlet state. It is still uncertain whether resonance energy migration occurs in the actual photosynthetic process, the number of chlorophyll molecules over which excitation energy could migrate during the life-time of the metastable state depending on whether the chlorophyll molecules are distributed in closely packed layers or at random.

The concept of diffusion of excitation energy through a system

is different from that of diffusion of electrons. If the electron and hole travel separately, the final effect is separation of charges; if they travel together, the result is migration of excitation energy without separation of charges, the electron-hole pair being represented as an exciton. It may be that the primary effect of light in photosynthesis is to set electrons free, in which case the photosynthetic unit could be considered to function by the exchange of electrons rather than excitations. The hypothesis that the primary process in photosynthesis is the excitation of mobile electrons was first put forward by Katz (69). The electron migrates until it becomes trapped and the trapped electron becomes in effect a chemical reducing agent. The positive hole remaining also migrates and becomes trapped elsewhere where it can function as an oxidising agent. Thus if the electron and positive hole escape from each other, they can take part in reduction and oxidation processes at sites relatively remote from the position of absorption of radiation, and the resulting chemical products will remain separated from each other.

Two hypotheses have therefore been put forward, one suggesting a charge transfer from chlorophyll to an active centre, and the other migration of resonance excitation energy. Livingston refutes the ideas of Katz, although he admits the existence of some of the components Katz suggests (70). Free electrons are in fact produced on illumination of chlorophyll-containing materials, and films of chlorophyll are photoconducting, the mobile carrier being the positive hole rather than the electron (71, 72). However, Livingston considers the spacial arrangement of the pigment enzymes and other attached

substances to be of primary importance, and that the above theory as originally proposed is over simplified.

Rabinowitch is in favour of the concept of energy transfer and estimates that the photosynthetic unit probably consists of 250 chlorophyll molecules (68). He visualises that energy migration occurs by means of an intramolecular exciton, without separation of charges, and that this exciton is involved in a chain of resonance transfers. The mechanism of the primary stage of photosynthesis is still in a highly speculative state, and it remains to be proved conclusively whether energy transfer or charge transfer actually occurs. Recent evidence is more in favour of the migration of excitation energy without separation of charges (73).

The binding energy of an exciton is small, and it will easily dissociate under the influence of an applied field, so that charge transfer occurs when a film of solid chlorophyll situated between metallic electrodes is illuminated in the presence of an applied voltage. Terenin and his co-workers have extensively studied the transport of charges released by light in layers of tetrapyrrolic pigments related to chlorophyll (72). The sign of the charge carriers is positive, either in air or in vacuo. The active light falls in the range of the absorption band of the dye and a photon of energy equivalent to 2 eV, or even less, is capable of detaching an electron from the lower filled band of the molecule. Since the carriers of photocurrent are positive holes, the electrons liberated by light must be kept in traps for some time, so that the current is transported mainly by an electron exchange between molecules at the

ground level.

Terenin has also shown that the photo-electromotive force in thallous and silver halides, as well as zinc oxide, can be sensitised by light absorbed by pigments and dyes deposited on their surface. The adsorption of chlorophyll on zinc oxide caused two bands to appear in the visible region of its spectrum at 4300°A and 6600°A when the photo-electromotive force was plotted against wavelength. Small sensitisation effects were also observed due to the adsorption of chlorophyll on thallous chloride and iodide. The adsorption of dye molecules creates additional electron levels on the surface of the crystal which can serve as suppliers of electrons to the conduction band or traps for electrons from the crystal. Alternatively, one can adopt the view that transfer of energy can occur from the adsorbed dye molecule excited by light to the electron of the crystal without any need for assuming transfer of an electron. In both cases the crystal must become conductive during exposure within the spectral range where the dye adsorption band is located if the electrons transferred fall into the conduction band of the crystal. Terenin used a condenser method originally described by Bergman (74) which enabled him to find the internal photo-electric effect in a semi-conductor without transmitting the current and applying electrodes, thus eliminating all secondary effects, such as polarization. Thin layers of the samples were pressed between insulating plates and illuminated with monochromatic light. It was also possible to determine the sign of the current carriers by this method.

Since the semiconductivity of zinc oxide, an n-type semiconductor,

could be sensitised by dyes of both n- and p-type, Terenin concluded that sensitisation was due to a transfer of energy from the dye to the electrons captured in holes at the surface of the semiconductor. It is difficult to see otherwise how adsorption of a p-type pigment could sensitise an n-type semiconductor (75), since a positive hole migrating in the pigment could not release an electron in, say, zinc oxide. Exciton migration in the pigment from the site of absorption of a quantum of light in the dye to the surface of the zinc oxide is a more plausible explanation. Thus sensitisation of the photo-conductivity and photo-electromotive force in inorganic semiconductors by chlorophyll and other dyes can be considered as an energy transfer to electrons trapped at the surface.

The object of the present work was to investigate the photo-conductivity of thin films of both thallous bromide and chlorophyll in vacuo and in oxygen. It was also hoped that experiments on films of chlorophyll deposited on thallous bromide both in vacuo and in oxygen might throw some light on the role of the substrate in the photo-oxidation of chlorophyll.

A second set of experiments was designed to determine the relationship between the dark and photoconductivity of chlorophyll and temperature. From a plot of the logarithm of the resistance of the specimen against the reciprocal of temperature it is possible to estimate the energy required to promote an electron from the highest filled to the lowest unfilled molecular orbital.

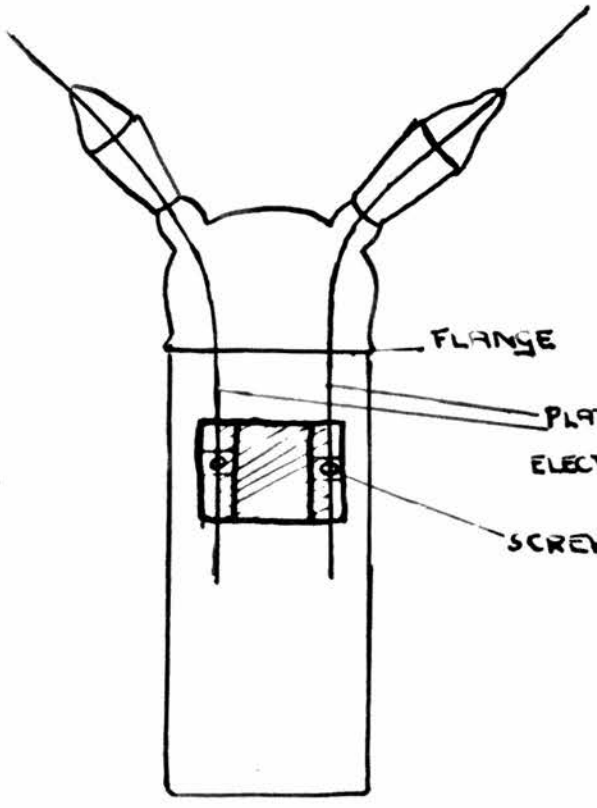
Experimental Methods

Initial experiments were carried out on the conductivity of thallous bromide both in the dark and on illumination.

The conductivity cell used was as shown in the diagram (fig. 25). The platinum leads from the cell were sealed into soda glass A 14 cones which were waxed into A 14 sockets. The cell was connected to a system which could be evacuated to less than 10^{-4} mm using an oil rotary and an oil diffusion pump, the final pressure being determined by means of a Pirani or an Ionisation gauge. Oxygen could be admitted to the system from a connected bulb at known pressures. The platinum leads were soldered externally to the leads from a Vibron Electrometer and a resistance measuring unit.

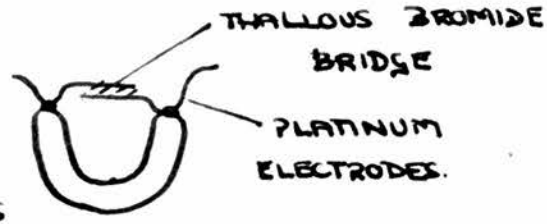
The Vibron electrometer is a vibrating condenser amplifier which measures small d.c. potentials in the range 1 millivolt to 1 volt from sources of very high internal resistance. By the addition of external fixed resistors of known value, it can also be used to measure small d.c. currents. Since it neither generates nor absorbs significant current from the component under test, it can be used for measurements which are far beyond the capabilities of the convential d.c. amplifier.

In order to use the Vibron electrometer for the measurement of small currents, a voltage drop was produced by passing the current through precision resistors. The resistance measuring unit used contained a set of high-stability resistors of values 10^6, 10^8 and 10^{10} ohms which were used to provide the voltage drop measured by the

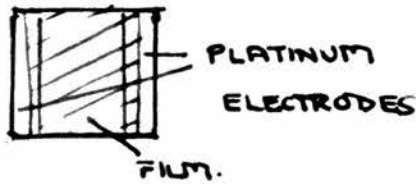


CONDUCTIVITY CELL

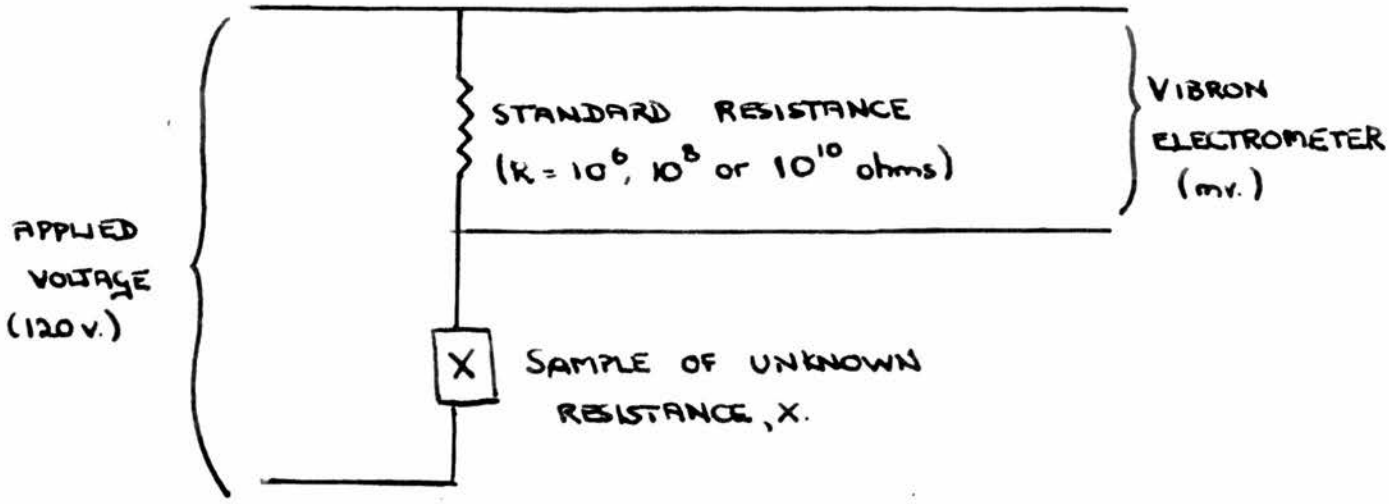
FIG. 25



SILICA BRIDGE. FIG. 26A



QUARTZ PLATE. FIG. 26B



CIRCUIT. FIG. 27

Vibron electrometer, and were connected to the electrometer by means of four flexible leads. By means of this unit, the electrometer could be used to measure currents ranging from 10^{-6} to 10^{-13} amp, the accuracy of the measurement being ± 2 per cent. The output from the electrometer was fed to a recorder which plotted directly the potential drop across the sample. The instrument was used with applied voltages between 12 and 120 volts.

The live lead to the electrometer and all points connected to it including the conductivity cell were well screened with copper foil to prevent the injection of a.c. voltages by stray capacitance coupling to the mains, and also to prevent the operator's movements causing fluctuations in the readings. The high input lead was made of polythene insulated "non-microphonic" coaxial cable.

Two types of support for the thallous bromide and chlorophyll samples were used. Initial experiments made use of a silica bridge to which was sealed two platinum leads as in the diagram (fig. 26A). Finely ground thallous bromide was pasted across these two electrodes placed 2 mm apart with a drop of water. The other ends of the electrodes were connected to the platinum leads in the cell.

The second type of support was a quartz glass plate, approximately 1½ cm square, at either end of which were painted and fired two platinum electrodes as shown (fig. 26B), using platinum paint. The electrodes were fired by placing the quartz plate for two hours in an oven stabilised at 600°C to ensure a shining surface and therefore good contact with the platinum leads from the cell, to which it was connected by two small screws. Approximately 0.05 g samples of

thallous bromide were deposited on the plate as a film across the two electrodes by adding a drop of water to the powdered thallous bromide. Approximately 10 mg samples of chlorophyll and pheophytin were also deposited on the plate between the electrodes in the form of thin films from ether solution. In both cases, the apparatus was pumped until a vacuum of 10^{-4} mm mercury was established after connecting the cell in position, thus ensuring that all moisture had been removed from the sample.

In order to operate the electrometer, the mechanical zero of the metre was first accurately set. Measurements could be made several minutes after switching on, when the instrument had reached a stable temperature. Voltage drops between 0.2 and 1000 millivolts could be read on the electrometer. A direct application of Ohms Law to the known value of the reference resistor and the voltage drop as read on the meter gave the value of the current. The circuit was as shown in the diagram (fig. 27).

After the dark current had been measured, the film was illuminated, and the photocurrent recorded when it had reached a steady value. When illumination was terminated, the dark current was again noted. For illumination, a 150 watt osira mercury lamp and 250 and 500 watt projector lamps were used in conjunction with a focussing device. A copper sulphate heat filter was introduced together with Chance coloured filters which could isolate the required regions of the spectrum. The intensity of the incident light could be fractionally reduced using calibrated grey filters.

In order to vary the temperature of the films, the cell was

surrounded by a beaker of water whose temperature was maintained at different values for fifteen to twenty minutes. When a steady reading was obtained on the electrometer, this was recorded, and the whole procedure was repeated for the same temperature. Each value recorded represented the mean of two or three readings taken for a given temperature. From the current obtained, the resistance of the sample was calculated and a graph of $\log_{10}$ Resistance against the reciprocal of temperature was plotted. From the gradient of the straight line obtained, the energy gap and activation energy for the conductivity of chlorophyll and pheophytin were obtained.

Experimental Results

Photoconductivity of thallous bromide

The first set of experiments was carried out on a thallous bromide bridge, prepared as described previously. The dark current dropped rapidly at first as the moisture remaining in the paste was removed, but eventually reached a steady value after about one hour's pumping. Pumping was continued for another hour before illuminating with white light from a 150 watt Osira mercury lamp.

Results

Dark current = 6×10^{-12} amp.

Photocurrent = 20×10^{-12} amp. The current rose to this value immediately on illumination, and remained steady at 20×10^{-12} amp indefinitely. The response was as fast as or faster than the pointer on the electrometer could move (fig. 28).

Variation of current with applied voltage

The applied voltage was varied from 12 to 120 volts, and the corresponding dark current and photocurrent was recorded.

INCREASE IN CURRENT ON ILLUMINATION OF A FILM OF THALLOUS BROMIDE

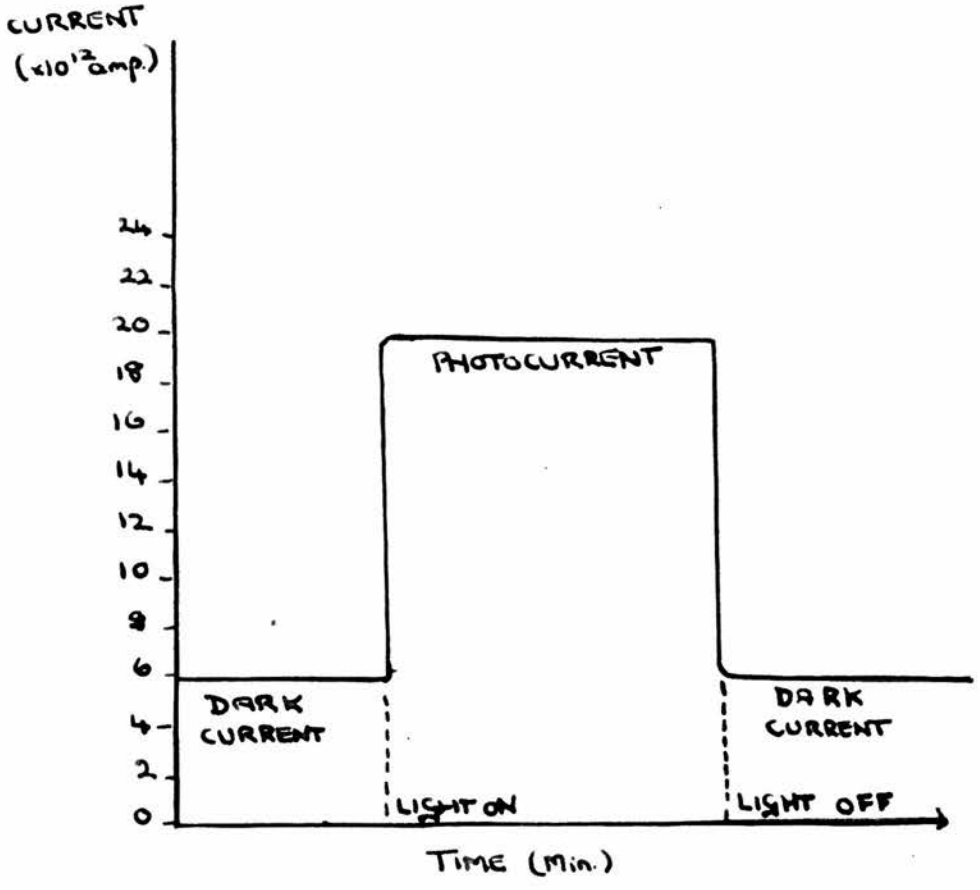


FIG. 28

TABLE 22.

Applied voltage (volts)	Dark current (x 10^{12} amp)	Photocurrent (x 10^{12} amp)
120	6.0	20.0
108	5.4	18.0
72	3.5	12.2
60	2.8	9.8
24	1.0	3.9
12	0.5	1.8

In both cases, a graph of applied voltage against current showed that the current was directly proportional to the applied voltage (fig. 29).

On applying zero voltage, a negative reading was obtained because the specimen had become polarized during the previous experiments and removal of the applied voltage caused the current to flow in the opposite direction to counteract this effect. The terminals were reversed and this current fell gradually to zero.

Spectral distribution of photocurrent

Various regions of the spectrum were isolated using Chance coloured glass filters, and the photocurrent was recorded for each filter.

VARIATION OF DARK AND PHOTOCURRENT WITH APPLIED VOLTAGE FOR THALLOUS BROMIDE.

FIG. 29

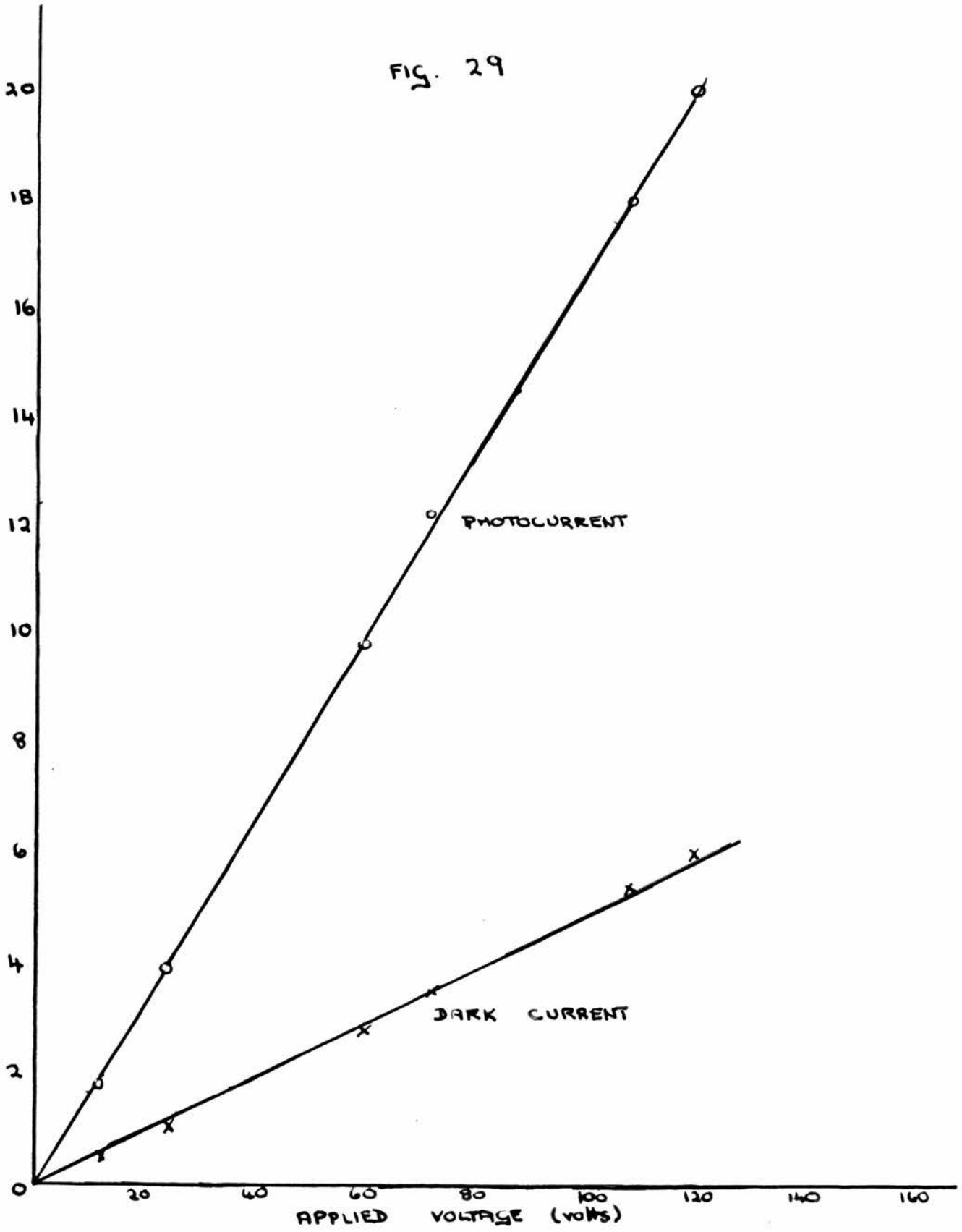


TABLE 23.

Filter	Region of spectrum	Dark current ($\times 10^{12}$ amp)	Photocurrent ($\times 10^{12}$ amp)
ORl	Above 6500°A	4.5	4.8
OY3	" 5500°A	4.5	5.0
OBlO	Isolates 4050 and 4360°A mercury lines	4.5	12.0
OXl	Isolates 3650°A mercury line	4.5	8.0
OX7		4.5	8.2

Since there is virtually no photocurrent using the ORl and OY3 filters the 5460°A (green) and 5790°A (yellow) lines of the mercury vapour spectrum have no effect. It is the 3650, 4050 and 4360°A lines that are responsible for this photoconductivity. Since the output of the mercury vapour lamp is such that the intensity of light of wavelength 3650°A equals approximately that of wavelengths 4050 and 4360°A together, it can be concluded from these results that blue light is rather more effective than near ultra violet light. This was in accordance with expectations, since it is the "tail end" region of the thallous bromide spectrum that is responsible for photoconductivity, and this extends into the blue (fig. 23).

Using the OBlO filter, it was found that the dark and photocurrents were proportional to the applied voltage, as previously proved using the whole range of the mercury spectrum.

If the terminals were reversed after any reading, there was an immediate increase in current, followed by a gradual fall to the value originally recorded (fig. 30). This was due to polarization effects after passage of the current for some time in one direction, as already discussed.

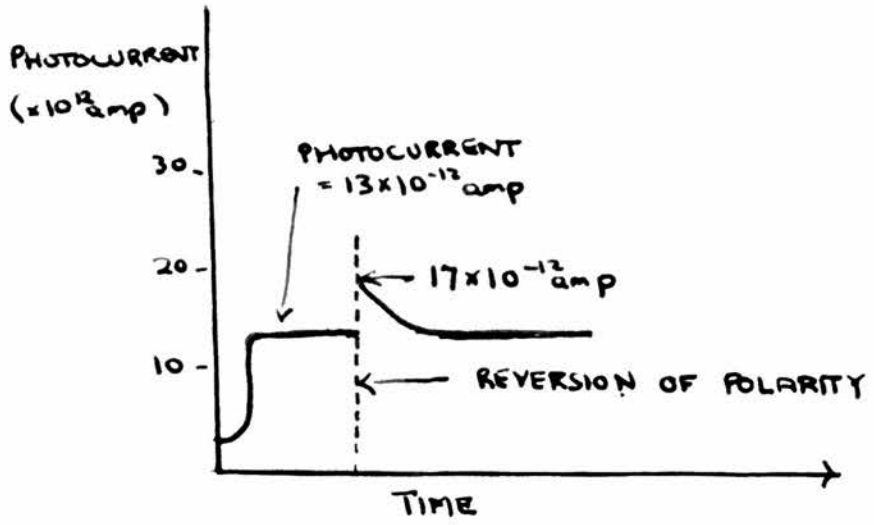
Photoconductivity in 50 mm oxygen

Using the OB10 filter, a steady photocurrent of 13×10^{-12} amp was maintained for some time. On admission of some oxygen there was an immediate drop to 9×10^{-12} amp, where the photocurrent remained steady. On evacuating the system again, the photocurrent immediately rose to its previous value. The reason for the fall in photocurrent is probably that oxygen withdraws some of the electrons from the conducting band of thallous bromide, which is an n-type semiconductor (fig. 31). The action is immediately reversible.

Relationship between photocurrent and light intensity

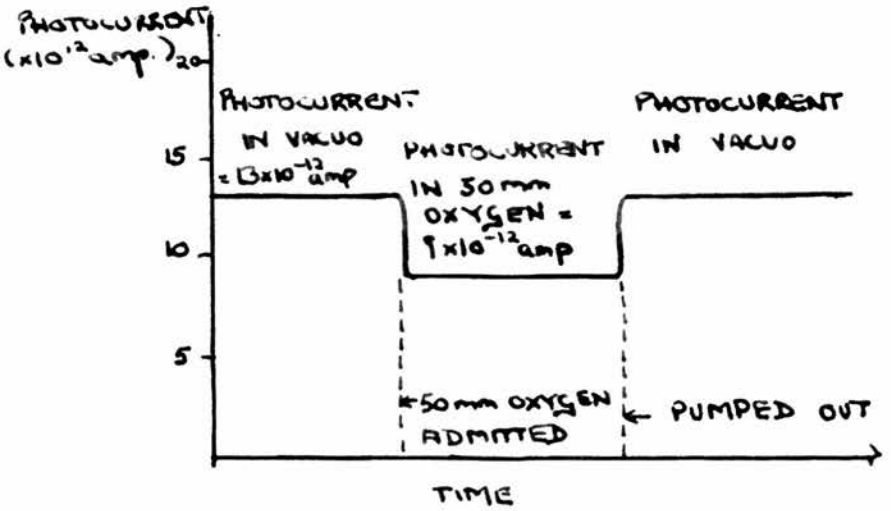
The 3650, 4050 and 4360 lines of the spectrum were each isolated in turn, and the intensity of the light falling on the sample was varied using Chance grey filters, the photocurrent being noted in each case.

THALLOUS BROMIDE FILM



REVERSION OF POLARITY

FIG. 30



CHANGE IN PHOTOCURRENT ON ADMISSION OF OXYGEN

FIG. 31

(1) 3650 line isolated using the OX1 filter

TABLE 24.

Grey filter	% transmission	Photocurrent (x 10^{12} amp)
No filter	100	4.5
1 ON 33	50	2.2
2 ON 33	25	1.1
3 ON 33	12.5	0.6
ON 10	5	0.3

A graph of photocurrent against percentage transmission was plotted. The straight line plot proved that the photocurrent was proportional to the light intensity (fig. 32A).

The 4050 line was isolated using an OB10 filter and a solution of iodine in carbon tetrachloride (7.5 g/litre in a 1 cm cell); the 4360 line was isolated using an OB10 filter and sodium nitrite solution (750 g/litre in a 1 cm cell) (86).

(2) 4360 line

TABLE 25.

Grey filter	% transmission	Photocurrent (x 10^{12} amp)
0	100	4.2
1 ON 33	57	2.4
2 ON 33	32	1.4
3 ON 33	19	0.8
ON 10	0.7	0.4

VARIATION OF PHOTOCURRENT WITH LIGHT INTENSITY

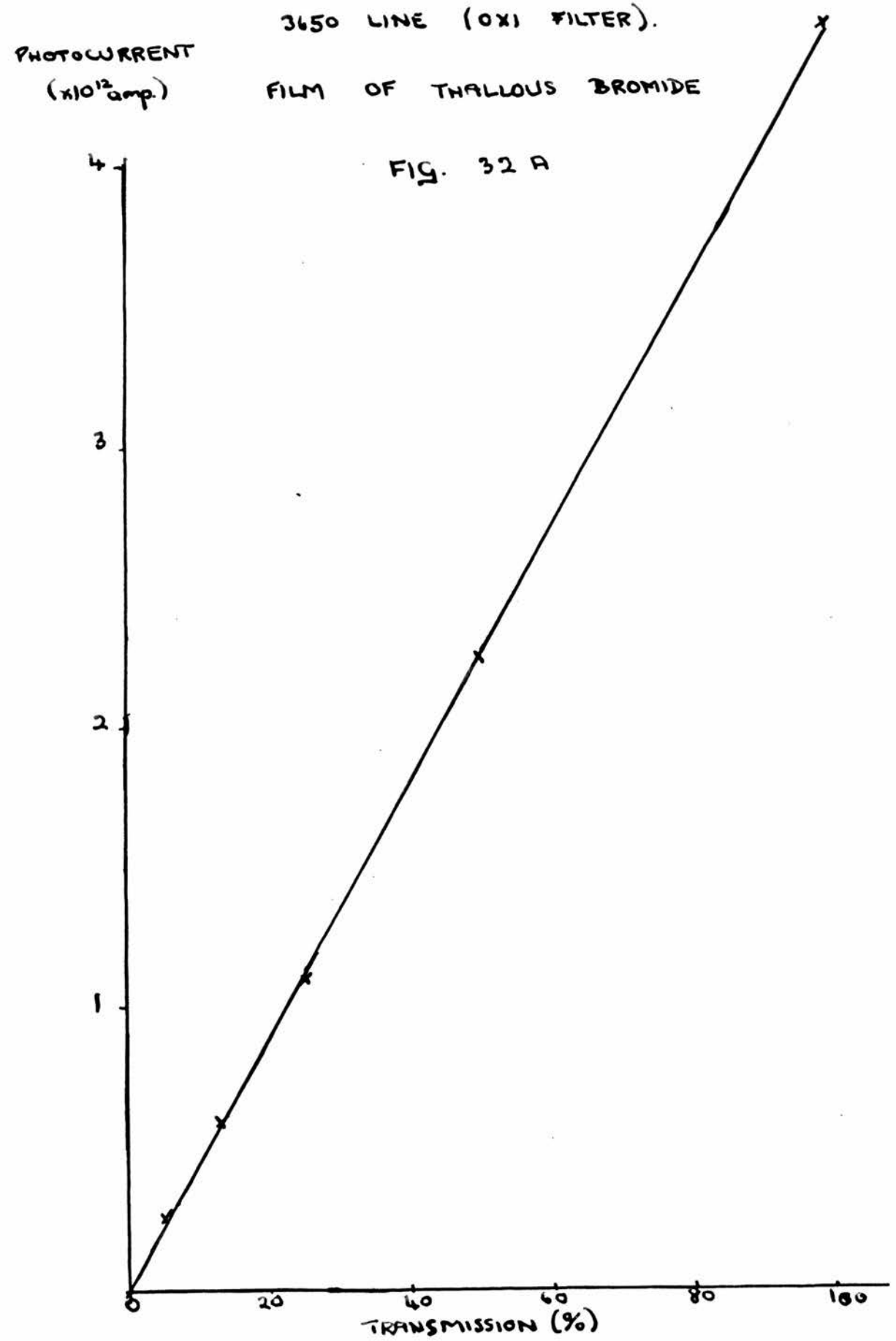
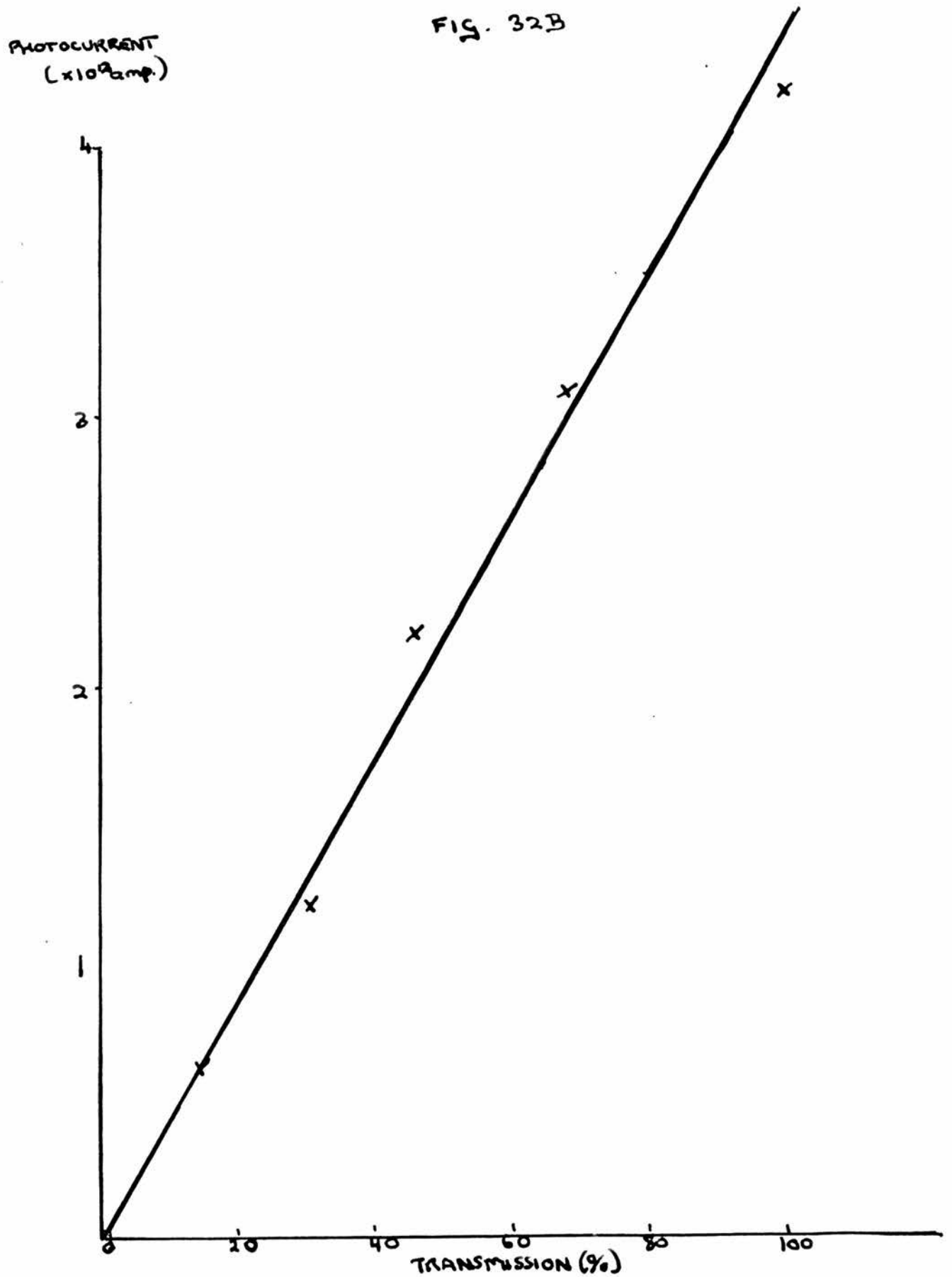


FIG. 32 A

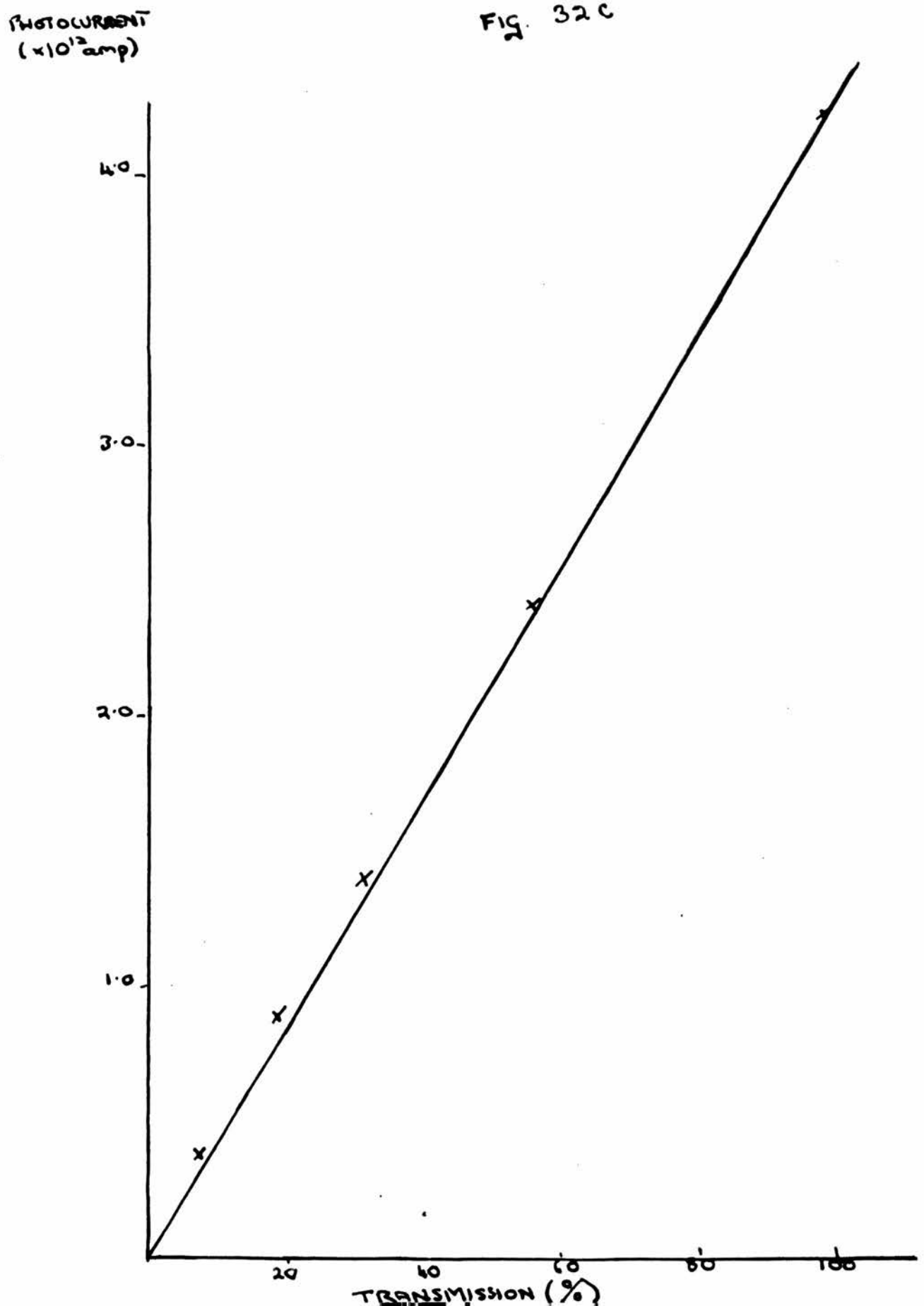
4050 LINE [0 BIO FILTER + SOLUTION OF IODINE IN CARBON TETRACHLORIDE.]

FIG. 32B



4360 LINE (0810 FILTER + SODIUM NITRATE SOLUTION)

FIG. 32C



(3) 4050 line

TABLE 26.

Grey filter	% transmission	Photocurrent (x 10^{12} amp)
0	100	4.2
1 ON 33	68	3.1
2 ON 33	46	2.2
3 ON 33	31	1.4
ON 10	15	0.6

In both cases, the graphs showed that the photocurrent was proportional to the percentage transmission (fig. 32B, C). Therefore for thallous bromide, the photocurrent produced is directly proportional to the intensity of the incident light falling on the conducting sample.

The above experiments were all repeated on a second sample of thallous bromide, and later on a film of 0.05 g of the substance on the quartz plate, prepared as previously described. Results of the same order were recorded in all cases.

Photoconductivity of thin films of chlorophyll

A bridge of approximately 10 mg of chlorophyll in wax form was deposited across the platinum electrodes from a very concentrated solution in ether. The initial dark current recorded was very high

due to the presence of water in the chlorophyll preparation, but after 3 to 4 hours pumping with the diffusion pump a steady dark current was maintained.

On illumination with the mercury lamp, the photocurrent produced was very small. However, a far greater rise in photocurrent could be produced by illuminating with a 250 watt tungsten lamp which gives a continuous spectrum in the visible region. The reason for this is probably that a greater effect is obtained with a source of light giving a continuous spectrum than with a mercury vapour lamp where light is emitted at certain discrete wavelengths, since chlorophyll absorbs continuously throughout the visible region of the spectrum.

Still better results were obtained by substituting the bridge for thin films of chlorophyll deposited across the platinum electrodes on the quartz plate. About 10 mg of chlorophyll were deposited from 2-3 drops of ether solution. This film was more easily prepared than the bridge and could be pumped free of solvent and combined or adsorbed water much more quickly. After 3 hours pumping a steady dark current of 2.1×10^{-12} amp was recorded. The total current flowing under illumination conditions was 3.2×10^{-10} amp, indicating more than a hundred fold rise in current on illumination.

Rise in photocurrent with time

In the case of illuminated films of chlorophyll in vacuo, the photocurrent did not instantaneously reach its maximum value. There was a gradual increase of current with time until a steady value was

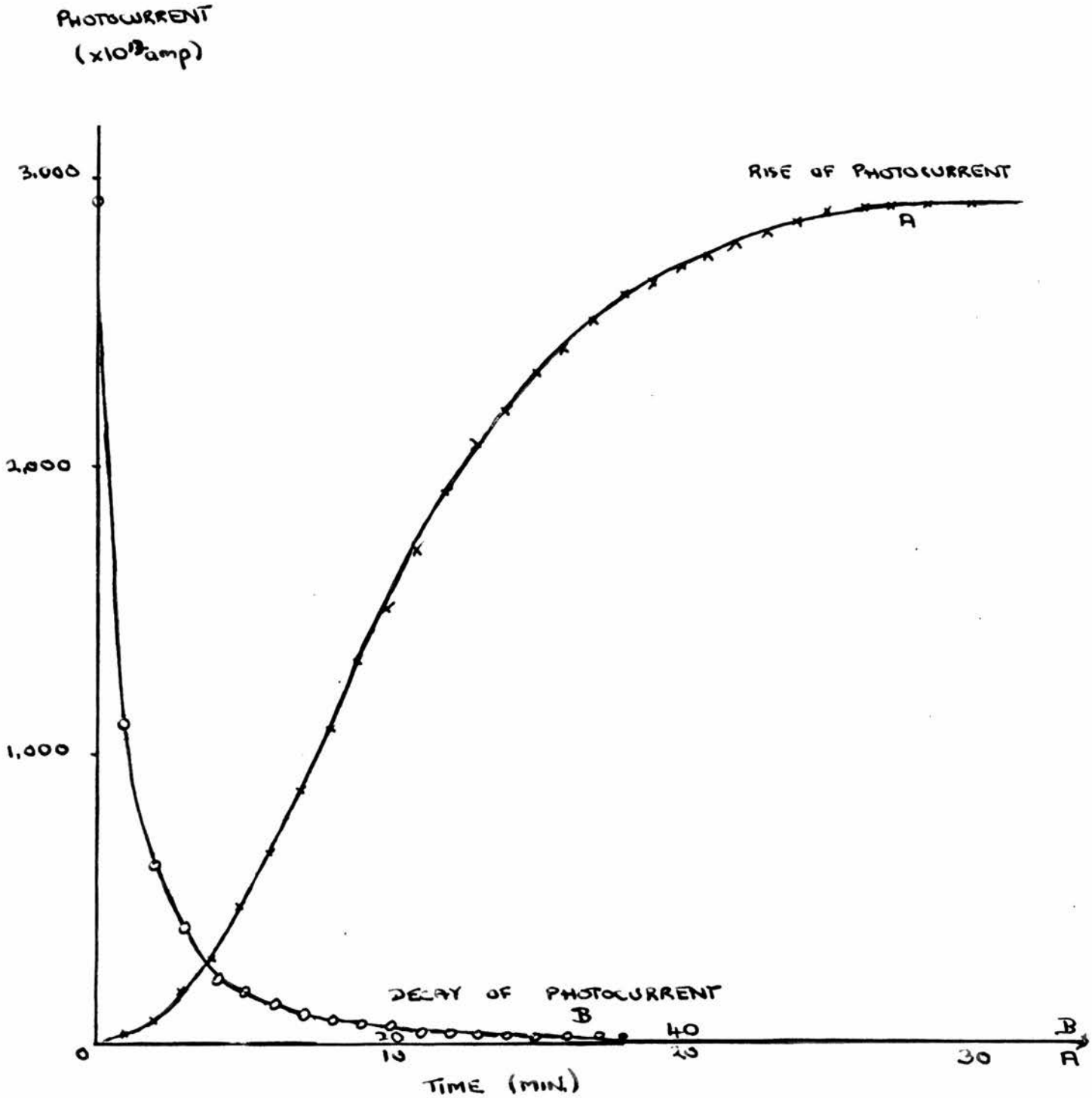
maintained after about 25 minutes. When illumination was ceased, the photocurrent decayed in a similar manner, reaching a steady value just below the original dark current after about 30 minutes. Graphs were plotted of rise of photocurrent with time and decay of photocurrent with time (fig. 33A).

TABLE 27. Rise of photocurrent with time.

	Time (min.)	Current ($\times 10^{13}$ amp)	Δc ($\times 10^{13}$ amp)	Time (min.)	Current ($\times 10^{13}$ amp)	Δc ($\times 10^{13}$ amp)
	0	19.5		15	2330	120
	1	45.0	25.5	16	2430	100
	2	90.0	45.0	17	2520	90
	3	190.0	100.0	18	2600	80
	4	300.0	110.0	19	2650	50
	5	480.0	180.0	20	2700	50
Maximum	6	660.0	180.0	21	2740	40
Rate	7	880.0	220.0	22	2780	40
	8	1100.0	220.0	23	2820	40
	9	1320.0	220.0	24	2850	30
	10	1520	200.0	25	2880	30
	11	1720	200.0	26	2880	
	12	1920	200			
	13	2080	160			
	14	2210	130	30	2920	
					steady	

RISE AND DECAY OF PHOTOCURRENT (NEW FILM)

Fig. 33



The column Δc represents the change in photocurrent per minute. The maximum rate of change of photocurrent with time came after about 8 minutes, after which there was a deceleration.

All attempts to fit these results into various mathematical expressions failed. It is probable that the rise of photocurrent in organic polynuclear compounds is a complex process which cannot be correlated with the mathematical formulae representing equivalent processes in inorganic ionic crystals. Although Fig. 33A is very similar in shape to some solid thermal decomposition curves, no expression could be found which gave a linear plot for a whole set of photocurrent/time measurements.

Decay of photocurrent with time (fig. 33B)

TABLE 28.

Time (Min)	Current ($x10^{13}$ amp)	Decay ($x10^{13}$ amp)	$\frac{1}{T}$ (Min^{-1})	Time (Min)	Current ($x10^{13}$ amp)	Decay ($x10^{13}$ amp)	$\frac{1}{T}$ (Min^{-1})
0	2920			17	19.5	2900.5	0.059
1	1160	1760	1.000	18	18.3	2901.7	0.056
2	560	2360	0.500	19	17.8	2902.2	0.053
3	340	2580	0.333	20	17.0	2903.0	0.050
4	262	2658	0.260	21	16.3	2903.7	0.048
5	180	2740	0.200	22	15.9	2906.1	0.045
6	140	2780	0.167	23	15.4	2904.6	0.043
7	100	2820	0.143	24	15.2	2904.8	0.042
8	76	2844	0.125	25	15.0	2905.0	0.040
9	61	2859	0.111	26	14.8	2905.2	0.039
10	51	2869	0.100	27	14.4	2905.6	0.037
11	42	2878	0.091	28	14.2	2905.8	0.036
12	32.2	2888	0.083	29	14.2	2905.8	0.034
13	26.0	2894	0.077				
14	24.0	2896	0.071				
15	22.0	2898	0.067				
16	20.8	2899.2	0.063	36	13.2	2906.8	0.028
					Steady		

On first sight, the decay current/time plot appeared to be exponential, but this was not in fact the case. However, for a

freshly prepared film, the plot of decay current against the reciprocal of time was found to be a straight line (fig. 34A). The significance of this result will be discussed later.

Considerable change in the photocurrent/time plots occurred as the films aged with consequent degradation of the chlorophyll. The following results were obtained using the same film seven days later.

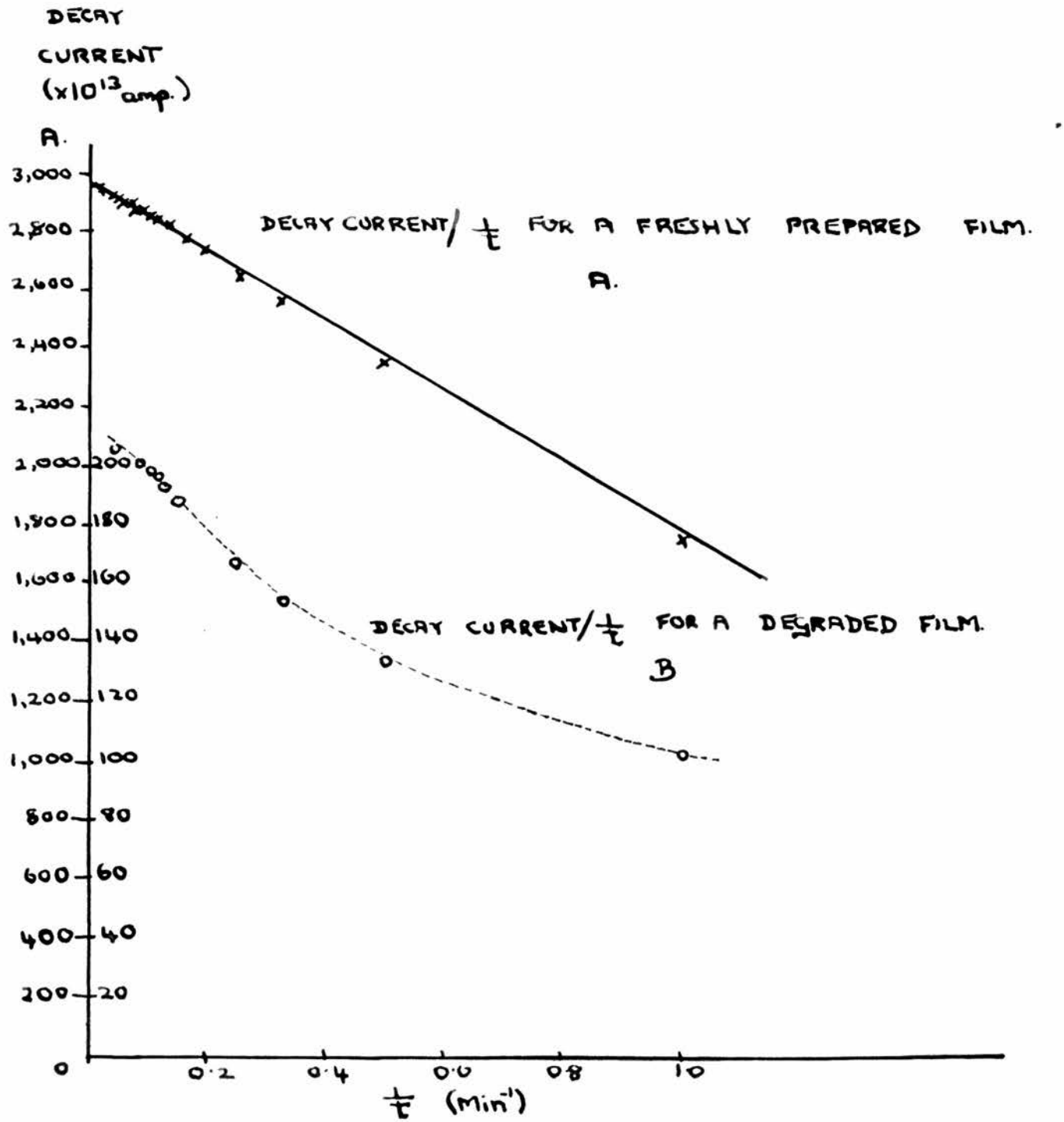
Rise of photocurrent (Film 7 days old)

TABLE 29.

Time (Min.)	Photocurrent ($\times 10^{13}$ amp)	Δc ($\times 10^{13}$ amp)	Time (Min.)	Photocurrent ($\times 10^{13}$ amp)	Δc ($\times 10^{13}$ amp)
0	12		13	172	10
1	26	14	14	180	8
2	38	12	15	188	8
3	50	12	16	194	6
4	62	12	17	200	6
5	76	12	18	206	6
6	90	14	19	212	6
7	104	14	20	215	3
8	116	12	21	218	3
9	128	12	22	220	2
10	140	12	23	222	2
11	150	10	24	"	0
12	162	12			
			28	222	
				Steady	

PLOT OF DECAY CURRENT AGAINST $\frac{1}{t}$

FIG. 34

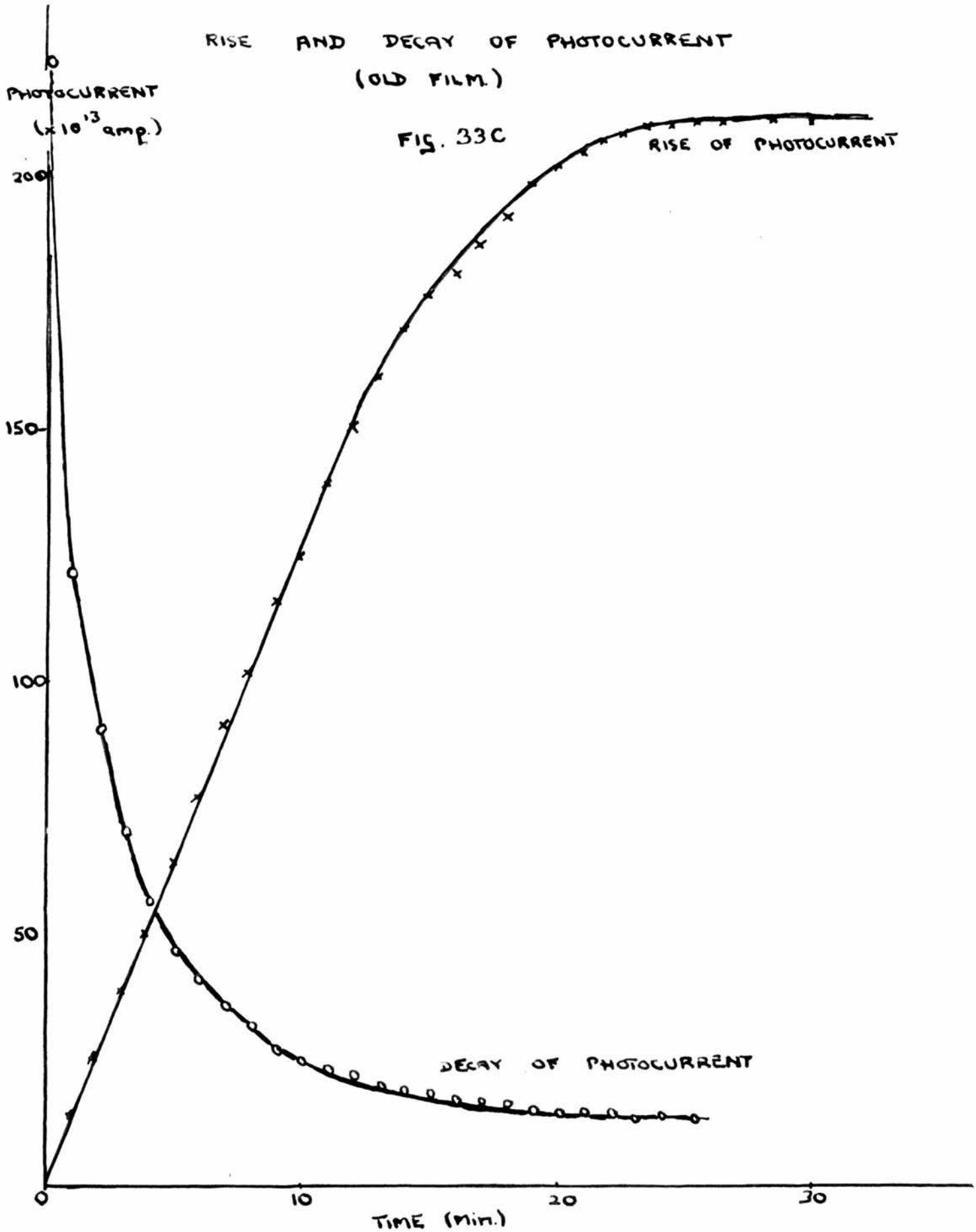


In this case, instead of there being an acceleration to a maximum rate of change of photocurrent after 9 minutes illumination, the increase in current proceeded at almost constant rate for the first 12 minutes before there was a deceleration (fig. 33C). It was also noted that as the film aged, the maximum photocurrent decreased rapidly. The same experiment was repeated on the same film another week later, and the maximum photocurrent was found to be only 38×10^{-13} amp. The shape of the photocurrent/time plot was again different, and this time the maximum rate period continued right up to 21 minutes after the commencement of illumination. It was found, however, that the maximum current was always reached within 30 minutes.

Decay of photocurrent (Film 7 days old)

TABLE 30.

Time (Min)	Photocurrent ($\times 10^{13}$ amp)	Decay ($\times 10^{13}$ amp)	$\frac{1}{T}$ (Min^{-1})	Time (Min)	Photocurrent ($\times 10^{13}$ amp)	Decay ($\times 10^{13}$ amp)	$\frac{1}{T}$ (Min^{-1})
0	222			14	17.8		
1	120	102	1.000	15	17.0	205.0	6.067
2	88	134	0.500	16	16.2		
3	68	154	0.333	17	15.6		
4	55	167	0.250	18	15.0		
5	46	176	0.200	19	14.2		
6	39	183	0.167	20	13.8	208.2	0.050
7	34	188	0.143	21	13.4		
8	30	192	0.125	22	13.0		
9	26	196	0.111	23	12.8	209.2	0.043
10	23.8	198.2	0.100	24			
11	21.8			25			
12	20.2	199.8	0.083	26	Steady		
13	18.8						


RISE AND DECAY OF PHOTOCURRENT
(OLD FILM.)
FIG. 33C
PHOTOCURRENT
(×10^13 amp.)
200
150
100
50
0
RISE OF PHOTOCURRENT
DECAY OF PHOTOCURRENT
0
10
20
30
TIME (Min.)

In this case the decay of photocurrent is not quite so rapid, but the plot is still not exponential. Nor is the graph of decay current against the reciprocal of time any longer linear (fig. 34B).

It was possible to reproduce the original curves on freshly prepared films. There was an acceleration in the rise of photocurrent to reach a maximum after about 8 minutes, followed by a deceleration, and this was gradually replaced by a constant rate portion as the film aged, and a progressive lowering of the maximum steady value of the photocurrent.

The linear relationship between the decay current and the reciprocal of time held for freshly prepared films, but not for aged films. These changes as the film aged were probably connected with the lack of uniformity of the molecules in the film.

Variation of photocurrent with applied voltage

Using a freshly prepared chlorophyll film, the applied voltage was varied between 12 and 120 volts and the dark current and the maximum steady values of the photocurrent at each applied voltage were recorded. It was found that, as for thallous bromide, both dark and photocurrents were directly proportional to the applied voltage (fig. 35A).

TABLE 31.

Applied Voltage (volts)	Dark current ($\times 10^{13}$ amp)	Photocurrent ($\times 10^{13}$ amp)
120	19.5	2920
108	17.5	2630
72	12.0	1750
60	9.5	1465
24	4.0	585
12	2.0	280

Spectral distribution of photocurrent

The spectral distribution of photocurrent was determined using the various filters.

TABLE 32.

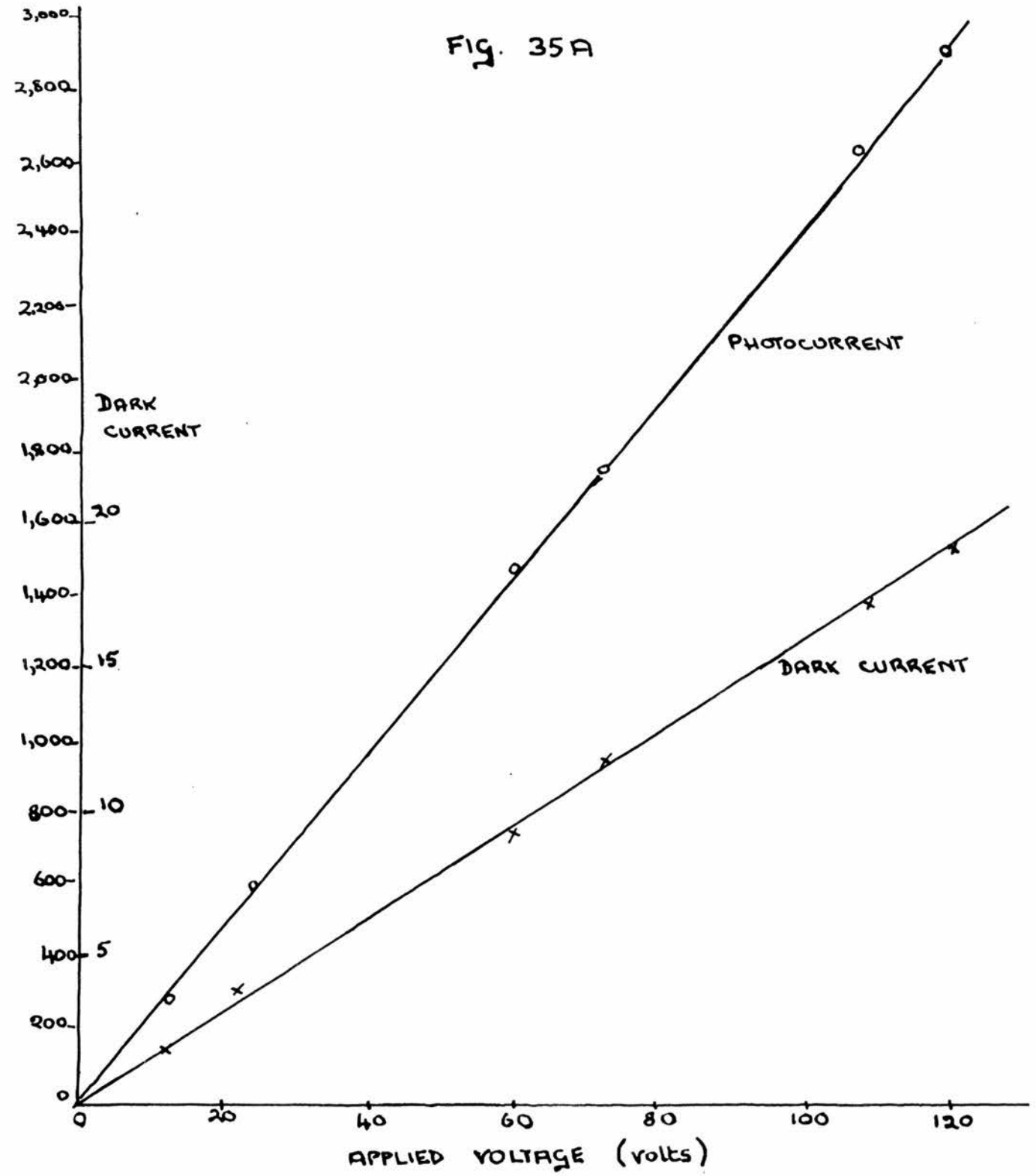
Filter	Range of spectrum	Photocurrent ($\times 10^{13}$ amp)	Region of spectrum	Corresponding photocurrent ($\times 10^{13}$ amp)
None	Whole	75.0		
ORl	Above 6500°A	1.1	Above 6500°A	1.1
OYl	" 6000°A	10.0	6000 - 6500°A	8.9
OY3	" 5500°A	70.0	5500 - 6000°A	60.0
OBlO	4000 - 4500°A	2.6	4000 - 4500°A	2.6
OXl	3500°A	0.1	3500°A	0.1

CURRENT
($\times 10^{13}$ amp)

VARIATION OF DARK AND PHOTOCURRENT WITH APPLIED VOLTAGE FOR A FILM OF CHLOROPHYLL

PHOTO-CURRENT

Fig. 35A



It was estimated by use of a selenium barrier cell, taking into account the relative selectivity of the cell at different wavelengths, that the lamp was emitting light in approximately the following relative intensities:

TABLE 33.

Range	Tungsten lamp	Mercury lamp
6500 - 8000	3	0
6000 - 6500	6	1.3
5500 - 6000	2.5	1.0
4000 - 4500	3	0.8
3500	1	1.0

The same experiment was performed using the mercury lamp. Thus correcting for these differences in output in different regions of the spectrum, the relative spectral distribution of photocurrent for chlorophyll is as follows:

TABLE 34.

Range of spectrum	Relative photocurrent ($\times 10^{13}$ amp)
6500 - 8000	12
6000 - 6500	89
5500 - 6000	1440
4000 - 4500	36
3500	6

These results do not correspond at all with those one would predict from the absorption spectra of solutions of chlorophyll in ether. One would have expected the maximum photoconductivity to occur with illumination in the regions 4000-4300°A and at 6600°A. Instead, these results show that the vast majority of the photocurrent is caused by light in the region 5500-6000°A. Possibly this has some connection with the triplet state absorption spectrum of solid films of chlorophyll.

The same spectral distribution was found for all preparations of chlorophyll, and also for aged films. For example, the following results were obtained on the same film several days later:

TABLE 35.

Region of spectrum	Photocurrent ($\times 10^{13}$ amp)	Corrected photocurrent ($\times 10^{13}$ amp)
Above 6500°A	3.0	4.0
6000 - 6500°A	12.0	12.0
5500 - 6000°A	19.0	42.0
4000 - 4500°A	24.5	9.0
3500°A	0	0

The spectral distribution is the same, although the maximum photocurrent between 5500 and 6000°A is greatly diminished.

Variation of photocurrent with light intensity

Using the tungsten lamp and the Chance filters, it was not possible to isolate light of any single wavelength. However, using the OY1 filter, it could be assumed that only light of wavelengths between 6000 and 6500°A was effective in producing a photocurrent since the photoconductivity achieved by light of wavelengths greater than 6500°A was very small. Thus using this filter it was possible to make an estimation of the relationship between the photocurrent produced and the intensity of the light responsible.

TABLE 36.

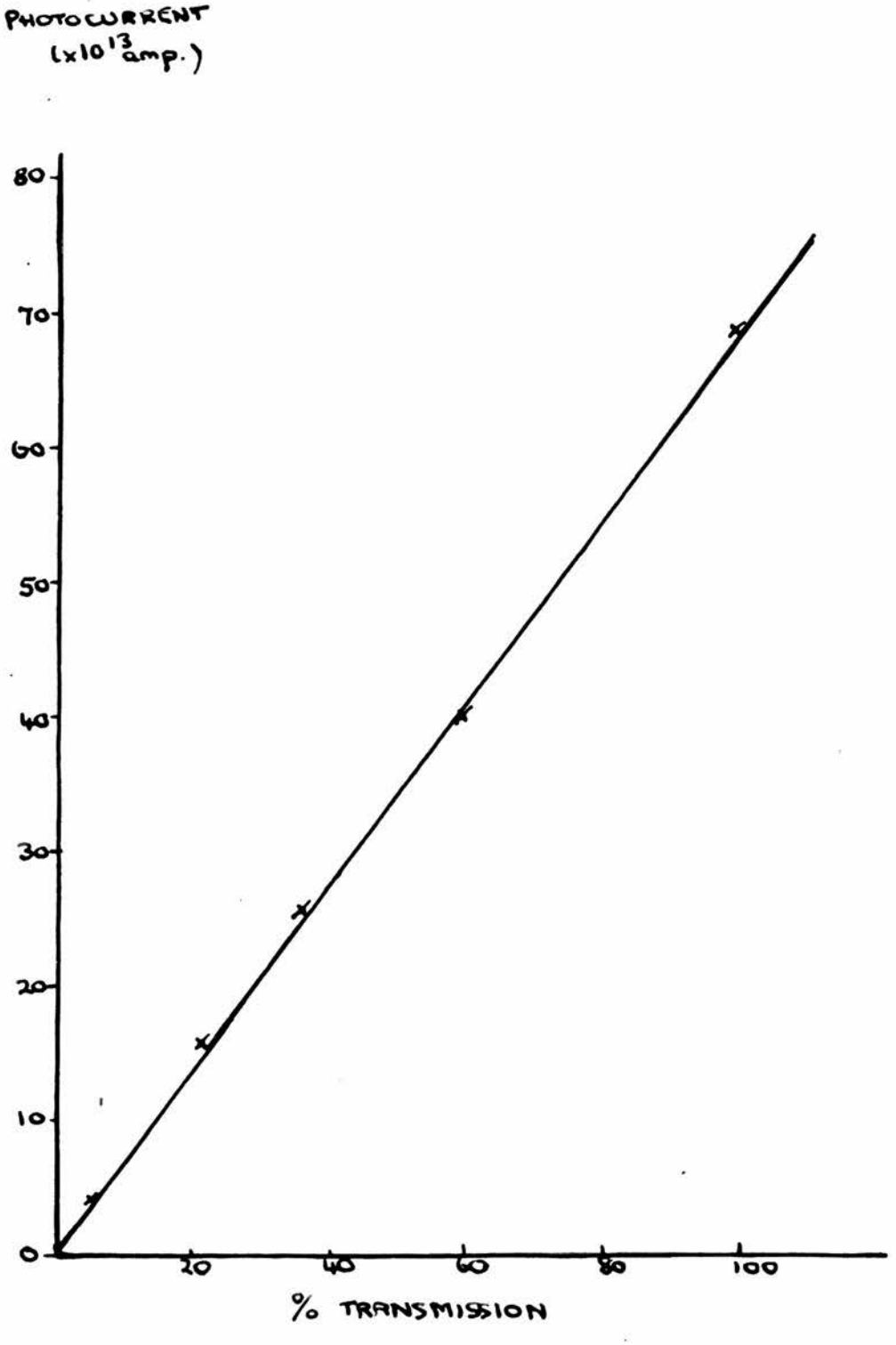
Grey filter	% Transmission	Photocurrent (x 10^{13} amp)
None	100	69.0
1 ON 33	60	40.0
2 ON 33	36	25.5
3 ON 33	22	15.7
ON 10	5	4.8

A straight line plot of photocurrent against percentage transmission was obtained, showing that the photocurrent was proportional to the intensity of the incident light (fig. 35B).

VARIATION OF PHOTOCURRENT WITH LIGHT INTENSITY

FOR A FILM OF CHLOROPHYLL

FIG. 35 B



Effect of admission of oxygen on the photocurrent

On admission of 50 mm oxygen to a freshly prepared film of chlorophyll, the photocurrent immediately dropped from 560 x 10^{-13} amp to 50 x 10^{-13} amp, and then gradually to 40 x 10^{-13} amp where it remained steady for 3 hours. The dark current dropped from 5.5 x 10^{-13} to 1 x 10^{-13} amp when oxygen was admitted.

The apparatus was evacuated overnight and the same experiment repeated the next day. On the second day, the dark current was 3.6 x 10^{-13} amp and the photocurrent 400 x 10^{-13} amp, falling immediately to 32 x 10^{-13} amp and then gradually to a steady value of 12 x 10^{-13} amp on admission of 50 mm oxygen.

After 3 hours illumination was interrupted and the dark current was found to be only 0.6 x 10^{-13} amp, rising to 0.9 x 10^{-13} amp on evacuation. The corresponding photocurrent was 300 x 10^{-13} amp.

Two deductions can be made from these observations:

(1) Oxygen quenches the photoconductivity of chlorophyll to a very great extent.

(2) From the diminished value of the maximum photocurrent on evacuation after each period of illumination in oxygen, it is apparent that oxygen is causing the destruction of some of the chlorophyll in the film.

The same experiment was repeated once more on a third day. In this case the photocurrent was only 180 x 10^{-13} amp falling eventually to 7.5 x 10^{-13} in the presence of oxygen. After evacuation,

the photocurrent had diminished to 70 x 10^{-13} amp.

The chlorophyll film was finally extracted in methanol. The methanolic extract gave a strong hydroperoxide reaction with ferrous thiocyanate, thus proving that oxidation of the film had occurred. It could therefore be concluded that destruction of the conjugated ring system by oxidation had considerably reduced the photoconducting properties of chlorophyll.

Photoconductivity of a film of chlorophyll on thallous bromide

Before determining the photocurrent for films of chlorophyll and thallous bromide together, it was necessary to repeat some of the previous experiments on thallous bromide using a film of this compound rather than a bridge, and illuminating with the tungsten rather than the mercury lamp. A paste of approximately 0.05 g powdered thallous bromide moistened with water was placed on the quartz plate across the platinum electrodes, and the apparatus was evacuated for 3 to 4 hours to 10^{-4} mm mercury, to give a steady dark current reading.

Spectral distribution of photocurrent of thallous bromide, using the tungsten lamp

TABLE 37.

Region of spectrum (^{o}A)	Photocurrent (x 10^{13} amp)	Corrected photocurrent (x 10^{13} amp)
6500	22	44
6000 - 6500	41	41
5500 - 6000	140	336
4000 - 4500	1300	2600
3500	112	672

These results were checked using the mercury vapour lamp:

TABLE 38.

Region of spectrum (^{o}A)	Photocurrent (x 10^{13} amp)	Corrected photocurrent (x 10^{13} amp)
6500	0	0
6000 - 6500	10	13
5500 - 6000	25	25
4000 - 4500	1000	800
3500	270	270

In both cases, the maximum photocurrent is in the region 4000 to 4500^{o}A, due to the 4050 and 4360^{o}A lines of the mercury vapour spectrum.

On admission of 50 mm oxygen to the system, the photocurrent fell from 1300 x 10^{-13} to 1080 x 10^{-13} amp. All results obtained previously were repeated using a film of thallous bromide illuminated with the tungsten lamp.

A film of chlorophyll was deposited from concentrated ether solution on top of the thallous bromide film. Both the dark current and photocurrent obtained from this combined film were proportional to the applied voltage.

Spectral distribution of photocurrent for thallous bromide + chlorophyll film

TABLE 39.

Region of spectrum (^{o}A)	Photocurrent (x 10^{13} amp)	Corrected photocurrent (x 10^{13} amp)
6500	10	20
6000 - 6500	62	62
5500 - 6000	170	408
4000 - 4500	290	580
3500	90	540

These results seem to demonstrate the combined photoconducting effects of both thallous bromide and chlorophyll. The adsorbed chlorophyll sensitises the thallous bromide so that it photoconducts in the region 5500 - 6000^{o}A. Photoconductivity is very marked in

the whole region 3500 to 6000°A using the combined film.

On admission of 50 mm of oxygen there was an immediate drop in photocurrent from 1000 x 10^{-13} amp to 520 x 10^{-13} amp, then gradually to 509 x 10^{-13} amp. The film was illuminated for 5 hours, and the photocurrent remained steady at this value.

Spectral distribution of photocurrent for thallous bromide + chlorophyll film after 5 hours illumination in 50 mm oxygen

TABLE 40.

Region of spectrum (°A)	Photocurrent (x 10^{13} amp)	Corrected photocurrent (x 10^{13} amp)
6500	0	0
6000 - 6500	12	12
5500 - 6000	20	48
4000 - 4500	190	380
3500	80	480

This distribution resembles that obtained for thallous bromide alone - the sensitising effect of chlorophyll in the region 5500 - 6000°A has disappeared completely. It therefore appears that oxidation of the chlorophyll film has completely removed its photo-conducting properties. It is to be expected that destruction of the conjugated ring system would have a drastic effect on the photo-conductivity of the substance.

Before extracting the film, with methanol, 1 atmosphere of oxygen was admitted to the system. The dark current was 24 x 10^{-13} amp and the photocurrent 500 x 10^{-13} amp, i.e. there was practically no difference in the effects of 50 mm and 1 atmosphere of oxygen on the current.

A methanolic extract of the film gave a strong hydroperoxidic reaction with ferrous thiocyanate proving that oxidation of chlorophyll had occurred.

These experiments were repeated on a freshly prepared film of chlorophyll on thallous bromide, and the above results were shown to be reproducible in every respect.

Photoconductivity of copper chlorophyll

The photocurrent was determined using a film of copper chlorophyll to see whether the presence of the stabilising co-ordinating copper atom had any pronounced effect.

The dark current obtained was 16 x 10^{-13} amp and the photocurrent 150 x 10^{-13} amp, as compared with dark currents of the order 30 x 10^{-13} amp and photocurrents in the region 1000 - 1500 x 10^{-13} amp using films of natural chlorophyll of approximately the same thickness.

Spectral distribution of photocurrent

TABLE 41.

Region of spectrum (^{o}A)	Photocurrent (x 10^{13} amp)	Corrected photocurrent (x 10^{13} amp)
6500	0	0
6000 - 6500	6	6
5500 - 6000	90	216
4000 - 4500	45	9
3500	1.5	9

Although the actual distribution of photocurrent was the same as for chlorophyll, the total photocurrent was considerably less. Perhaps the stabilising copper atom caused a decrease in the number of current carriers available. There was the usual large drop in photocurrent on admission of oxygen.

Variation of conductivity with temperature

The relationship between conductivity and temperature for a semiconductor is:

$$K = K_o \exp^{-\Delta\epsilon/2kt} \quad \Omega^{-1} cm^{-1}$$

where K is the specific conductivity and $\Delta\epsilon$ the energy gap for intrinsic semiconductivity.

This may be written as:

$$\log_{10} R = \log_{10} R_o + \frac{\Delta\epsilon}{4.606\, kt}$$

where R is the resistance of the specimen in ohms (81, 82). In all the following runs, $\log_{10} R$ was plotted graphically against $\frac{1}{T}$, and was obtained from the slope of the graph and expressed in electron volts.

The temperature was varied using a water bath, as previously described. In all cases, after each heating of the chlorophyll film, there was an irreversible increase in resistance, so that in each successive run on the same specimen, the results lay at a slightly higher resistance than the previous one. This is probably due to slight degradation of the chlorophyll on heating, and for this reason the temperature was never raised much above 50°C.

Cooling runs were used in all cases so that the results obtained were for the specimen in the state of decomposition reached at the maximum temperature. Each reading recorded was the average of two or three taken when the current was stable at any given temperature, i.e. after each reading, the specimen was heated a little and the temperature was allowed to fall back to its previous value, where it was kept steady for several minutes before taking a second reading.

In all cases the applied voltage was 120 volts.

Film 1. Variation of dark current with temperature

TABLE 42.

Temp. (^{o}A)	$\frac{1}{T} \times 10^{3}$	Current ($\times 10^{13}$ amp)	$\log_{10} R$
311.0	3.218	212.0	12.753
310.0	3.225	178.0	12.829
307.5	3.253	126.0	12.979
303.0	3.300	53.6	13.350
298.2	3.354	21.5	13.747
295.7	3.383	13.8	13.939

From the graph (fig. 36A), the plot of $\log_{10} R$ against $\frac{1}{T}$ is a straight line.

$$\text{Slope of the graph} = \frac{0.82}{0.122 \times 10^{-3}} = \frac{\Delta\epsilon}{4.606K}$$

$$K = 1.3805 \times 10^{-16}$$

$$1 \text{ e.v.} = 1.6021 \times 10^{12} \text{ erg.}$$

$$\therefore \quad \Delta\epsilon = \frac{0.82}{0.122 \times 10^{-3}} \times \frac{4.606 \times 1.3805 \times 10^{-16}}{1.6021 \times 10^{-12}}$$

$$= 2.68 \text{ e.v.}$$

i.e. the energy gap for the dark conductivity is 2.68 e.v.

A maximum percentage error of 4 per cent was estimated from the slope of the graph

$$\therefore \quad \Delta\epsilon = 2.68 \pm 0.11 \text{ e.v.}$$

VARIATION OF DARK CURRENT WITH TEMPERATURE

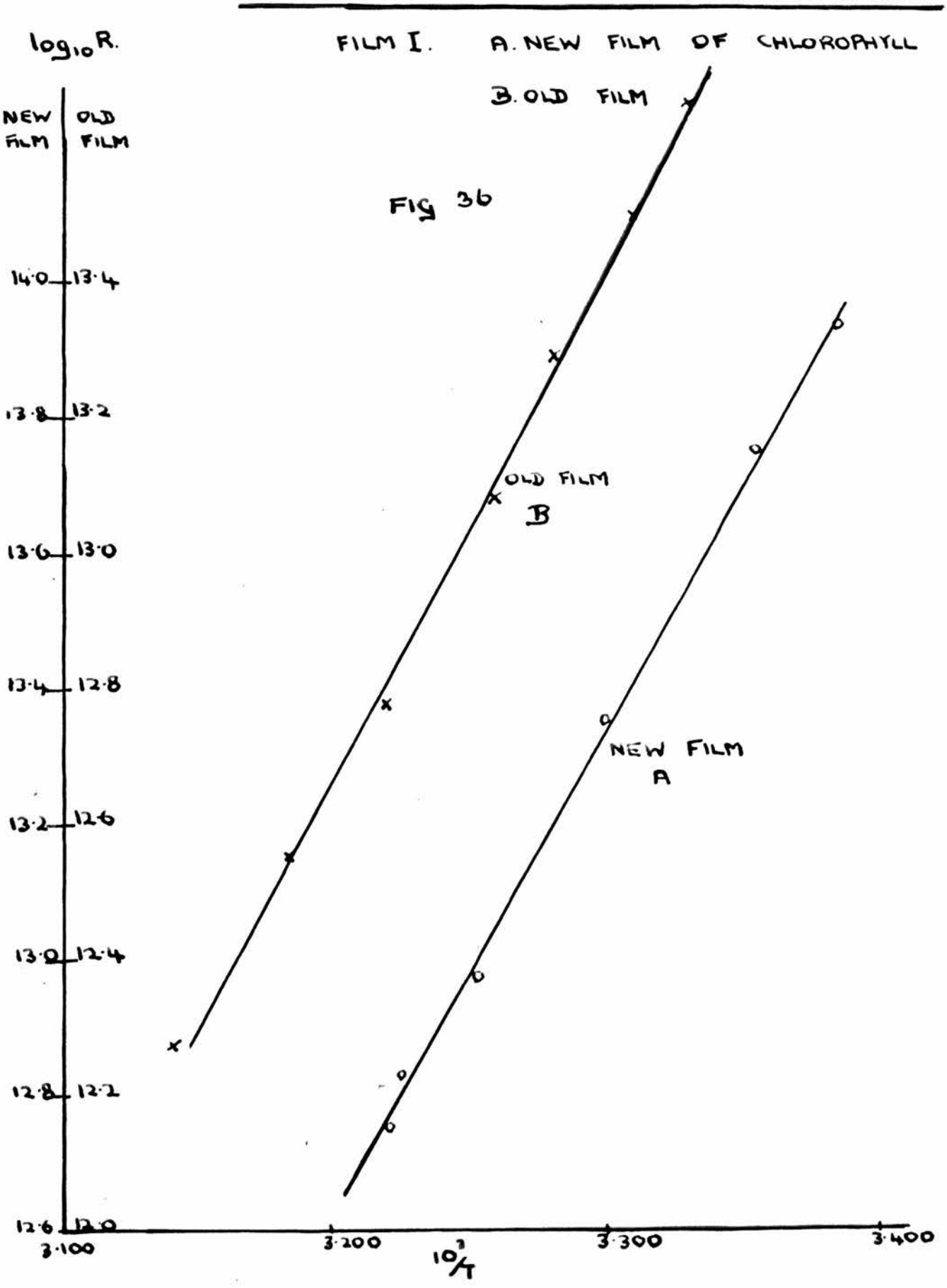


Fig 36

The activation energy E can also be calculated from the graph using the relationship $\sigma = \sigma_0 e^{-E/RT}$ (64) representing the variation of the conductivity of the specimen with temperature.

This may be written as:

$$\log_{10} R = \log R_0 + \frac{E}{2.303RT}$$

$$\therefore \text{Slope of the graph} = \frac{0.82}{0.122 \times 10^{-3}} = \frac{E}{2.303R}$$

$$\therefore E = 30.76\ K \pm 1.2\ K\ cal.$$

An energy 2E is, however, required to excite an electron from the top of the highest filled band to the bottom of the lowest unfilled band (80).

The same experiment was repeated four days later on this film.

TABLE 43.

Temp. (^oA)	$\frac{1}{T} \times 10^3$	Dark current ($\times 10^{13}$ amp)	$\log_{10} R$
318.2	3.143	6.4	12.273
314.1	3.184	3.4	12.548
310.7	3.218	2.0	12.778
307.2	3.256	1.0	13.079
304.8	3.281	0.62	13.287
302.2	3.309	0.37	13.511
300.4	3.329	0.26	13.664

$$\text{Slope of the graph (fig. 36B)} = \frac{1.13}{0.150 \times 10^{-3}} = \frac{\Delta\epsilon}{4.606K}$$

$$\therefore \quad \Delta\epsilon = 2.99 \pm 0.12\ e.v.$$

Only a small difference was found indicating that the energy gap increased as the film degraded. This is thought to be due to increased disorder in the system (81). It has also been suggested that the increased resistance of the specimen is associated with the formation of traps, formed by the decomposition products, which reduce the number of charge carriers. However, in view of the experimental error as shown by the graph, not much difference in slope could be obtained.

Variation of photocurrent with temperature

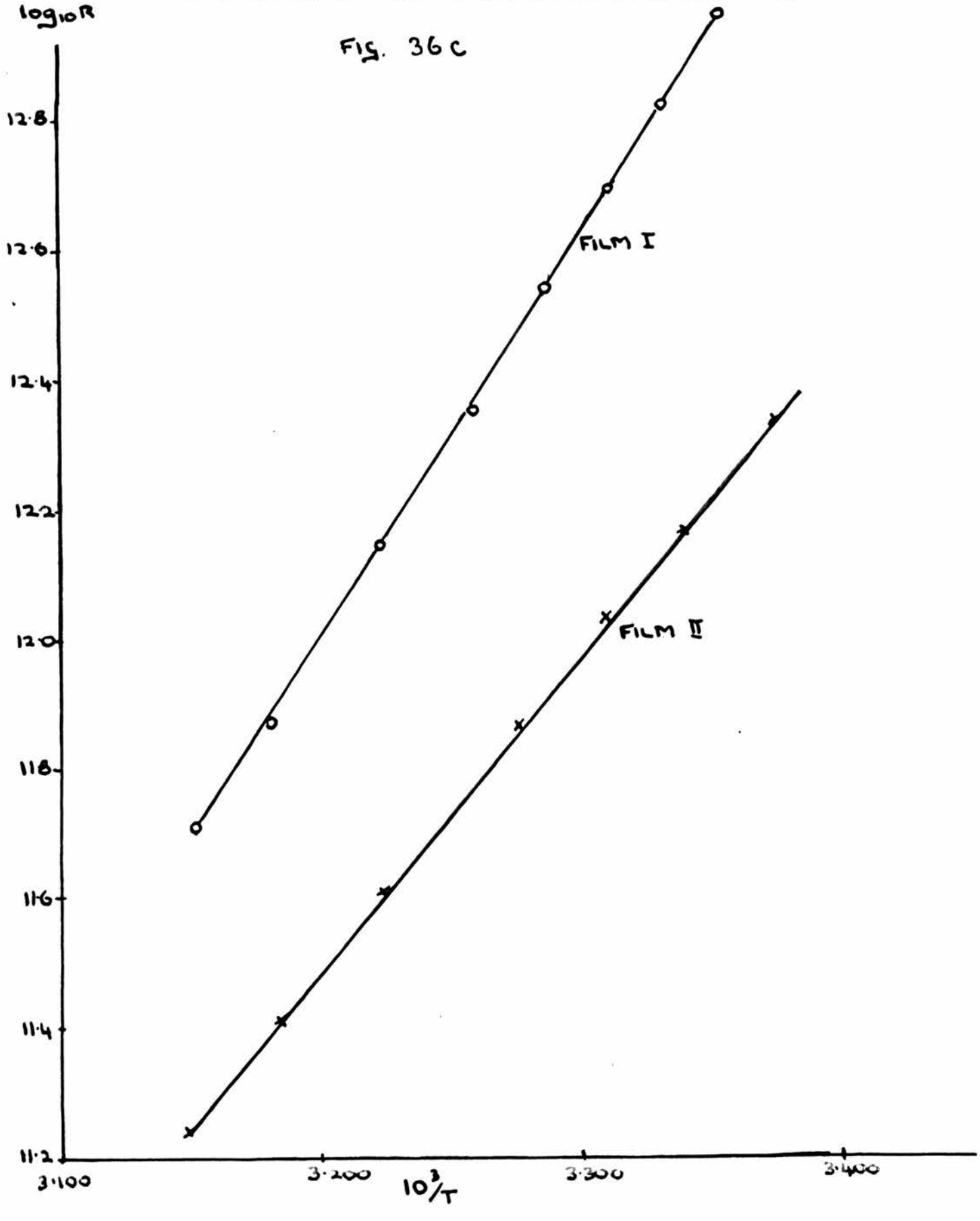
In this case the change in photocurrent with temperature was measured. The photocurrent was first allowed to reach its maximum steady value (after at least 30 min.) and then the temperature was varied as before. After adjusting each temperature, a steady reading was maintained for several minutes.

TABLE 44.

Temp. (^{o}A)	$\frac{1}{T} \times 10^{3}$	Photocurrent ($\times 10^{13}$ amp)	$\log_{10} R$
317.2	3.151	2300	11.717
314.4	3.181	1620	11.870
310.0	3.225	850	12.150
307.0	3.258	530	12.355
304.2	3.287	340	12.545
302.0	3.311	240	12.699
300.2	3.332	180	12.824
298.2	3.354	130	12.965

VARIATION OF PHOTOCURRENT WITH TEMPERATURE

FIG. 36 C



Slope of the graph (fig. 36C) $= \frac{1.0}{0.160 \times 10^{-3}}$ $\therefore$ $\Delta\epsilon =$ 2.48 e.v.

The activation energy E = 28.6 K cal.

This was somewhat lower than the value of $\Delta\epsilon$ obtained for the dark current. Since by this stage the film was certainly to some extent degraded, these experiments were repeated on a new film.

Film 2. Dark current variation with temperature (fig. 37A)

TABLE 45.

	Temp. (^{o}A)	$\frac{1}{T} \times 10^{3}$	current ($\times 10^{13}$ amp)	$\log_{10} R$
1st heating	314.2	3.183	270.0	12.576
	307.7	3.250	125.0	12.982
	303.0	3.301	56.0	13.331
	297.2	3.364	21.0	13.752
2nd heating	311.5	3.210	245.0	12.690
	306.0	3.270	104.0	13.065
	302.7	3.304	56.0	13.331
	298.5	3.350	28.8	13.620
	294.2	3.399	13.5	13.949

1st heating $\Delta\epsilon = 2.60 \pm 0.10$ e.v. E = 29.9 $\pm$ 1.2 K cal.

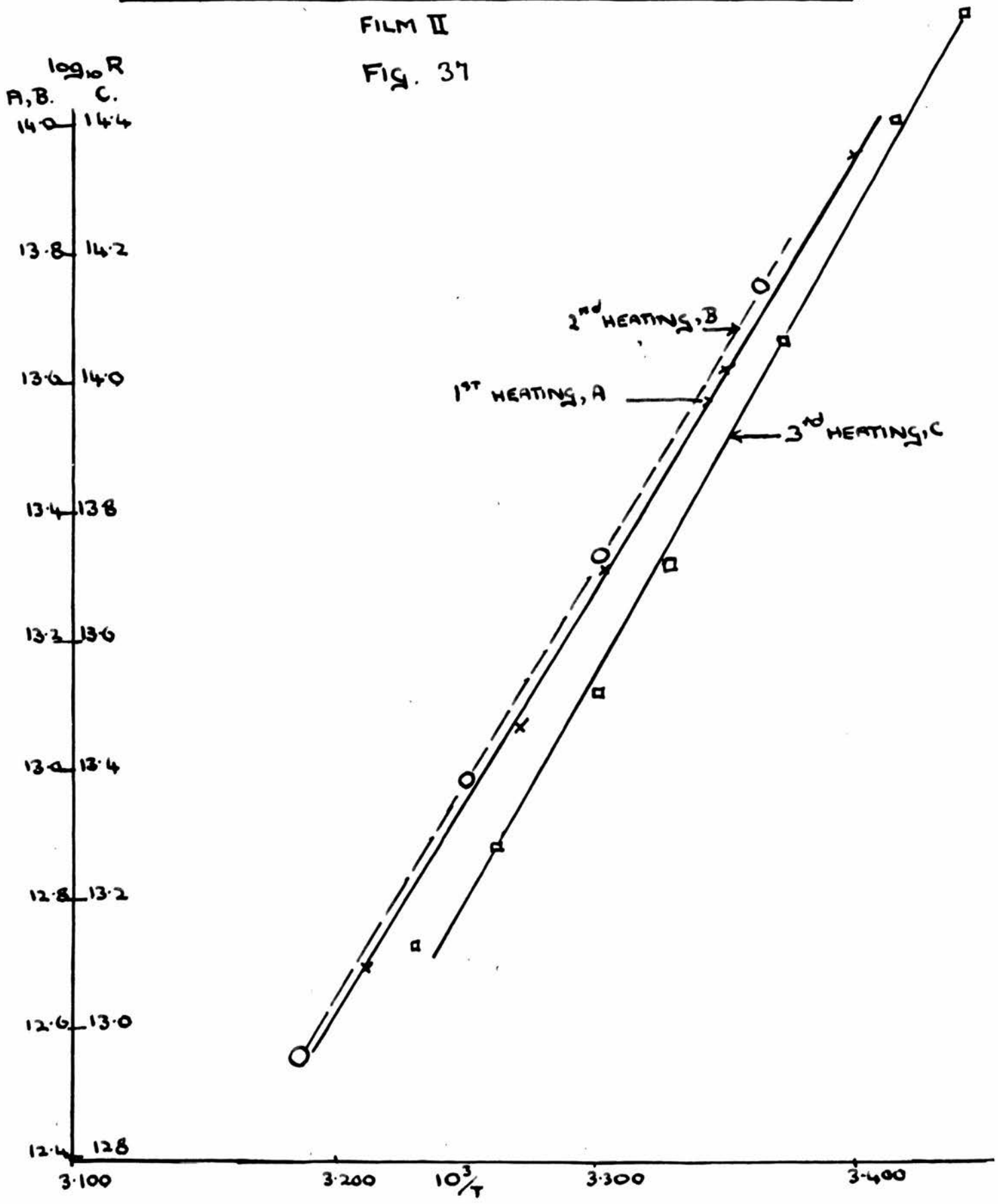
2nd heating $\Delta\epsilon = 2.62 \pm 0.10$ e.v.

Very little change in the energy gap occurred on a second heating although there was the usual slight irreversible increase in resistance.

VARIATION OF DARK CURRENT WITH TEMPERATURE.

FILM II

Fig. 37



Variation of photocurrent with temperature (fig. 36C)

TABLE 46.

Temp. (^{o}A)	$\frac{1}{T} \times 10^{3}$	Photocurrent ($\times 10^{13}$ amp)	$\log_{10} R$
317.5	3.150	6890	11.241
314.0	3.185	4600	11.416
310.0	3.225	2950	11.689
305.2	3.276	1620	11.870
302.2	3.309	1120	12.030
299.0	3.344	820	12.166
296.3	3.375	220	12.342

$\Delta\epsilon = 1.71 \pm 0.07$ e.v.

This value was considerably lower than that obtained for the previous film, due to the fact that this film was freshly prepared. The experiment was repeated and a value of $\Delta\epsilon = 1.52 \pm 0.06$ e.v. was obtained. The activation energies were calculated to be 19.7 ± 0.8 and 17.5 ± 0.7 K cal. respectively.

The film was heated once more, and a third value of $\Delta\epsilon = 2.64$ e.v. was obtained. A considerable increase in the resistance of the sample was observed in this case. A fourth heating gave $\Delta\epsilon = 2.78$ e.v. for the dark current and 2.00 e.v. for the photocurrent, showing that degradation of the film was occurring.

These experiments were repeated on a third film.

	Dark current	Photocurrent
1st heating	$\Delta\epsilon$ = 2.61 ± 0.10 e.v.	$\Delta\epsilon$ = 1.66 ± 0.66 e.v.
2nd heating	$\Delta\epsilon$ = 2.65 ± 0.11 e.v.	$\Delta\epsilon$ = 1.76 ± 0.70 e.v.

Average values of = 30.2 ± 1.2 K cal. and 19.7 ± 0.8 K cal.

The results for the three films can be summarised as follows:

Dark current	Freshly prepared film	Degraded film
1st film	$\Delta\epsilon$ = 2.68 e.v.	2.99 e.v.
2nd film	2.60	2.78 e.v.
	2.62	
	2.64	
3rd film	2.61	
	2.65	

The average value of $\Delta\epsilon$ for the dark current variation with temperature is $\Delta\epsilon$ = 2.63 ± 0.11 e.v. with a corresponding activation energy of 30.2 ± 1.2 K cal.

Photocurrent	Freshly prepared film	Degraded film
1st film		2.48 e.v.
2nd film	$\Delta\epsilon$ = 1.71 e.v.	2.00 e.v.
	1.52	
3rd film	1.66	
	1.76	

The average value of $\Delta\epsilon$ for the photocurrent variation with temperature is 1.66 ± 0.07 e.v. The corresponding value of E = 19.1 ± 0.76 K cal.

It can be concluded from these results that the energy gap involved is always less for the photocurrent than for the dark current. It appears that photoconductivity proceeds via some state of lower energy than dark current semiconductivity. This at once suggests that photoconductivity is associated with energy transfer in the triplet state of chlorophyll.

Conductivity of films of pheophytin

A film of pheophytin was prepared in the same manner as for chlorophyll, and the rise and decay of photocurrent with time were recorded as below:

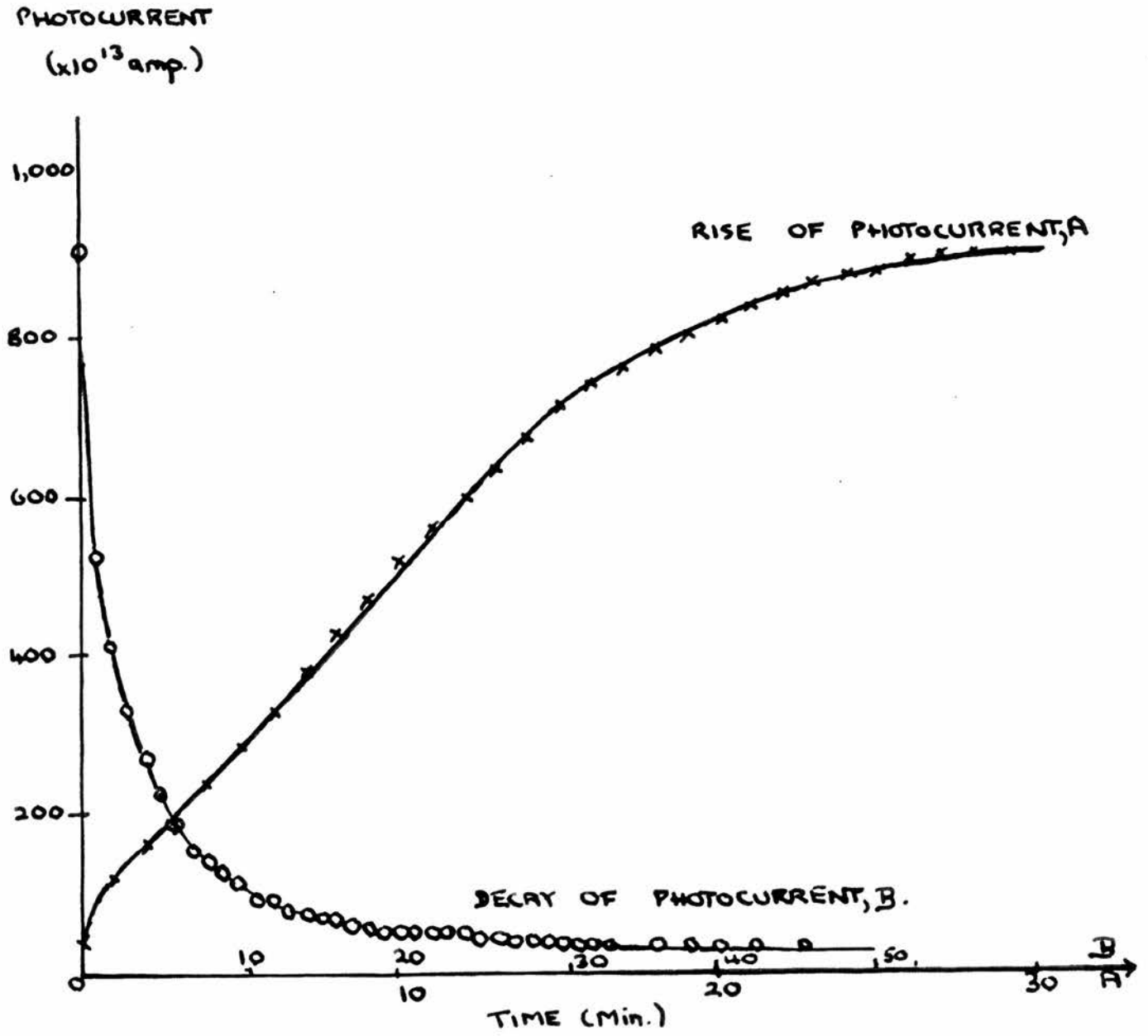
Rise of photocurrent with time (fig. 38A)

TABLE 47.

Time (Min.)	Current ($\times 10^{13}$ amp)	Δc	Time (Min.)	Current ($\times 10^{13}$ amp)	Δc
0	37		15	710	32
1	120	83	16	740	30
2	160	40	17	760	20
3	192	32	18	782	22
4	232	40	19	804	22
5	282	50	20	824	20
6	325	43	21	840	16
7	380	55	22	856	16
8	428	48	23	870	14
9	473	45	24	880	10
10	515	42	25	890	10
11	560	45	26	898	8
12	600	40	27	902	4
13	640	40	28	904	2
14	678	38	29		
			30	steady	

RISE AND DECAY OF PHOTOCURRENT FOR A FILM OF PHEOPHYTIN

Fig. 38



The Shape of the photocurrent/time plot was slightly different from that for chlorophyll. There was an initial fast response when the current probably changed faster than the pointer could move, followed by a constant rate period up to 15 min., then a deceleration until a constant steady value was maintained. This plot showed some resemblance to that obtained for degraded chlorophyll.

Decay of photocurrent with time (fig. 38B)

TABLE 48.

Time (Min.)	Current (x 10^{13} amp)	Decay (x 10^{13} amp)	Time (Min.)	Current (x 10^{13} amp)	Decay (x 10^{13} amp)
0	904		23	42.0	862
1	520	384	24	40.0	864
2	410	494	25	38.0	866
3	336	568	26	37.0	867
4	274	630	27	36.0	868
5	224	680	28	35.0	869
6	190	714	29	34.0	870
7	162	742	30	33.0	871
8	140	764	31	32.5	871.5
9	123	781	32	32.0	872
10	110	794	33	31.5	872.5
11	93	811	34	31.0	873
12	87	817	35		
13	79	825	36	30.4	873.6
14	72	832	37		
15	67	837	38	30.0	874
16	62	842	39		
17	58	846	40	29.8	874.2
18	54	850	41	29.6	874.4
19	51	853	42	29.5	874.5
20	48.2	855.8	43		
21	46.0	858	44	29.4	874.6
22	44.0	860	45	29.3 steady	874.7

There was a deceleration until a constant value was finally reached for the dark current after about 45 minutes. However, this decay was not exponential, nor was the decay current proportional to the reciprocal of time.

Aged films of pheophytin showed an even bigger fast response on illumination followed by the constant rate period. The maximum photocurrent was much diminished and was reached in a shorter time, i.e. approximately 20 minutes.

Both the dark current and photocurrent were found to be proportional to the applied voltage. The spectral distribution of photocurrent was similar to that for chlorophyll.

Film 1. Variation of dark current with temperature (fig. 39A)

TABLE 49.

Temp. (^{o}A)	$10^{3}/_{T}$	Dark current ($\times 10^{13}$ amp)	$\log_{10} R$
308.6	3.240	270	12.648
306.3	3.265	208	12.761
302.3	3.309	117	13.011
300.3	3.331	84	13.143
296.5	3.378	46	13.416
295.8	3.381	40.8	13.469

Slope of the graph = $\frac{0.8}{0.142 \times 10^{-3}}$ $\Delta\epsilon = 2.20 \pm 0.09$ e.v.

Activation energy E = 25.8 ± 1.0 K cal.

VARIATION OF DARK AND PHOTOCURRENT WITH TEMPERATURE

FOR A FILM OF PHEOPHYTIN. FILM I

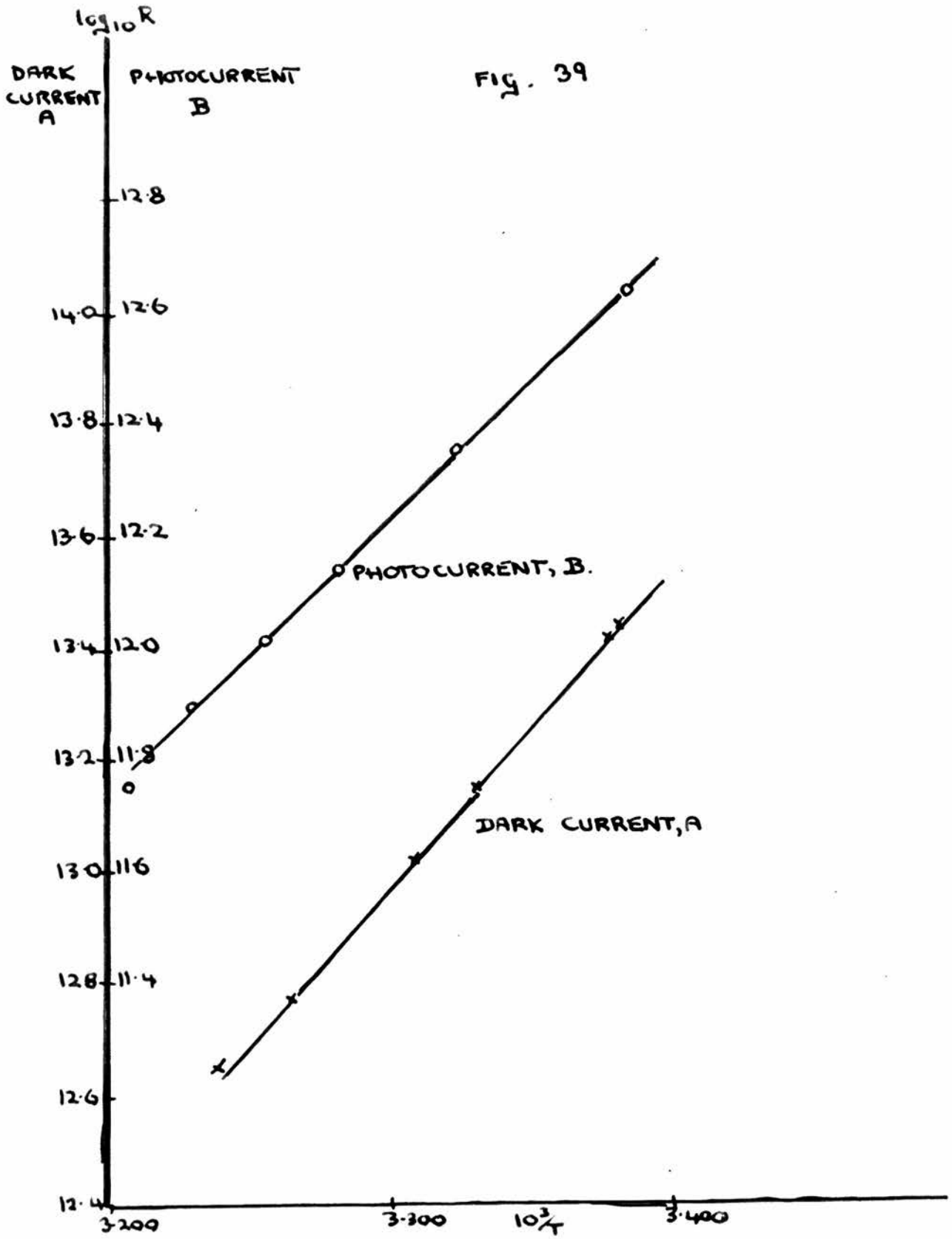


FIG. 39

Variation of photocurrent with temperature (fig. 39B)

Table 50.

Temp. (^{o}A)	$\frac{10^3}{T}$	Photocurrent ($\times 10^{13}$ amp)	$\log_{10} R$
311.7	3.207	2160	11.745
309.5	3.230	1540	11.892
307.2	3.256	1180	12.007
304.8	3.281	880	12.135
301.0	3.322	540	12.347
295.5	3.386	280	12.632

$$\text{Slope} = \frac{0.75}{0.14 \times 10^{-3}} \qquad \Delta\epsilon = 2.13 \pm 0.09 \text{ e.v.}$$

$$E = 24.5 \pm 1.0 \text{ K cal.}$$

For pheophytin, the energy gap appeared to have practically the same value for both the dark and photocurrent variations with temperature.

These experiments were repeated on the same film three weeks later when it had undergone considerable degradation.

The $\log_{10} R/\frac{1}{T}$ plots tended to become non-linear at high temperatures, probably because pheophytin had undergone considerable decomposition. The resistance of the specimen was also greatly increased.

$\Delta\epsilon$ was 3.36 e.v. for the dark current and 2.89 e.v. for the photocurrent for these degraded films.

The variations of dark and photocurrent with temperature were measured once more on a second freshly prepared film.

Film 2. Dark current (fig. 40A)

TABLE 51.

Temp. (^{o}A)	$10^{3}/_{T}$	Dark current (x 10^{13} amp)	$\log_{10}$ R
312.8	3.197	734	12.213
310.0	3.225	538	12.348
307.5	3.253	385	12.494
303.8	3.293	278	12.721
300.7	3.326	132	12.959
299.2	3.342	104	13.062
297.8	3.357	81	13.171

$$\text{Slope} = \frac{1.1}{0.18 \times 10^{-3}} \qquad \Delta\epsilon = 2.42 \pm 0.10 \text{ e.v.}$$

$$E = 30.0 \pm 1.2 \text{ K cal.}$$

Photocurrent (fig. 40B)

TABLE 52.

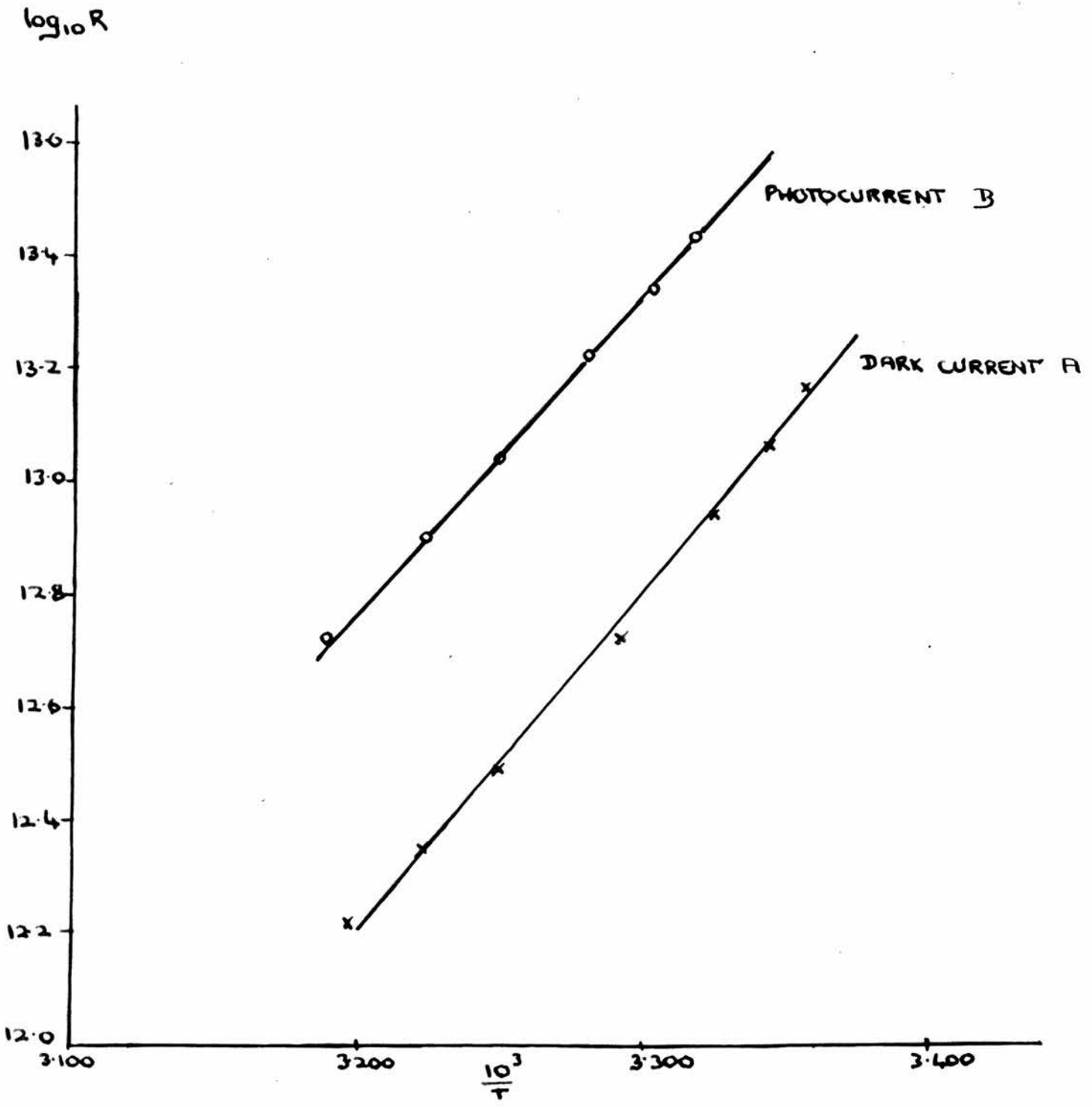
Temp. (^{o}A)	$10^{3}/_{T}$	Dark current (x 10^{13} amp)	$\log_{10}$ R
313.5	3.190	2280	11.721
310.0	3.225	1500	11.903
307.6	3.251	1100	12.038
304.8	3.281	720	12.222
302.8	3.303	540	12.347
301.5	3.216	430	12.446

$\Delta\epsilon = 2.27 \pm 0.09$ e.v. $\qquad E = 26.1 \pm 1.0$ K cal.

VARIATION OF DARK AND PHOTOCURRENT WITH TEMPERATURE

FILM II (PHEOPHYTIN)

Fig 40



These results agree very well with those obtained for the first film. It appears that both the dark current and the photocurrent proceed via the same energy level in pheophytin.

Oxidation of a film of pheophytin

For a freshly prepared film, the dark current was 25×10^{-13} amp and the photocurrent 187×10^{-13} amp. On admission of 50 mm oxygen there was an immediate drop in photocurrent to 110×10^{-13} amp, and then a continual gradual fall to 32×10^{-13} amp during the next 5 hours. The initial drop was not nearly so great as that obtained for chlorophyll under the same conditions.

On evacuation the photocurrent rose to 62×10^{-13} amp and the dark current was 10.8×10^{-13} amp, showing that destruction of part of the film had occurred (fig. 41).

The oxidised film was extracted in methanol and gave a strong hydroperoxidic reaction with ferrous thiocyanate, proving that oxidation had occurred. In contrast to films of chlorophyll illuminated in oxygen, oxidation of pheophytin was accompanied by a progressive drop in photocurrent (fig. 41).

Spectroscopic measurements

It has been suggested that the energy gap for semiconductivity is associated with the "absorption edge" found in the solid state spectra of organic materials (82, 83). The solid state spectrum

CHANGE IN PHOTOCURRENT ON ILLUMINATION OF A FILM OF PHEOPHYTIN IN OXYGEN

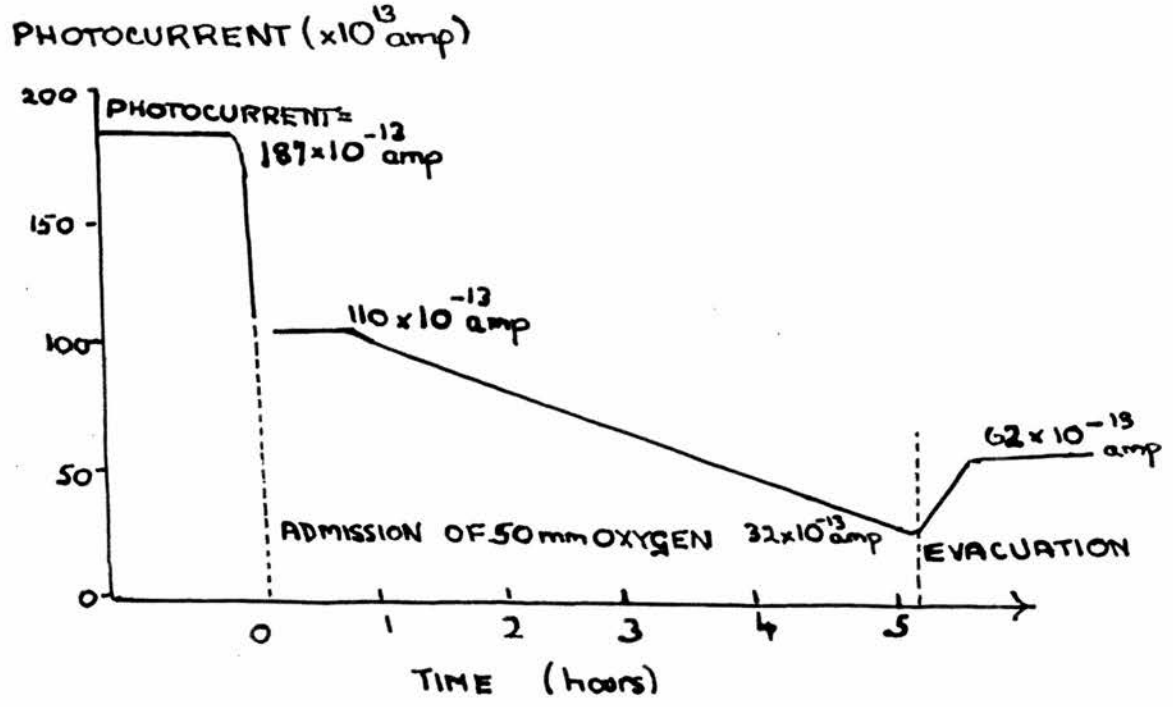


FIG. 41

$\frac{n}{2}^{th}$ and $\left(\frac{n}{2}+1\right)^{th}$ LEVELS FOR THREE CONJUGATED MOLECULES

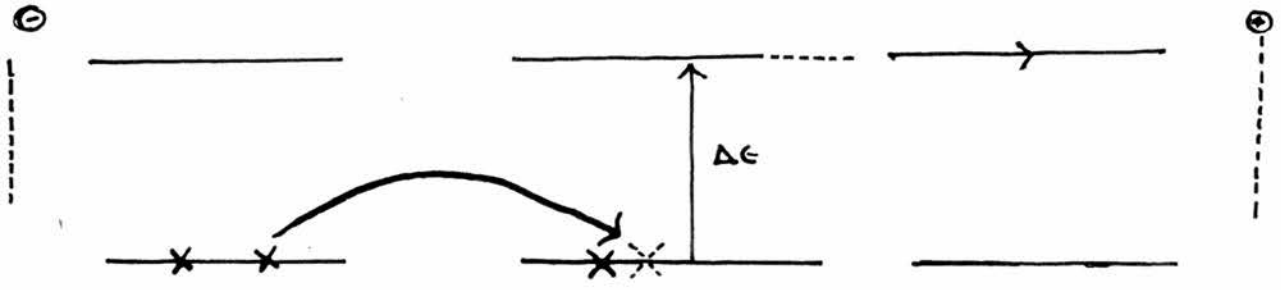


FIG 42

of chlorophyll shows an absorption edge at approximately $5000^{\circ}A$.

The energy quantum $h\nu$ associated with this wavelength can be calculated as follows:

$$1 \text{ e.v.} = 1.6021 \times 10^{-12} \text{ erg.}$$

$$h = 6.6254 \times 10^{-27} \text{ erg sec.}$$

$$\lambda = 5000^{\circ}A = 5000 \times 10^{-8} \text{ cm.}$$

Velocity of light $= 2.998 \times 10^{10}$ cm sec^{-1}

$$\therefore \nu = \frac{2.998 \times 10^{10}}{5000 \times 10^{-8}}$$

and the energy $h\nu$ associated with a wavelength of

$$5000^{\circ}A = \frac{6.6254 \times 10^{-27}}{1.6021 \times 10^{-12}} \times \frac{2.998 \times 10^{10}}{5000 \times 10^{-8}}$$

$$\simeq 2.5 \text{ e.v.}$$

This is close to the value of 2.63 e.v. obtained for the dark current conductivity of chlorophyll.

Discussion

Conductivity of thallous bromide

This work has shown that films of thallous bromide exhibit primary photoconductivity when illuminated with light in the visible and near ultra violet regions of the spectrum, i.e. in the "tail end" of its absorption spectrum. The films showed photoconductivity immediately on illumination, indicating that the formation of F-centres in the crystal prior to illumination was not necessary. There was an instantaneous rise in photocurrent and saturation was reached within the first minute. Similarly, the original dark current was reached within a minute after illumination had ceased.

It was found in all cases that both the dark and photocurrents were proportional to the applied voltage. The electrons released by a quantum of light drift a certain distance towards the anode before being trapped, and this effect was recorded on the electrometer. Suppose the distance between the electrodes is 'd' and an electron of charge e moves a distance x before being trapped. Had the electron moved right across the film, then the charge recorded would be e. Therefore for a distance x the measured charge will be $\frac{ex}{d}$. Now for weak fields, the distance x that the electron moves before being trapped is proportional to the applied voltage. However, if the field is increased indefinitely, the photocurrent will reach a maximum given by $\frac{nex}{d}$ where n is the number of electrons released. If the voltage is large enough, then the saturation current will be ne.

For thallous bromide films, the maximum photocurrent was proportional to the intensity of the incident light. According to Mott and Gurney (77) this indicates that the excited electron is captured at some crystal imperfection or other form of trap so that it is in a state of higher energy than it was originally. The crystal tends to be left in a metastable condition after illumination has ceased, and the total current passing is proportional to the light absorbed.

On admission of oxygen to the system, the photocurrent diminished slightly. An electron transfer between a semiconductor and an adsorbed gas is reflected by a change in conductivity. Thus a decrease in the photoconductivity of thallous bromide on the adsorption of oxygen implies electron transfer from the semiconductor to the gas causing ionisation of the oxygen on the surface of the film:

$$O_2 + 2\epsilon \rightarrow 2O'$$

Removal of electrons causes a decrease in the number of charge carriers with a consequent fall in conductivity.

Since oxygen has no tendency to form positive ions, the reverse process, i.e. transfer of electrons from the adsorbed oxygen to the thallous bromide thus filling up positive holes in the semiconductor, does not apply.

One can therefore conclude that thallous bromide is an n-type semiconductor under these conditions. This is to be expected since it was prepared in the presence of excess thallium (see experimental methods, Part I) and should therefore contain an excess of interstitial thallium ions.

The polarization effects shown by thallous bromide after a direct current has flowed through the sample for some time have already been discussed. Polarization always occurs when an ionic solid is placed under the influence of an applied field due to distortion of the electronic structure of the crystal.

One may conclude from these experiments that the thallous bromide as prepared is an n-type semiconductor showing primary photo-conductivity on illumination in the blue and the near ultra violet.

Conductivity of chlorophyll

The conductivity of chlorophyll appears to be a more complex phenomenon than that of thallous bromide and the observed results are therefore more difficult to explain. Eley and his co-workers (76) have concluded that semiconductivity in the porphyrin series is associated with the mobile π electrons of the conjugated double bond system rather than the presence of electronegative nitrogen, since this effect is observed in substances of the anthracene type, but not in some nitrogen-containing compounds.

No explanation has been found for the slow increase in photo-current, the maximum saturation current being reached after 25 minutes. Decay back to the original value took approximately the same time after illumination had ceased. Nelson (71) has reported that for ethyl chlorophyllide films there were two time constants of response. The fast response was less than one second and the slow response such that a steady state was reached in 10-15 minutes, the two being easily

distinguishable. This effect was observed in the present work for films of pheophytin, and also to a lesser extent for degraded films of chlorophyll. However, for freshly prepared films of chlorophyll there was no such effect, there being an acceleration in the rate of change of photocurrent up to a maximum rate 8 minutes after the commencement of illumination, followed by a deceleration in this rate until the saturation current was achieved after approximately 25 minutes. That this current was proportional to the intensity of absorbed light was confirmed by Nelson.

For freshly prepared films, the decay of photocurrent was found to be proportional to $\frac{1}{t}$ where t is the time that has elapsed since illumination ceased. An analagous relationship was found between the decay of the phosphorescence of certain inorganic compounds and the reciprocal of time: Randall and Wilkins found that the intensity of phosphorescence emission was proportional to $\frac{1}{t}$ where t was the extent of the decay period (78). They explained this delayed emission by assuming that the electron spends a certain amount of time in traps or metastable states before returning to the ground state. Now the intensity of phosphorescence emission is due to the release of electrons from traps at a certain depth and is proportional to the rate of release of electrons from these traps.

But the rate of release of an electron from a trap $\propto$ $\frac{1}{\text{time electron spends in the trap}}$.

When there is a continuous distribution of trap depths, at time t during phosphorescence, most of the light emission is due to traps in

which an electron spends a mean time 't', since the shallower traps are nearly all emptied by this time and the deeper traps release electrons too slowly to contribute much to the phosphorescence. Therefore, if there is an equal number of traps at all depths, phosphorescence $\propto \frac{1}{t}$. An exactly similar explanation can account for the fact that decay of photocurrent occurs inversely with time. In freshly prepared films where decay $\propto \frac{1}{t}$ it can be assumed that there is a uniform distribution of trap levels. However, this relationship does not hold for degraded films or for pheophytin. If the traps are not uniformly distributed, then the photocurrent decays as an inverse power of $t > 1$

The most surprising results were obtained on investigation of the spectral sensitivity of the distribution of photocurrent. The main bulk of the photocurrent was invariably caused by light of wavelength 5500-6000°A which does not coincide at all with the maxima at 4300 and 6600°A found for the absorption spectra of chlorophyll solutions. Even taking into account the shift of 800°A towards the red found in the absorption spectra of unimolecular films of the substance (79) the maximum photocurrent should fall in the region of 5000°A, and not between 5500 and 6000°A.

The most likely explanation is that this observation is connected with the solid-state triplet state absorption spectrum of chlorophyll. It seems likely that chlorophyll photoconducts by means of its lowest excited state, i.e. the first triplet level. It is therefore to be expected that the spectral distribution of photocurrent would bear more relation to the triplet state absorption spectrum of chlorophyll

than to that of its ground state. The maximum absorption of the triplet state in solution occurs between 4000 and 5000°A, which would shift to between 5000 and 6000°A in the solid state.

Nelson (71) states that the action spectrum for ethyl chlorophyllide does bear a resemblance to its absorption spectrum in solution, only with a great distortion of relative frequencies and a displacement towards the red. He says that absorption in the blue and the red is weakened relative to that in the green because absorption is so great in these regions that light is only absorbed closely adjacent to the surface. The effect per photon absorbed is thus relatively small due to a higher rate of recombination of the electrons and positive holes formed because of their higher concentration and proximity. It is, however, doubtful whether this explanation alone could account for the vast majority of the photoconductivity occurring in the green.

The very large decrease in photocurrent observed on admission of oxygen to the system was interesting. It is well known that interaction of excited pigment molecules with oxygen can quench the triplet state, frequently with the formation of addition compounds. If photoconductivity does in fact proceed via the triplet state, then shortening of the mean life of the metastable state would inevitably reduce the photocurrent. This interaction resulted finally in photooxidation because methanolic extracts of the illuminated film gave a hydroperoxidic reaction with the reagent.

The quenching action of oxygen on the metastable state of chlorophyll makes it necessary to use a photoconducting substrate to obtain a measurable rate of oxygen uptake when oxidising films of

solid chlorophyll. When adsorbed on the glass walls of the reaction vessel, the pigment oxidises only at an extremely slow rate. This is probably because the oxygen quenches the triplet state to a considerable extent. However, when a sensitising substrate such as thallous bromide is present, continual transfer of excitation energy can occur from the substrate to the chlorophyll so that the number of molecules in the metastable state is constantly replenished. Because of their longer mean life, these activated pigment molecules have more chance of encountering oxygen molecules and the oxidation proceeds at a measurable rate.

It was of importance in this connection that the photoconductivity of films of chlorophyll deposited on thallous bromide in the presence of oxygen did not drop by more than the amount observed for thallous bromide alone.

The drop in the photoconductivity of pheophytin on admission of oxygen was not so great as for chlorophyll under similar conditions, and during illumination there was a steady decrease in photo-conductivity instead of a steady value being maintained as was the case for chlorophyll. This fall was probably more rapid for pheophytin because it oxidises more readily than chlorophyll due to the decreased conjugation resulting from the removal of magnesium from the molecule.

Theory of the energy gap

Conductivity is associated with the π electrons of the aromatic

nucleus. According to Eley (80) for a molecule of n atoms there are n π-electrons and n levels, each of which contain two electrons. The highest filled level is the $\frac{nth}{2}$ level and the lowest unfilled level is the $(\frac{n}{2} + 1)^{th}$ level. In order to transport an electron into the excited state, it is therefore necessary to excite a single electron thermally or optically from the $\frac{nth}{2}$ to the $(\frac{n}{2} + 1)^{th}$ level.

In a crystal or a solid film, these levels merge into bands and an energy 2E is required to excite an electron from the top of the highest filled band to the bottom of the lowest unfilled band. E is defined by the equation $\sigma = \sigma_0 \exp^{-E/RT}$ which represents the variation of conductivity σ with temperature T. The current is carried by means of electrons in the upper band and possibly positive holes in the top of the lower filled band.

Fig. 42 shows the $\frac{n}{2}^{th}$ and $(\frac{n}{2} + 1)^{th}$ levels for three conjugated molecules. On absorption of a photon $h\nu$ (or by thermal excitation) an electron is excited into the empty level, and may tunnel through the energy barrier into the neighbouring molecule causing electronic conductivity. The positive hole remaining in the filled $\frac{n}{2}^{th}$ level may also move towards the cathode. The mobility of the excited electron will depend on the ease of tunnelling through the potential barrier between adjacent molecules. The positive hole conductivity may be confined simply to the particular molecule where it is formed.

$\Delta\epsilon$ represents the energy required to transport an electron from the highest filled to the lowest unfilled level, which may be an excited singlet or a triplet state. The photo-excitation of electrons into the conduction band can take place with any quantum of energy

greater than 3 e.v. giving rise to electrons and positive holes and if these migrate together they constitute an exciton capable of transferring energy.

The energy gaps determined for several films of freshly prepared chlorophyll agreed very well and were of the order 2.6 e.v. This value agrees very well with those obtained by other workers for substances of this type, e.g. natural haemoglobin 2.66 e.v., denatured haemoglobin 2.89 e.v. (82). These values are higher than those found for cyclic hydrocarbons, possibly because in the case of chlorophyll the phytyl part of the molecule may have the effect of a non-polar diluent in the film and thus interfere with the movement of charge carriers. The overall result is a lowering of conductivity. On ageing, both the resistance and the energy gap of the films increased, since degradation caused increased disorder in the arrangement of the molecules with consequent reduction in conductivity. This increase in the energy gap on degradation is also observed in haemoglobin, where denaturation has been observed to raise $\Delta\epsilon$ from 2.63 e.v. to 2.89 e.v.

The values of $\Delta\epsilon$ obtained for the photoconductivity were very much lower, the average value being 1.6 e.v. This is in accordance with results reported by Vartanyan who studied the d.c. dark and photoconductivity of layers of many dyes deposited from solution onto the gap between metallic electrodes. He found that $\Delta\epsilon$ was generally much lower for photoconductivity measurements than that calculated from the temperature dependance of dark current (83). The most likely explanation is that pigments photoconduct by means of their

triplet states which lie at a lower level than the first excited singlet state. This hypothesis is supported by the results of flash photolysis measurements on chlorophyll preparations. On exposure to an intense flash of light, chlorophyll is produced in the metastable triplet state (84) and would therefore presumably photoconduct from this level. The normal ground state absorption of these pigments is not observed immediately after a photolytic flash due to depopulation of the ground state.

It was surprising that while chlorophyll showed decreased values for photoconductivity, pheophytin did not, the $\Delta\epsilon$ values being almost the same for both dark current and photocurrent variation with temperature. Flash spectroscopy again offers an explanation for this. Solutions of pheophytin exhibit a reverse effect to chlorophyll, for illumination by an intense flash does not result in depopulation of the ground state (84), so that it could be predicted that photo-conductivity and dark conductivity would proceed by the same excited level on increasing the temperature.

Vartanyan (83) and Eley (82) both point out that the wavelength λ corresponding to $\Delta\epsilon$ is frequently close to the wavelength of the long-wave absorption edge of the solid dye. For chlorophyll $\lambda \simeq 5000^{\circ}A$ giving a $\Delta\epsilon$ value of 2.5 e.v., which is close to the calculated dark current value of 2.63 e.v.

This work raises once more the fundamental question asked by Szent-Györgyi as to whether semiconductivity may not underlie the electronic mobility present in certain aspects of living systems (85). It appears to play a major role where a photon is involved in the

primary process causing electronic excitation. This applies particularly to substances containing complex systems of conjugated double bonds, such as chlorophyll in photosynthesis and rhodopsin in vision. It has already been firmly established that energy can transfer from one excited molecule to another over considerable distances, and the study of the photo- and semiconductivity of chlorophyll is therefore of vital importance to the understanding of the complex mechanism of photosynthesis.

Bibliography

Part I

1. Fischer and Wenderoth. Annalen 537 170 (1939).
2. Woodward. J.A.C.S. 82 3800 (1960).
3. Zscheile and Comar. Bot. Gaz. 102 463 (1940).
4. Conant, Hyde, Moyer and Dietz. J.A.C.S. 53 359 (1931).
   Conant, Dietz, Bailey and Kammerling. J.A.C.S. 53 2382 (1931).
5. Rabinowitch, "Photosynthesis" (Interscience 1945) Vol. I, Chapter 16, p. 461.
6. Rabinowitch, loc. cit., Vol. II, Part II, Chapter 37B, p. 1773.
7. Fischer and Pfeiffer. Annalen 555 94 (1944).
8. Dunicz, Thomas, Van Pee and Livingston. J.A.C.S. 73 3388 (1951).
9. Weller. J.A.C.S. 76 5819 (1954).
10. Rabinowitch and Weiss. P.R.S. A162 251 (1937).
11. Strain in "Functions and properties of chloroplast pigments" in "Photosynthesis in Plants" (1949), pp. 133-178.
12. Ashkinazi, Glikman and Dain - Compt. Rend. (Doklady). Acad. Sci. U.S.S.R. 73 743 (1951).
13. Jablonski - Z. Physik. 94 38 (1935).
14. Lewis and Kasha. J.A.C.S. 66 2100 (1944).
15. Lewis and Calvin. J.A.C.S. 67 1232 (1945).
16. Kasha and Becker. J.A.C.S. 77 3669 (1955).
17. Livingston, Porter and Windsor. Nature 173, 485 (1954).
18. Livingston and Ryan. J.A.C.S. 75 2176 (1953).

19. Livingston and Knight. J. Phys. Chem. 54 703 (1950).

20. Krasnovsky and Vojnovskaya. Compt. Rend. (Doklady) Acad. Sci. U.S.S.R. 73 1239 (1951).

21. Calvin and Dorough. J.A.C.S. 70 699 (1948).
Calvin. 71 4024, 4031 (1949).

22. Weiss. J. Soc. Dyers and Colourists 65 135 (1949).

23. Krasnovskii. Doklady Akad. Nauk. S.S.S.R. 60 421 (1948).

24. Evstigneev and Gavrilova. Doklady Akad. Nauk. S.S.S.R. 92 381 (1953), 95 381 (1954), 103 97 (1955).

25. Livingston, Watson and McArdle. J.A.C.S. 71 1542 (1949).

26. Evstigneev, Gavrilova and Krasnovskii. Doklady Akad. Nauk. S.S.S.R. 70 261 (1950).

27. Livingston and Weil. Nature 170 750 (1952).
Livingston, Watson and McArdle. J.A.C.S. 71 1542 (1949).
Fernandez and Becker. J. Chem. Phys. 31 467 (1959).

28. Becker and Kasha. J.A.C.S. 77 3669 (1955).

29. Lonie. Ph.D. Thesis, Edinburgh 1950.
Lawrie. " " " 1952.
McFarlane. " " " 1950.

30. Wager. P.R.S. B87 386 (1913).

31. Sunners. Ph.D. Thesis, Edinburgh 1957.

32. Zscheile and Comar. Bot. Gaz. 102 463 (1940).
Comar and Zscheile. Plant Physiol. 17 198 (1942).

33. Griffiths and Jeffrey. Ind. Eng. Chem. Anal. Ed. 17 448 (1945).
Le Rosen. Ind. Eng. Chem. Anal. Ed. 14 165 (1942).

34. Willstatter. Annalen 354 205 (1907).

35. Willstatter and Stoll. Annalen 378 18 (1911).
36. Willstatter. Annalen. 387-8, 332 (1912)
37. Hans Fischer. Chem. Review 20 65 (1937).
38. Lawrie. Ph.D. Thesis, Edinburgh 1952.
39. Edgerton et al. Analytica chimica Acta 10 422 (1954).
40. Ovensten and Rees. Analyst 75 204 (1950).
Kotanur and Jelling. J.A.C.S. 63 1432 (1941).
41. Young, Vogt and Nieuwland. Ind. Eng. Chem. Anal. Ed. 8 198 (1936).
42. Cartlidge and Tipper. Analyt. Chim. Acta 22 106 (1960).
43. Cumming and Kay, "Quantitative Chemical Analysis" (10th Ed.), p. 273.
44. Climie. Ph.D. Thesis, Edinburgh, 1956.
45. McLean. Ph.D. Thesis, Edinburgh, 1959.
46. Taylor. Can. J. Chem. 36 1213 (1958).
47. Sidorov and Terenin, "Optics and Spectroscopy", Vol. viii, No. 4, 254 (1960).
48. Hubacher. Anal. Chem. 21 945-7 (1949).
49. Holt and Jacobs. Plant Physiol. 30 553 (1955).
50. Bellamy, "The Infra Red Spectra of Complex Molecules", p. 120.
51. Sheppard. Discussions of the Faraday Society 9 322 (1950).
52. Zscheile, Comar and Harris. Plant Physiol. 19 627-37 (1944).
53. Christiane. Genest. Deut. Lebensm. Rundschau 51 200-3 (1955).
54. Fujimori and Livingston. Nature 180 1036 (1957).
55. Rabinowitch, loc. cit., Vol. II, Part I, Chapter 21, p. 618.
56. Franck and Livingston. J. Chem. Phys. 9 184 (1941).
57. Bolland. Quart. Rev. 3 1 (1949).
58. Bateman. Quart. Rev. 8 147 (1954).

59. Lonie. Ph.D. Thesis, Edinburgh, 1950.

60. Rabinowitch, "Photosynthesis", loc. cit., Vol. II, Part II, p. 1282.

61. Bateman, Hughes and Morris. Faraday Society Discussions 14 190 (1953), T.F.S. 52 89 (1956).

62. Krasnovskii. Doklady Akad. Nauk. S.S.S.R. 58 617 (1947).

Part II

63. Mott and Gurney, "Electronic Processes in Ionic Crystals", p. 101.

64. Mott and Gurney., loc. cit., p. 158.

65. Gurney and Mott. P.R.S. A164 151 (1958).

66. Szent Györgyi. Nature 148 157 (1941).

67. Emerson and Arnold. J. Gen. Physiol. 16 191 (1930).

68. Rabinowitch, loc. cit., Vol. II, Part II, p. 1282.

69. Katz, "Photosynthesis in Plants". Iowa State College Press, 1949, pp. 287-292.

70. Livingston. Quart. Rev. 14 192 (1960).

71. Nelson. J. Chem. Phys. 27 864 (1957).

72. Terenin and Putzeiko. J. Chem. Phys. 55 681 (1958).
Terenin, Putzeiko and Akrimov. Discuss. Faraday Soc. 27 83 (1959).
Terenin, Putzeiko and Akrimov. J. Chim. Physique 54 716 (1957).

73. Rabinowitch. Discuss. Faraday Soc. 27 161 (1959).

74. Bergman. Z. Physik. 100 50 (1936).

75. Terenin, Putzeiko and Akrimov. J. Chim. Physique 54 716 (1957).

76. Eley, Parfitt, Perry and Taysum. T.F.S. 49 79 (1953).

77. Mott and Gurney, loc. cit., p. 123.

78. Randall and Wilkins. P.R.S. A184 390 (1945).

79. Jacobs and Holt. J. Chem. Phys. 20 1326 (1952).

80. Eley and Parfitt. T.F.S. 51 1536 (1955).

81. Cardew and Eley. Faraday Soc. Discussions 27 115 (1959).

82. Eley and Spivey. T.F.S. 56 1432 (1960).

83. Vartanyan. Izvest. Akad. Nauk. S.S.S.R. Ser. Fiz. 20 1541-7 (1956).

84. Livingston, Porter and Windsor. Nature 173 485 (1954).

85. Szent-Gyorgyi. Faraday Soc. Discussions 27 111 (1959).

86. Bowen and Wokes, "Fluorescence of Solutions", p. 62.

In conclusion, the author would like to express her thanks to Dr Mowbray Ritchie for his advice and encouragement during the course of this work, and to D.S.I.R. for the grant which made the work possible; also to Professors Hirst and Cottrell for the provision of laboratory and technical facilities.