XXXVII. PHOTO-OXIDATION OF PLASMA.

A NOTE ON ITS SENSITISATION.

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PLASMA can be made to take up oxygen when irradiated in the manner described in a previous paper [Harris, 1926], but the rate at which this occurs in the case of clear plasma is extremely slow. Exposure of 10 cc. of fresh goat's plasma in a dilution of 1 in 5 to the light of the quartz mercury vapour lamp ($\frac{1}{2}$ kw.) at a range of 12.5 cm. causes an absorption usually of 4 mm.³ of oxygen per minute, *i.e.* 0.04 volume %; at this rate it would require an exposure of 25 minutes to enable plasma to take up 1 % of its own volume of oxygen. When these volumes of oxygen are again converted into masses the magnitudes are such that the analytical chemist either loses or ignores them. However, a variation appeared in the results with different samples of freshly drawn plasma and it was suspected that the discrepancies were due to incomplete centrifugalisation. The effect of the latter can be seen in the two examples:

Centrifugalisation O ₂ uptake: vols. % per minute	Goat 1		Goat 2	
	1 hour	3 hours	1 hour	3 hours
	0.065	0.030	0.060	0.029

It appears that a trace of blood-pigment may double the uptake. Irradiation of clear plasma and of a simultaneous sample which had undergone slight haemolysis gave the figures 0.04 and 0.08 respectively, another pair 0.04 and 0.07, showing that the presence of blood pigment in an amount which is only just visible may double the velocity of oxygen uptake.

As haematoporphyrin had been found to act as a powerful sensitiser in other biological experiments [Hausmann, 1910; Harris, 1925], it was decided to test whether it enhanced the oxidative action of light on plasma. Haematoporphyrin alone was first irradiated ($H\phi^n$, Fig. 1); it showed a small absorption of oxygen of the same low order as the plasma. When, however, the plasma and haematoporphyrin were together exposed to light, the rate of oxygen absorption was seen to be increased thirty- to forty-fold (Fig. 1, steep curves).

These curves (Fig. 1) reveal the characteristics common to the majority of photo-chemical reactions, *viz.* a period of induction lasting here nearly a minute and corresponding to the biological latent period, an intermediate

steadily maintained change or tonic state, and lastly a period of deduction. By suitably adjusting the physical conditions the induction period could be reduced to less than half a minute, which is comparatively short, so that it is unlikely that we are here dealing with an autocatalytic reaction. It must, however, be pointed out that it was impossible completely to obliterate the induction period and, as a general rule, it was found that when the duration of the induction period was 20 seconds, say, then the deduction period was 20 seconds or rather more, so that there was merely a definite lag in the response as a whole behind the irradiation.

Fig. 1. Velocity of the photo-oxidation of haematoporphyrin and plasma separately and together.

The rise in the haematoporphyrin curve (Fig. 1, $H\phi^n$) shows that haematoporphyrin exhibits the absorption and re-radiation phenomenon referred to in the previous paper [Harris, 1926] and shown by any coloured substance, whereas the plasma does not do so to any appreciable extent. An examination of the plasma and haematoporphyrin curves shows that the chemical intensity of the radiations of the mercury vapour lamp increased as the lamp warmed up: it is therefore necessary in comparative experiments to run the lamp some minutes until the amperage is constant before giving an exposure.

Since the above experiments were carried out, there has appeared a preliminary notice of a method devised by Gaffron [1925] for estimating small quantities of haematoporphyrin in human serum in terms of the oxidation occurring on exposure to daylight or to the light of a metal-filament lamp; the method is said to be so sensitive that one-millionth of a gram of haematoporphyrin per cc. of serum may be detected.

In view of the light-absorbing properties and powerful optical sensitisation of chlorophyll, as well as the analogy of the position occupied by it in respect to plant life to that which haematoporphyrin holds in the animal kingdom, it was decided to test this sensitiser next. The only specimen available was a suspension in olive oil of the mixed chlorophylls from nettles. Olive oil, when agitated in water, was found to take up 10 volumes of O_2 % per minute's irradiation, whereas the olive oil and chlorophyll took up 170. When added to plasma, they caused a fifty-seven-fold acceleration in the oxygen uptake, but on account of the presence of the olive oil it was impossible to apportion the respective effects to plasma (40 ?) and oil (17 ?). The outstanding feature of the effect of chlorophyll was the extent to which the photo-chemical threshold had been shifted into the red end of the spectrum, which brings this into line with its effect on the sensitisation of silver salts in panchromatic photographic plates as well as with the optimum action to red light in photosynthesis.

Although a very pure sample of carotene was available, difficulties were encountered in measuring its photo-sensitisation on account of its great insolubility. Experiments with carotene dissolved in chloroform and also with carotene suspended in olive oil gave a small ($\times 2$) increased rate of photo-oxidation which was probably not more than could be attributed to the oxidation of carotene itself.

A consideration of the curves of Fig. 1 shows that we are dealing with a complex set of reactions. Reference to the figure makes it clear that the dark state is one of equilibrium so that oxidation of plasma in the light does not belong to the type of photo-reaction in which the light acts as a catalyst accelerating a reaction already taking place in the dark, as is the case with cod-liver oil when agitated with air. The photo-oxidation of plasma, on the contrary, appears to involve a true endo-actinic stage, the light energy actually bringing about a change in the chemical potential rendering possible a reaction which would not otherwise occur (in the dark); the oxidation of plasma in the light becomes then a photo-chemical reaction of the simplest type. The theory of high energy chemistry recently advanced by Baly [1925] may be interesting in this connection. On the inclusion of haematoporphyrin the reacting system becomes very complicated; the net effect of this substance is to accelerate the velocity of the photo-oxidation of plasma. Whether the effect of the haematoporphyrin is simply a catalytic one might be determined by carrying the photo-oxidation of plasma to completion, using different concentrations of haematoporphyrin on equal quantities of substrate. As the present paper deals especially with the velocities of reaction, the effect of

varying concentrations of haematoporphyrin on the rate of photo-oxidation of plasma only is presented (Fig. 2). At low concentrations the curve is practically linear and the velocity of the photo-chemical reaction is directly proportional to the mass of sensitiser; at higher concentrations, a limit appears to be reached (*ca.* 0.0025 g. for 10 cc. plasma + 37 aq.) above which addition of this sensitiser has no further effect upon the velocity of reaction—a phenomenon analogous to the saturation current in photo-electricity. In all these experiments the emission of the light source was maintained constant; information relating to the energetics of the reaction might be obtained from a study of the effects of varying quantities of light upon a photo-chemical system consisting of substrate (10 cc. plasma) just saturated with sensitiser (0.0025 g. haematoporphyrin). It is interesting to note the similarity of this curve to that giving the relation between photosynthesis and light intensity [Boysen Jensen, 1918].

Fig. 2. Effect of concentration of sensitiser on the velocity of the photo-oxidation of plasma.

When one attempts to understand the mode of action of this photo-sensitiser, one is struck by its two outstanding optical properties. In the first place, solutions of haematoporphyrin exhibit very powerful absorption of light, as may be seen in the transmission spectrum given with the mercury arc and appearing in an earlier paper [Harris, 1925]; the absorption is nearly complete in the ultra-violet (practically all below $\lambda = 436\mu\mu$), and regional in the visible spectrum; it is also very marked in the infra-red, as shown by Hartridge and Hill [1914]. In the second place, on examining such solutions in ultra-violet light they display a beautiful pink fluorescence. It appears, therefore, that haematoporphyrin absorbs ultra-violet energy ($\lambda = 200 - 436\mu\mu$) and again radiates some of this energy in a limited spectral

band (*ca.* 0.7μ), thus becoming self-luminous in ultra-violet. The spectral locus of this emission would be interesting in the possible applicability of the principle of resonance to the photo-oxidation of plasma alone by means of radiant energy from specific spectral bands.

The effect of a few dyes exhibiting optical properties similar to those of haematoporphyrin may be quoted:

Dye (1 % aq. solution)	Volumes of O ₂ taken up by 100 vols. plasma exposed to 1 minute's radiation*					
	Ruby	Canary	Green	Blue	U-V	Hg arc/aq.
Methylene blue	0	0.15	0.06	0.11	0.25	0.67
Eosin	0	0.08	0.09	0.24	0.40	0.76
Erythrosin	0	0.69	0.66	0.53	0.75	1.77

* These figures also show that there is a good deal of overlap in these commercial glasses which are further complicated by different depths of colour.

The effects appear maximal for the regions of maximum absorption of light by the sensitiser. It is probable that fluorescence alone does not account for the difference in effect of different dyes. When the dye uranin, which exhibits a strong green fluorescence, was added to the plasma the rate of oxidation was increased only five-fold.

Turning to the chemical properties of haematoporphyrin, perhaps its most remarkable feature is the possession of an atom of iron. Upon testing a simple inorganic salt of iron in the same way as haematoporphyrin a parallel but much smaller accelerating action on the photo-oxidation of plasma was revealed. This is seen in Fig. 2, in which the two curves can be seen to belong

to the type expressed by the equation $y = x^{\frac{1}{n}}$; the entire curve is given in the case of FeCl_3 , but only the first part of the haematoporphyrin curve is shown.

The position of iron in the sensitisation of this photo-reaction appears to be unique: similar properties were not shown to be possessed by other metals producing catalytic effects in other reactions, *e.g.* salts of platinum, nickel and palladium were ineffective—very likely because they are powerful precipitants of protein. Experiments to be described later show that it is essential that the iron should be in an ionisable form and capable of passing easily from the ferric to the ferrous state—possibly by the addition of a valency electron under the action of light. In view of the less stable character of the Fe'' atom, one might expect ferrous salts to be more effective than the ferric forms; ferrous sulphate (0.1 cc. of *M*/20 solution) was found to be 33 % more powerful than equimolar amounts of ferric chloride. Ferrous ammonium citrate was then subjected to examination on account of its reputed compatibility in pharmacy and its deep colour: 0.5 g. of this salt when added to 10 cc. plasma and also to the control showed a rate of oxygen absorption expressed in volumes % per minute when exposed to the filtered radiations from the mercury vapour lamp as follows:

Ruby	Canary	Signal green	Blue	U-V	Hg arc
0	0	0	0.50	1.25	1.85

The photo-dynamic action of this salt is confined to the blue and ultra-violet which is also the spectral region of its maximum absorption.

The other scale-preparation of iron—iron and quinine citrate—behaved in a similar manner but was more sensitive, presumably on account of the peculiar optical properties possessed by the quinine, thus:

Per minute's irradiation through filters	Vols. O ₂ taken up by 100 cc. plasma containing iron and quinine citrate		
	1 : 10,000	1 : 1000	1 : 100
Ruby, Canary or Signal green	0	0	0
Blue	0.09	0.71	1.59
U-V	0.30	0.75	1.70
Hg arc	0.80	0.88	2.10

This double salt may therefore be usefully employed for photo-sensitisation; it must be noted, however, that the concentration necessary to produce a given acceleration is practically ten times that of haematoporphyrin. It is interesting to speculate as to whether the extraordinary efficiency of haematoporphyrin is due to the exaggeration of the catalytic and optical properties possessed by iron and quinine respectively or to the possession of a third undefined factor. A comparison of the above compounds indicates that the more complex the ion in which the iron is joined the more effective is it as a sensitiser. Now the ferric form is very stable, whereas the bivalent form may be made to absorb oxygen from the air and link it coordinatively. When, however, the iron is joined in a complex ion it can be easily brought into each of the oxidation stages, as shown by Baudisch [1918], visible and ultra-violet radiations then possessing the power to reduce the trivalent iron of certain complex compounds into the bivalent form. Just [1907] carried out interesting investigations leading one to assume the formation of the peroxide FeO_3 as an intermediary product. Later experiments by Baudisch and Welo [1924] show that initially one molecule of oxygen is added to one molecule of ferrous salt. The oxygen molecule, thus activated, is able to link certain compounds in a coordinative manner and make them more active. Another feature of interest, if we accept the electromagnetic theory of light, is the relation of iron compounds to their state of oxidation. Hilpert [1909] has shown that white ferrous hydroxide, which is practically non-magnetic, becomes black in colour and strongly ferro-magnetic on absorption of oxygen, but on further absorption of oxygen it turns into the red and weakly magnetic ferric hydroxide. Baudisch and Welo [1924] made several observations on the affinity of the magnetic iron with the para-magnetic gas O_2 and on light absorption by complex ions, which are very interesting in connection with the experiments described in this paper.

In view of the susceptibility of the iron-plasma system to light and the extensive use made of potassium ferricyanide in estimations of the oxygen capacity of blood, it became a matter of importance to examine its effect on plasma. As it is well known that solutions of this salt rapidly deteriorate in

the light, suitable controls had to be performed, *e.g.* using 1 cc. of saturated $K_3Fe(CN)_6$ and 10 cc. plasma:

Blood-tube B	Control tube C	O ₂ absorbed/min. B-C, mm. ³
Plasma	H ₂ O	+ 6
H ₂ O	Ferricyanide	- 22
Plasma + ferricyanide	Ferricyanide	- 17

Two points are brought out here, viz. (1) ferricyanide absorbs oxygen in the light at a much faster rate than does plasma and (2) the plasma protects or removes some of the ferricyanide from oxidation. The same may be seen in the figures obtained from different controls given by the blood of another goat:

Plasma	H ₂ O	+ 2
Ferricyanide	H ₂ O	+ 10
Plasma + ferricyanide	H ₂ O	+ 5

The absence of any sensitising action by the iron in the above experiment is to be ascribed to the absence of free Fe ions from the plasma + ferricyanide system. It is, however, noteworthy that the presence of cyanide *per se* does not inhibit the photo-oxidation of plasma, as seen in the following experiment giving the number of mm.³ of oxygen absorbed by 10 cc. of plasma per minute's irradiation:

Plasma	3.0
Plasma + 0.25 g. NaCN	3.4
Plasma + NaCN + 0.1 g. haematoporphyrin	234.0

The addition of 0.25 g. sodium cyanide (CO₂-free) did not prevent the photo-oxidation of plasma nor its eighty-fold increase in velocity by the sensitiser. The system presented here must be different from the purely chemical one of Fleisch [1924], who was able to inhibit the aerobic oxidation of succinic acid by means of cyanides, or the biological system of Warburg and Meyerhof [1912], who found that the increase of oxidation by the eggs of the sea-urchin caused by the addition of iron salt is inhibited in the same way as normal respiration by cyanide and narcotics. Fleisch, however, found that the anaerobic oxidation of succinic acid which proceeds in the presence of methylene blue is unaffected by cyanides, and further Dixon and Thurlow [1924] found that the oxidation of hypoxanthine aerobically is not affected by cyanides. Corresponding to these two types of cyanide susceptibility we have the suggestion of Evans [1923] that there are two kinds of respiratory processes in plain muscle, one of which is inhibited by cyanide and the other is not. Warburg's system [1921], although containing a metallic catalyst, is susceptible to cyanide, whilst the plasma + haematoporphyrin system investigated in the present paper is insensitive to cyanide.

SUMMARY.

The velocity of oxidation of plasma in light may be increased fifty-fold in the presence of minute amounts of certain sensitising substances. Haematoporphyrin and chlorophyll caused a very powerful acceleration, whilst many dyes and ionic iron show a marked though somewhat smaller effect.

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