XXXVI. THE ACTION OF LIGHT ON BLOOD¹.

By DANIEL THOMAS HARRIS.

From the Departments of Physiology and Biochemistry, University College, London.

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INTRODUCTION.

THIS investigation was begun with the object of determining the effect of light, if any, on the respiratory function of blood. Briefly, its effect was to interfere with the respiratory function, since, although it caused absorption or liberation of oxygen by various components of the blood, these were irreversible in character.

In order that the phenomena to be described may fall within the compass of a short paper, the presentation of hypotheses is left over to allow of a fuller treatment of the dynamics of the responses to light to which this paper is confined. Although the photo-chemical reactions appearing here are either imperfectly or not at all understood, many interesting comparisons with oxidation and reduction phenomena [Hopkins, 1925] present themselves to the biochemist and it is hoped that in his hands the easily controlled method about to be detailed will prove to be yet another useful means for the examination of intermediary processes in oxidation.

The influence of radiant energy of short wave-length upon the dissociation of CO₂ from solutions of haemoglobin and suspensions of red blood cells has been examined by Straub and Gollintzer-Meier [1923], who consider Einstein's formula to be quantitatively applicable to the change. The present paper deals only with oxygen.

EXPERIMENTAL METHODS.

The chief components of the apparatus used are diagrammatically represented in Fig. 1, the essential measuring instrument, the blood-gas apparatus of J. S. Haldane [1896, 1920], appears on the right-hand side; this was found to give very constant results when suitable precautions were adopted. The placing of a thick glass screen in front of the burette and levelling tubes as recommended by Douglas and the viewing of the fluid levels through magnifying lenses contributed greatly to constancy and accuracy in reading the burette. The latter consisted of a 1 cc. pipette graduated in large divisions of 0.01 cc. so that it could be read easily to 0.001 ± 0.0005 cc.

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Fig. 1. The quartz blood-tube *B* and control tube *C* coupled to a Haldane constant-pressure gas volumeter.

In the earlier experiments the oxygen capacity of 1 or 2 cc. of blood was determined by the ferricyanide method, using flasks of a shape described in a previous paper [1920]. Some of these were made of quartz and others of glass covered with an electrolytic coating of copper so that a comparison of the capacities in the light and in the dark could be made. Slight differences were recorded which became more obvious with the use of a thinner burette with a maximum range of only 0.25 cc. This, however, was soon abandoned on account of its inconstancy due to varying capillarity, and a return to the 1 cc. burette was rendered practicable by working with larger quantities of fluid (10 cc.). In order to pick up as much radiant energy as possible the blood and control flasks were then made tubular in shape and of about the same length as the mercury-vapour lamp. The final form of tubes evolved is shown in the diagram (Fig. 1), in which the lower tube is seen slightly displaced to the left for clearness; both were made of quartz with a total capacity of about 200 cc. each.

The tubes were wholly immersed in an electrically heated water-bath and the temperature was maintained electrically and by hand-control at 37.0° ; during the experiments the temperature was not allowed to vary more than 0.01° . A quartz window 12.5 cm. square was fitted into one side of the thermostat and a similar one of glass into the opposite side; distilled water was used in the bath and was frequently renewed since the transparency to

light of short wave-length is appreciably lowered by soluble impurities and floating particles cause much scattering of light [Pidduck, 1909]. The water was kept circulating by a suitably placed propeller and the tubes were actively rocked so as to spread the blood out in a film for maximum light absorption. All readings were made while the blood was being agitated; this is extremely important if constant penetrability and absorption of radiant energy is to be maintained.

The air spaces above the fluids in tubes *B* and *C* (Fig. 1) were always equalised; this could be checked in a preliminary experiment by arranging that large variations in temperature of the bath should not affect the burette reading.

The source of radiant energy found to be most suited to this work was an air-cooled quartz mercury vapour lamp of the vacuum type ($\frac{1}{2}$ kw.). This was placed close to the quartz window and its height adjusted by trial so that the blood and control tubes *B* and *C* (Fig. 1) were equally irradiated; this was easily determined by placing distilled water in *B* and *C* and adjusting the lamp until no change occurred in the burette reading when the light was switched on. For an approximate estimation of the efficiency of the various regions of the spectrum in eliciting the phenomena to be described glass filters in common use were inserted in turn against the quartz window inside the bath. These filters consisted of the usual photographic ruby and canary, also signal green and blue glass and lastly cobalted quartz; consequently, their use must be regarded only as a first approximation in spectral localisation.

Two goats were kept especially for the purpose of providing uniform samples of fresh blood. The usual procedure was to draw the blood from the jugular vein into a paraffined syringe and either transfer it directly to the blood-tube or centrifuge it under paraffin, using a mixture of 1 part of sodium fluoride and 3 parts of potassium oxalate as an anti-glycolytic and anti-coagulating agent.

The blood-tube *B* (Fig. 1) could be attached to a manometer and either to a vacuum pump or to a nitrogen reservoir containing commercial nitrogen kept over alkaline pyrogallol solution. In this way the gases could be thoroughly washed out by repeated exhaustion and filling of the blood-tube with nitrogen, and it was possible to admit air to give any desired oxygen tension up to 150 mm. Hg. The tubes were vigorously agitated in the dark until equilibrium was established between the blood and the gas mixture and a perfectly constant reading of the burette was obtained. Some minutes' exposure to the radiations of the mercury vapour lamp was then made and time given afterwards for return to a dark equilibrium. Samples of the gas in the blood-tube were withdrawn for analysis at the end of the experiment and the oxygen content determined in a Haldane air analysis apparatus: the CO_2 was usually inappreciable and the O_2 in close agreement with the initial tension of O_2 admitted into the apparatus in those experiments in which no transfer of oxygen occurred.

Control experiments. After the volumes of fluid in the blood and control tubes had been adjusted tests were carried out on the effects of variation in temperature of the bath, and it was found that the maximum error due to the variation permitted in the experiment was $\pm 0.05 \text{ mm.}^3$, which is considerably less than the personal error in reading the burette. The height of the mercury lamp was then permanently fixed so as to have an equal effect on the tubes *B* and *C* (Fig. 1) when containing the appropriate amounts of distilled water only.

The effect of inclusion in tube *B* of salts having the same concentration as in the blood was examined. The lowest curve (Fig. 2) shows that in an atmosphere of nitrogen, Ringer's fluid maintains a state of gaseous equilibrium both in the dark and on exposure to the radiations of the mercury arc. In air a slight but negligible absorption of oxygen takes place.

Fig. 2. Irradiation of solutions of a dye with the same colour as blood and of a solution of the saline constituents of blood.

A solution of micro-carmine matched to the colour of goat's blood and subjected to the radiations of the mercury arc shows no absorption of oxygen (Fig. 2). The two curves show the purely physical phenomenon of the local heating of the tube *B* containing this stable dye, the control tube *C* containing distilled water; the temperature of the bath, meanwhile, was maintained at 37.0° . The sharp rise in the curves (Fig. 2) after switching on the light is caused by the local heating of the tube *B* by the ultra-violet and visible radiations absorbed by the carmine and transformed into heat; the plateau represents the steady temperature maintained when the light energy absorbed is equal to the transformed heat energy radiated; switching off the light causes a drop in the curve to the original dark level. The examination of some fluorescent dyes in this manner might reveal interesting peculiarities.

Fig. 4 shows the effect of irradiation at low oxygen tensions. Whereas at tensions of oxygen above 20 mm. the curves show a constant upward tendency corresponding to evolution of gas from the blood, at 10 mm. and below the curves slope downwards, indicating an uptake of gas. When tensions of oxygen intermediate between these two sets were examined the dark equilibrium was maintained practically level (Fig. 5) irrespective of exposure to light. It appeared, therefore, that either two processes were at work or two different

Fig. 4. Irradiation of blood at low tensions of oxygen.

Fig. 5. Irradiation of blood at 15 mm. tension of oxygen.

constituents were involved, the one maintaining the ascendancy at high oxygen tensions (Fig. 3) and the other at low oxygen tensions (Fig. 4), a balance of effects being realised at 15 mm. tension of oxygen (Fig. 5).

On separating the blood into its two components by centrifugalisation and exposing each component separately to light, it was seen (Fig. 6) that the yielding up of gas was a property of the corpuscles, whilst the taking up of gas was inherent in the plasma. That the gas taken up by the latter is oxygen may be seen from an examination of the two lower curves (Fig. 6), since if the plasma is exposed to pure nitrogen the dark equilibrium remains practically level irrespective of irradiation. It may be mentioned that the complete removal of oxygen was a matter of some technical difficulty. In these experiments the screw clips at α and β (Fig. 1) were closed so that the oxygen might be removed from the burette and its indicating fluid; this usually required from 10 to 12 washings with nitrogen. Oxygen, too, appears to be the gas evolved from the corpuscles in the top curve (Fig. 6) since no evolution takes place after the corpuscles have first been deprived of oxygen (second curve from top, Fig. 6). It appears that some reduced derivative of haemoglobin is formed under the action of light; although methaemoglobin is said to be a product of the action of light on blood this does not seem to satisfy the direction of oxygen transfer.

Fig. 6. Irradiation of corpuscles and plasma separately.

OXYGEN UPTAKE OF PLASMA IN ULTRA-VIOLET LIGHT.

From the above experiments it appears that, provided there is a trace (0.5 %) of oxygen present in the gaseous medium in contact with the plasma, the taking up of oxygen proceeds in the light. It is immaterial whether serum or plasma be used, so that fibrinogen at least is not necessarily involved in the process. Under the conditions of these experiments serum was found to take up oxygen at a steady rate of 0.03 volume % per minute for at least half an hour.

Reference to Fig. 2 shows that the oxygen uptake of plasma in the light cannot be ascribed to the saline constituents since the absorption curve obtained with a Ringer's solution is practically level. A buffered glucose solution ($p_H = 7.4$) took up no oxygen during fifteen minutes' irradiation. By a process of exclusion and without resort to the methods of analytical chemistry, it would appear that the constituents of plasma responsible for the oxygen uptake are either the proteins or fatty substances. That the latter, if present, should exhibit the phenomenon was anticipated especially with regard to the unsaturated forms of fat. Thus it was found that 5 cc. of a sample of cod-liver oil in 42 cc. of a buffered phosphate solution were capable of taking up 0.4 volume % per minute in the dark when the emulsion was agitated with air at 37°, whilst on exposure to the mercury lamp 9.4 volumes of oxygen % per minute were absorbed. In this case, however, the effect of the light was to accelerate 23-fold the velocity of a reaction which was already proceeding in the dark, and in this respect it differed from the effect observed in the case of plasma. Olive oil, on the other hand, in a dilution of 0.5 cc. in 46.5 cc. water showed no appreciable uptake in the dark but a very similar figure in the light, viz. 10.4 volumes of oxygen % per minute.

In order to carry the investigation of the photo-oxidation of plasma further, it became necessary to find means for amplifying the small response of 0.04 volume of oxygen % per minute. Since the effect was confined to the ultra-violet region, the possibility of extending the photo-chemical threshold into the visible spectrum was entertained. The methods of bringing this about will be described in a subsequent paper.

SUMMARY.

Irradiation of blood with ultra-violet light causes it to give up oxygen at high oxygen tensions and to take up oxygen at low tensions. At about 15 mm. tension of oxygen, there is gaseous equilibrium, no net change occurring on passing from the dark into the light.

The taking up of oxygen by blood in ultra-violet light is a property of the plasma.

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