LXIX. THE ULTRA-VIOLET SPECTRUM OF OXYHAEMOGLOBIN.

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(Received February 5th, 1934.)

A PROMINENT band in the near ultra-violet spectrum of oxyhaemoglobin was discovered by Soret [1883] who referred to it as the "great" ultra-violet band. Gamgee [1895] pointed out that this band was also found in haemoglobin, carbon monoxide haemoglobin, nitrous oxide haemoglobin, haemochromogen, haematin hydrochloride, methaemoglobin and haematoporphyrin, but was absent from the spectra of bilirubin, hydrobilirubin and urobilin. The position of the peak of this oxyhaemoglobin band in the wave-length scale was found by Rost *et al.* [1909] to be 4100 Å. Newcomer [1919] placed it at 4140 Å. and Schumm [1921] at 4135–4140 Å.

In the case of carbon monoxide haemoglobin the absorption is greater, and the band is shifted towards the red, while the spectrum of acid haematin shows a similar band with the absorption not so pronounced and extending into the ultra-violet, obliterating a small peak at 3650 Å. which is characteristic of the oxyhaemoglobin spectrum.

Oxyhaemoglobin also has two bands with peaks at 3400 Å. and 2700 Å., and beyond 2400 Å. the absorption becomes general. Warburg and Negelein [1929] on measuring the absorption of light by the extent of chemical change which the absorbed energy produced, fixed the peak of the "great" band at 4300 Å. and found another prominent band at 2700 Å. in the case of oxyhaemoglobin, the latter band being lacking in all pure haem compounds.

Newcomer [1919] concluded that the similarity between the spectra indicated the presence of a common radical, but Hicks and Holden [1929] could not find any relationship between the valency of the iron in the pigments and their ultra-violet spectra. Anson and Mirsky [1930] state that pigments of similar chemical structure produce spectra of the same general form and of the same dimensions.

Macallum and Bradley [1930] pointed out that the absorption band occurring at 4100 Å. for crystalline oxyhaemoglobin of the horse does not appear in the spectrum of washed corpuscle suspensions containing the pigment in its normally occurring form. These observations have been verified and extended to the blood pigments of other species.

The instrument employed was a Bellingham and Stanley quartz spectrograph. The ultra-violet spectrum from 2100 to 4500 Å. was extended over 18 cm. upon the negative. The absorption measurements were determined by the use of a photoelectric recording photometer developed in this laboratory by Macallum and Coates [1933].

The blood was drawn from the animal into sodium citrate. In the case of the corpuscle suspensions the blood was diluted with mammalian saline so that the pigment concentration was adjusted to a predetermined value (0.2 % haemoglobin), these values being determined by Newcomer's method. In the case of the haemoglobin, the corpuscle suspension was laked with ether, the haemoglobin values being adjusted as stated above.

Typical results may be illustrated by the data obtained from rabbits' blood (Fig. 1).

1. *Unlaked blood.* The absorption curve shows a slight elevation about 4500 Å. then a fall towards 4300 Å.; between 4300 and 4000 Å. there is another slight rise terminating about 4000 Å. There is another slight rise at 3900 Å. The curve then becomes level to 3500 Å.

2. *Laked blood.* The absorption curve is fairly level to 4200 Å. where it rises sharply to a peak about 4100 Å. and then drops off gradually to the original level at 3900 Å. The curve is then level to 3700 Å. where it recedes slightly and then rises at 3600 Å. and comes back to the original level at 3500 Å. The laked blood at the height of the great ultra-violet band (4100 Å.) shows about 40 % absorption for the solution used.

In comparing the laked and unlaked oxyhaemoglobin, it is seen that the laked shows greater absorption at any given wave-length.

Fig. 1.

Oxyhaemoglobin from other species. For the sake of convenience the beginning of the great ultra-violet band has been designated A, the peak B and the end C, the figures representing Å.

1. *Laked blood.*

	A	B	C
Rabbit	4200	4100	3900
Goose	4300	4100	3910
Human	4300	4100	3850
Turtle	4300	4100	3900
Horse	4200	4100	3800

2. *Unlaked blood.* The absorption spectra do not show any marked band. The spectrum of rabbit-oxyhaemoglobin, in the visible region, was examined

both in the corpuscle suspension and in the laked blood and the position of the α and β bands determined. The position of the bands was fixed as follows:

	α band (Å.)	β band (Å.)
Laked blood	5780	5400
Unlaked blood	5780	5400

This experiment was repeated several times on rabbits' blood and indicates that there is no difference in the spectral bands of oxyhaemoglobin inside or outside the corpuscle.

DISCUSSION.

Our observations have been made on oxyhaemoglobin solutions, and they have served to confirm the observations previously recorded. The great ultra-violet absorption curve in the oxyhaemoglobin solutions shows in most cases an origin about 4300 Å., a rise to a peak about 4100 Å. and then a drop back to 3900 Å. The unlaked blood does not show this great ultra-violet band in the region of 4100 Å. The rest of the curve remains much the same except that there is a slight rise in some cases at 4100 Å.; in all cases where this latter absorption occurred there was slight haemolysis, and it is believed that this absorption is due to traces of oxyhaemoglobin outside the corpuscle.

There is the possibility that the absence of the great ultra-violet band from the corpuscle suspensions might be due to the masking effects of the blood-serum, the corpuscle wall, material mixed with haemoglobin in the cell or material combined with haemoglobin in the cell but removed by ether-laking. As to the serum it has been shown by Lewis [1917] that the ultra-violet absorption spectrum of serum has two peaks at 2800 Å. and 2400 Å.; it represents several superimposed curves and does not resemble that of a pure compound, being due chiefly to protein. Furthermore, we have found that the great ultra-violet band of oxyhaemoglobin exists in laked whole blood as well as in aqueous solutions of crystalline oxyhaemoglobin.

The question as to whether the corpuscle wall itself may be a factor can be eliminated by the fact that blood laked by freezing and containing the corpuscle "ghosts" still displays the band in the ultra-violet. Removal of the "ghosts" by centrifuging does not affect the absorption band.

Cell stromata evidently play no part since the laked blood containing corpuscle contents displays the spectrum of pure oxyhaemoglobin.

Finally, since our early experiments were carried out on ether-laked samples it might be possible that this treatment had removed a lipid fraction which was combined with the oxyhaemoglobin in the corpuscle and that lack of this portion might be responsible for the absence of the band under question. However, the fact that laking brought about by the physical processes of freezing and thawing, which do not remove any constituent of the cell, still gives the same spectrum as the ether-laked blood indicates that the presence of the band is independent of the absence of any ether-soluble substance.

Our results indicate that there is an essential difference between oxyhaemoglobin inside and outside the corpuscle. If the presence of absorption bands is to be interpreted as indicating a condition of desaturation then it must be assumed that oxyhaemoglobin outside the corpuscle is in a state of unsaturation while in its naturally occurring state in the cell it enters into combination with some of the cell constituents. The combination must be rather labile since it is disrupted by any force which alters the osmotic balance of the cell.

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