REMARKS ON SPECTROPHOTOMETRIC CURVES OF BLOOD PIGMENTS*

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Photographs of blood spectra, owing largely to the varying sensitivity of plates to different wave-lengths of light, give an inadequate idea of the spectrum as a whole. Anyone who has attempted to photograph a mixture of methaemoglobin and oxyhaemoglobin, or a so-called neutral solution of methaemoglobin, will have recognized the difficulties. The spectrophotometer, which gives accurate measurements of absorption in different parts of the spectrum, would seem, provided certain constants are maintained, to be free from objection. The curves obtained, however, do not represent the blood spectrum seen by the eye, and in this respect at least are disappointing. In view of the increasing use of the spectrophotometer as an instrument of precision in analysis and research, some observations made in the course of other investigations may not be without interest.

THE INSTRUMENT

The spectrophotometer employed consisted of a Hilger large-model constant deviation wave-length spectrometer and a Nutting polarization photometer giving a triple field. The large-model spectrometer is less convenient to use than the small model, but owing to the greater dispersion it is more delicate, especially for the observation of absorption towards the red end of the spectrum. Its average accuracy over the visible spectrum is about one Ångström unit. The light source was a 100 c.p. pointolite lamp, 63 cm. from the collimator slit. The width of this slit, which controls resolution and affects the height, and to some extent the width, of the absorption bands in the curves, should be as narrow as the light source will allow. In most of my experiments it was 0.05 mm. The ocular slit was 0.1 mm. wide, and covered wave-lengths of 2.1μ in the yellow and 1.2μ in the blue. The internal diameter of the cells used was 1 cm.

TECHNICAL DIFFICULTIES

When a convenient solution of blood showing well-separated oxyhaemoglobin bands is examined spectroscopically, the blue of the spectrum appears clear, and on diluting the solution the α band is the last to go; but when a curve of the absorption coefficients of the solution is plotted, the β band of the oxyhaemoglobin appears to be denser than the α band, and the limb of the β band towards the blue does not reach zero, and is followed by increasing absorption as the violet is approached. The whole curve presents a somewhat tilted appearance. This relative increase in absorption towards the violet is not wholly natural, and is difficult to explain. It is obtained with solutions of crystallized haemoglobin and other blood pigments, even when these are plotted against dilute serum; but it is not seen in spectrophotometric curves of substances of low molecular weight, such as potassium permanganate or even eosin. It was at first thought to be due to the increased scattering of light of diminishing wave-length by the large molecules of haemoglobin. But this explanation is not wholly satisfactory. The graphs of absorption coefficients of particulate matter, such as suspensions of micrococci or a mastic emulsion, are straight lines, the inclination of which varies with the concentration; whereas the haemoglobin absorption increases more towards the violet end than visual observation indicates. It may possibly be due to the residual

yellow colour of blood solutions. Ordinary colour screens are not sufficiently labile to counteract this effect. Silver colloid solutions, which show increasing absorption towards the violet, are more serviceable, and I have found dilutions of electrargol especially useful as a control in the spectrophotometry of blood pigments. By using a dilution which will give zero at λ 500 μ against an oxyhaemoglobin solution, a spectrophotometric curve is obtained which presents fairly accurately the blood spectrum seen by the eye.

METHAEMOGLOBIN IN NORMAL BLOOD

Most of my work has been done on ox blood, crystallized or more or less purified, but near the end of my researches three experiments were made on human blood, and they illustrate the value of curves of this type. Blood was obtained from the ears of three different persons in the usual way, and was diluted to a convenient concentration for examination. To 5 c.cm. of the dilution was added one drop of strong ammonia solution, and to another 5 c.cm. one drop of water. The two solutions were straightway placed in identical cells; the ammoniacal dilution was put in the beam of the polarized light, and the neutral solution in the non-polarized beam of the spectrophotometer. The difference in the absorption coefficients was then taken. On plotting, the curve showed slight rises in the red and in the position of the two oxyhaemoglobin bands. From an analysis of the curves of oxyhaemoglobin and of acid and alkaline methaemoglobins it is difficult to avoid the conclusion that in these three individuals a trace of methaemoglobin is normally present in the blood. Whether this is universally the case further research, for which I have not at present the opportunity, will show. There seems, however, no obvious reason why oxyhaemoglobin and methaemoglobin should not normally exist in a balanced state, and a minute concentration of methaemoglobin be in equilibrium with the oxyhaemoglobin of normal blood.

Memoranda

MEDICAL, SURGICAL, OBSTETRICAL

SURVIVAL FOR SEVEN DAYS AFTER RUPTURE OF AORTIC ANEURYSM

Rupture of an aneurysm of the aorta is not a common occurrence, but survival for seven days thereafter must be very rare. Only the post-mortem findings in the following case could have led me to believe that such a happening was possible.

The patient, a man aged 65 years, first consulted me on October 15th, 1931, complaining of pain radiating down the inner aspect of the left thigh from the vicinity of the saphenous opening. This I diagnosed as an anterior crural neuritis, and treated it with sodium salicylate and potassium iodide, therewith affording considerable relief.

On October 22nd I was called to see the patient, who informed me that, as he was strolling round his room, he suddenly "sat down on the floor." He felt no sensation of discomfort, but in view of the fall he felt alarmed, and went to bed. Two days later it was evident that he had had an enormous haemorrhage into the tissues of his left abdominal wall. The skin from the spine to the mid-axillary line, and from the crest of the ilium to the scapula, was black with effused blood. The left iliac fossa was occupied by a tense mass, which stretched up under the costal margin. The only symptom referable to the abdomen was a troublesome hiccup, which persisted till the end. He survived till October 28th (seven days), when he died suddenly after some slight movement in bed.

Post mortem I found in the abdominal cavity a large quantity of serous fluid and a lesser, though considerable,

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