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# A SPECTROSCOPIC METHOD FOR THE STUDY OF HÆMOGLOBIN IN DILUTE SOLUTIONS.

By F. G. HALL.

(*From the Physiological Laboratory, Cambridge.*)

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## Introduction.

The comparison microspectroscope has been used by several investigators for the study of hæmoglobin [Bürker, 1911]. Krogh [1919] described a method and Krogh and Leitch [1919] used such an instrument to study hæmoglobin in fishes' blood. More recently the microspectroscope has been employed in the study of respiratory pigments by Keilin and Hill. The author is indebted to both Dr Keilin and Mr Hill for suggestions which have led to the use of this instrument as herein described. The following description involves no new use of the microspectroscope. The purpose of this paper is to outline a procedure rather than to describe the use of any particular instrument. Perhaps in this way it may serve others who may be interested in a relatively simple technique for making oxygen dissociation curves on limited quantities of blood.

## The microspectroscopic comparator.

A Zeiss microspectroscopic eyepiece was arranged as shown in Fig. 1. A colorimeter plunger was attached to the tube of the eyepiece. A colorimeter cup was held in a fixed position some centimetres below. Between the fixed cup and the fixed plunger a movable cup ($C_1$) and a movable plunger ($P_1$) could be raised or lowered by means of a rack and pinion. Thus the sum of the depths of solutions in movable and fixed cups was always constant regardless of the position of the movable cup and plunger. A depth of 20 mm. was found a convenient one. Consequently it will be seen that when a solution of fully oxygenated hæmoglobin was placed in one cup and of fully reduced hæmoglobin in the other, one could obtain a spectrum of hæmoglobin representing any degree of oxygenation by

simply varying the relative depth of the solutions in the two cups. The ratio of depths of the two hæmoglobin solutions to one another was read on a vernier scale. A tonometer ($T$) containing the hæmoglobin solution under investigation was placed in front of the side opening of the eyepiece

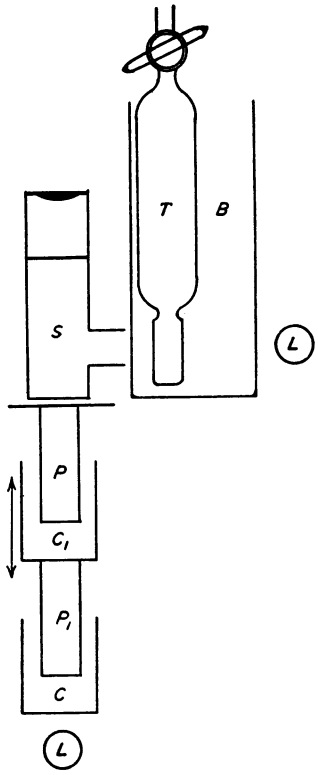


Fig. 1. Diagram to show the relative positions of the spectroscopic eyepiece ($S$), the tonometer ($T$), the colorimeter plungers ($P$, $P_1$) and the colorimeter cups ($C$, $C_1$). The tonometer was kept in a glass water bath ($B$). Two lights were fixed in the position ($L$) and their intensity regulated with resistances so that the comparison spectra were of equal intensity.

(Fig. 1). In this manner the two spectra could be matched and the degree of oxygen saturation of the unknown determined.

A nitrogen container (Fig. 2) was constructed by inverting a large bottle and filling it about one-third full with a concentrated solution of sodium hydrosulphite. Nitrogen from a supply tank was bubbled through the hydrosulphite solution and allowed to stand for some hours before using. Samples were withdrawn from time to time and analysed with a

Haldane gas analysis apparatus. No detectable quantity of oxygen was ever found. A manometer ($M$) was arranged to show the pressure within the nitrogen container or within the tonometer as desired. This could be accomplished by manipulation of the stopcock ($S_2$).

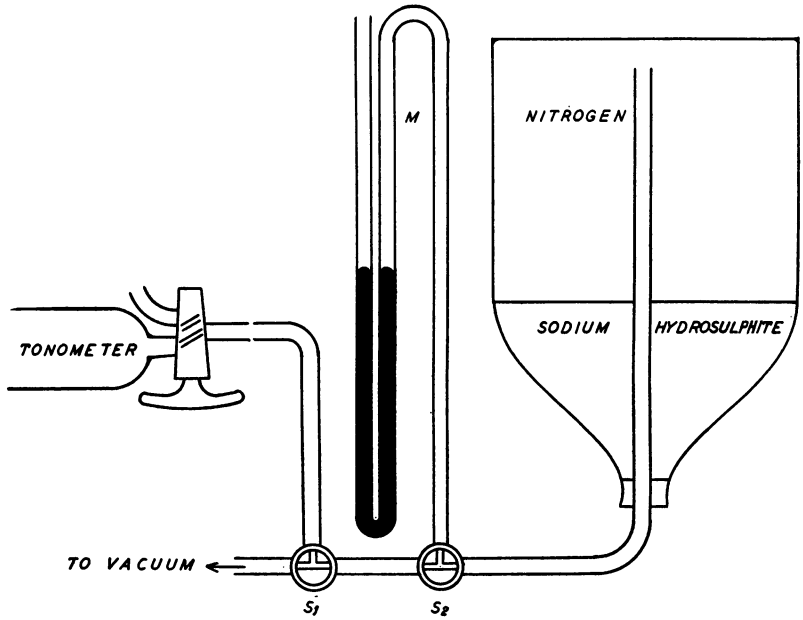


Fig. 2. Diagram to show how tonometers were filled with gas mixtures of known oxygen pressures. Two stopcocks ($S_1$, $S_2$) were manipulated so that the tonometer could be alternately evacuated and refilled with nitrogen. Pressures were read on manometer ($M$).

The tonometer was constructed somewhat after that used by Redfield [1930], except that the cell was blown from one piece of glass. The cell was approximately 2 cm. in diameter and 1 cm. deep. The total capacity of the tonometer was about 35 c.c.

## Procedure.

3 c.c. of a dilute hæmoglobin solution were placed in the tonometer. The tonometer was then evacuated three times, refilling with nitrogen each time. By gentle shaking the dissolved gases could be removed from the solution and the hæmoglobin fully reduced. Fig. 2 shows the position of the stopcocks in relation to the vacuum line, nitrogen supply and tonometer. It was found more convenient to draw the nitrogen from the

manometer by manipulation of a stopcock ($S_2$) than directly from the nitrogen container. After the last evacuation the pressure in the tonometer was left somewhat below atmospheric, and a measured quantity of air was admitted from a gas burette. The temperature of the air in the gas burette and the atmospheric pressure were noted. The tonometer was then shaken for 10 min. in a constant temperature bath. It was then quickly removed to the glass water bath in front of the spectroscope and a reading taken at once.

The standard hæmoglobin solution placed in the colorimeter cups was prepared as follows. Since the depth of the solution in the cups was twice that in the tonometer the hæmoglobin solution was diluted to one-half that in the tonometer. One portion was placed in the lower cup and about 5 mg. of sodium hydrosulphite added. The other portion was saturated with air and placed in the upper cup.

### Calculations of the oxygen tensions.

If $V_{\text{Ton.}}$ is the capacity of the tonometer in c.c., $V_B$ the volume of hæmoglobin in c.c. which has been admitted into the tonometer and freed of dissolved and combined oxygen, $V_A$ the quanity of air in c.c. introduced into the tonometer at room temperature ($T_1$) and atmospheric pressure ($P$) in mm. of Hg, and $V_{O_2(c+d)}$ the c.c. of oxygen which is combined with hæmoglobin plus that which is physically dissolved in the hæmoglobin solution after equilibration at temperature $T_2$, then the partial pressure of oxygen ($pp$) in mm. of Hg will be:

$$pp = \frac{0{\cdot}2094\,V_A - V_{O_2(c+d)}}{V_{\text{Ton.}} - V_B} \times \frac{T_2}{T_1} \times P.$$

Since the quantity of physically dissolved and combined oxygen is small, $V_{O_2(c+d)}$ may be approximated without significant change in values for oxygen tensions.

It was thought desirable to compare the spectroscopic method with the van Slyke-Neill manometric method using similar samples of blood. Hæmoglobin from sheep blood was prepared free from stroma protein by the use of ether and salt as described by Adair [1925] and kept in a $M/15$ phosphate buffer solution of $p$H 6·8. One portion was diluted fifty times with the phosphate buffer solution. A sample of whole blood after having been hæmolysed with a minimal quantity of distilled water was diluted fifty times with the phosphate buffer solution of $p$H 6·8. The concentrated hæmoglobin solution was used for the van Slyke technique and equilibrated in Barcroft tonometers. The two

diluted samples treated in a similar manner described above were analysed with the spectroscopic comparator. Three dissociation curves thus obtained are shown in Fig. 3. The two methods seem to check satisfactorily

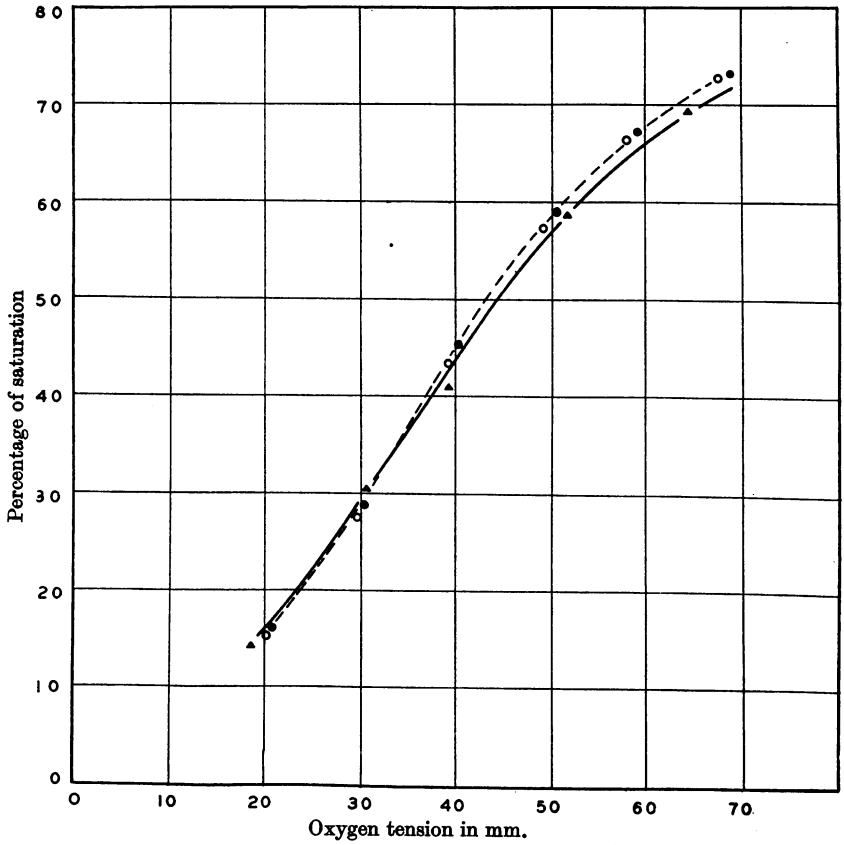


Fig. 3. Three dissociation curves obtained by three different methods. Sheep blood at *p*H 6·8 was used.

▲ represent analyses made with a van Slyke manometric apparatus on a hæmoglobin solution prepared by the Adair method.

o represent analyses made with the comparison spectroscope on whole blood hæmolysed and diluted fifty times with phosphate buffer solution *M*/15 and *p*H 6·8.

• represent analyses made with the comparison spectroscope on the hæmoglobin solution prepared by the Adair method and diluted fifty times with the phosphate buffer solution.

with one another. There appears to be no difference in curves obtained with hæmolysed whole blood and with hæmoglobin prepared by the Adair method.

There are certain limitations to the spectroscopic method. Determinations of oxygen saturation between 0 and 20 p.c. or between 80 and 100 p.c. cannot be made accurately. Also an error is introduced if appreciable methæmoglobin is formed since the absorption bands of methæmoglobin are quite similar to those of oxyhæmoglobin.

The advantages of the method are: a small quantity of blood is required and it is apparently not necessary to prepare a purified hæmoglobin solution. The *p*H, temperature, and oxygen tension can be controlled accurately and easily.

## SUMMARY.

A spectroscopic method is described whereby dissociation curves can be made on small quantities of hæmoglobin.

I wish to thank Prof. Barcroft and Mr Adair for laboratory facilities and for many helpful suggestions.

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