Technical and experimental errors in the spectrophotometric determination of oxygen saturation

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SYNOPSIS The method of Deibler, Holmes, Campbell, and Gans (1959) is a quick, accurate, and reliable method for the determination of oxygen saturation. The results should be calibrated against those obtained by the Van Slyke method, and the setting, sensitivity, and reliability of the spectrophotometer should be carefully established. This is especially necessary to meet the stringent requirements for accurate setting at the isobestic point 805 m μ . This method is superior to a method using saponin as a haemolytic agent and reading at only one wavelength.

Now that respiratory problems are being increasingly investigated in the laboratory there is need for a simple but reliable method for the determination of blood oxygen saturation based on standard equipment. This need has largely been met, for example, by Hickam and Frayser (1949), Wade, Bishop, Cumming, and Donald (1953), and Deibler *et al.* (1959). However, in attempting to set up a similar method we experienced a number of difficulties, not mentioned in the published texts, which are discussed below.

METHODS

The spectrophotometric method of Hickam and Frayser (1949) was used to begin with but that described by Deibler and her colleagues (1959) was later adopted. The results with both were compared against oxygen saturations determined by Van Slyke's constant volume method with which there should be close agreement.

In the method of Hickam and Frayser the blood is haemolysed with saponin and the optical density determined at 660 m μ . Fully oxygenated blood is treated in the same way and read at the same wavelength. The resulting optical density difference is converted into an oxygen difference in volumes per cent by use of the relationship:

Oxygen difference in vol. % = a (optical density difference) + b where a and b are constants.

In the method of Deibler *et al.* blood samples are drawn into heparinized syringes, capped, and then stored in ice until analysed. A drop of mercury is added to each

sample then 0.05 ml. of the Triton X¹ 100 solution (33% v/v Triton X 100 in 0.1 molar borax solution) is added to each millilitre of blood. The syringe is capped again and shaken gently by hand. An aliquot of the sample is transferred to a standard cuvette in which the light path is reduced to 1 mm. Readings of optical density are then made, the first at 650 m μ and then at 805 m μ in a suitable spectrophotometer. The percentage saturation is then calculated from the following formula:—

$$\text{Percentage saturation} = 100 \left[\frac{1 - D^{650}/D^{805} - 0.491}{3.738} \right]$$

where D^{650} = optical density at 650 m μ
and D^{805} = optical density at 805 m μ

The figures 0.491 and 3.738 represent constants for the method.

To obtain a range of oxygen saturations human blood was equilibrated with appropriate gas mixtures in tonometers rotated in a water bath at 37°C. A Unicam spectrophotometer (model SP 600) was used for the optical density determinations and the standard Van Slyke manometer for the gasometric determinations.

DIFFICULTIES

HAEMOLYSIS Saponin as used in Hickam and Frayser's method (1949) was found to be unsatisfactory since the efficacy of haemolysis and hence the quantity required varies from one sample to another. Turbidity may also occur. These disadvantages arise because saponin is not a pure chemical substance but a biological product containing variable amounts of the haemolytic substance. The use of Triton X 100 is a marked improvement; first it is of known chemical structure, secondly it is extremely active: for complete

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¹Triton X 100 can be obtained from Messrs. V. A. Howe Ltd., 46 Pembridge Road, London, W.11.

haemolysis only 0.05 ml. of Triton X 100 per millilitre of blood is necessary. Hence turbidity of the solution and dilution are not important factors, confirming the experience of Deibler *et al.*

HAEMOGLOBIN DETERMINATIONS Accurate determination of haemoglobin is necessary for conversion of oxygen capacity to saturation. The M.R.C. grey wedge photometer was not sufficiently accurate (2% difference on the average between duplicates). Accordingly total haemoglobin was determined by conversion to cyanmethaemoglobin as described by Drabkin and Austin (1935-36). Duplicates then agreed to less than 1%.

OPTICAL DENSITY DETERMINATION AT 650-660 $m\mu$ Readings of optical density are reliable at this wavelength as it is on a flat part of the characteristic absorption curves of the pigments. However, reading only at one wavelength, as in the method of Hickam and Frayser, did not eliminate the effects of interfering substances such as bilirubin and lipids. These authors claimed that there was little difficulty on this account but we believe it to be a common trouble at this wavelength.

OPTICAL DENSITY DETERMINATION AT 805 $m\mu$ It is essential that the isobestic point of haemoglobin and reduced haemoglobin (805 $m\mu$) should be accurately given by the spectrophotometer. At this wavelength the absorption curve of oxyhaemoglobin undergoes an acute minimum; a shift in wavelength of 100 Angstrom units gives almost 10% change of transmission. On being set at 805 $m\mu$ our instrument was about 100 Angstrom units out when checked by an 805 $m\mu$ narrow band metal dielectric interference filter (Barr and Stroud). Checking with the didymium glass filter supplied with the instrument did not reveal this defect in setting. This filter checks the instrument throughout a wide range of wavelengths but is not sensitive enough to show small shifts of

FIG. 1. Comparative determinations of oxygen saturation by manometric and spectrophotometric methods.

setting which become very important at the 805 $m\mu$ minimum. Apart from the faulty setting our instrument showed a declining sensitivity over a period of time shown by the stringent requirements of the method of Deibler *et al.* and also by the need for a wider slit width to obtain a balance on 'check'. Moreover the micro-ammeter was excessively unstable on 'dark current'. Subsequently these deficiencies were corrected by the manufacturer and then replicable readings were obtained at 805 $m\mu$.

RESULTS

The table summarizes a representative sample of results obtained by the spectrophotometric and gasometric methods.

Figure 1 represents the results of comparative determinations of the percentage oxygen saturation by the manometric and Deibler spectrophotometric

TABLE

COMPARISON OF RESULTS OBTAINED BY GASOMETRIC AND SPECTROPHOTOMETRIC METHODS

Case No.	Blood O ₂ Capacity (vol. %, cyanide method)	Ratio of D650/D805 Optical Densities	% O ₂ Saturation (Deibler <i>et al.</i>)	% O ₂ Saturation (Van Slyke)
1	21.90	3.533	18.62	16.87
2	19.20	2.921	35.00	33.85
3	22.45	2.666	41.76	40.32
4	20.82	2.463	47.25	49.07
5	27.37	2.112	56.60	52.62
6	25.16	1.737	66.50	60.90
7	20.51	1.447	74.43	71.20
8	27.48	1.374	76.30	73.50
9	18.58	1.290	78.62	80.14
10	18.19	1.099	83.72	83.85
11	16.33	0.762	95.71	95.59
12	21.87	0.553	98.40	100.34

FIG. 2. Calibration curve between D650 and D805 and oxygen saturation determined by Van Slyke's method.

methods. The calibration curve and its 95% confidence limits were computed as the regression line of the spectrophotometer values on the Van Slyke values, the calculations being on the assumption that the Van Slyke estimates were correct. The maximum variation in duplicate values was 0.334 vol. % and compared well with the accuracy that Van Slyke (Peters and Van Slyke, 1932) suggests should be obtained (0.25 vol. %).

The correlation coefficient was calculated to be 0.995 and the regression line

$$y = 0.9604x + 3.5572.$$

Figure 2 shows a calibration curve between D650/D805 and percentage oxygen saturation by Van Slyke's method, from which can be calculated the extinction coefficients of reduced and oxyhaemoglobin. The coefficients obtained in this way should agree with those determined by spectrophotometric means. The regression line can be calculated as

$$y = -0.0359x + 4.095.$$

Substituting at two values of saturation and D650/D805 the extinction coefficients can be calculated from the equation of Deibler *et al.* The calculated and known values agree to within 1%. This provides an independent check of the method.

DISCUSSION

The method of Deibler *et al.* gave results that had a high degree of correlation with those obtained by Van Slyke's method after the initial difficulties had been overcome.

In agreement with other workers we found that the spectrophotometric method gave slightly higher values than the gasometric method. In our hands this variation was greatest between the 50 and 70% levels of saturation. A complete explanation for this finding is not available but a partial one may be that some of the samples came from patients with secondary polycythaemia due to chronic lung disease. For example, in case 6 the haemoglobin was 130% and the error 6% as compared with the expected 3%. Normally only about 0.4% of the pigment of the red cell is present as methaemoglobin (Van Slyke, Hiller, Weisiger, and Cruz, 1946) but various authors have commented upon increases up to 5% in secondary polycythaemia. Normally the equilibrium haemoglobin $\rightleftharpoons$ methaemoglobin is kept to the left by reductive enzymes; when the haemoglobin mass increases one might expect the capacity of the enzymes to be approached and the percentage of methaemoglobin increased. This leads on the one hand to overestimation of the oxygen capacity and on the other, due to absorption at 650 m μ , to a falsely high oxygen saturation by the spectrophotometric method.

Bosman (1962) recalls two cases of toxic methaemoglobinemia with cyanosis and secondary polycythaemia in which gross discrepancies between spectrophotometric and gasometric saturations were observed.

When we became aware of this problem we made one or two spot determinations of methaemoglobin which was usually greater than 2%, but we were unable to correlate the level with the degree of secondary polycythaemia. To increase accuracy of blood oxygen determination beyond the levels reported here would necessitate more detailed investigation of these pigments.

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REFERENCES

- Bosman, A. R. (1962). Personal communication.
- Deibler, G. E., Holmes, M. S., Campbell, P. L., and Gans, J. (1959). *J. appl. Physiol.*, **14**, 133.
- Drabkin, D. L., and Austin, J. H. (1935). *J. biol. Chem.*, **112**, 105.
- Hickam, J. B., and Frayser, R. (1949). *Ibid.*, **180**, 457.
- Peters, J. P., and Van Slyke, D. D. (1932). *Quantitative Clinical Chemistry*, vol. 2, p. 321. Williams and Wilkins, Baltimore.
- Van Slyke, D. D., Hiller, A., Weisiger, J. R., and Cruz, W. O. (1946). *J. biol. Chem.*, **166**, 121.
- Wade, O. L., Bishop, J. M., Cumming, G., and Donald, K. W. (1953). *Brit. med. J.*, **2**, 902.