Blood Oxygen Determination by Gas Chromatography

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One hundred blood specimens have been analyzed for blood oxygen content simultaneously by the Van Slyke gasometric technic and gas chromatographic technic. The two technics agree within ±0.83 Vol. %.

The gas chromatography instrument must be standardized by comparing its results with another blood oxygen procedure such as the Van Slyke technic.

Suggestions are made for the improvement of the gas chromatography equipment.

DEVELOPMENTS in diagnostic procedures such as cardiac catheterization have changed the requirements for blood oxygen determinations. These procedures sometimes require that a large number of blood specimens be taken for oxygen determination. Where the patient is an infant, there is not a large enough circulating blood volume for each of these specimens to be 6 ml., as in replicate determinations by the classic Van Slyke technic, or even 2 ml., as in most of the spectrophotometric technics. The instability of blood specimens for oxygen determination, combined with the large number of specimens taken in a short time during cardiac catheterization, makes the Van Slyke technic for blood oxygen determination impractical.

Several other procedures have been reported. Cuvet oximeters (1), and oxygen tension measurements, either by the bubble equilibration method (2) or the oxygen electrode (3), have been tried and each has been rejected for general use because of some major difficulty. Spec-photometric methods for blood oxygen determination have been used widely (4-7). Specimen size required for the spectrophotometric technic is still larger than is desirable, however, and it has been shown that in lower values of oxygen capacity the values for oxygen

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content are in error (8). Spectrophotometric technics also are not compatible with the injection of dyes such as Evans green, during cardiac catheterization studies.

Gas chromatography technics, which have been used so successfully by the industrial chemist, should be applicable to the determination of blood oxygen. These technics are inherently sensitive. The equipment is simple to operate, and accurate quantitative analyses may be obtained quickly. It is the purpose of this communication to report blood oxygen determinations performed with one of the first commercially produced pieces of clinical gas chromatography equipment. These determinations are compared with blood oxygen determinations performed by the classic Van Slyke gasometric technic.

Materials and Reagents

Van Slyke Determination

Equipment used:

The reagents used are those recommended by Dr. Van Slyke (9) with the exception of the gas-releasing mixture. The final concentrations of materials in the latter reagent as used in these laboratories are: dodecyl sodium sulfate, 0.67%; potassium ferricyanide, 2.67%; and lactic acid (85%), 0.40%.

Gas Chromatography Determination:

Equipment used:
1. Fisher Clinical Gas Partitioner (Fisher No. 6-390, fitted with the Fisher thermal stabilizer Model 27, Fisher No. 11-134-200).
2. Hamilton Syringe with Chaney Adapter (0.1 ml.)
3. Helium tank with good two-stage gas pressure regulator (such as Fisher No. 11-565-25).

This gas chromatography equipment was used as specified in the manufacturer’s instruction manual after the following modifications were made:
1. The silica gel column was emptied.
2. The molecular sieve column was replaced with a longer one, 10 feet in length.
3. The chart recorder speed was changed from 1 in./min. to 2 in./min.
4. The original instruction manual specified the use of 85% lactic acid solution in the gas-releasing mixture. The same volume of a 1:10 dilution of the 85% lactic acid was used instead.

**Methods**

Blood specimens of 6- to 8-ml. size were drawn into 10-ml. syringes which had been rinsed inside with heparin solution. These large quantities of blood were taken in order that simultaneous determinations could be performed. Each syringe of blood, free of all air, was closed with a syringe cap (BD 425) filled with mercury, shaken thoroughly, and submerged in an ice-water mixture until the oxygen determinations were performed.

Van Slyke oxygen determinations were performed by the classic procedure (9) using two Thomas manometric gasometers. Two technologists simultaneously determined blood oxygen on 1-ml. aliquots of the same blood specimen with these gasometers. The results agreed within 0.5 vol.% or the determination was repeated. The average result, in vol.% oxygen, was reported to the surgeon.

Gas chromatography determinations were performed using the modified Fisher Clinical Gas Partitioner. The instrument was brought to equilibrium with a helium flow rate of 100 ml./min. and a thermistor current of 7 ma., as recommended by the manufacturer. The reaction chamber was charged with a gas-releasing mixture containing saponin, ferricyanide, lactic acid, and octyl alcohol, and the dissolved air was allowed to sweep entirely out of the system. The recorder was adjusted to the zero line and a 0.1-ml. aliquot of the next blood sample was injected. A single injection of each sample was used in this study. After about five sample injections, the reaction chamber was emptied and recharged with fresh gas-releasing mixture.

Figure 1 is an example of the type of recording obtained. A base line (dashed line) was drawn from the point of injection to the end of the sample recording, and the trailing edge of each peak was extrapolated to the base line. The area representing the oxygen peak (cross-hatched) was measured to the nearest 0.01 sq. in. with a planimeter. This area in square inches was compared with the Van Slyke results in vol.% for each specimen.

* A later instruction manual for this equipment recommended the use of 0.1 N HCl instead of the lactic acid solution. This change does eliminate the foaming in the reaction chamber.
Fig. 1. Type of tracing obtained on injection of 0.1 ml. blood specimen into the Fisher Clinical Gas Partitioner. Zero time is the time of injection, indicated by a sharp swing of the needle. The dashed line indicates the baseline drawn to aid in area measurement. The area measured for the calculation of oxygen content of the blood specimen is cross-hatched.

Fig. 2. Each point on the graph represents one blood specimen analyzed simultaneously by the Van Slyke and the gas chromatographic techniques for blood oxygen determination. \( \sigma_y = 0.83 \) VOL. %
Results

Each of 100 blood specimens was analyzed as it was received from the cardiac catheterization laboratory. Figure 2 shows the relationship between the results of the two technics for these blood specimens. Each point represents one blood specimen, with the Van Slyke results on the ordinate in vol.% and the gas chromatography results on the abscissa in square inches of oxygen peak area. The oxygen concentration ranges from 8 to 18 vol.%. The solid line shows the calculated straight line fit for these data; its formula is \( Y_v = 4.14X + 0.66 \), where \( Y_v \) represents the Van Slyke result in vol.% and \( X \) represents the gas chromatography result in square inches of oxygen peak area. Twice the standard error of estimate, calculated from these data, defines the range which includes 95% of the data. The broken lines, calculated to fall 0.83 vol.% above and below the solid line, enclose this area.

Discussion

A blood oxygen procedure should be rapid, accurate, and should require a small amount of blood. With respect to these points, the new method and the Van Slyke technic compare as follows.

1. With respect to rapidity, the gas chromatography technic averages 8 min. per determination, and the Van Slyke technic averages 20 min. per determination. For about 5 specimens, one report can be returned to the catheterization team each 6 min. with the gas chromatography procedure.

2. With respect to accuracy, the data show that the Van Slyke and the gas chromatography results do not agree closely enough for them to be accepted interchangeably by the thoracic surgeon who must interpret them. The study does not show whether one procedure is more accurate. Since Van Slyke determinations have been so well accepted, we have assumed that the gas chromatographic technic must be improved further.

3. With respect to the amount of blood required, the gas chromatography method necessitates 0.8 to 1.0 ml. of blood for two determinations, should a repeat be necessary. Van Slyke determinations, as they are performed in these laboratories, require at least 3.5 ml. of blood for duplicate determinations, with more required if repeat is necessary. At least 2 ml. of blood is required for a single spectrophotometric determination (7).

Several things must be improved if the gas chromatography tech-
nic is to become a practical procedure for blood oxygen determination:

1. The present gas chromatography instrument must be standardized by comparing its results with the results from another procedure such as the Van Slyke. This is a distinct disadvantage. It had been hoped that a standardization curve could be constructed from the data obtained by injecting volumes of air containing known amounts of oxygen. A curve prepared in this manner, however, does not agree with the curve prepared by a Van Slyke standardization. This may indicate that all of the oxygen is not released from the blood specimen in the chromatography reaction chamber.

2. The number of mechanical movements required for routine use of this gas chromatography instrument should be reduced. The instrument is not "technician-proof." Many simple errors or omissions in technic are possible which would damage the instrument sufficiently that it would not operate.

3. The analysis of the gas chromatography recorder data could be speeded and its accuracy increased if the recorder were fitted with an integrator. This is not possible until the base line drift of the instrument has been eliminated. It must be determined how much of this drift is due to inadequate temperature control and how much to the change of helium flow rate.

4. The bubble column, which is used for measurement of the gas flow rate, is slow and awkward. A better device should be developed, especially if helium flow rate is to be corrected continuously. At the time this paper was prepared, the instrument was not commercially available. The Fisher Scientific Co. has returned it to the development laboratories to be redesigned.

References