# *Calculation of Blood $O_2$ Content from Optically Determined Hb and $HbO_2$*

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The Co-Oximeter is an instrument of considerable utility in measuring Hb and $HbO_2$. For $O_2$ contents calculated from these measurements to be compatible with those determined by the Van Slyke procedure, it is necessary to use 1.30 as the $O_2$-binding capacity for Hb and to correct $HbO_2$, other than at unity, by reference to a calibration curve. The scales must be set with blood of the species to be studied, and special precautions are necessary if actual Hb differs greatly from that used for adjusting the scales and constructing the calibration curve.

THE CO-OXIMETER, Model 182 (Instrumentation Laboratories, Boston), requires less than 1 ml of blood for analysis and automatically provides a digital display of Hb, $HbO_2$, and HbCO approximately 15 seconds after sample introduction. Reproducibility of measurements is excellent. Maintenance problems are minor. However, $O_2$ content calculated from Hb and $HbO_2$ determined by the Co-Oximeter is significantly greater than that determined by the Van Slyke procedure. The following report documents this discrepancy and suggests an empiric resolution.

## Material and Methods

The Co-Oximeter was operated as suggested in the instruction handbook provided by the manufacturer. In daily checks, only minor adjustments were necessary for the Hb scale to indicate correctly zero or the values of standards provided by the manufacturer and for the $HbO_2$ scale to indicate zero or unity with blood samples treated either with dithionite to remove $O_2$ completely or with 100 per cent $O_2$ to fully saturate Hb. The operator introduces the sample and pushes a button. The instrument automatically dilutes and mixes the blood with a hemolyzing agent; it warms and delivers the mixture to a cuvette, where absorbances at 548, 568, and 578 $m\mu$ are determined; it then enters the latter into a computational matrix which solves simultaneously the three equations relating Hb, $HbO_2$, and HbCO, and displays the solutions on a digital readout. Cleaning of the system is automated, with provision for zero checks and manual adjustments if necessary. Results were reproducible to within less than 1 per cent for each measured entity, independent of technician experience. Oxygen content of blood (ml/100 ml) analyzed by the Co-Oximeter was calculated as suggested by the manufacturer from Hb (g/100 ml), $HbO_2$ (fraction), $P_{O_2}$ (mm Hg, electrodes 37 C), and an $O_2$-binding factor for Hb of 1.39:

$$O_2 \text{ content} = P_{O_2} \times 0.0031 + Hb \times 1.39 \times HbO_2 \quad (1)$$

The Van Slyke procedure used for determination of $O_2$ content of blood was identical in all known respects to that described in 1924 by Van Slyke and Neill.[1] Briefly, this method involves direct measurement of the decrease in gas pressure resulting from chemical absorption of previously released $O_2$. $O_2$ content of blood is then calculated by application of standard gas laws and physical constants to the measured pressures, volumes, and temperature. The technique does not involve complicated transducers or electronic circuits, and has not been challenged for absolute accuracy since its introduction. However, each determination requires approximately 5 ml of

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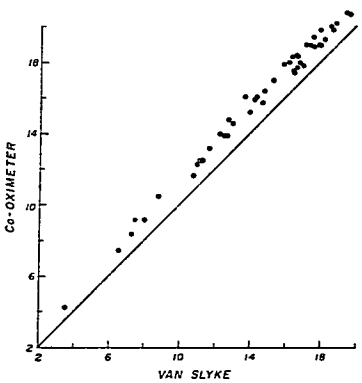


FIG. 1. Blood $O_2$ content by the Van Slyke procedure and calculated from Hb and $HbO_2$ determined by the Co-Oximeter using an $O_2$-binding factor for Hb of 1.39 and $P_{O_2}$ by electrodes. The solid diagonal is the line of identity. Van Slyke procedure values are all less than those calculated.

blood and from 30 to 45 minutes for completion. The procedure must be modified if volatile anesthetics are present in the blood, and the difference between duplicates varies considerably with the experience of the technicians involved, in our laboratory averaging $1.5 \pm 1.5$ (SD) per cent.[2]

In the present studies, blood was drawn from antecubital veins of nonsmoking adults into syringes containing heparin. The $HbO_2$ desired was obtained by exposure to an appropriate $P_{O_2}$. The residual gas was expelled, and the blood was mixed with the aid of glass beads and analyzed in duplicate by both the Co-Oximeter and the Van Slyke procedure. $P_{O_2}$ of blood was determined by calibrated electrodes at 37 C. On ten occasions, the internal consistency of each method was checked by analyzing a mixture of equal parts of two previously analyzed samples. In each instance, the $HbO_2$ of the components was unity in one and less than 0.95 in the other. Predicted values for the mixture were calculated from the results of the original analyses of the two components on the assumption that the mixture contained equal amounts of each and that $O_2$ was neither added nor lost in preparation of the mixture. In addition, 14 blood samples were equilibrated at $P_{O_2}$ 200 to 660 mm Hg to provide samples with Co-Oximeter values of unity for $HbO_2$. Several of these were left undisturbed until the erythrocytes had settled out and divided into separate samples in order to provide samples with Hb concentrations above and below normal. These were analyzed by Co-Oximeter and the Van Slyke procedure and the Hb obtained by Co-Oximeter was compared with that calculated after rearrangement of equation (1) and insertion of the Van Slyke $O_2$ content, $P_{O_2}$, and $HbO_2$ of unity. The same technique was used after equilibration at a lower $P_{O_2}$ to provide samples with different Hb values but the same $HbO_2$ at a value less than unity.

TABLE 1. Differences for Van Slyke Procedure and Co-Oximeter between Observed and Predicted Results of Analysis of Mixtures (N = 10) Prepared from Samples Analyzed Previously

| | Difference (Observed − Predicted) | |
|---|---|---|
| | Mean | SE |
| Van Slyke | | |
| $O_2$ content, ml/100 ml | 0.11 | 0.09 |
| Co-Oximeter | | |
| Hb, gm/100 ml | −0.04 | 0.04 |
| $HbO_2$ (fraction) | 0.022* | 0.002 |
| $O_2$ content, ml/100 ml | 0.34* | 0.07 |

* Significant difference between observed and predicted values ($p < 0.05$) by $t$ test, paired data.

## Results

In each instance, $O_2$ content was greater by the Co-Oximeter method than by the Van Slyke procedure (fig. 1). The results of the tests for internal consistency of each method are summarized in table 1. With the Van Slyke procedure, differences between observed and predicted values for the mixture were insignificant. With the Co-Oximeter, differences between observed and predicted values were insignificant for Hb but not for $HbO_2$. For each mixture, the observed $HbO_2$ value was greater than expected, and calculated $O_2$ content was greater than predicted. In all sam-

ples, HbCO by Co-Oximeter was less than 1 per cent.

The demonstrated internal inconsistency of $HbO_2$ and $O_2$ content by Co-Oximeter leads to an examination of the accuracy of the Hb and $HbO_2$ scales. The Hb scale of the Co-Oximeter is adjusted at the factory on the basis of Hb standards analyzed by cyanmethemoglobin procedures. Studies in our laboratory utilizing the standardized cyanmethemoglobin method [3] demonstrated excellent agreement between Hb values by this method and by the Co-Oximeter. However, for blood with $HbO_2$ values of unity, the Hb value calculated after rearrangement of equation (1) from Van Slyke $O_2$ content, $HbO_2$, and $P_{O_2}$ was less than by Co-Oximeter, and the relationship was dependent on Hb concentration. The ratio of calculated Hb value to Hb value determined by Co-Oximeter averaged 0.934 (table 2). Accordingly, actual $O_2$-binding capacities using Hb values determined by Co-Oximeter are: Hb × 0.934 × 1.39, or Hb × 1.30. The use of 1.30 lessened, but did not eliminate, the difference between $O_2$ content values by Co-Oximeter and by the Van Slyke procedure. The difference between Co-Oximeter observed and predicted values for the mixtures remained. Accordingly, we conclude that $HbO_2$ values provided by the Co-Oximeter were in error at values other than unity and zero, and a calibration curve relating $HbO_2$ by Co-Oximeter and by the Van Slyke procedure was constructed (fig. 2). The latter values were calculated after rearrangement of equation (1) by inserting Van Slyke $O_2$ content, $P_{O_2}$, and the product of Co-Oximeter Hb × 1.30. Calculation of $O_2$ content using Co-Oximeter Hb × 1.30, Co-Oximeter $HbO_2$ corrected from this curve, and $P_{O_2}$ yielded values similar to and not significantly different from those by the Van Slyke procedure (mean −0.07, ±0.05 SE, N = 46).

These considerations apply equally to Co-Oximeter determinations involving dog blood. It is necessary, however, to check and adjust the unity and zero settings of the $HbO_2$ scale with blood of the species to be studied. Finally, there is for each species an effect of Hb on this setting and the calibration curve. This can be lessened by setting the $HbO_2$ scale with blood that has a Hb concentration similar to that expected. For extreme ranges of Hb (<5 and >20 g/100 ml), additional calibration curves or other approaches are recommended.

TABLE 2. Hemoglobin Determinations by Van Slyke Procedure and Co-Oximeter

| Blood Sample | Hemoglobin, g/100 ml | | |
|---|---|---|---|
| | Van Slyke | Co-Oximeter | Ratio* |
| 1 | 9.0 | 9.5 | 0.947 |
| 2 | 10.5 | 11.2 | 0.938 |
| 3 | 11.4 | 12.2 | 0.934 |
| 4 | 11.8 | 12.6 | 0.937 |
| 5 | 11.8 | 12.4 | 0.952 |
| 6 | 12.2 | 13.4 | 0.910 |
| 7 | 12.5 | 13.4 | 0.933 |
| 8 | 12.5 | 13.3 | 0.940 |
| 9 | 12.6 | 13.9 | 0.906 |
| 10 | 12.8 | 13.7 | 0.934 |
| 11 | 13.1 | 14.1 | 0.929 |
| 12 | 13.6 | 14.5 | 0.938 |
| 13 | 13.7 | 14.5 | 0.945 |
| 14 | 23.4 | 25.2 | 0.929 |
| MEAN | | | 0.934 |
| SD | | | 0.010 |

* Ratio = $\frac{\text{Van Slyke}}{\text{Co-Oximeter}}$.

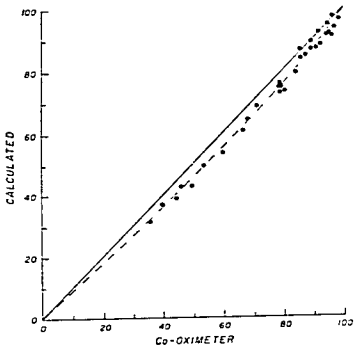


FIG. 2. Calibration curve relating $HbO_2$ determined by Co-Oximeter and $HbO_2$ calculated from $O_2$ content by the Van Slyke procedure, Hb determined by Co-Oximeter, and $O_2$-binding factor of 1.30 and $P_{O_2}$ determined by electrodes. The solid diagonal is the line of identity. The dashed line connecting zero and 100 was drawn as the line of best visual fit. At values other than unity, $HbO_2$ by Co-Oximeter was greater than by calculation.

## Discussion

The foregoing, while stressing the utilitarian features of the Co-Oximeter, suggests that calculation of $O_2$ content from Co-Oximeter-determined Hb and $HbO_2$ and an $O_2$-binding factor for Hb of 1.39 leads to erroneously high values. Although an empiric approach that resolves discrepancies between $O_2$ contents calculated by the Co-Oximeter method and those determined by the Van Slyke procedure has been evolved, the basis for the problems encountered cannot presently be explained. The two factors believed to be involved will be treated separately.

First, for samples with an apparent $HbO_2$ of unity, calculation of $O_2$ content from Hb determined by the current cyanmethemoglobin method and a factor of 1.39 for the $O_2$-binding capacity of Hb yields values greater by approximately 7 per cent than those obtained by the Van Slyke procedure. Possible explanations for this difference include systematic errors in the cyanmethemoglobin standard or method, the binding-capacity factor, or the Van Slyke procedure or in the assumption that all of the Hb of the sample binds $O_2$ to the greatest theoretic extent under the conditions conventionally used to prepare blood with an $HbO_2$ of unity. Unfortunately, none of these possibilities can be unequivocally eliminated from consideration.

The current accepted standard for determination of Hb is the cyanmethemoglobin procedure, approved in 1967 by the International Committee for Standardization in Haematology (ICSH). This is an optical technique. ICSH has defined relevant physical aspects of the procedure and provides for availability to clinical laboratories of a standard solution of cyanmethemoglobin.[2] This development followed acceptance by the International Union of Pure and Applied Chemistry of a new molecular weight for Hb (64,458) based on current knowledge of the structure of the molecule.[3] One molecule of Hb is considered to have four Fe atoms, each capable of binding one molecule of $O_2$. It follows that the total binding capability of 1 mole of Hb (64,458 g) is 4 moles of $O_2$ ($4 \times 22.4$ liters) and, therefore, 1 g of Hb is capable of binding 1.39 ml of $O_2$. Previously, a similar approach using a higher molecular weight for Hb based on Fe analysis yielded a value of 1.36.[4] The widely used value of 1.34 is based on studies of the CO-combining power of Ox hemoglobin, carried out before 1900.[5] Apparently, neither the Hb standard nor any of these $O_2$-binding factors have been confirmed by direct determinations of actual $O_2$ content. The Van Slyke procedure, however, has been validated by determination of $O_2$ in standard solutions of $H_2O_2$ and seems an unlikely source of systematic errors.[6] Furthermore, it seems unlikely that our results reflect only a peculiarity in the application of the Van Slyke procedure in our laboratory since they have been confirmed in analysis of duplicates by other technicians, using other equipment, in two other locations in this institution. Theoretically, the presence of HbCO could give rise to discrepancies of the type discussed, because Hb in this form is undetected by the Van Slyke procedure for $O_2$ content but is detected and added to the total Hb in the Co-Oximeter approach. This possibility is believed excluded in the present studies because, by circumstance, Co-Oximeter analysis, and direct manometric procedures for HbCO, HbCO did not exceed 1 per cent in any of the samples. A value of approximately 7 per cent would be required to explain the results. Finally, it is difficult to relate these problems to the use of too low a $P_{O_2}$ in the preparation of samples having an $HbO_2$ of unity because increase in $P_{O_2}$ from 200 to 660 mm Hg and prolongation of exposure did not alter the results. The question remains, however, whether these conditions result in $O_2$ binding to Hb to the extent that is theoretically possible.

A separate, unrelated factor is considered responsible for the discrepancies that necessitate the use of the calibration curve relating $HbO_2$ by the Van Slyke procedure and by Co-Oximeter. The need for this approach and independence of this problem from that involving total Hb and the binding factor is most evident in the studies utilizing a mixture of two previously analyzed samples. In these, the internal inconsistency of the $HbO_2$ scale of the Co-Oximeter was clearly demonstrated by the occurrence on each occasion of a value for $HbO_2$ of the mixture greater than that predicted from Co-Oximeter analysis of the components. This problem presumably is based

on an instrumental shortcoming, possibly involving errors in wavelengths, cuvette design, light detection, computational matrix, or electronic circuitry. It should be emphasized that this appears to be an inherent, systematic error which reproduces very well and, while a nuisance, is not a serious deterrent to the use of the instrument. In our experience, this instrumental error is reproduced with less variance than occurs in the random errors of the Van Slyke procedure required for its detection and quantitation.

## References

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## Surgery

**"SCALP-VEIN" INFECTION** Intravenous indwelling scalp-vein needles from a group of patients with neoplastic disease were cultured. Twenty-four of the 74 needle cultures were positive; microorganisms generally considered pathogenic grew from the material on seven of these needles. The latter organisms included *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans,* α-hemolytic streptococcus, and group D streptococcus. Infected scalp-vein needles were the bacteriologic sources of septicemia in one patient and cellulitis in another. There was a trend toward increasing incidence of growth with increasing duration of needle placement. (*Lowenbraum, S., and others: Infection from Intravenous "Scalp-Vein" Needles in a Susceptible Population, J.A.M.A. 212: 451 (April) 1970.*)

**CANDIDA SEPSIS** Five of 22 surgical patients being treated with parenteral nutrition developed *Candida albicans* septicemia. Two died of the infection. In all of these patients factors known to predispose to or promote *Candida* sepsis could be demonstrated. Failure of weight gain in an infant, fever otherwise unexplained, or the known presence of *Candida* infection elsewhere should alert the physician to the possibility of *Candida* septicemia. Regular periodic blood cultures may reveal organisms in the mildly symptomatic patient and facilitate early diagnosis. Treatment is removal of the central venous catheter. Amphotericin B therapy may also be required in severe cases. (*Ashcraft, K. W., and Leape, L. L.: Candida Sepsis Complicating Parenteral Feeding, J.A.M.A. 212: 454 (April) 1970.*)

**PARENTERAL NUTRITION** The concept of an artificial gut system which can provide prolonged nutrition to patients incapable of enteric feeding is described. Concentrated nutrients are safely introduced into the circulation via an arteriovenous shunt, and delivered by either a day-mode (portable pump) or night-mode (gravity feed) delivery system. The system has been designed to be operated by the patient in his own home, and may prove effective in maintaining the health of patients who have chronic bowel diseases. (*Scribner, B. H., and others: Long-term Total Parenteral Nutrition: The Concept of an Artificial Gut, J.A.M.A. 212: 457 (April) 1970.*)