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Laboratory Report

Normal Hemoglobin–Oxygen Affinity

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Hemoglobin–oxygen affinity is known to vary in a number of disease states. The authors measured the continuous affinity of blood from healthy subjects and, using mathematical data reduction techniques, calculated coefficients for rational function models of average normal affinity, ±2 standard deviations, and 95 per cent confidence limits. Average normal P_{50} was 27.10 torr, with a two-standard-deviation range of 25.85 to 28.35; P_{50}'s of the 95 per cent confidence limits were 26.69 and 27.53 torr. The affinity usually accepted as standard lay between or very near to the 95% confidence limits of normal throughout its range. It is concluded that the range of normal affinity is narrow and that, for most practical purposes, standard affinity adequately represents normal affinity. There should be little difficulty in distinguishing from normal the shifts that occur in certain disease conditions. (Key words: Blood, oxyhemoglobin dissociation curve; Oxygen, oxyhemoglobin dissociation curve.)

HEMOGLOBIN–OXYGEN AFFINITY is the continuous relationship between hemoglobin–oxygen saturation and oxygen tension (P_{O_2}) under standardized conditions of *p*H (7.40), P_{CO_2} (40 torr), and temperature (37 C). Data collected by Severinghaus[1] and expressed in the form of an "oxyhemoglobin dissociation curve," a table, and a special slide rule are usually accepted as standard affinity and are assumed to represent normality. However, the normality of this standard affinity has not been confirmed, and the statistical variance about normality has not been measured.

Recently developed techniques[2] facilitate the direct measurement of hemoglobin–oxygen affinity and permit estimation of statistical properties of the affinity of a group of blood samples. We measured the continuous hemoglobin–oxygen affinity of blood from 15 normal subjects and calculated the mean affinity, standard deviation, and 95 per cent confidence limits about the normal mean. These results were compared with standard affinity.

Method

None of the subjects smoked, had recently been subjected to high altitude, or had any condition expected to alter hemoglobin–oxygen affinity. Heparinized blood samples were drawn and immediately tonometered to P_{O_2} 0 torr and P_{CO_2} 40 torr. Oxygen uptake, P_{O_2} and *p*H were then measured continuously to a P_{O_2} of approximately 300 torr on a Radiometer Corp. Model DCA-1 dissociation curve analyzer to which had been fitted a *p*H electrode. Final P_{O_2}, P_{CO_2}, and *p*H were measured on a Radiometer Corp. Model BMS3 blood–gas analyzer.

Numerical processing methods previously described[2] were used to estimate P_{CO_2} and to reduce these measured data to standard conditions (P_{CO_2} 40 torr and *p*H 7.40); to calculate the four coefficients each of the mathematical rational functions (eq. 1) representing average hemoglobin–oxygen affinity, plus and minus two standard deviations, and 95 per cent confidence limits; and to plot each of these functions as an oxyhemoglobin dissociation curve. The seven-coefficient rational function which Kelman fitted to dissociation curve data[3] was used to calculate standard affinity. All calculations and plots were per-

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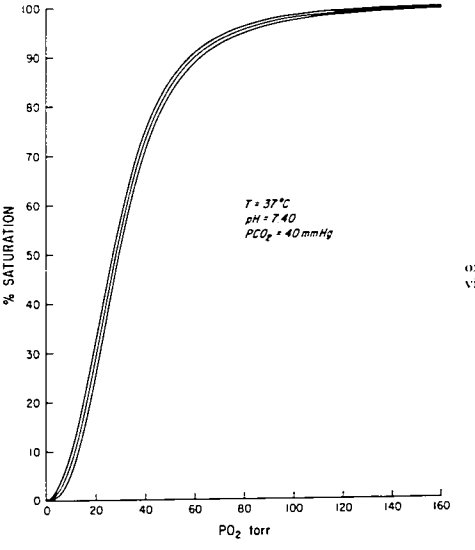

FIG. 1. Average normal hemoglobin–oxygen affinity and two-standard-deviation range.

formed on a Xerox Data Corporation Sigma-3 computer.

$$S = \frac{0.25A_1P + 0.5A_2P^2 + 0.75A_3P^3 + A_4P^4}{1 + A_1P + A_2P^2 + A_3P^3 + A_4P^4} \quad (1)$$

where

S = saturation,
A_i = coefficients
$P = P_{O_2}$.

Results

The oxygen tension at 50 per cent saturation (P_{50}) for average normal hemoglobin–oxygen affinity was 27.10 torr, with a standard deviation of 0.625 torr. Thus, the two-standard-deviation range of average normal P_{50} was from 25.85 to 28.35. The continuous variance of average normal affinity is represented by a plot of its two-standard deviation range in figure 1.

The 95 per cent confidence limits of average normal affinity were 26.29 and 27.53 torr at 50 per cent saturation, and are plotted along with standard affinity in figure 2. The maximum discrepancy between normal and standard affinities occurred at approximately 21 torr, which corresponded to 35 per cent saturation, where standard affinity was approximately 3.75 per cent saturation greater than average normal and 2.75 per cent saturation greater than the upper 95 per cent confidence limit.

Rational function coefficients for all of the measured curves are contained in Table 1.

Discussion

To evaluate normality of the hemoglobin–oxygen affinity of a blood sample, it is necessary to know the range of normality, not just its mean value. Apparently the range of normality of hemoglobin–oxygen affinity is narrow. The P_{50}'s of approximately 95 per cent of normal specimens lie within a range of 2.0 torr. This may be illustrated in terms of oxygen delivery in a hypo-

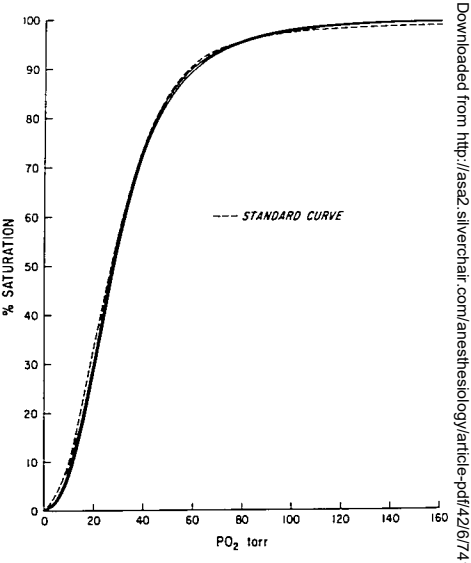

FIG. 2. Ninety-five per cent confidence limits of average normal hemoglobin–oxygen affinity with "standard" affinity superimposed.

thetical patient with normal affinity. Assuming arterial and venous blood P_{O_2}'s of 80 and 35 torr, respectively (and assuming that both arterial and venous blood P_{CO_2}'s and pH's are 40 torr and 7.40), the arteriovenous saturation difference, or oxygen exchange fraction, would amount to 28.81 per cent, on the average, and would range from 27.04 to 30.47 per cent. Thus, the range of variation in exchange fraction among hypothetical patients having normal affinity would be only 3.4 per cent, or 12 per cent of the exchange fraction itself. This is remarkable uniformity and suggests accurate homeostatic control.

Dramatic affinity changes have been observed in some disease states, suggesting that homeostatic mechanisms that are capable of maintaining close tolerances in health are either overcome or modified in disease. It is intriguing that pathologic variations in affinity often act toward compensation of decreased cardiac output or arterial hypoxemia by permitting release of oxygen at higher-than-normal tensions, thus preserving tissue oxygenation and tissue and venous blood P_{O_2}'s.

Present evidence points to organic phosphates, especially 2,3-diphosphoglycerate (DPG), as mediators of this homeostasis.[4,5]

TABLE 1. Coefficients of Rational Function Model of Hemoglobin–Oxygen Affinity

	A_1	A_2	A_3	A_4
Average normal	$.697101 \times 10^{-2}$	$.156576 \times 10^{-2}$	$-.195468 \times 10^{-4}$	$.238890 \times 10^{-5}$
+1 standard deviation	$-.100593 \times 10^{-1}$	$.153636 \times 10^{-2}$	$-.152770 \times 10^{-4}$	$.159592 \times 10^{-5}$
−1 standard deviation	$.499686 \times 10^{-2}$	$.219088 \times 10^{-2}$	$-.333697 \times 10^{-4}$	$.302752 \times 10^{-5}$
+95 per cent confidence limit	$-.443996 \times 10^{-2}$	$.166832 \times 10^{-2}$	$-.190093 \times 10^{-4}$	$.197852 \times 10^{-5}$
−95 per cent confidence limit	$-.499618 \times 10^{-4}$	$.189974 \times 10^{-2}$	$-.250895 \times 10^{-4}$	$.243786 \times 10^{-5}$

with increased levels of DPG being associated with decreased hemoglobin–oxygen affinity. A seemingly inconsistent finding that hemoglobin–oxygen affinity can decrease during a single passage through the coronary system, in the presence of angina, without accumulating additional intraerythrocytic DPG[6] is probably explained by the demonstration that erythrocytes normally carry an intracellular reserve of DPG on their cell walls. This intracellular reserve can be mobilized rapidly to combine with hemoglobin and thus lower hemoglobin–oxygen affinity.[7]

Whatever the mechanisms, it follows that neither standard nor normal affinities are adequate under these circumstances, and one result of this research might be to simplify the clinical method of determining the normality or abnormality of hemoglobin–oxygen affinity. It certainly is feasible, in the clinical environment, to measure hemoglobin concentration and standard blood–gas factors, including P_{O_2}, P_{CO_2}, and pH. Relatively automatic and economical machines that measure oxyhemoglobin saturation of blood are now also available. The formulas and coefficients for the two-standard-deviation limits of affinity could be substituted into a blood–gas calculation program[8] that estimates oxyhemoglobin saturation or blood oxygen content mathematically from blood–gas data and hemoglobin concentration, using a digital computer or programmable desk calculator. This would result in rapid estimation of the limits of normal saturation or oxygen content, compensated for the patient's ventilatory and biochemical state, and at the measured P_{O_2} of the blood sample. Comparison of measured saturation or oxygen content against these calculated limits would suggest the extent to which the patient's affinity varied from normal. Although requiring the use of a computational device, the technique should prove more expeditious than measurement of the P_{50} or of the entire affinity relationship by usual methods.

Since standard hemoglobin–oxygen affinity is, for the most part, statistically and physiologically indistinguishable from normal affinity, and since the range of normality is narrow, there would seem to be no objection to continued use of current tables, graphs, slide rules, and formulas for most purposes. Certainly there should be no difficulty in recognizing as abnormal gross affinity changes observed in some disease states.

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