Effect of Varying Carbon Dioxide Tensions on the Oxyhemoglobin Dissociation Curves Under Hypothermic Conditions *

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THE EFFECT on the oxyhemoglobin dissociation curve of increasing carbon dioxide tension at body normal temperature has been known since Bohr [2] described the gradual drift of the sigmoid curve to the right and downward as the carbon dioxide tension increased. Hypothermia has been shown to have the reverse effect on the curve by causing it to move to the left and upwards.[1] A review of the literature failed to reveal data demonstrating whether these two opposing effects on the oxyhemoglobin dissociation curve could be made to negate each other, either partially or completely.

Therefore, a method was devised for determining the effects of simultaneous changes of pCO_2 and temperature on the dissociation curve. The older method of leaving gases of known concentration in contact with blood in a sealed tonometer has the following disadvantages:

1. The time required for equilibration is long.
2. It is difficult to assure that a random specimen of blood obtained for analysis from the tonometer contains an average number of red blood cells in accordance with the hematocrit of that particular blood.
3. Lowering the temperature of the sealed tonometer results in some condensation of the water vapor with consequent variability in the hematocrit during equilibration between the gas mixture and the blood.
4. Determination of the exact end-point of equilibration is difficult.
5. There is time for glycolysis and significant oxygen consumption by the erythrocytes to occur because of the protracted period for equilibration.

An apparatus was first constructed on the Severinghaus principle [3] using multiple open tonometers at one time, each perfused by a different gas mixture and each being rocked by a multilocked belt drive. By this method a constant water vapor tension was in part assured, for the gas was saturated at the temperature of the water bath, and equilibration of the blood proceeded at a quicker rate than in the sealed Barcroft tonometers. However, in the Severinghaus apparatus, when drawing specimens of blood through the sampling tubes (previously placed through the stop-

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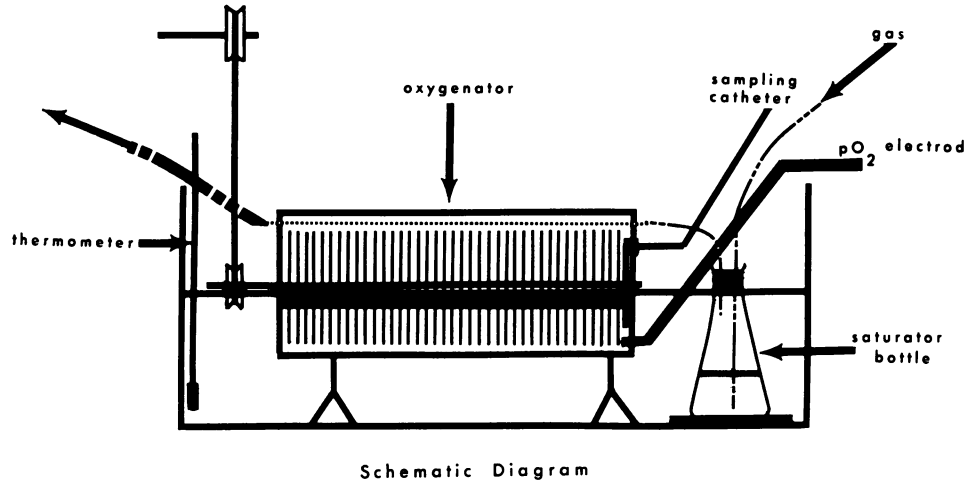

FIG. 1. Schematic diagram of Kay-Cross oxygenator used as a tonometer.

pers at the ends of the tonometers), hematocrits and oxygen contents were found to vary, giving evidence of sludging of red blood cells at the periphery of the flask. Furthermore, time for equilibration was still too long.

Therefore, a blood oxygenator was modified for use as a tonometer. This instrument was found to mix the blood thoroughly without undue hemolysis, and gave a larger surface area of blood exposed per minute to the gases. This shortened the time for equilibration and insured that all samples had the same hematocrit.

Method

The apparatus consisted of a Kay-Cross disc oxygenator, 13 inches in length containing 54 flat discs, 4.5 inches in diameter, which rotated on the central axis (Fig. 1). Flat discs were used because hemolysis was less than with convoluted discs. A polarographic pO_2 electrode and a sampling catheter entered the cylinder through one of the endplates. The cylinder contained 450–500 cc. fresh heparinized dog blood. The volume of heparin used caused negligible dilution of the blood. An aliquot of the blood was used to determine the hemoglobin and the methemoglobin. These values were obtained on the Beckman Spinco apparatus for micro-determinations. The hemoglobin was estimated at 542 m.u. of wave length, the methemoglobin at 635 m.u. of wave length. The error was about 0.5 per cent on these estimations. Sulfhemoglobin was not estimated because of its stable nature under the conditions of the experiment. Carboxyhemoglobin similarly was not estimated, for the apparatus was exposed to light during the equilibration and sufficient time elapsed for its conversion to hemoglobin.

The gas mixtures first passed through a water-vapor saturator bottle immersed in the same temperature controlled water bath as the oxygenator, then flowed into the cylinder via a small bored tube containing multiple orifices, and escaped through an opening in the opposite endplate. The gas flow was maintained at a rate sufficient to produce a steady influx into the cylinder, but not sufficiently great to cause an increase of pressure within the cylinder. The gas mixtures were analyzed by the Scholander method and the partial pressures of each gas calculated, taking into account the water vapor and barometric pressures. Values were also estimated for the solubility of oxygen in plasma at varying tem-

peratures. These values were obtained by assuming a constant ratio between the solubility of oxygen in water, obtained from tables, and its solubility in plasma for all temperatures. This dissolved oxygen fraction was subtracted from the oxygen content as obtained from the Van Slyke analysis and the amount in combination with hemoglobin used to estimate its saturation as a percentage of the oxygen capacity. The amount of methemoglobin was subtracted to give the true hemoglobin value.

The discs were allowed to rotate slowly presenting the blood to the flowing gases, until the polarograph indicated a constant value for the pO_2 of the blood. Some difficulty in stabilization of the polarograph was encountered when the higher tensions of oxygen were being used. However, with a little time stability was achieved. Three samples of blood were then drawn in syringes prepared with mineral oil. The volume of oil used was negligible.

Van Slyke. The syringe was sealed, the sample mixed, and then kept in an ice bath while awaiting analysis, the interval between the drawing of the sample and the analysis being kept to a minimum. Two technicians were used for both Scholander and Van Slyke analysis, and the accuracy of each was checked by duplicate and triplicate estimations. These errors were considered in the establishment of confidence limits on each point on the curves as shown later.

Hematocrit. The second sample was used to determine the hematocrit, so as to estimate the amount of mixing of the blood being obtained. Plasma hemoglobins were not estimated because previous experience with this type of oxygenator had been shown to yield negligible hemolysis. After centrifugation the plasma was always found to be clear.

pH. The third sample was used to determine the pH, using a Beckman Model G apparatus with a glass electrode, which was frequently standardized at the temperature of the water bath during the procedure.

Carbon dioxide tensions were selected for convenience at approximately 20, 40, 60, and 70 mm. Hg partial pressure by using gas mixtures containing various proportions of oxygen and CO_2. Each mixture thus contained oxygen at varying tensions approximately 5.0, 20, 50, 80, and 100 mm. Hg, and a curve could be constructed for each carbon dioxide isobar by plotting percentage saturation of the hemoglobin against the partial pressure of the oxygen. Temperatures also were selected for convenience at 15, 23, 30, and 37° C., and four curves, one for each carbon dioxide level, were plotted for each isotherm. Some difficulty was encountered in having exactly the same carbon dioxide tension for a family of oxygen points, because

1) There were slight variations in the carbon dioxide contents of the tanks (as analyzed by the Scholander method);

2) The water vapor pressure varied at different temperatures; and

3) The barometric pressure varied during the running of an experiment due to rapid weather changes.

However, the carbon dioxide tensions for each curve were kept constant within about 3.0 to 5.0 mm. Hg over-all range from the highest to the lowest tension.

The total ionic and protein contents of the plasma were not estimated in this experiment and their effects on the solubility coefficient of oxygen in the plasma were neglected.

The data obtained from very anemic dogs were excluded because the dissolved oxygen fraction under these circumstances would have exceeded the predicted value in view of the lowered red cell mass.

Results

The curves, expressing percentage saturation of hemoglobin against partial pressure of oxygen, for the various carbon di-

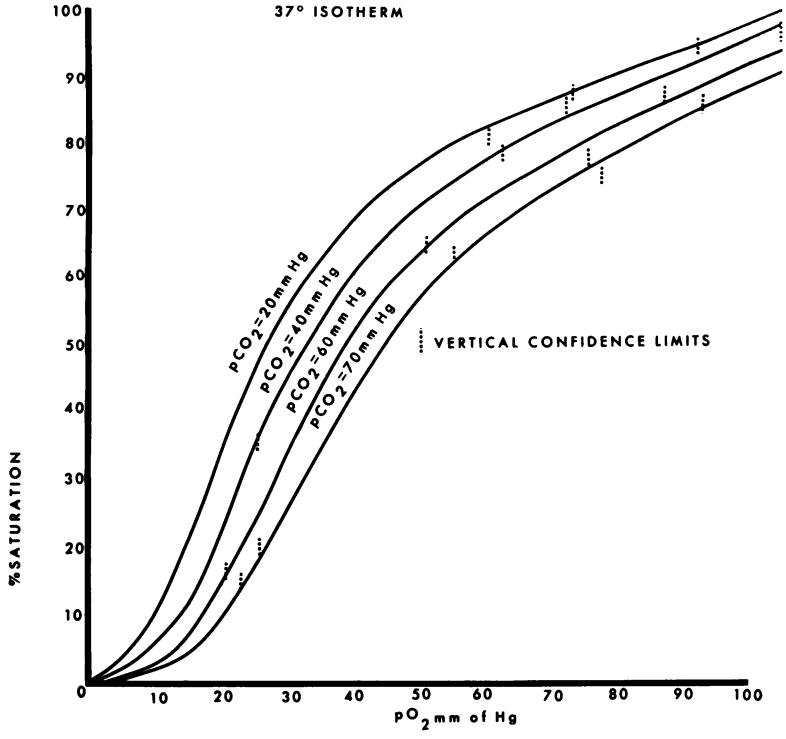

FIG. 2. Oxyhemoglobin dissociation curves with varying pCO_2 at 37° C.

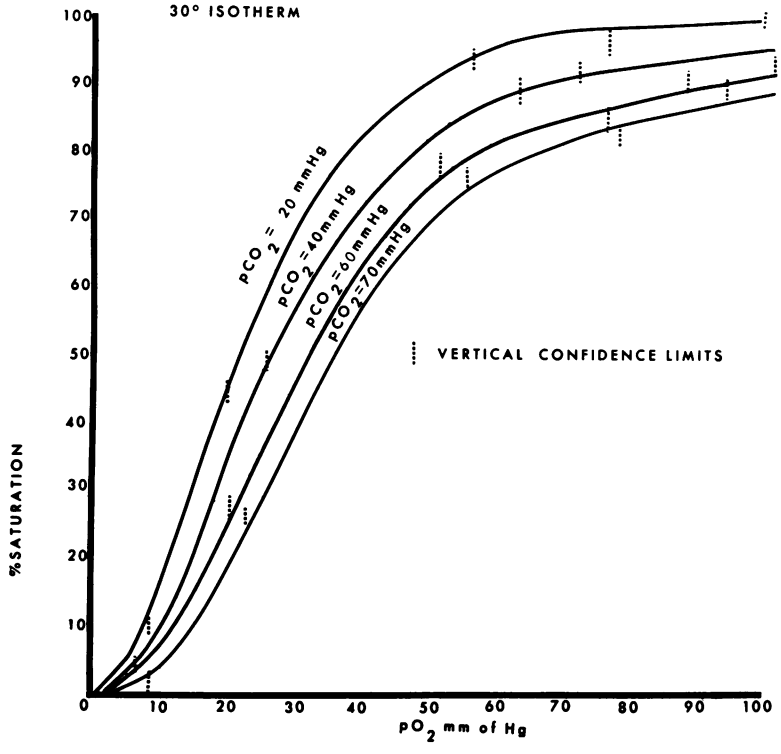

FIG. 3. Oxyhemoglobin dissociation curves with varying pCO_2 at 30° C.

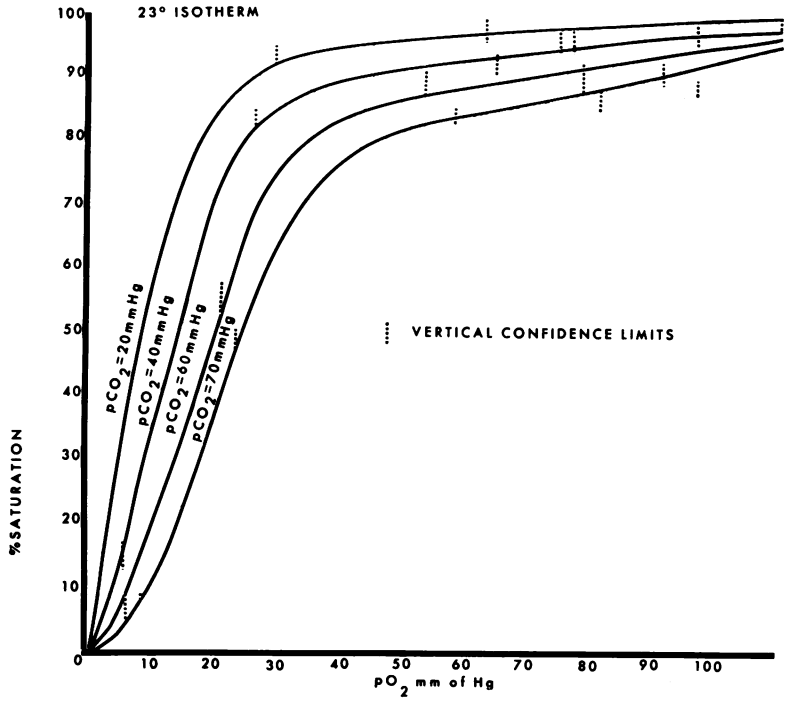

FIG. 4. Oxyhemoglobin dissociation curves with varying pCO_2 at 23° C.

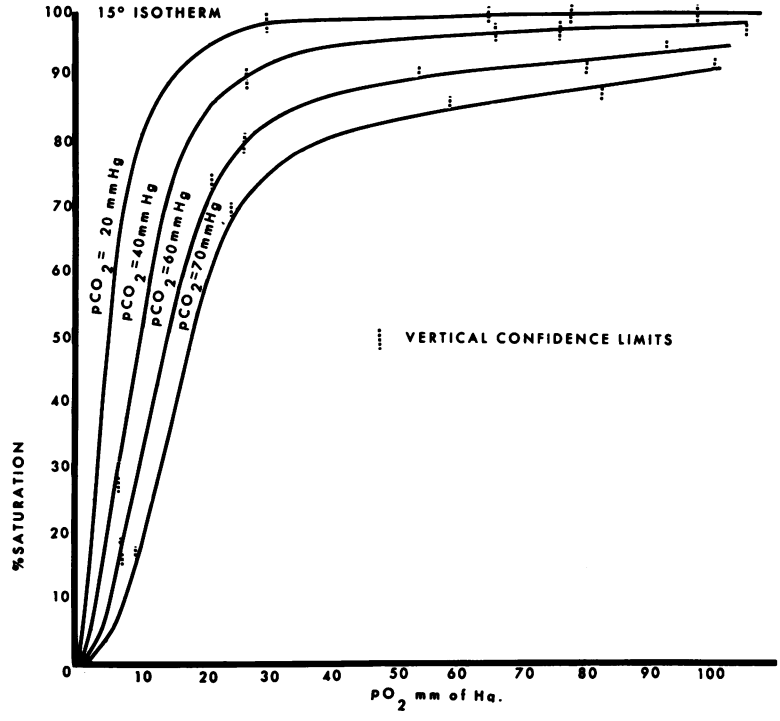

FIG. 5. Oxyhemoglobin dissociation curves with varying pCO_2 at 15° C.

oxide isobars at the four isotherms are shown in Figures 2–5. The vertical limits include an error of ± 0.15 per cent in the hemoglobin estimation, in addition to the Van Slyke errors which were ± 0.15 Vols. per cent for one operator, and ± 0.10 Vols. per cent for the other. The horizontal confidence limits include corrections for errors in the Scholander gas analysis (± 0.4 per cent for each operator), but graphically the horizontal errors were so small as to be negligible.

The values plotted in Figures 2–5 have been corrected for hematocrit variations. By using this method for equilibration, corrections of this type have been kept to a minimum. However, if the hematocrit of a particular blood is low, its oxygen content will be correspondingly low, for the amount of oxygen dissolved in the relatively increased plasma compartment cannot compensate for the deficiency due to the decreased number of red cells. Consequently, the percentage saturation obtained must be increased in proportion to the deficiency in the hematocrit. The converse is true if the hematocrit is not corrected. Not only is the position of the curve lost but also its shape, because shape is determined by the relative positions of the various points in the pCO_2: 60 mm./Hg curve, as shown in Figure 6. Corrections of this type returned this curve in Figure 6 to that shown in Figure 1. The hematocrit variations do not apply to relationships between different curves because the oxygen contents are expressed as a percentage of the oxygen capacity of that particular blood. However, the variations in the hematocrit between curves will affect the relative positions of points on the curve, because they are referred to one particular oxygen capacity.

Discussion

The results obtained indicate that an opposing influence to hypothermia is ex-

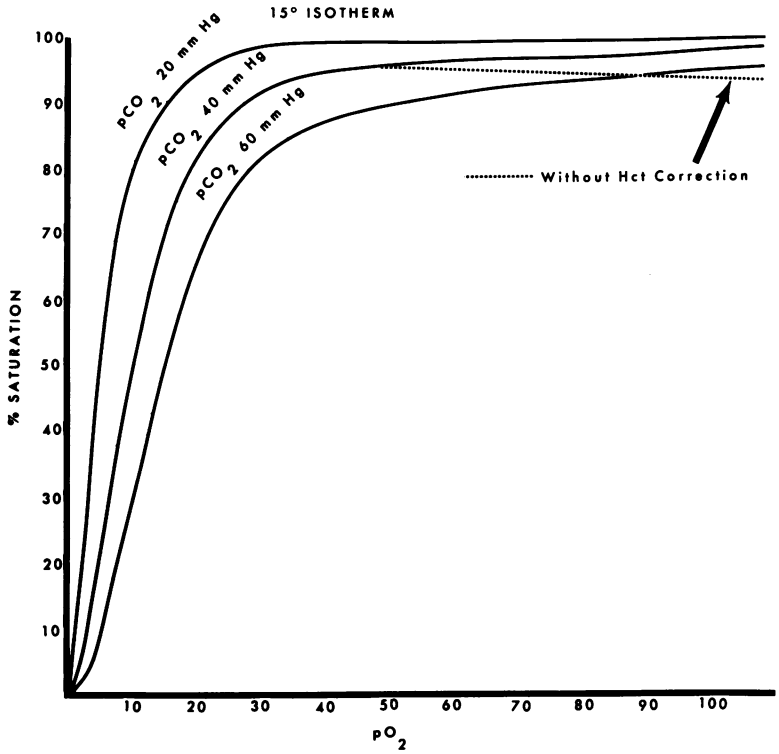

FIG. 6. Oxyhemoglobin dissociation curves with varying pCO_2 at 15° C., showing curves corrected and uncorrected for hematocrit.

TABLE 1. *Percentage Oxygen Saturation of Blood at 100 and 35 mm./Hg pO_2 with Varying pCO_2 and Temperatures*

pCO_2: mm./Hg	20		40		60		70	
pP_2: mm./Hg	100	35	100	35	100	35	100	35
Temperature								
37° C.	99	63	97	55	94	45	90	35
30° C.	99	78	98	65	92	56	88	49
23° C.	99	92	98	87	96	79	92	71
15° C.	99	99	99	92	96	83	91	78

erted by a rising carbon dioxide tension. Conversely, lower carbon dioxide tensions reinforce the effect of hypothermia, and move the curve upward and to the left.

The advent of deep hypothermia as an accepted technic in cardiovascular surgery requires that a reappraisal be made of the oxyhemoglobin dissociation curves at low temperatures so that a fuller understanding may be obtained of the relationships between the blood and tissue oxygen. The factors determining the passage of oxygen from the blood into the tissues are: 1) the gradient in oxygen tensions between the plasma and the tissue; and 2) the willingness of the red cells to give up oxygen.

The availability of oxygen from the red cell under any particular set of circumstances can be determined by studying the oxyhemoglobin dissociation curve applicable to those conditions and, especially, by calculating the degree of saturation at 35 mm./Hg (normal tissue oxygen tension). Normally blood enters the arterial end of the capillary with an oxygen tension of about 100 mm./Hg, and leaves the venous end with a tension of 35 mm./Hg. The amount of oxygen available to the tissues from oxyhemoglobin depends upon the difference in saturation of the blood at 100 mm./Hg and at 35 mm./Hg of oxygen tension. For example, at 37° C. blood with a pCO_2 of 40 mm./Hg is 97 per cent saturated at 100 mm./Hg oxygen tension; at 35 mm./Hg oxygen tension the same blood is 55 per cent saturated. Thus, between the arterial and venous end of the capillary it is possible for this blood to give up 42 per cent of its contained oxygen. However, at 15° C. the blood would be 99 per cent saturated at 100 mm./Hg oxygen tension and 92 per cent saturated at 35 mm./Hg oxygen tension. Therefore, at this temperature only 7.0 per cent of the contained oxygen is available for uptake by the tissues (1/6th as much as at 37° C.). The availability of oxygen under other conditions of pCO_2 and temperature can be determined from Table 1.

These limitations of oxygen dissociation make it clear that the A-V oxygen difference at low temperature depends more upon the tenacity of the red cell for oxygen than the demands of the tissues. A small A-V oxygen difference may represent an imposed restriction of diffusion and not a satisfied oxygen demand. A standard estimation of oxygen consumption, therefore, probably bears little or no relationship to oxygen requirement, and may be largely a measurement of the consumption of the oxygen dissolved and transported in the plasma, rather than that carried by hemoglobin. The classical use of the A-V oxygen difference as a measure of the adequacy of oxygen supply and the efficiency of oxygen extraction, therefore, does not obtain at low temperatures. However, the uptake of oxygen can be improved by increasing the pCO_2 of the arterial blood (Table 1)

and it may, therefore, be advantageous to increase the pCO_2 and thereby, the efficiency of oxygen transfer.

The great increase in the amount of carbon dioxide dissolved in the plasma at lower temperature raises possible hazards. If carbon dioxide were administered in increased percentages, the dissolved fraction would increase further and difficulty with bubbles might become a serious factor as the blood was warmed. This also applies to the dissolved oxygen but because of a much lower solubility coefficient of oxygen in comparison to that of the carbon dioxide, and its much smaller changes with temperature, its bubble forming potential compared with the dissolved carbon dioxide is minimal. On the other hand, if careful control of changes in blood temperature were exerted, bubble formation could probably be prevented and thus the desirable effect of increasing oxygen dissociation at low temperatures could be obtained.

Summary and Conclusions

An experiment was designed to investigate the effects of different carbon dioxide tensions on the oxygen dissociation curve of blood at various temperature levels using a rotating disc oxygenator as a tonometer. Variations in hematocrit were shown to be important in the construction of the curves obtained.

A rising carbon dioxide tension moves the dissociation curve to the right and flattens it in shape at all the temperatures studied, and raising the carbon dioxide tension opposes the effect of a simultaneous fall in temperature.

At low temperatures (23° to 15° C.) the oxygen dissociation curve moves so far to the left that, at normal arterial blood and tissue oxygen and carbon dioxide tensions, there is so great a tenacity of hemoglobin for oxygen that tissue oxygen demands probably cannot be met by oxygen transported by hemoglobin. Arterio-venous oxygen differences no longer can be used as a valid estimate of adequate tissue oxygenation. Dissolved plasma oxygen becomes increasingly significant as a source of tissue oxygen under these conditions.

If bubble formation were prevented by careful temperature control, increased carbon dioxide tension might be clinically desirable in profound hypothermia to increase oxygen dissociation at low temperatures.

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