Does Anesthesia Alter Hemoglobin Dissociation?

Theodore C. Smith, M.D.* Ethan T. Colton, III, M.D.,†
Marjoram G. Behar, Ph.D.‡

That anesthesia may alter hemoglobin oxygen affinity was tested in 19 groups of six to eight individuals. Paired venous and arterial blood samples were analyzed for oxyhemoglobin content, pH, base excess and PO\textsubscript{2}. Venous oxygen tension was calculated from the standard dissociation curve, allowing for body temperature. In conscious man this calculated PO\textsubscript{2} agreed well with the directly-measured PO\textsubscript{2}. During general anesthesia, however, there was usually a positive difference of measured PO\textsubscript{2} minus calculated PO\textsubscript{2} unrelated to agent, duration, or degree of anesthesia. The shift in dissociation ranged from less than one torr in a group inhaling diethyl ether to 12 torr during Ethane anesthesia, with intermediate values in other groups and with other agents. The average direction and degree of the shift were sufficient to compensate for a Bohr effect on hemoglobin dissociation resulting from a pH of 7.6. (Key words: Hemoglobin; Dissociation.)

Arterial oxygen tension of anesthetized man is regularly and significantly lower than expected from knowledge of the inspired oxygen concentration, the ventilation, and preoperative pulmonary status.\textsuperscript{1,4} The alveolar-arterial difference for oxygen (A-aDO\textsubscript{2}) is typically increased three- to tenfold during anesthe sia even in healthy young adults. Brodie and Coburn\textsuperscript{5} recently demonstrated that a moderate increase in carboxyhemoglobin could shift the hemoglobin dissociation curve in a direction such as to augment an A-aDO\textsubscript{2} arising from either anatomic shunting or ventilation-perfusion maldistribution. Nunn suggested that anesthesia might also alter the shape of the curve, contributing to the observed arterial-alveolar difference for oxygen.\textsuperscript{6} A shift to the left would increase pre-existing A-aDO\textsubscript{2} and a drift to the right would result in a decrease. It is possible that either the anesthetic or the state of anesthesia alters hemoglobin-oxygen affinity. Xenon is known to bind to hemoglobin.\textsuperscript{7} Both xenon and cyclopropane are known to bind to myoglobin, thus altering its tertiary structure.\textsuperscript{8}

In the course of measurement of cerebral flow, pulmonary blood flow, and cardiac output in both volunteers and patients over the last five years, we have amassed considerable data bearing on hemoglobin dissociation. While these experiments were planned and executed without thought to the present problem, they included careful measurements of oxygen content and partial pressure, acid-base status, and hemoglobin or hematocrit in paired venous and arterial samples. These analyses have been supervised by one of us (M. G. B.) over this entire period.

Hypothesize that anesthesia and anesthetics do not alter oxyhemoglobin dissociation. Therefore, pairs of blood oxygen tension and hemoglobin saturation values from anesthetized subjects' blood should lie on the standard hemoglobin dissociation curve when corrected for pH, temperature, and base excess by the standard factors. The venous blood samples from awake and anesthetized man provide the best test of the hypothesis since they lie on steeper portions of the dissociation curve. If the points form a curve indis-
tangible from the normal curve, the hypothesis may be accepted, but if not, anesthesia either alters the hemoglobin affinity or invalidates corrections for acid-base disturbance and temperature. The latter may be tested by examining varied acid-base conditions. The data indicate a slight but real rightward (not leftward) shifts of dissociation during anesthesia with five different agents. The usual acid-base and temperature corrections minimize scatter and are probably valid.

Methods

Blood samples were drawn anaerobically from brachial or femoral artery, and by the internal jugular vein, right atrium, or pulmonary artery, into individually-ground, heparinized glass syringes, then promptly sealed and iced. The heparin solution in the deadspace of the syringe was flushed out once or twice, to avoid dilution of the samples. Oxygen content measurements were made by the technique of Van Slyke and Neill, or, in the presence of considerable dissolved anesthetic gases, by the polarographic method of Klingenmaier, Behar, and Smith. Hemoglobin saturation of venous blood was calculated from oxygen content of the simultaneously drawn venous and arterial pair, corrected for dissolved oxygen and minor differences in hemoglobin, whenever arterial PO2 exceeded 150 torr. In the few instances during anesthesia (and in awake air-breathing and hypoxia studies) with lower arterial PO2, venous oxygen capacity was measured after tonometry or calculated from hemoglobin content. Hemoglobin was measured by the cyanmethemoglobin method. Carbon dioxide and oxygen tensions and pH were measured with Instrumentation Laboratory micro or ultra-micro electrodes, corrected for differences in temperature between electrode and patient by the nomogram of Hedley-Whyte et al. or by the nomogram of Severinghaus when the oxygen tension was below 150 torr. Blood of most subjects was equilibrated with a known gas mixture in an Instrumentation Laboratory rotating tonometer and the correction factor for difference in electrode response between gas and the patient's blood on that day used to correct the oxygen tension measurements. Hemoglobin saturation was occasionally measured spectrophotometrically, as a confirming check on hemoglobin and oxygen content. The measured values were averaged in groups of six or more individuals subjected to similar experimental conditions. The results are given as a shift along the PO2 axis (positive indicating a shift rightward) induced by the condition studied, and displayed graphically as a point at the measured saturation displaced from the standard pH of 7.4 curve by the observed ΔPO2.

Results

Six groups of six or more men and one group of women provided the sets of data shown as solid dots in figure 1a, documenting the reliability of the methods in conscious man. Thin lines connect sets from the same group of subjects. For example, from a study of cerebral blood flow during deliberate hypoxia, we show venous data during breathing of air and both arterial and venous data during breathing of oxygen at three connected points. For another study breathing 0.3 per cent carbon monoxide for diffusing capacity measurements, we show venous samples during inspiration of 20 and 100 per cent oxygen as connected dotted circles. The average data fall nicely on the standard hemoglobin dissociation curve between 65 to 90 per cent saturation. Two conditions studied are known to affect the curve: hypoxia and inhalation of carbon monoxide. The arterial and venous hypoxia points (the two lowest PO2's to saturations of figure 1a) are to the right of the curve. This is consistent with a moderate rise in erythrocytic 2,3-diphosphoglycerate (2,3-DPG) found by Lanfant during hypoxia. (However, no data exist to indicate that our brief severe hypoxia will produce prompt elevation of 2,3-DPG. Lanfant studied chronic mild hypoxia.) Breathing carboxyhemoglobin reduced the oxygen capacity of the blood by approximately 12 per cent, and caused a shift of the hemoglobin dissociation curve to the left approximately 4 torr (circled points in figure 1a). This is just the magnitude expected from the known effect of carbon monoxide on hemoglobin oxygen affinity.
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**Fig. 1.** (left), segment of the standard hemoglobin dissociation curve with points representing observed mean values from eight groups of awake subjects. The arterial and venous points from the experiment of deliberate hypoxia are the lowest two, connected by a light line to the normoxic venous points of the same subjects. A light line connects different observations in the same group of subjects. (right), all of the data from anesthetized subjects superimposed on two dissociation curves. The heavier line to the left is the standard curve. The light line to the right is the effect of the mild hypoxia of altitude with an increase of 2,3-diphosphoglycerate. Each point is the mean of six or more individuals.

All of the data from various anesthetics are shown in figure 1b. The heavier curve is the standard dissociation curve; the lighter line is that caused by a 1-mM increase in 2,3-DPG. The average shifts associated with anesthesia are listed in the table.

Data obtained during studies of halothane anesthesia are shown in figure 2a. These data contain the only major departure from uniform behavior in the five anesthetic drugs studied. In one group of men studied one hour, two hours, and three hours after anesthesia (three closely-grouped connected points), the measured points of oxygen tension and saturation fell almost exactly on the hemoglobin dissociation curve. In puzzling con-

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<th>Table 1. Average Shifts in Hemoglobin Dissociation</th>
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<td>Agent</td>
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</tr>
<tr>
<td>None</td>
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<tr>
<td>Halothane</td>
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<td>Ethrane</td>
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<td>Diethyl ether</td>
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<td>N_{2}O</td>
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<td>CH_{3}Cl</td>
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* Each group represents six to eight subjects. Some groups provided two or three sets of data as duration of anesthesia, acid-base balance, depth of anesthesia, etc., were altered. ΔP_{O2} is the average difference between observed and calculated oxygen tensions of venous samples. P_{aO2} is the usual method of describing hemoglobin affinity for oxygen, the tension at which hemoglobin is 50 per cent saturated. P_{aO2} ranges 24 to 27 torr in normal man. Data from conscious man excludes carbon monoxide and hypoxia data.
Fig. 2. Data from five anesthetics superimposed on the standard dissociation curve. The dotted circles in the upper left panel are values during carbon monoxide inhalation. The single triangle in the lower left panel is the value for halothane-supplemented N₂O anesthesia, while the rest are values for d-tubocurarine-supplemented N₂O anesthesia. A light line connects observations from the same group of subjects.

Contrast, groups of six men studied two years earlier (three points farthest to the right of the curve, connected by a thin line) and one year later (two connected points) evidenced clear shifts in hemoglobin dissociation. Furthermore, in a study of diffusing capacity by the steady-state carbon monoxide uptake method (the dotted circles connected by a light line), the pairs of values for saturation and tension lay on the predicted hemoglobin dissociation curve despite the presence of 12 to 15 per cent carboxyhemoglobin. This suggests that halothane anesthesia caused a rightward shift of approximately the same magnitude as the leftward shift of 12 per cent carboxyhemoglobin. On the average, during halothane anesthesia (excluding only the carbon-monoxide breathers), we found a 5.8-torr shift at 77 per cent saturation.

Diethyl ether and Ethane anesthesia provided the data for the points in figure 2b. The diethyl ether measurements were made
during anesthesia of light or light-and-moderate depth and show only slight shifts to the right in two of the sets. The Ethane anesthetics include a group of three sets, studied at hypocarbic, normocarbic, and hypercarbic, with an average $\Delta P_{O_2}$ of 10.4 torr, and a group studied at two tensions of alveolar Ethane equivalent to light and moderate anesthesia, with an average $\Delta P_{O_2}$ of 3.8 torr.

Results from nitrous oxide anesthesia with various states of hyperventilation are shown in figure 2c. In one group the inspired carbon dioxide was varied while ventilation was kept constant to study the conditions of normocarbic (pH 7.4), moderate hypocarbic (pH 7.8), and severe hypocarbic (pH 7.8). Jugular venous oxygen content and tension fell, but there was no systematic difference in the $\Delta P_{O_2}$ as alkalosis increased. In another group pH was varied by alkaline infusion, creating a pH of 7.8 while the $P_{CO_2}$ was 20 torr. The $\Delta P_{O_2}$ was slightly greater in this group of six men than in the group hyperventilated to a pH of 7.8. In other studies we varied the time of anesthesia over three hours, and varied $P_{CO_2}$ by altering total ventilatory volume. Both produced approximately the same $\Delta P_{O_2}$ of +4 torr. The only exceptional behavior during nitrous oxide anesthesia occurred only in a single experiment, involving six subjects under halothane-supplemented nitrous oxide anesthesia, shown as a triangle in figure 2c, in which a $\Delta P_{O_2}$ of −0.1 torr was found.

Results from cyclopropane experiments are shown in figure 2d. Five groups of six or more healthy young adult volunteers produced the eight data points. In every instance, the average $\Delta P_{O_2}$ had a positive sign. The increases ranged from one to 9 torr over saturations of 64 to 94 per cent. There was no dose-related effect. The greatest shift was seen with 8 per cent cyclopropane, where 68 per cent saturation gave a $P_{O_2}$ of 46 torr, 10 torr above predicted. The smallest shift, one torr, was seen with a cyclopropane concentration of 36 per cent. Concentrations of 12, 20, and 50 per cent gave intermediate $\Delta P_{O_2}$’s. During inspiration of 20 per cent cyclopropane in 25 per cent oxygen or in 80 per cent oxygen, alteration of the $P_{CO_2}$ by hyperventilation ($P_{CO_2}$ 20 torr) or by spontaneous ventilation ($P_{CO_2}$ 50 torr) produced shifts of +3 and 5 torr.

Discussion

With the exception of one group of eight men during prolonged halothane anesthesia, one group of six breathing nitrous oxide-halothane, and another group of six breathing diethyl ether, 33 of 36 sets of data (collected from 18 groups of six to eight subjects or patients) showed decreased affinities for oxygen amounting to an increase of approximately 3 to 4 torr in the oxygen tension calculated to produce 50 per cent saturation, the $P_{50}$. A systematic error producing this effect could be produced by a major error in measurement of body temperature, but the thermistor and Yellow Springs thermometer combination were frequently calibrated against a N.B.S. standard mercury thermometer, and were not in error by enough to account for more than one-tenth of the observed shift. Errors in pH measurement sufficient to account for this shift would be of the magnitude of 0.2 units. Considering the excellent correlation of $CO_2$ tension and the measured blood pH, it is inconceivable that a systematic error in pH accounting for as much as one-tenth of the observed shift existed. A systematic error in saturation to account for the data would require that venous content be regularly low by one volume per cent while arterial content is measured accurately. In fact, the methods are at least tenfold better, internally consistent, and consistent with the spectrophotometric data. The finding that values from awake men lie within one torr of the normal $P_{50}$ further buttresses our argument that this shift is real.

A recent editorial reviewed 2,3-diphosphoglycerate metabolism and hemoglobin’s oxygen affinity in various clinical conditions, and showed that both rise in conditions known to produce hypoxia. Perhaps localized areas of blood stasis and tissue hypoxia may be heralded by this shift. An increase in erythrocytic organic phosphorus of the order of only 100 micromolar would account for the observed differences. We are preparing to study this question.
This survey was prompted by observations of abnormal A-aD_{2} values developed in ostensibly healthy men during general anesthesia. We have found an effect of anesthesia on the oxyhemoglobin dissociation curve such as to reduce the magnitude of an A-aD_{2} due to physiologic shunting by a rightward shift in the hemoglobin dissociation curve. Since anesthetists customarily and frequently induce states of mild hyperventilation yielding pH values of 7.5 to 7.6, this demonstrated decrease in the oxygen affinity of hemoglobin serves the unintended function of compensating for hyperventilation-induced increase in hemoglobin’s oxygen affinity. The occurrence of a shift of similar magnitude resulting from a broad spectrum of anesthetic techniques, during various acid–base balance values, and over widely varied depths of anesthesia, offers little help in identifying the cause of the abnormality. An obvious but unsatisfying suggestion is that there must be something in the state of anesthesia other than the physical presence of the anesthetics themselves which alters hemoglobin affinity. It is known from x-ray crystallography that xenon and cyclopropane bind to crystallized hemoglobin. If such sites are saturated by less than an anesthetic concentration, one would not see dose-related changes, but the shift then is to the left.

Whatever the cause of this interesting abnormality, it tends to improve oxygenation in patients, and has more theoretical than practical importance. However, since hypoxemia is known to stimulate 2,3-DPG synthesis, it may be that areas of stagnant hypoxia will be reflected by a rise in P_{50} of mixed erythrocytes. Therefore, further experiments will need to examine not only in vitro effects of anesthetics on hemoglobin, but in vivo effects as well.

References