The Absence of an Effect of Halothane on Blood Hemoglobin O_2 Equilibrium in Vitro

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The effects of halothane on blood hemoglobin–oxygen equilibrium *in vitro* were studied. Mean P_{50} of blood equilibrated with gas which did not contain halothane was 26.53 ± 0.27 torr. Mean P_{50} of blood equilibrated with 1 to 2 per cent halothane was 26.79 ± 0.35 torr ($P > 0.5$). The study did not show any significant effect of halothane on the affinity of hemoglobin for oxygen. (Key words: Hemoglobin dissociation; Halothane; P_{50}; Oxygen dissociation curve.)

IN A RETROSPECTIVE REVIEW of their work with human volunteers, Smith *et al.*[1] reported a substantial increase in the hemoglobin P_{50}, the P_{O_2} for half-saturation of hemoglobin at standard pH and temperature, as a result of administration of general anesthesia to man. Some anesthetics appear to bind to the hemoglobin molecule,[2,3] and it has been suggested that such binding might alter the physical configuration and the physiologic properties of the protein.[4] The physical shape of hemoglobin does change with oxygenation.[5] Recently, Laasberg and Hedley-Whyte demonstrated binding of halothane to the β chain of hemoglobin.[3] It is, then, of interest to know whether anesthetic agents alter the affinity of hemoglobin for oxygen *in vitro*. This does not appear to have been tested previously.

Methods

We drew blood from each of six volunteers into a heparinized glass syringe, then divided the sample into four aliquots. To each syringe we added the appropriate gas mixture to achieve approximately $P_{CO_2} = 40$, $pH = 7.4$, and $P_{O_2} = 25$–30. For equilibration with halothane, the anesthetic was added in concentrations sufficient to result in gas phase concentrations of 1 to 2 per cent at equilibrium. We then rotated all samples in a tonometer at 37 C for 45 minutes. The gas phase was then ejected and analyzed for halothane, and the blood phase stored anaerobically in ice until the following determinations were made.

Blood P_{O_2}, P_{CO_2} and pH were measured with appropriate electrodes. The gas/blood correction factor of the P_{O_2} electrode was determined by a modification of the method of Hulands *et al.*,[6] in which water equilibrated with air at 37 C was compared with air. Using their terminology, $\emptyset$, the gas/liquid factor, was calculated as:

$$\emptyset_b = 1 + 1.8 \, (\emptyset_w - 1)$$

where subscripts "b" and "w" refer to blood and water, respectively.

During this study we encountered considerable difficulty in determining the P_{O_2} values of those bloods equilibrated with halothane. We conducted a separate series of experiments from which we concluded that halothane is polarographically reduced by the oxygen electrode.[7] Some halothane, then, is read electrically as oxygen. The magnitude of the error is dependent upon several factors, and

Table 1. Results*

Subject	Without Halothane						With Halothane						
	P_{O_2} (torr)	P_{CO_2} (torr)	pH	C_{HCO_3} (vol %)	Per Cent Saturation	P_{50} (torr)	P_{O_2} (torr)	P_{CO_2} (torr)	pH	C_{HCO_3} (vol %)	Per Cent Saturation	P_{50} (torr)	ΔP_{50} (torr)
Subject 1	25.5 27.3	35.0 38.8	7.440 7.408	9.38 9.76	40.9 51.9	26.7 26.7	40.0 39.0	26.8 —	7.476 7.402	14.62 13.53	77.7 71.9	27.1 27.2	+ 0.4 + 0.5
Subject 2†	15.9 18.3	40.2 36.7	7.372 7.330	3.20 4.95	20.8 28.8	26.0 26.8	18.2 20.8 21.2	41.4 35.3 35.0	7.369 7.408 7.394	4.26 5.48 5.88	27.2 31.9 34.2	25.6 28.1 27.3	− 0.4 + 1.3 + 0.5
Subject 3	24.6	33.7	7.400	7.34	45.2	26.6	18.7	39.0	7.398	4.31	26.5	26.9	+ 0.3
Subject 4†	28.7 26.8	30.0 35.8	7.452 7.421	9.93 9.91	60.7 53.4	25.6 26.0	33.4 28.0 24.9	33.0 29.8 26.7	7.380 7.428 7.444	10.40 8.96 7.06	64.4 53.1 47.2	26.4 28.2 27.7	+ 0.8 + 2.2 + 1.7
Subject 5	13.8	27.2	7.464	—	19.6	26.0	23.9 19.9	44.7 39.6	7.302 7.325	— —	42.0 33.0	24.4 24.4	− 1.6 − 1.6
Subject 6	22.4	40.5	7.341	—	32.0	26.4	25.3 22.5	41.5 39.9	7.314 7.352	— —	39.8 34.6	27.4 27.6	− 1.0 − 0.8
Mean						26.53						26.70	+ 0.18
SD						± 0.82						± 1.26	± 1.20
SE						± 0.27						± 0.35	± 0.33

* Each value for P_{O_2}, pH, P_{CO_2}, C_{HCO_3}, and per cent saturation (IL) is a mean of duplicate determinations.
† Determinations were made on more than one day.

can be reduced by maintaining the polarizing voltage of the electrode at 0.55 volts rather than the customary 0.68 v, and by frequently grinding the cathode surface of the electrode with a wet arkansas stone. Subsequent work was done with a polarizing voltage of 0.55 v and a carefully polished electrode. Calibrating gases were read immediately before and immediately after reading each blood P$_{O_2}$. Saturation was determined from the measurements of the contents and capacities of samples either by an IL CO-oximeter or by the method of Klingenmaier et al.[8] as modified by Behar and Severinghaus,[9] in which a sample of blood is mixed with 50 times its volume of CO-saturated, O$_2$-free water and its P$_{O_2}$ measured. Oxygen content, P$_{O_2}$, P$_{CO_2}$ and pH for all samples were determined in duplicate. A correction for dissolved oxygen in every sample was made by subtracting from the determined oxygen content an amount equal to 0.0031 P$_{O_2}$ vol per cent. P$_{50}$ was calculated as $26.6 \times M/C$ where M is the measured P$_{O_2}$ of the initial sample and C is the P$_{O_2}$ calculated for that sample from the measured saturation, pH, and base excess, using the Severinghaus blood–gas calculator.[10]

The concentration of halothane in the gas phase of the equilibrated sample was measured either by gas chromatography or by infrared spectrophotometry.

Results

The results are listed in table 1.

The mean P$_{50}$ of the blood samples equilibrated with gas which did not contain halothane was 26.53 ± 0.27 (SE) torr. The mean P$_{50}$ of blood samples containing halothane was 26.79 ± 0.35 torr. Standard error of difference was 0.33. These results failed to show a significant difference by Student's t test ($P > 0.5$).

Discussion

Inasmuch as halothane binds to the β chain of hemoglobin,[2] it might be expected that the physiologic properties of the protein would be altered. We conclude from this study, however, that the affinity of hemoglobin for oxygen remains unchanged in the presence of halothane. Any change in P$_{50}$ during halothane anesthesia must be related to something other than a direct effect of the agent upon the hemoglobin molecule.

Halothane (Fluothane) supplied by Ayerst Laboratories.

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