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Blood Carbonic Anhydrase Activity in Anesthetized Man

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CARBONIC anhydrase is an enzyme found in many mammalian tissues but is especially concentrated in erythrocytes, renal cortex, and gastric mucosa. Carbonic anhydrase in erythrocytes (there is none in plasma [1]) serves to catalyze the reaction

$$CO_2 + H_2O \rightleftharpoons H_2CO_3.$$

The majority of carbon dioxide dissolved in blood at the tissue level is hydrated with the aid of carbonic anhydrase to H_2CO_3 which in turn promptly dissociates into H^+ and HCO_3^- without the aid of enzyme activity. Only a minority of carbon dioxide is transported to the lungs as physically dissolved CO_2 and as carbamino compounds. At the lungs, carbonic anhydrase catalyzes the above reaction in the reverse direction in erythrocytes to produce CO_2 which diffuses into alveoli. It has been calculated that in the absence of carbonic anhydrase, 200 seconds are required at 38° C. for the above reaction to come within 10 per cent of equilibrium.[2] Since blood remains approximately one second in pulmonary capillaries, a 200-fold increase in the rate of the reaction is required for attainment of equilibrium and elimination of CO_2. Actually, carbonic anhydrase may produce as much as a 7,500-fold acceleration of the reaction.[2]

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In the kidney, carbonic anhydrase plays an important role in acidification of urine. Plasma CO_2 diffuses into renal tubular cells and is hydrated to form H_2CO_3, again a carbonic anhydrase-accelerated reaction.[3] The resultant H_2CO_3 dissociates into HCO_3^- and into H^+ which is actively excreted into the tubular lumen in exchange for Na^+. H^+ is either excreted in urine as such, or is buffered, or finally, combines with filtered HCO_3^- to form CO_2 which diffuses back into plasma. When renal carbonic anhydrase is inhibited, large quantities of $NaHCO_3$ are lost in the urine resulting in an osmotic diuresis and eventually in metabolic acidosis. Since K^+ competes with H^+ in the exchange mechanism for Na^+,[3] depression of H^+ excretion also may lead to increased urinary loss of K^+.

In the parietal cells of gastric mucosa the formation of H_2CO_3 from plasma CO_2 by carbonic anhydrase activity serves as an important source of H^+ for the production of HCl. Administration of carbonic anhydrase inhibitors depresses or may even eliminate HCl secretion by gastric mucosa.

Considering the physiological importance of carbonic anhydrase, it is interesting that the effects of anesthetics on activity of this enzyme have not been examined with but one exception.[4] This one report described a 20 per cent decrease in blood carbonic anhydrase activity during thiobarbiturate anesthesia but did not include unanesthetized controls, hematocrit determinations, or other anesthetic agents. The

present study was instituted to provide information on carbonic anhydrase activity under clinical conditions in the presence of anesthesia produced with three commonly used agents.

Methods

Twenty-nine patients, 20 female and 9 male, scheduled for elective operation were studied. They were evenly distributed in age between 11 and 82 years. No selection of patients was made except to exclude those with thyroid disease and those who had received acetazolamide (Diamox), sulfonamides, iodides (in any form), bromides, or heavy metal compounds, all of which influence carbonic anhydrase activity.[5-7] Transfusions and intravenous fluids were not administered during the period of study.

All patients received atropine or scopolamine, 0.4–0.6 mg., and pentobarbital or secobarbital, 50–150 mg., as premedication at least 45 minutes prior to the induction of anesthesia. Immediately prior to induction, a 10 cc. venous forearm sample of blood was obtained in a heparin-moistened syringe which had been specially prepared to assure chemical purity. Exactly 30 minutes after induction of anesthesia and prior to the start of operation, a second venous sample was obtained, each patient thus serving as his own control.

In 10 patients ether was the anesthetic, in 10, cyclopropane, and in 9, thiopental-nitrous oxide. In all cases care was taken to assure adequate ventilation with insertion of pharyngeal airways or endotracheal tubes as indicated. Respirations were manually controlled with cyclopropane and were spontaneous or assisted with ether and with thiopental-nitrous oxide. At the time the second blood sample was obtained, depth of anesthesia was Plane 2 of Stage III as judged by clinical signs. Ether anesthesia was induced using nitrous oxide-oxygen (4:1 liters per minute) on a semiclosed system, the nitrous oxide being discontinued after 4–5 minutes, and the oxygen being continued thereafter at one liter per minute to assure almost complete removal of nitrous oxide and nitrogen at the end of 30 minutes. Cyclopropane anesthesia was induced starting with a system filled with 100 per cent oxygen to which 50 per cent cyclopropane in oxygen was added, the concentration of cyclopropane being reduced as the induction proceeded. Maintenance of cyclopropane anesthesia was on a semiclosed system of one liter per minute of oxygen with 200–250 cc. cyclopropane per minute to assure gradual denitrogenation. Thiopental-nitrous oxide anesthesia was administered by fractional doses of 2.5 per cent thiopental and nitrous oxide (3.5–4.0 liters per minute) with one liter per minute of oxygen. Adequate depth of anesthesia for the proposed surgery and for insertion of artificial airways when indicated was maintained. No other drugs including muscle relaxants were used at any time.

Blood samples were also drawn from healthy male controls aged 21 to 39 years. Controls rested on cots for one hour, the first sample being drawn after 30 minutes of rest and the second at the end of the hour.

Blood specimens were immediately transferred to a chilled Erlenmeyer flask, from which, within 2 minutes, exactly 2.0 ml. were transferred by pipette to a 100 ml. volumetric flask containing *ca.* 50 ml. chilled distilled water. The flask was then filled to 100 ml. with chilled distilled water, thus obtaining a 1:49 dilution of hemolyzed blood in water.

Carbonic anhydrase activity was measured using the Krebs-Roughton Warburg technique.[8] In this technique carbonic anhydrase activity is measured by measuring manometrically the rate of dehydration of H_2CO_3 at constant *p*H and temperature by measuring the rate of CO_2 production. This technique was employed in preference to others because (1) it requires low concentrations of phosphate buffer, thereby avoiding possible inhibition of carbonic anhydrase seen in the presence of high concentrations of phosphate[9]; (2) *p*H indicators with possible inhibitory effects are not required; (3) the effects of changes in *p*H on carbonic anhydrase activity are eliminated by maintaining a constant *p*H; (4) low temperatures, which may influence carbonic anhydrase activity,[10] are eliminated.

The Warburg respirometer employed consisted of a manometer and a flask, the flask being made up of a reaction chamber and a side-arm chamber. The graduated, U-shaped manometer was open to room air at one end and connected by an air-tight stopcock at the other end to the flask. The manometric fluid, Brodie's Solution[5] (10,000 mm. Brodie's

Solution = 760 mm. Hg), could be added or removed from the U-tube via an opening at its base. Solutions could be placed separately in either the reaction chamber of the flask or in the side-arm and were prevented from running together until such time as mixing was indicated by the angle of the side-arm.

With the flask upright, 1.5 ml. of buffer solution (40 millimoles Na_2HPO_4 and 26.7 millimoles KH_2PO_4 per liter) and 0.2 ml. of diluted blood were added to the reaction chamber and 0.5 ml. of substrate (0.05 molar $NaHCO_3$) was placed in the side-arm. The respirometer was then placed in a 30° C. water bath and shaken through a small arc 27 times per minute until there was no longer any change in pressure in the manometer attached to the flask. At the same time a thermobarometer (a respirometer containing, instead of buffer and substrate, an equal volume of distilled water) was placed in the water bath and shaken to record changes in pressure secondary to variations in temperature and barometric pressure. When pressure changes were no longer noted, both manometers were adjusted to the zero mark and removed with flasks attached from the bath. The buffer with the previously added blood specimen was then mixed with the substrate and the respirometers were returned to the water bath where shaking was resumed. Pressure readings were taken every 15 seconds until a pressure of 100 mm. Brodie's Solution had been obtained. Each blood sample was run in duplicate and preceded by a blank in which 0.2 ml. distilled water instead of diluted blood was placed in the reaction chamber.

Pressure readings were corrected for atmospheric pressure and temperature change by subtracting thermobarometer from respirometer readings. Corrected pressure readings were used in calculations without conversion to volume since the volumes of the four respirometers employed differed from a mean value of 16.986 cc. by an average of only 0.8 per cent. Rate of pressure increase was thus directly proportional to rate of CO_2 evolution. Krebs and Roughton,[8] in their determinations of carbonic anhydrase activity, selected a reading between 60 mm. and 80 mm. from which CO_2 evolution for a standard period was calculated. In the present study corrected pressure readings were plotted against time and the rate of pressure increase calculated from the time required for the curve to reach the 70 mm. mark. Enzymatic activity was expressed as the rate of pressure increase of the enzyme catalyzed reaction less that of the blank (uncatalyzed reaction) as shown in the formula below:

$$\text{Enzymatic activity} = P_E \text{ (mm./second)} - P_B \text{ (mm./second)}$$

where P_E (mm./second) refers to rate of pressure increase in respirometers containing buffer, substrate, and diluted blood and P_B (mm./second) refers to rate of pressure increase in respirometers to which only buffer, substrate, and distilled water were added.

Part of each blood sample was used to measure hematocrit. From carbonic anhydrase activity found prior to anesthesia and from the percentage change in hematocrit occurring during the 30 minutes of anesthesia an expected value for carbonic anhydrase activity due to change in hematocrit was calculated. The difference between the expected change in carbonic anhydrase activity due to hematocrit and the actual values found after anesthesia was used to calculate for each subject percentage changes in carbonic anhydrase activity resulting from effect of anesthesia on enzyme activity. Since physiologic effects are related to change in both enzyme concentration and to activity of the enzyme, percentage change in carbonic anhydrase activity without correction for hematocrit was also determined.

In each of the three groups of subjects receiving anesthesia, as well as in the controls, the mean percentage change in carbonic anhydrase activity and standard error of the mean percentage change were calculated both with and without correction for hematocrit. The significance (if any) of observed changes in anesthetized patients was determined by comparison with control patients. This was done by calculating the significance of differences of mean percentage changes between groups receiving anesthesia and the control group. The significance (if any) of observed changes associated with one type of anesthesia compared to another was determined by calculating the significance of differences of mean

TABLE 1. Carbonic Anhydrase Activity During Ether Anesthesia

Carbonic Anhydrase Activity (mm./sec.)		Not Corrected for Hematocrit		Percentage Change in Hematocrit	Corrected for Hematocrit	
Before Anesthesia	During Anesthesia	Change (mm./sec.)	Percentage Change		Change (mm./sec.)	Percentage Change
1.130	1.030	−.100	−8.9	0	−.100	−8.9
.860	.790	−.070	−8.1	0	−.070	−8.1
.806	.763	−.043	−5.3	+2.5	−.063	−7.6
.720	.755	+.035	+4.9	0	+.035	+4.9
.665	.587	−.078	−11.7	−2.2	−.063	−9.7
.610	.595	−.015	−2.5	−4.8	+.014	+2.4
.730	.685	−.055	−6.2	+1.2	−.054	−7.3
.737	.680	−.057	−7.7	0	−.057	−7.7
.660	.650	−.010	−1.5	−2.3	+.005	+0.8
.730	.662	−.068	−9.3	+10.0	−.141	−17.6
		Mean: −5.6 ± 1.5%			Mean: −5.9 ± 2.1%	

percentage changes between groups receiving anesthesia.

Results

Results are summarized in tables 1–4. When the findings were uncorrected for changes in hematocrit, the mean decrease in carbonic anhydrase activity of 5.6 per cent associated with ether anesthesia was statistically significant ($P < 0.01$). The mean decrease of 8.4 per cent associated with thiopental-nitrous oxide was also significant ($P < 0.001$). The change observed with cyclopropane was not significant ($P > 0.05$), nor was the change noted in control subjects ($P > 0.05$).

Following correction for changes in hematocrit, the decreases in carbonic anhydrase activity found with ether (5.9 per cent) and with thiopental-nitrous oxide (6.4 per cent) were also statistically significant at, respectively, values of < 0.01 and < 0.05. Changes associated with cyclopropane and changes in control subjects were not statistically significant.

Patients receiving thiopental-nitrous oxide showed a significantly greater decrease in carbonic anhydrase activity than did patients receiving cyclopropane, not only when values were uncorrected for hematocrit ($P < 0.01$) but also when they were ($P < 0.05$). The decrease in carbonic anhydrase activity observed

TABLE 2. Carbonic Anhydrase Activity During Thiopental-Nitrous Oxide Anesthesia

Carbonic Anhydrase Activity (mm./sec.)		Not Corrected for Hematocrit		Percentage Change in Hematocrit	Corrected for Hematocrit	
Before Anesthesia	During Anesthesia	Change (mm./sec.)	Percentage Change		Change (mm./sec.)	Percentage Change
.755	.623	−.132	−17.5	+1.2	−.141	−18.5
.665	.615	−.050	−7.5	0	−.050	−7.5
.810	.790	−.020	−2.5	0	−.020	−2.5
.687	.670	−.017	−2.5	0	−.017	−2.5
.690	.665	−.025	−3.6	−8.1	+.031	+4.1
.755	.660	−.095	−12.6	−11.4	−.009	−1.2
.687	.667	−.020	−2.9	−1.1	−.012	−1.8
.670	.570	−.100	−14.9	−4.7	−.131	−19.5
.740	.655	−.085	−11.5	−2.7	−.065	−9.0
		Mean: −8.4 ± 1.9%			Mean: −6.4 ± 2.7%	

TABLE 3. Carbonic Anhydrase Activity During Cyclopropane Anesthesia

Carbonic Anhydrase Activity (mm./sec.)		Not Corrected for Hematocrit		Percentage Change in Hematocrit	Corrected for Hematocrit	
Before Anesthesia	During Anesthesia	Change (mm./sec.)	Percentage Change		Change (mm./sec.)	Percentage Change
.640	.580	−.060	−9.4	−8.5	−.006	−1.0
.730	.785	+.055	+7.5	+10.4	−.021	−2.6
.510	.545	+.035	+6.9	− 2.4	+.047	+9.4
.635	.642	+.007	+1.1	−6.4	+.046	+7.7
.655	.625	−.030	−4.6	−4.9	+.002	+0.3
.545	.540	−.005	−0.9	0	−.005	−0.9
.665	.680	+.015	+2.3	+3.5	−.008	−1.2
.755	.722	−.033	−4.4	−5.3	+.007	+1.0
.672	.642	−.030	−4.5	0	−.030	−4.5
.627	.665	+.038	+6.1	−2.5	+.054	+8.8
		Mean: +0.1 ± 1.8%			Mean: +1.9 ± 1.6%	

in patients anesthetized with ether was significantly greater than that observed in the cyclopropane group, again regardless of whether the values were ($P < 0.01$) or were not ($P < 0.05$) corrected for hematocrit. There was no significant difference between decreases in activity observed in ether and in thiopental-nitrous oxide groups ($P > 0.05$).

There was no correlation between changes in carbonic anhydrase activity and patients' age, sex, or the amount of preanesthetic medication.

Discussion

The decreases in blood carbonic anhydrase activity found with ether and with thiopental-nitrous oxide anesthesia could be due either to a direct effect of the anesthetics on carbonic anhydrase or they could be due to an indirect effect mediated by a physiological or biochemical response to the anesthetics. The direct effects of anesthetics on other enzyme systems, when studied using clinically effective concentrations, are negligible, at least insofar as the enzymes involved in carbohydrate metabolism are concerned.[11,12] Nevertheless, ether has been demonstrated to have a significant inhibitory effect in anesthetic concentrations on at least one enzyme system, namely, that responsible for the metabolism of pentobarbital.[13] The possibility cannot be excluded that ether and thiopental-nitrous oxide have a direct depressant effect on the enzyme carbonic anhydrase, but neither can it be proven on the basis of the present study.

TABLE 4. Carbonic Anhydrase Activity—Control Subjects

Carbonic Anhydrase Activity (mm./sec.)		Not Corrected for Hematocrit		Percentage Change in Hematocrit	Corrected for Hematocrit	
Before Anesthesia	During Anesthesia	Change (mm./sec.)	Percentage Change		Change (mm./sec.)	Percentage Change
.630	.685	+.055	+8.7	0	+.055	+8.7
.645	.652	+.007	+1.1	−1.1	+.014	+2.2
.712	.700	−.012	−1.7	−1.0	−.004	−0.6
.795	.827	+.032	+4.0	0	+.032	+4.0
.650	.652	+.002	+0.3	+3.7	−.022	−3.3
.647	.697	+.050	+7.7	+1.3	+.042	+6.4
.700	.717	+.017	+2.4	+1.2	+.009	+1.3
		Mean: +3.2 ± 1.5%			Mean: +2.7 ± 4.1%	

It is interesting to note that the inhibition of carbonic anhydrase by the anesthetics studied is in the reverse order of their ability to produce reflex release of epinephrine and norepinephrine. As Price, *et al.*[14] have demonstrated, blood levels of catechol amines are increased to a greater extent with cyclopropane than they are with ether, and the increases with ether, though erratic, are greater than with thiopental. In the present study, carbonic anhydrase activity was depressed most with thiopental and least with cyclopropane, with ether occupying an intermediary position. Although the effects of epinephrine and norepinephrine on carbonic anhydrase activity have not been reported, the fact that epinephrine is known to increase activity of other enzyme systems,[15] together with the fact that a sympathomimetic agent such as ephedrine has been demonstrated to increase carbonic anhydrase activity both in man[16] and animals,[17] raises the possibility that the anesthetics studied might, in fact, have a direct inhibitory effect on carbonic anhydrase activity but that this is offset by concurrent reflex release of catechol amines, notably epinephrine.

Inhibition of carbonic anhydrase involves both hydration of CO_2 and dehydration of H_2CO_3. In dogs given acetazolamide the rate of hydration of CO_2 is so decreased that the P_{CO_2} of blood reaching the lungs is significantly higher than is the P_{CO_2} of mixed venous blood removed and given sufficient time *in vitro* to achieve equilibrium.[18] This is because with acetazolamide-induced carbonic anhydrase inhibition the time required for transit of blood from tissue to lungs is no longer adequate for attainment of equilibrium. At the pulmonary capillary level, on the other hand, the rate of dehydration of H_2CO_3 is so impaired by acetazolamide that again equilibrium cannot be achieved in the time in which blood is exposed to alveolar membranes. As a result, arterial blood specimens in acetazolamide-treated dogs have a P_{CO_2} higher than simultaneously measured alveolar P_{CO_2},[19,20] because equilibrium is still being approached after blood has left the lungs. In man, elevation of arterial P_{CO_2} (Pa_{CO_2}) over alveolar P_{CO_2} (PA_{CO_2}) associated with carbonic anhydrase inhibition produced by sulfonamides[21] or by acetazolamide has been reported,[19,22] though the A-a P_{CO_2} difference usually becomes apparent only in the exercising, not in the resting, individual. In view of the A-a P_{CO_2} difference noted during general anesthesia by Nunn[24] and by Severinghaus, *et al.*,[25] the question arises as to whether this A-a P_{CO_2} difference is due to carbonic anhydrase inhibition produced by the anesthesia, or whether, as has generally been hypothesized, it is due to unequal alveolar ventilation. It has been estimated by Roughton[2] that *in vivo* erythrocyte carbonic anhydrase accelerates by 7,500-fold the conversion of H_2CO_3 to H_2O and CO_2. Since only a 200-fold increase is necessary to come within 10 per cent of equilibrium,[2] large excess of enzyme is normally present and considerable carbonic anhydrase inhibition is necessary to produce a significant effect on CO_2 transport. The degree of inhibition of carbonic anhydrase activity observed during ether and thiopental-nitrous oxide is so slight that it is most unlikely that carbonic anhydrase inhibtion is responsible for A-a P_{CO_2} differences noted during anesthesia.

The question also arises as to whether carbonic anhydrase inhibition induced by anesthetics is in any way responsible for alterations in renal functions produced by anesthesia. Galdstone[26] found in humans that acetazolamide increased urinary concentration of Na^+, K^+, and HCO_3^- and had no significant effect on urinary Cl^- concentration. Berliner and Orloff,[27] in their review, concluded that carbonic anhydrase inhibitors increase urinary flow, urinary excretion of Na^+, K^+, HCO_3^-, and H_2O and urinary *p*H, and decrease urinary ammonia and titratable acid. These are not the alterations in renal function seen during general anesthesia. There is usually a decrease in urinary flow[28,29] and a decreased rate of excretion and urinary concentration of Na^+ and K^+.[28] Since even when 99 per cent of renal carbonic anhydrase is inhibited the remainder is adequate to hydrate CO_2 at 18-34 per cent of control values,[30] it is unlikely that the modest carbonic anhydrase inhibition seen with general anesthetics has any significant effect upon renal tubular function.

There is not adequate information on the effect of general anesthetics on gastric secretion of HCl to determine whether carbonic

anhydrase inhibition associated with anesthesia alters HCl production during anesthesia or not.

Summary

In 29 patients under clinical conditions, there was a statistically significant decrease in blood carbonic anhydrase activity associated with ether and with thiopental-nitrous oxide anesthesia but not with cyclopropane anesthesia. Possible causes of this inhibition are discussed. While such carbonic anhydrase inhibition is statistically significant, it is probably not physiologically significant insofar as carbon dioxide transport or renal function are concerned.

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DIAPHRAGMATIC HERNIA Untreated diaphragmatic hernia in infants has a 75 per cent mortality. Decreased mortality is due to early recognition, immediate operation, improved anesthesia, and good postoperative care. Life is threatened as a result of mechanical impairment of the cardiorespiratory system. There may be under-development of the left lung, and mediastinal shift which impairs the function of the right lung. Almost half the patients have symptoms during the first 48 hours after birth. Dyspnea occurs in 90 per cent of patients, cyanosis in 78 per cent, and vomiting in 31 per cent. Preoperatively, the infant's condition can be improved by clearing the airway, administering oxygen, elevating the patient about 30 degrees, and turning him toward the side of the hernia. Endotracheal anesthesia is recommended. At the conclustion of operation the lung should be expanded by positive pressure, but vigorous inflation should be avoided because of the risk of pneumothorax on the unobserved opposite side. Pulmonary hypoplasia may prevent immediate expansion, and a chest tube with underwater seal drainage should be used. Progressive expansion takes place. Postoperative care should include gastric suction, humidity, oxygen and clearing the airway. (*Kiesewetter, W. B., Gutierrez, I. Z., and Sieber, W. K.: Diaphragmatic Hernia in Infants Under One Year of Age, A. M. A. Arch. Surg. 83: 561 (Oct.) 1961.*)

ASEPSIS Greatest source of air contamination in the operating room is probably the respiratory tracts of the occupants. Improved masks and proper air conditioning are the most effective means of reducing air contamination. (*Pecora, D. V., and Brook, R.: Some Factors Affecting Operating Room Asepsis, A. M. A. Arch. Surg. 83: 161 (Aug.) 1961.*)