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Research Article

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COMPARISON BETWEEN THE TIME AVAILABLE AND THE TIME REQUIRED FOR CO_2 EQUILIBRATION IN THE LUNG *

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We have previously shown that the injection of sodium bicarbonate into the pulmonary artery of a dog enclosed in a body plethysmograph results in the evolution of gas in the alveoli which is measurable as an increase in the pressure in the plethysmograph (1). By comparing the time elapsed between injection and gas evolution following injection of a bicarbonate solution with the analogous time interval following injection of an inert gas, ether (2), a value for the over-all rate of the reactions leading to CO_2 liberation in the lung can be obtained. Relatively small doses (5 mg per kg) of a carbonic anhydrase inhibitor, acetazolamide, given intravenously, markedly suppressed the evolution of CO_2 after bicarbonate injection. This observation suggested to us a method for carrying out experiments to investigate the relationship between the time available and the time required for CO_2 equilibration in the lungs. These experiments form the basis of the present report.

METHODS

Four dogs weighing 16 to 20 kg were used in these experiments. They were anesthetized with pentobarbital sodium in an initial dose of 25 mg per kg; this was usually supplemented by 100 mg about one hour later. A tracheal tube was inserted. A shortened no. 9 cardiac catheter (capacity 0.9 ml) was introduced via the external jugular vein into the pulmonary artery. The dog was then enclosed in a body plethysmograph, and just before the start of the actual experimental period, spontaneous breathing was arrested with appropriate doses of succinylcholine chloride and ventilation was maintained by a Starling pump. The pulmonary artery pressure [1] and the plethysmograph pressure [2] were recorded by using strain gauges and a direct-writing oscillograph.[3] The electrocardiogram was monitored continuously. The details of the calibrating and the recording procedure are reported elsewhere (2).

After checking the plethysmograph for air tightness and calibrating it, an injection of 0.5 ml of 1 : 4 ether in alcohol was made into the pulmonary artery to determine the circulation time from the pulmonary artery to the site of gas exchange (2). This was followed by the injection, in the same manner, of 3 ml of a saturated solution of sodium bicarbonate (approximately 1.2 M at room temperature 20° C). At least duplicate control injections of both ether and bicarbonate were made. An injection of 10 or 20 mg of sodium acetazolamide (Diamox sodium) was then made into the pulmonary artery, and 10 to 12 minutes later duplicate ether and bicarbonate injection records were obtained. The process was then repeated using 10- to 20-mg increments of sodium acetazolamide until a total dose had been reached which markedly suppressed gas evolution.

Analysis of records. All the records obtained were analyzed by determining the time taken for half the gas evolved to appear, as well as by determining the total volume of gas liberated. In every pair of ether and bicarbonate injection records, the time taken for half the gas following ether injection to appear was then subtracted from the time taken for half the gas following bicarbonate injection to appear, yielding a value for the time taken by the reactions leading to CO_2 liberation to reach half-completion (1). This value was termed reaction half-time.

We have found that the rate of evolution of CO_2 as seen in the plethysmographic pressure record after bicarbonate injection is essentially a logarithmic process over at least 3 cycles of half-time. The curves showing a decrease in gas volume liberated after Diamox, however, deviated from the logarithmic course at some point along the rise. In the curves showing a decrease in gas volume following the minimal effective total dose of Diamox, the initial point of deviation from the logarithmic course was located on the curve, and a tangent was drawn to the curve at this point. This marked the time when a fraction of the injected bicarbonate first left

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[1] Statham Model P23D.

[2] Statham Model PM97 TC ± 0.05-350 ± 0.05 PSID.

[3] The Grass Instrument Co., Quincy, Mass.

TABLE I

Analysis of CO_2 evolution curves for HCO_3^- transit time

Dog*	Median capillary transit time	Range of transit time		Percentage deviation from median transit time	
		Minimum	Maximum	Minimum	Maximum
	sec	*sec*	*sec*	%	%
15	1.28	0.68	1.94	−41	+51
	1.90	1.26	2.62	−34	+38
16	1.80	1.20	2.50	−33	+40
	1.58	1.14	2.10	−24	+28
19	1.96	1.00	2.92	−49	+49
	1.72	1.16	2.72	−33	+58
20	3.52	2.52	4.52	−28	+28
	3.88	3.14	4.62	−19	+19
Mean	2.21			−33	+39

* These numbers correspond to those in Table IV of reference 1.

the gas exchange vessels. Another tangent (a horizontal line) was drawn to the peak of the curve. This was the point where gas evolution ceased, hence where the last fraction of injected bicarbonate left the vessels of gas exchange. A tangent to the curve was then drawn having a slope equal to the average slope of the two previously drawn tangents. This point on the curve then marked the median time at which injected bicarbonate left the gas exchange vessels. When the median arrival time of ether obtained from the immediately preceding or immediately following ether injection curve was subtracted from the time interval between injection and median exit time of bicarbonate from the vessels of gas exchange, the remaining figure gave the median time spent by the injected bicarbonate in the gas exchange vessels. The scatter of bicarbonate transit times [4] around the median value was obtained from the scatter of exit times, obtained by the above measurement, and from the scatter in the arrival times of any injected substance, obtained from the scatter of arrival times around the median arrival time for ether. Thus the shortest time spent by injected bicarbonate in the gas exchange vessels was obtained by subtracting the longest ether arrival time from the earliest bicarbonate exit time, and the longest transit time for bicarbonate was obtained by subtracting the shortest ether arrival time from the latest bicarbonate exit time.

RESULTS

The median time spent by bicarbonate in the pulmonary vessels of gas exchange averaged 2.2 seconds. The individual results can be seen in Table I. The figures under the heading "Range of transit time" include the scatter of the ether arrival time and of the bicarbonate exit time in the manner described above. This deviation averaged − 33 per cent and + 39 per cent of the median bicarbonate transit time value.

Figure 1 shows the results of a typical experiment. The curve to the extreme left is that following the injection of ether; the next curve is that of the control bicarbonate injection; the third curve shows the effect of 40 mg of sodium acetazolamide on the evolution of CO_2 from bicarbonate, and the fourth and fifth curves show the effect of a total dose of sodium acetazolamide of 60 mg and 140 mg respectively, given in increments of 10 to 20 mg. It can be seen that the smaller dose of inhibitor affects only the rate of CO_2 liberation, whereas larger doses slow the rate and also decrease the volume of gas liberated. The significance of this observation will be discussed later.

Because of the possibility that the alveolar capil-

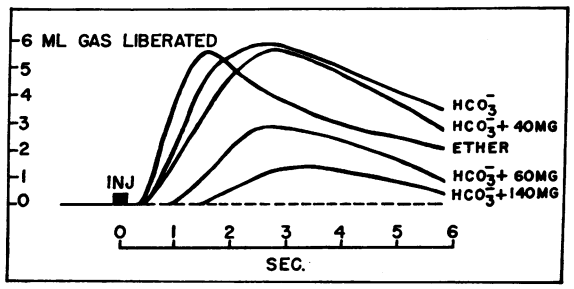

FIG. 1. RESULTS OF AN EXPERIMENT SHOWING RECORDS OF INJECTION OF ETHER AND SODIUM BICARBONATE INTO THE PULMONARY ARTERY OF A DOG BOTH BEFORE AND AFTER THE ADMINISTRATION OF ACETAZOLAMIDE IN A TOTAL DOSE OF 40, 60, AND 140 MG.

[4] Transit time here is defined as the time the bicarbonate ion spends in contact with the gas exchange surface.

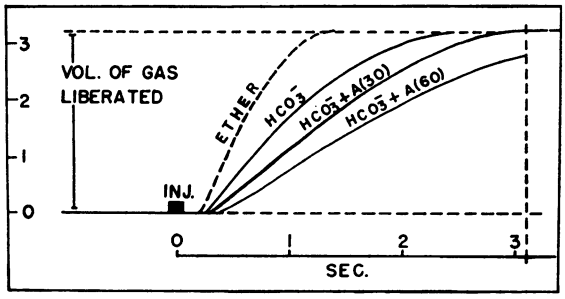

FIG. 2. SCHEMATIC REPRESENTATION OF THE EFFECT OF ACETAZOLAMIDE ON THE RATE AND AMOUNT OF CO_2 LIBERATION IN THE LUNG AFTER INJECTION OF A BICARBONATE SOLUTION. The vertical dashed line to the right represents the time of exit of injected bicarbonate from the vessels of gas exchange. The curve labeled "HCO_3^-" illustrates the fact that at the normal rate of evolution of CO_2 from bicarbonate, the reaction is completed before the injected bicarbonate leaves the gas exchange vessels. The curve labeled "$HCO_3^- + A$ 30" illustrates the fact that moderate slowing of the reaction with 30 mg of acetazolamide still permits gas evolution to be complete. In the curve labeled "$HCO_3^- + A$ 60" a degree of slowing is produced by a total dose of 60 mg of acetazolamide which prevents completion of the reaction before the bicarbonate leaves the gas exchange vessels and as a result gas volume decreases. The rate of evolution of ether gas is drawn for comparison. All the curves are drawn arbitrarily to show the relative rates of CO_2 evolution with varying degrees of carbonic anhydrase inhibition; these doses of acetazolamide are not actual figures from an experiment, but they represent the correct order of magnitude of the dose required to decrease the volume of gas evolved from bicarbonate.

lary gradient for CO_2 may be reversed so as to permit re-entry of CO_2 into the blood before the evolution of gas from the injected bicarbonate is complete, we feared that the apparent peak of the curve might not reflect accurately the end of CO_2 evolution. In order to exclude such a possible source of error, we set up an experiment in which the single injection of bicarbonate solution was immediately followed by a constant infusion of a less concentrated bicarbonate solution at such a rate as to permit the recording of a definite plateau at the peak of the curve. Comparison of the initial, median, and peak points obtained by such a method showed close agreement with the analogous points obtained by a single injection of bicarbonate solution.

DISCUSSION

Pulmonary "capillary" transit time for injected bicarbonate. The dependence of CO_2 evolution from injected bicarbonate on carbonic anhydrase suggested that partial inhibition of the enzyme might give information on the pulmonary capillary transit time for bicarbonate. The theoretical basis for this possibility may best be explained with reference to Figure 2. The volume of CO_2 liberated from injected bicarbonate is determined by *1*) the rate at which CO_2 evolution proceeds and *2*) the time the bicarbonate spends in the vessels where gas exchange takes place. When stepwise inhibition of carbonic anhydrase is brought about by giving repeated small doses of acetazolamide, the rate of evolution of CO_2 is slowed, but the volume of gas evolved is not affected so long as the transit time remains longer than the time taken by the reaction to be completed. As soon as the reaction is sufficiently slowed to prevent its equilibration within the time the blood spends in the capillaries, equilibrium cannot be reached by the time the blood leaves the capillaries, and a decrease in gas volume evolved is observed. The curve showing the first decrease in volume can be analyzed, as described previously, and the capillary transit time for bicarbonate calculated.

Pulmonary reaction space for bicarbonate. The results obtained by using the procedure just described showed that injected bicarbonate spends an average of 2.2 seconds going through the vessels of gas exchange, with a range of from 1.28 seconds to 3.88 seconds around the median value. In this connection, Cain and Otis (3) recently presented data from which they calculated the time available for bicarbonate to react in the lungs during carbonic anhydrase inhibition to be 5.4 seconds in dogs. These workers, however, pointed out that their figure may be overestimated because of the marked effects of small errors in the assumed values for intracellular pH and the fraction of bicarbonate which reacted. Our results, obtained by a different and more direct method, lend support to the interpretation which Cain and Otis applied to their findings.

The figure of 2.2 seconds for bicarbonate transit time in the pulmonary exchange vessels is much higher than the accepted value of 0.7 seconds (4) for erythrocyte transit time (this value for the erythrocytes pertains to humans and would be expected to be even smaller in a smaller animal such as the dog). In another series of experiments,

the injection of trishydroxymethylaminomethane (Tris) into the pulmonary artery caused uptake of CO_2 which continued for an average of 2.6 seconds; and more recently, injection of sodium carbonate in three dogs caused CO_2 uptake which continued for an average of 2.9 seconds, confirming the discrepancy shown between the transit time for bicarbonate and erythrocytes, and indicating that all three injected substances spend from 2 to 3 seconds in contact with the gas exchange surface.

We cannot determine with certainty from our experiments the exact nature of the mechanism which permits the bicarbonate ion a longer transit time through the gas exchange vessels. One possible explanation would be that gas exchange may not be limited to the anatomic capillaries of the lungs. Staub (5) has shown that in cat lungs ventilated with 100 per cent oxygen and prepared for histologic examination by a quick-freezing technique, there is evidence of oxygenation of the blood in vessels before the anatomic capillaries are reached. We investigated this possibility by comparing the rates of evolution of ether in the alveoli after its injection into the pulmonary artery when the vehicle for injection was alcohol or alternatively kerosene. Kerosene has been shown to lodge in vessels larger than the pulmonary capillaries (6); thus ether dissolved in kerosene would be expected to leave the blood stream by diffusing through the walls of vessels larger than the capillaries. Under such conditions the rate of appearance of ether gas in the alveoli was much slower than that observed when ether was permitted to reach the capillaries (Figure 3), suggesting that under normal conditions, the major fraction of gas evolution occurs preferentially through the walls of capillaries.

The other and more likely explanation for the longer transit time for bicarbonate is that there is a large pericapillary dilution space for CO_2 or bicarbonate in the lung, thus effectively delaying its transit. Such an explanation is in agreement with the finding by DuBois, Fenn, and Britt (7) of a capacity for CO_2 storage in the isolated bloodless lungs of dogs. More recently, Sackner, Feisal, and DuBois (8) measured the pulmonary tissue and blood space for CO_2 by observing the rate of change in pressure in a body plethysmograph after the inhalation of 15.4 percent CO_2 in air, and they have concluded that the initial volume uptake of CO_2 is greater than can be accounted for by the physical solution of CO_2 in the pulmonary tissues and its solution in and chemical combination with the blood in the pulmonary capillaries, suggesting that CO_2 is buffered in lung tissues, as postulated by DuBois, Fenn, and Britt (7). On the basis of studies of the transit time of various substances through the pulmonary capillary bed, and of the contributions of bicarbonate ions and dissolved CO_2 to expired CO_2, using isotopes of carbon and large doses of acetazolamide, Chinard, Enns, and Nolan have concluded that the alveolar-capillary membrane is impermeable to bicarbonate ions (9). This, however, does not preclude equilibration of plasma bicarbonate with pericapillary tissue bicarbonate, occurring via CO_2 which, being readily diffusible through the capillary wall, enters the tissue space and there recombines with water under the influence of carbonic anhydrase, recently demonstrated in lung tissue (10–12), to form carbonic acid which instantaneously dissociates into hydrogen ions and bicarbonate ions. This mechanism is an example of "catalyzed diffusion" (13). Our observations on the transit time of bicarbonate through the lung capillaries, as well as the figures calculated by Cain and Otis for the time available for bicarbonate to react in the lung, can best be explained by the presence of a large distribution space for the bicarbonate-CO_2 system in the lung tissues.

SUMMARY

The factors determining whether CO_2 equilibration will occur in the lung are the rate of the reactions leading to CO_2 evolution and the time the bicarbonate ions spend in contact with the gas exchange surface. If the rate of the reactions

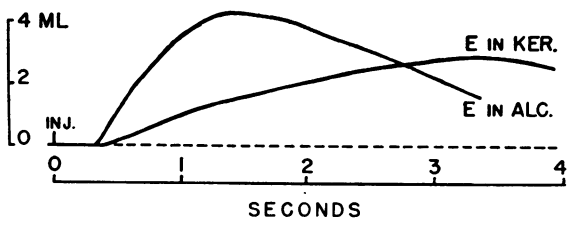

FIG. 3. COMPARISON BETWEEN THE RATE OF EVOLUTION OF ETHER GAS IN THE LUNG WHEN THE VEHICLE FOR INJECTION OF ETHER IS ALCOHOL OR ALTERNATIVELY KEROSENE.

involved in CO_2 liberation is slowed gradually by repeatedly giving small doses of acetazolamide, a point will be reached where the evolution of CO_2 cannot be completed in the time the blood spends in the pulmonary capillaries, and the amount of CO_2 produced decreases sharply. In applying this principle, we used a body plethysmograph as a manometer to determine the rate and magnitude of CO_2 evolution, and we found that the bicarbonate ions spend an average of 2.2 seconds in contact with the gas exchange surface in dogs, a much longer time than the erythrocyte transit time through the pulmonary capillaries. We think that this prolongation of bicarbonate transit time is caused by the presence of a large pericapillary dilution and reaction space for the bicarbonate-CO_2 system, and we suspect that this prolongation plays a role in the completion of CO_2 equilibration in the lungs.

REFERENCES

1. Soni, J., Feisal, K. A., and DuBois, A. B. The rate of intrapulmonary blood gas exchange in living animals. J. clin. Invest. 1963, **42**, 16.
2. Feisal, K. A., Soni, J., and DuBois, A. B. Pulmonary arterial circulation time, pulmonary arterial blood volume, and the ratio of gas to tissue volume in the lungs of dogs. J. clin. Invest. 1962, **41**, 390.
3. Cain, S. M., and Otis, A. B. Carbon dioxide transport in anesthetized dogs during inhibition of carbonic anhydrase. J. appl. Physiol. 1961, **16**, 1023.
4. Roughton, F. J. W. The average time spent by the blood in the human lung capillary and its relation to the rates of CO uptake and elimination in man. Amer. J. Physiol. 1945, **143**, 621.
5. Staub, N. C. Gas exchange vessels in the cat lung. Fed. Proc. 1961, **20**, 107.
6. Howell, J. B. L., Permutt, S., Proctor, D. F., and Riley, R. L. Effect of inflation of the lung on different parts of pulmonary vascular bed. J. appl. Physiol. 1961, **16**, 71.
7. DuBois, A. B., Fenn, W. O., and Britt, A. G. CO_2 dissociation curve of lung tissue. J. appl. Physiol. 1952, **5**, 13.
8. Sackner, M. A., Feisal, K. A., and DuBois, A. B. Pulmonary tissue space for nitrous oxide and carbon dioxide during inspiration. Fed. Proc. 1962, **21**, 441.
9. Chinard, F. P., Enns, T., and Nolan, M. F. Contributions of bicarbonate ion and of dissolved CO_2 to expired CO_2 in dogs. Amer. J. Physiol. 1960, **198**, 78.
10. Berfenstam, R. Carbonic anhydrase activity in fetal organs. Acta Paediat. (Uppsala) 1952, **41**, 310.
11. Chinard, F. P., Enns, T. and Nolan, M. F. The permeability characteristics of the alveolar-capillary barrier. Trans. Ass. Amer. Phycns. In press.
12. Maren, T. Personal communication.
13. Jacobs, M. H., and Stewart, D. R. The role of carbonic anhydrase in certain ionic exchanges involving the erythrocyte. J. gen. Physiol. 1942, **25**, 539.