Journal of Clinical Investigation
Vol. 42, No. 1, 1963

THE RATE OF INTRAPULMONARY BLOOD GAS EXCHANGE IN LIVING ANIMALS *

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(Submitted for publication June 11, 1962; accepted September 1, 1962)

The respiratory function of the blood depends on a series of reversible reactions of oxygen and carbon dioxide with blood. The optimal performance of this function requires that the rate of these reactions be such as to permit them to approach completion within the time the blood spends in the vessels of gas exchange. In the case of carbon dioxide, experiments by Roughton (1) and more recently by Forster (2), using different methods, have suggested that the reactions involved may be slow enough to limit the excretion of CO_2 in the lung. Most of the work in this field has been performed *in vitro,* and because of the difficulty in carrying over the information obtained to living systems, we have devised a technique by which some aspects of the reactions leading to CO_2 liberation in the lung can be studied in living animals.

The technique depends upon the use of a highly sensitive body plethysmograph as a manometer for the instantaneous detection and the quantitation of the magnitude and the rate of evolution or absorption of gas in the lung after the injection of acid, bicarbonate, alkali, or an amine buffer into the pulmonary artery, and on the availability of a method (3) for distinguishing the time spent in transit to the site of gas exchange from the time occupied by the reactions leading to gas liberation in the lung. The present report describes the effects of injection of lactic acid, sodium bicarbonate, and trishydroxymethylaminomethane (Tris) and the modification of these effects by breathing oxygen and by the administration of carbonic anhydrase or of a carbonic anhydrase inhibitor (acetazolamide). Using these methods, we have obtained information on the rate of evolution and absorption of CO_2, as well as on the time required for the equilibration of CO_2 in the lungs (4).

* Supported by Contract H 4797 with the U. S. Public Health Service.

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‡ This work was done during the tenure of an Established Investigatorship of the American Heart Association.

METHODS

Experiments with lactic acid and Tris. The experiments were performed on dogs weighing 19 to 23 kg. They were anesthetized with intravenous sodium pentobarbital, 25 mg per kg, and a cuffed endotracheal tube was inserted. A shortened no. 9 cardiac catheter (capacity 0.9 ml) was introduced via an external jugular vein into the pulmonary artery. The dogs were then enclosed in a body plethysmograph, and just before the actual experiment was begun, spontaneous respiratory movement was abolished with appropriate doses of succinylcholine chloride, and ventilation was maintained with a Starling pump. Pulmonary artery and plethysmograph pressures were measured using Lilly capacitance manometers, and the output recorded on a 4-channel direct-writing oscillograph.[1] In some of the experiments a strain gauge was used to measure pulmonary arterial pressure [2] or plethysmographic pressure.[3] The electrocardiogram was monitored continuously. The dimensions of the plethysmograph and the characteristics of the detecting-recording system have been described elsewhere (3).

After calibrating the plethysmograph and testing it for air-tightness, a small amount (0.5 ml of 1.2 M solution) of lactic acid was instilled into the catheter and, during a pause in ventilation, flushed in rapidly with 3 ml of a 0.9 per cent sodium chloride solution. The time and duration of injection were recorded by means of a magnet attached to the plunger of the injecting syringe and moving within a coil, and also by means of the accompanying rise in the plethysmographic pressure record. The subsequent rise in plethysmographic pressure caused by the evolution of gas was recorded, and after its completion, ventilation was resumed. Similarly, a record was obtained of the injection of 1 ml of 1 M Tris.

The time from the injection to the evolution of gas after injection of acid consists of two components, *1*) the time occupied by mixing, bulk flow, and transit to the site of gas exchange and *2*) the time occupied by the reactions leading to gas liberation. In order to distin-

[1] The Grass Instrument Company, Quincy, Mass.

[2] Statham Model P23D.

[3] Statham Model PM97 TC ± 0.05–350 ± 0.05 PSID.

guish between these two components, an injection of 0.5 ml of a 1:4 solution of ether in alcohol or ether in a lipid emulsion was made, as described previously (3). Other inert gases, such as isoprene, isopropyl chloride, and acetylene have been tested and found to give results essentially identical with those obtained using ether.

A series of injections of ether, lactic acid, and Tris was given, first while ventilating the animal with air, then after 10 to 15 minutes of ventilation with 100 per cent oxygen. The purpose of ventilation with oxygen was to suppress the evolution of oxygen from hemoglobin, caused by the acidification of the blood.

In three dogs, carbonic anhydrase (20 to 100 mg) was injected by placing a solution of the powdered enzyme in the catheter space in front of the Tris.

Sodium acetazolamide (Diamox sodium) was administered intravenously in a dose of 20 to 50 mg per kg, and the series of injections of ether, lactic acid, and Tris was repeated, first after ventilation with air and later after ventilation with 100 per cent oxygen. In one dog, injections of more concentrated acid (2.4 M and 4.8 M) were given to determine the effect on the quantity and rate of gas evolution. In this dog, additional injections of acid were given into the right atrium instead of into the pulmonary artery, to determine whether prolonging the path, and thus the circulation time of the injected acid, altered the amount or rate of gas evolution.

Experiments with sodium bicarbonate. In another series of experiments, after obtaining the pulmonary arterial circulation time with injection of ether, 3 ml of 1 M sodium bicarbonate was injected at the same site in the pulmonary artery. This caused a rise in plethysmographic pressure. At least duplicate records were obtained of both ether and bicarbonate injections. In some dogs, the injections were repeated after ventilating the animal with 100 per cent oxygen for 10 to 15 minutes.

The experiments were repeated after the intravenous administration of 100 to 200 mg of sodium acetazolamide. In one dog the administration of acetazolamide was followed by bicarbonate injections every minute to determine the onset of action of intravenously injected acetazolamide. In two dogs, injections of bicarbonate were continued for up to 6 hours after the administration of acetazolamide to determine the duration of action of the drug.

Analysis of records. All the records were analyzed by determining the time from the point of injection to the point at which half the gas volume evolved had appeared. The total volume of gas liberated was also determined. In each pair of curves, the time taken for half the gas volume after ether injection to appear was subtracted from the time taken for half the CO_2 volume to appear. This yielded a value for the time taken by the reaction and gas evolution to reach half-completion. This figure was termed reaction half-time.

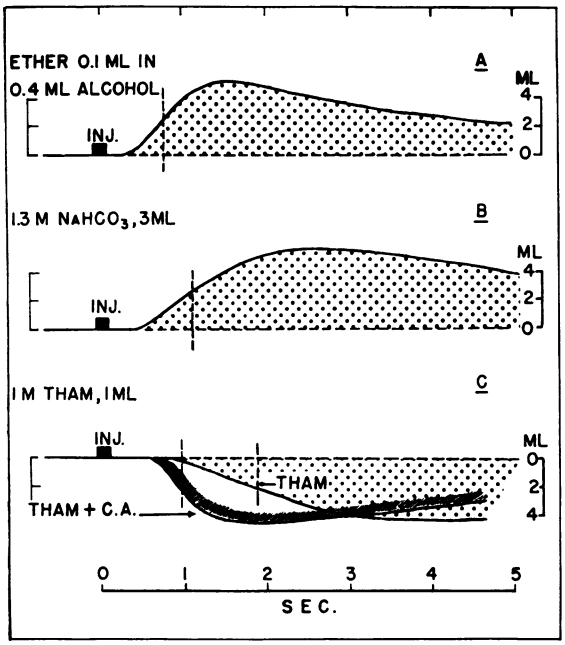

FIG. 1. RECORD OF THE INJECTION OF ETHER, SODIUM BICARBONATE, TRIS (THAM), AND TRIS (THAM) WITH CARBONIC ANHYDRASE INTO THE PULMONARY ARTERY OF A DOG.

TABLE I

Ether circulation time from pulmonary artery to vessels of gas exchange

Dog no.*	Breathing air	Breathing O_2	Breathing O_2; after acetazolamide	Breathing air; after acetazolamide
	sec	*sec*	*sec*	*sec*
4	0.40	0.65	0.68	1.20
5	0.87	0.64	1.11	1.00
6	1.60	1.84	1.56	1.12
8	0.89	1.32	1.32	1.04
12	0.68	0.72	0.72	0.88
Mean	0.88	1.03	1.07	0.84
	Average of the means 0.96 sec			

* These numbers correspond to those in reference 3.

RESULTS

Injection of ether. The injection of ether, produced as before (3) an S-shaped rise in plethysmographic pressure (Figure 1A). The time taken to reach 50 per cent of the maximal deflection was taken to indicate the arrival time of 50 per cent of the ether in the vessels of gas exchange. This time is called the "median arrival time of ether," and the values obtained are listed in Table I. The mean value was 0.96 second. The ether circulation time did not change during the series of injections of lactic acid, Tris, or bicarbonate.

Injection of lactic acid, breathing of air. The

injection of lactic acid into the pulmonary artery produced an S-shaped rise in plethysmographic pressure. The time taken for 50 per cent of this rise to occur was, on the average, 0.40 second longer than the median arrival time of ether. Compared to the ether curve, the curve obtained after acid injection had a more gradual rise, as if it were tipped over to the right and thus elongated (Figure 1B). The average volume of gas liberated was 3.0 ml BTPS (body temperature and pressure saturated) per 0.5 ml of 1.2 M acid injected.

Lactic acid, breathing of oxygen. When the lactic acid injections were repeated after ventilation with 100 per cent oxygen, the pressure curves appeared earlier and were steeper, and less gas was evolved. The mean reaction half-time was 0.19 second. Statistically this was not found to be significantly different from the value of 0.40 second observed while breathing air. The volume of gas evolved, however, averaged 2.1 ml, a statistically significant decrease ($p \cong 0.001$).

Effect of increasing the concentration of acid. In dog 12, 0.5 ml of 1.2 M, 2.4 M, and 4.8 M lactic acid were injected into the pulmonary artery. The volumes of gas evolved were 1.7 ml, 3.8 ml, and 6.8 ml, respectively, indicating proportionality of volume response within the limits of accuracy of the method. The time for half the rise to occur was not significantly altered by changes in gas volume evolved.

Injection of Tris. The injection of 1 ml of 1 M Tris was followed by the absorption of gas, causing a drop in plethysmograph pressure (Figure 1C). The curves were more drawn out than those following ether or lactic acid injection. The half-time of the reaction averaged 1.01 seconds, and was unaltered by ventilation with oxygen. The volume of gas absorbed averaged 5.6 ml on air and 5.3 ml on oxygen; the difference in volume was not statistically significant.

Injection of Tris and carbonic anhydrase. The injection of carbonic anhydrase almost simultaneously with Tris resulted in a marked acceleration of the rate of gas absorption, this being half completed in 0.16 second. The volume of gas absorbed, however, was unaltered.

Effect of acetazolamide. Neither the volume of gas evolved after acid or absorbed after Tris injection, nor the rate of the process was altered significantly by the administration of acetazolamide. In dog 12, after acetazolamide, the injec-

TABLE II

A. The rate and magnitude of gas liberation after injection of lactic acid and their modification by oxygen breathing and acetazolamide
*B. Effect of varying concentration of acid and site of injection**

			Before acetazolamide				After acetazolamide			
			Acid; breathing air		Acid; breathing oxygen		Acid; breathing air		Acid; breathing oxygen	
	Dog	Volume injected	Volume of gas	Reaction half-time	Volume of gas	Reaction half-time	Volume of gas	Reaction half-time	Volume of gas	Reaction half-time
		ml	*ml*	*sec*	*ml*	*sec*	*ml*	*sec*	*ml*	*sec*
A.	4	1.0	7.8	0.40	5.6	0.25	7.8	0.00	5.0	0.40
	5	1.0	6.5	0.25	2.8	0.09	7.5	0.56	4.5	−0.15
	6	0.5	3.7	0.31	2.9	0.68	2.2	0.91	3.5	0.46
	8	1.0	2.6	0.87	1.9	0.00			1.4	0.48
	12	0.5	2.3	0.18	1.7	−0.06	4.0	−0.04	3.0	0.38
	Mean per 0.5 ml		3.0	0.40	2.1	0.19	3.5	0.36	2.4	0.31
	SD		1.1	0.26	0.9	0.31	0.9	0.43	1.1	0.15
	SE		0.5	0.12	0.4	0.14	0.4	0.21	0.5	0.06
B.	12									
	Acid 2.4 M 0.5 ml into P.A.				3.8	0.68				
	Acid 4.8 M 0.5 ml into P.A.				6.8	0.72	10.7	1.36		
	Acid 2.4 M 0.5 ml into R.A.						4.3	2.8		
	Acid 4.8 M 0.5 ml into R.A.						10.2	2.4		

* P.A. = pulmonary artery; R.A. = right atrium.

TABLE III

The rate and magnitude of gas absorption after injection of Tris and their modification by oxygen breathing and acetazolamide; effect of simultaneous injection of carbonic anhydrase (C.A.) and effect of injection into the right atrium

Dog	Volume injected	Before acetazolamide: Tris; breathing air: Volume evolved	Before acetazolamide: Tris; breathing air: Reaction half-time	Before acetazolamide: Tris; breathing oxygen: Volume evolved	Before acetazolamide: Tris; breathing oxygen: Reaction half-time	After acetazolamide: Breathing air: Volume evolved	After acetazolamide: Breathing air: Reaction half-time	After acetazolamide: Breathing oxygen: Volume evolved	After acetazolamide: Breathing oxygen: Reaction half-time
		ml	*sec*	*ml*	*sec*	*ml*	*sec*	*ml*	*sec*
4	1.0	7.8	1.20	5.6	0.92	7.8	0.16	5.0	0.72
5	1.0	4.0	0.61	4.5	0.79	6.5	0.67	3.5	0.44
6	0.5	3.5	1.99	2.8	1.78	1.7	0.68	2.2	0.32
Tris + C.A.	1.0			5.2	0.50				
8	1.0	3.5	0.67	6.5	0.88			4.7	1.76
Tris + C.A.	1.0			6.5	−0.18				
12	0.5	2.8	0.60		0.68	1.2	0.92	2.7	0.44
	1.0	4.3							
Tris + C.A.	1.0	5.2			0.14				
Tris	1.0	4.3							
Tris into R.A.	1.0					3.1			
Mean per ml		5.6	1.01	5.3	1.00	4.5	0.61	4.5	0.74
SD		1.9	0.61	0.9	0.49	2.1	0.31	0.7	0.59
SE		0.8	0.27	0.4	0.19	1.0	0.12	0.3	0.26

tion of acid or Tris into the right atrium instead of into the pulmonary artery did not significantly affect the rate or volume of gas evolution or absorption.

The results of the above experiments using injections of lactic acid and of Tris can be seen in Tables II and III.

Injection of bicarbonate. The results of bicarbonate injection can be seen in Table IV. The evolution of gas was half completed in an average of 0.44 second. This is not significantly different from the reaction half-time obtained with the injection of lactic acid. The volume of gas evolved averaged 4.8 ml. Neither the rate of gas evolution nor the volume evolved was influenced by prior ventilation with 100 per cent oxygen, indicating that the gas evolved did not contain oxygen liberated from hemoglobin.

Effects of acetazolamide. Acetazolamide, in a dose of approximately 5 mg per kg, markedly suppressed the volume of gas evolved from bicarbonate. This action appeared, in the one dog tested, within 3 minutes after administration of the drug. The action had not disappeared when bicarbonate was given as late as 6 hours after the injection of a single large dose of sodium acetazolamide.

DISCUSSION

The method that we used to study the reactions of CO_2 in the lung depended on injecting a substance which initiated reactions leading to the evolution or absorption of CO_2 in the pulmonary vessels of gas exchange. The rate and volume of gas moving across the vascular wall were determined by means of a sensitive body plethysmograph. The time from injection to complete gas

TABLE IV

Time for evolution of 50 per cent of CO_2 from HCO_3^- (relative to ether)

Dog	Reaction half-time	Volume evolved
	sec	*ml gas/ml solution*
13	0.31	0.7
14	0.28	1.9
15	0.45	1.7
16	0.54	2.0
17	0.52	0.4
18	0.60	1.1
19	0.37	1.3
20	0.67	1.5
21	0.25	1.1
22	0.42	0.9
Mean	0.44	1.3
SD	±0.14	±0.5

evolution consisted of two components. The first was the time spent in transit from the site of injection to the site of gas exchange. This component was determined from the record of the injection of an inert gas, ether, at the same site in the pulmonary arterial circulation; the feasibility of this determination is dependent upon theoretical considerations of the movement of inert gases across the pulmonary capillary wall (5). A detailed report of the method has been published (3).

The second component is the time taken by the reactions leading to gas liberation. After the injection of a substance into the pulmonary artery, there is a process of mixing and bulk flow in the conducting vessels, and in the case of certain substances, chemical reactions and movement of ions between plasma and erythrocytes are initiated. Upon arrival at the site of gas exchange, the movement of respiratory gases into and out of the blood results in re-equilibration of gas tensions and further shifts of electrolytes to re-establish the balance of electric charge between plasma and red cells. In cases where the reactions involved are slowed down sufficiently to prevent their completion within the time spent in the pulmonary capillaries, the reactions continue after the blood has left the vessels of gas exchange.

The main chemical reactions involved in the carriage of CO_2 by the blood can be summarized as follows:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+. \quad [1]$$

This reaction obeys the law of mass action, and its general direction in the tissues is to the right and in the lungs to the left. Some of the CO_2 entering the blood in the tissue capillaries is carried as a carbamino compound with hemoglobin, and a small fraction is carried in physical solution. The major fraction of CO_2 is carried in the plasma as bicarbonate, although hemoglobin plays the major role in its buffering (6). The right-hand reaction in Equation 1 can be considered instantaneous. The left-hand reaction, in contrast, is far less rapid, and in the absence of carbonic anhydrase, which is present in red blood cells but not in the plasma, this is one of the factors which limit the rate of formation of CO_2 from plasma bicarbonate. Another limiting factor may be the time required for the passage of bicarbonate and chloride ions through the erythrocyte membrane. In the presence of normal carbonic anhydrase activity, the contribution of the different forms in which CO_2 is carried to expired CO_2 is proportional to the fraction carried in each form, i.e., equilibrium between the various forms occurs within the limits of the time available for gaseous exchange in the lungs (7).

Injection of acid. The injection of acid resulted in an increased hydrogen ion concentration in the plasma. This shifted reaction (Equation 1) to the left, resulting in an increase in plasma and red cell CO_2 tension, followed by CO_2 evolution at the site of gas exchange. The evolution of CO_2 following acid injection is the final step in a series of reactions which include an initial combination of hydrogen ions and bicarbonate ions in the plasma to form carbonic acid, which hydrolyzes, yielding water and CO_2. The CO_2 then enters the erythrocyte and recombines with water to form carbonic acid which instantaneously dissociates into hydrogen ions and bicarbonate ions. The hydrogen ions are buffered largely by hemoglobin; the bicarbonate ions diffuse back into the plasma and in doing so, create an electric field which favors the migration of chloride ions into the erythrocyte to maintain the pre-existing equilibrium of electric charges. When the blood reaches the vessels of gas exchange in the lungs, the reverse reactions occur and CO_2 is liberated. The processes involved in the liberation of CO_2 in our experiments were 50 per cent complete in 0.40 second; hence this figure may be indicative of the time required for these processes to occur spontaneously. The injection of lactic acid, however, even though some lactic acid enters the blood during muscular exercise, may not initiate reactions comparable to those occurring during CO_2 liberation under resting conditions because the concurrent artificial acidification of the plasma produces excessive amounts of plasma carbonic acid which then dissociates into water and CO_2 without the need for carbonic anhydrase, hence without entering the erythrocyte. The time constant for the change of carbonic acid to CO_2 is approximately 0.01 second, a much shorter time than we could measure. The failure of carbonic anhydrase inhibition to alter the rate and volume of CO_2 liberation confirms this pathway for CO_2 liberation resulting from acid injection.

Effect of ventilation with oxygen. It seemed possible that part of the gas appearing in the plethysmographic pressure record after acid injection was not carbon dioxide. This is because acidification of the blood results in a shift to the right in the oxyhemoglobin dissociation curve, so that a higher oxygen tension exists at any given oxygen saturation except at values near complete saturation. This could then slow the uptake of oxygen by the blood, or if the alveolar-capillary oxygen gradient were reversed, even cause a movement of oxygen from the blood into the alveolar gas, in either case producing an increase in plethysmographic pressure. To test this possibility, the animal was ventilated for 10 to 15 minutes with 100 per cent oxygen. This results in a high alveolar oxygen tension, which insures normal movement of oxygen into the blood to saturate fully the hemoglobin. The records obtained after ventilation with oxygen showed that the volume of gas evolved was significantly decreased (2.1 ml versus 3.0 ml), the difference presumably representing the volume of O_2 contributing to the plethysmographic pressure rise when acid is injected while breathing air.

Injection of Tris. Trishydroxymethylaminomethane is a hydrogen ion acceptor, and when injected into the blood causes reaction (Equation 1) to move to the right. This results in enhanced uptake of CO_2 by the blood. By decreasing CO_2 elimination from the blood, and by promoting the absorption of alveolar CO_2 by the blood, Tris thus caused a decrease in plethysmographic pressure. This drop in pressure proceeded more slowly than the pressure rise after acid injection, possibly reflecting the fact that the hydration of CO_2 in an alkaline medium is slower than the dehydration of carbonic acid in an acid medium. Another possible explanation for this phenomenon may be that the washout rate of Tris from the pulmonary circulation is longer than that of acid because of the possibly larger dilution space for Tris in the lung, caused by its ability to penetrate into cells (8); thus the buffering action of Tris would be available for a longer period of time than that of substances confined to the vascular system.

Did the acid and Tris reactions reach an equilibrium? The following evidence is presented in support of the view that the reactions of injected lactic acid and Tris in the blood reached equilibrium during both the initial transport phase and the subsequent gas exchange period. *a*) Increasing the path traversed, and thus the time available for the reaction of acid with the blood, by injecting acid into the right atrium resulted in the evolution of the same volume of gas as when the acid was injected into the pulmonary artery. *b*) Increasing the concentration of acid injected (1.2 M, 2.4 M, and 4.8 M) resulted in proportionate increases, within the limits of accuracy of the method, in gas volume evolved, indicating that the time available was adequate for the reactions involved to approach completion. *c*) Marked acceleration of the rate of uptake of gas by Tris, produced by the almost simultaneous injection of carbonic anhydrase, did not increase the volume of gas absorbed, indicating that the time available in the gas exchange vessels was not a limiting factor in the uptake of gas by the buffered blood.

Criticism of the use of lactic acid. The use of an injection of lactic acid to initiate reactions leading to CO_2 evolution in an effort to determine the rate of this evolution in a living animal may be criticized on the following grounds. *1*) Injection of 1.2 M lactic acid into the blood stream may produce hemolysis. Hemolysis *in vitro* may be observed when concentrations of acid as small as $\frac{1}{16}$ molar are added to blood. This could influence the rate of CO_2 liberation by liberating carbonic anhydrase or hemoglobin from the erythrocyte into the blood stream. That these were not significant factors in our experiments is suggested by the fact that low and high concentrations of acid yielded the same rate, and that acetazolamide did not alter the rate of CO_2 liberation after acid injection. *2*) The study of the rate of CO_2 liberation is complicated by the fact that the pH change induced by acid injection causes oxygen to be liberated. This, however, can be suppressed by ventilating the animal with 100 per cent oxygen. *3*) Since the likelihood is great that CO_2 liberation after acid injection does not proceed along the normal pathways for CO_2 liberation, it may be argued that the method sheds no light on these normal reactions. It is true that lactic acid increases in the blood during muscular exercise and thus that part of the CO_2 eliminated in the lung under such conditions may be derived in a manner similar to that which we suspect operates in our experiment. That this may account

for some but not all of the CO_2 excreted during exercise is attested to by the fact that carbonic anhydrase inhibition interfers but slightly with CO_2 transport during muscular exercise (9).

Injection of bicarbonate. The injection of 1 M bicarbonate solution into the pulmonary artery initiates reactions leading to the evolution of CO_2 which can be summarized in Equation 1 read from right to left. A small fraction of injected bicarbonate combines with plasma hydrogen ion. The major fraction enters the erythrocytes where it combines with hydrogen ions dissociated from the hemoglobin. This movement into the red cell is associated with an outward movement of chloride ions into the plasma, to maintain the balance of electric charges. In the presence of normal carbonic anhydrase activity, all these reactions reach equilibrium within the time of transit from the pulmonary artery to the vessels of gas exchange. Thus the time elapsed from injection to the evolution of half the gas after bicarbonate injection, corrected for the time spent in mixing and transit by subtracting the analogous interval in the ether injection record, gives a figure for the rate of evolution of CO_2 in the lung. The near identity of the values obtained with bicarbonate and with lactic acid confirms the view that the two techniques measure similar time-dependent processes. Both values are in agreement with the results of West, Holland, Dollery, and Matthews (10), using radioactive oxygen-labeled CO_2, on the rate of transfer of CO_2 from the alveolar air to the blood.

In contrast to the injection of lactic acid, bicarbonate injection results in the evolution of only CO_2. This was suspected from the observation that the addition of bicarbonate to blood *in vitro* caused only a slight increase in pH; this, if anything, would tend to decrease the oxygen tension required to produce a given oxyhemoglobin saturation, thus enhancing the uptake of oxygen by hemoglobin. The suspicion was confirmed by the fact that prior ventilation of the dog with 100 per cent oxygen did not decrease the volume of gas liberated after injection of bicarbonate.

Effect of acetazolamide. In a dose as small as 3 to 5 mg per kg, acetazolamide almost completely suppressed the evolution of CO_2 from injected bicarbonate. That this suppression was not complete, however, was shown by the fact that when a solution of 2M potassium bicarbonate was injected, gas evolution occurred. This effect may have been caused by the fact that, since the potassium salt of bicarbonate is more soluble than the sodium salt, a larger total dose of bicarbonate ions could be administered in this form. (The injection of potassium bicarbonate causes death from cardiac arrhythmia and arrest within minutes after injection, presumably from the large single dose of potassium ions.)

Because of the relative abundance of bicarbonate ions in the plasma, the addition of a large dose of hydrogen ions, as in the injection of acid, can produce a high enough concentration of carbonic acid so that dissociation to CO_2 proceeds quickly and without the need for carbonic anhydrase. On the other hand, since the concentration of hydrogen ions in the plasma is normally small, the addition of bicarbonate ions does not produce the same mass action effect. Thus the evolution of CO_2 from bicarbonate is largely dependent for rapid equilibration on the normal activity of intracellular carbonic anhydrase.

The use of bicarbonate in these experiments thus provides an answer to the criticisms of the use of lactic acid for the same purpose, inasmuch as hemolysis does not occur, the pH change induced is small, and its direction does not complicate the picture as to the nature and volume of gas evolved. Moreover, chemical considerations of the possible reactions of bicarbonate ions in the blood and the observed dependence of the reaction on normal carbonic anhydrase activity make it almost certain that the reactions initiated by bicarbonate injection proceed along the physiological pathways for CO_2 liberation. This suggests that the values obtained for the over-all rate of these processes are probably valid estimates of these rates in the living animal.

SUMMARY

A method has been developed for the study of the rate of evolution or of absorption of CO_2 in the lung. This is based on the injection into the pulmonary artery of substances which initiated reactions leading to the liberation or the absorption of CO_2 in the lung and on determining the rate and amount of gas evolution or absorption by using a sensitive body plethysmograph. The time spent in transit from the point of injection to the site of

gas exchange is determined from the injection of a solution of an inert gas, ether, at the same point in the pulmonary artery.

The evolution of gas after the injection of 1.2 M lactic acid took, after substracting transit time, 0.40 second to reach half-completion. The gas evolved was estimated to consist of two-thirds CO_2 and one-third oxygen. Tris led to the absorption of gas and this reaction took 1.01 seconds for half-completion. Simultaneous injection of carbonic anhydrase accelerated this reaction so that it took only 0.16 second for half-completion. Neither gas evolution nor gas absorption was prevented by sodium acetazolamide. The injection of 1 M sodium bicarbonate in the same manner was followed by the evolution of CO_2 which took 0.44 second for half-completion after the bicarbonate entered the vessels of gas exchange. This reaction was almost completely suppressed by sodium acetazolamide. Evidence is presented that the reactions involved in the movement of gas across the alveolar-capillary membrane reach equilibrium within the time the bicarbonate spends in the conducting portion of the pulmonary arterial tree and the vessels of gas exchange.

Reasons are given for the belief that this method measures the over-all rate of the reactions involved during the liberation, under physiological conditions, of CO_2 in the lung.

ACKNOWLEDGMENTS

The authors express their thanks to Dr. Robert E. Forster II for advice concerning the mechanism of reactions and for his suggestion concerning the use of lactic acid as a means of displacing CO_2 from the blood.

Dr. Marvin A. Sackner participated during some of the experiments.

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