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THE INFLUENCE OF ALTERATIONS IN ACID-BASE BALANCE UPON TRANSFERS OF CARBON DIOXIDE AND BICARBONATE IN MAN[1]

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The output of carbon dioxide by the lungs is primarily dependent upon the oxidative metabolism of the organism, and may, indeed, be used as a measure of oxidative processes under appropriate conditions (1 to 3). It is well recognized, however, that augmentation or depletion of the large quantity of CO_2 stored within the body, which occurs during alkalosis or acidosis, may produce alterations in respiratory CO_2 output, independent of oxidative metabolism (4 to 7). Some attempts have been made to establish quantitative relationships between such changes in the output of CO_2 by the lungs and fluctuations of acid-base equilibrium within the organism. Shaw (8) measured the respiratory exchange of cats subjected to artificial ventilation with CO_2-rich mixtures, and correlated the CO_2 exchange of the whole animal with variations in blood CO_2 content. From such observations, the amount of CO_2 absorbed by the tissues could be estimated. Irving and his coworkers (9, 10) made direct determinations of the CO_2 content of various tissues of dogs and cats overventilated with air and with CO_2 enriched mixtures. They attempted to account for the net CO_2 exchange of the whole organism in terms of altered CO_2 content of blood, muscle, bone, and viscera. Applications of Shaw's technique in an effort to determine the CO_2 capacity of the human body has been reported by Adolph, Nance, and Shiling (11). Their results were inconclusive, as were those of similar studies by Brocklehurst and Henderson (12), because it proved impossible to attain the equilibrium state required by the conditions of the experiments (13). In all of these investigations, acid-base change was induced either by overbreathing or by ventilation with CO_2-rich mixtures, procedures which primarily altered the concentration of dissolved CO_2 in the body (14). No comparable studies have been reported concerning the influence of primary alteration in bicarbonate ion concentration upon respiratory CO_2 production.

The present study deals with the effects of acidosis and alkalosis upon respiratory CO_2 output in the post-absorptive state, when the oxidative metabolic mixture is relatively constant. Concentration of dissolved CO_2 in the body was increased by rebreathing, and decreased by overbreathing; concentration of bicarbonate ion was altered by sodium bicarbonate infusion, and by ammonium chloride ingestion. The respiratory production of CO_2 was found to be strikingly altered during primary change of the concentration of dissolved CO_2 but was little influenced by change of serum bicarbonate.

EXPERIMENTAL PROCEDURES AND METHODS

The experiments involving measurement of respiratory exchange were carried out on normal male adults (the same subject was used in all but 2 experiments). The fasting subject came to the laboratory at about 8 a.m. and rested for one-half hour under basal conditions. The basal respiratory exchange was determined over a 10-minute period by the open circuit method, with the subject breathing through a rubber mouthpiece into a Tissot spirometer. Analyses of the expired air for CO_2 and oxygen were carried out in duplicate by means of a modified Haldane apparatus. The usual precautions were taken to avoid leaks and the apparatus was checked from time to time by analyses of atmospheric air.

Venous blood samples for determination of serum CO_2 content were obtained anaerobically, without stasis, immediately before the initiation of the procedure designed to alter acid-base equilibrium. In the overventilation experiments, as soon as the blood was in the syringe, the subject began to breathe into the spirometer at a ventilation rate about twice normal. After 5 to 10 minutes of overbreathing, a second venepuncture was made and the spirometer disconnected as soon as the blood sample had been obtained. The rebreathing experiments were carried

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out in similar fashion, except that a Douglas bag without valves, containing 80 to 100 liters of a 4.36 to 5.75 per cent CO_2-air mixture was substituted for the Tissot spirometer. Samples of air from the Douglas bag were taken for analysis shortly before and immediately after the period of rebreathing.

In the ammonium chloride experiments, measurement of respiratory exchange was carried out over 10-minute periods, under resting conditions, at intervals (usually 45, 60, and 90 minutes) after the ingestion of 10 grams of NH_4Cl in 0.5 gram enteric coated tablets. Blood samples for serum CO_2 determination were taken 10 to 20 minutes before and immediately after each collection of expired air.

Measurement of respiratory exchange during sodium bicarbonate infusion proved impracticable. Untrained subjects could therefore be employed and most of the experiments were carried out on convalescent patients. The volume of distribution of bicarbonate ion was studied following the administration of sodium bicarbonate intravenously as a 4 per cent solution in distilled water. The dose ranged from 10 to 14 grams given over a period of 10 to 45 minutes. In order to insure its quantitative administration, the bicarbonate solution was followed by 200 to 300 cc. of normal saline given through the same infusion set. Blood samples were obtained just before and at one or more intervals, 25 to 120 minutes after the end of the infusion. Complete urine collections were made over the period between each pair of blood samples. Determinations were made of serum CO_2 content, serum chloride concentration, and, in some cases, serum sodium concentration. The urine specimens, preserved and, in some instances, collected under mineral oil, were analyzed for total CO_2 content, chloride concentration, and, in some experiments, sodium concentration. In all experiments, the extracellular fluid volume of the subject was determined by the thiocyanate method (15). Thiocyanate was usually administered on the evening before the bicarbonate infusion, so that change in extracellular fluid during the course of the experiment could be estimated from change in serum SCN concentration, as well as from the alterations in concentration of chloride and sodium in the serum.

All chemical determinations were carried out in duplicate. Serum CO_2 content was determined by the method of Van Slyke and Neill (16), serum chloride by the Hald modification of Patterson's micromethod (16), serum sodium by the method of Hald (17), urine sodium by the method of Butler and Tuthill (16), and urine chloride by the modified Volhard-Harvey titration (16). Thiocyanate was determined colorimetrically with ferric nitrate (18).

CALCULATIONS

The magnitude of change in respiratory CO_2 output produced by altered acid-base equilibrium was calculated as follows:

1. The respiratory quotient of the basal period was calculated from the CO_2 output and oxygen consumption of that period.

2. The oxygen consumed during the period of acid-base change was multiplied by the R. Q. of the basal period to give the oxidative or "metabolic" CO_2 production during acid-base change. The metabolic CO_2 production was then subtracted from the total CO_2 output during the period of acid-base change to give the non-metabolic CO_2 production. The value for non-metabolic CO_2 thus obtained represented the net increase or decrease of CO_2 in the body as a whole, since urinary excretion during the brief periods of overventilation or rebreathing was negligible, and the CO_2 content of the urine following ingestion of ammonium chloride proved to be insignificant.

Change in the amount of CO_2 contained within the extracellular fluids was calculated by multiplying change in the concentration of total CO_2 in the serum by the extracellular fluid volume. In all of the experiments, except those dealing with the effects of NH_4Cl ingestion, blood for estimation of serum CO_2 content was drawn immediately before and after the period over which respiratory exchange was measured. Consequently, the calculated alteration in extracellular fluid content occurred during the same interval over which the CO_2 balance of the whole organism was being measured. The difference between extracellular change and change in the content of the body as a whole was allocated to the tissues.

In the ammonium chloride experiments, the first blood sample was drawn at least 10 minutes before collection of expired air began, in order to avoid possible overventilation during the measurement of respiratory exchange. The change in serum CO_2 content over the 10 minute respiratory exchange period was interpolated from the alteration observed over the longer interval, which was usually 20 minutes and never exceeded 30 minutes.

The volume of distribution of administered bicarbonate ion was calculated by means of the following formulae:

$$ECF_2 = \frac{ECF_1 \times [Cl]_1 + \Delta Cl}{[Cl]_2}, \qquad (1)$$

where ECF_2 is the volume in liters of extracellular fluid at the end of the experimental period,

ECF_1 is the volume in liters of extracellular fluid at the beginning of the experimental period,

$[Cl]_1$ is the concentration of serum chloride in milliequivalents per liter at the beginning of the experiment,

ΔCl is the amount of chloride in milliequivalents retained during the experiment,

and $[Cl]_2$ is the concentration of serum chloride in milliequivalents at the end of the experiment.

ECF_2 was also calculated from change in serum sodium and change in serum SCN by analogous formulae, substituting [Na] or [SCN] for [Cl].

$$\Delta ECF = ECF_2 - ECF_1, \qquad (2)$$

where ΔECF is the change in liters in the volume of extracellular fluid during the experiment.

$$V_{HCO_3} = \frac{\Delta CO_2 - \Delta ECF[CO_2]_2}{[CO_2]_2 - [CO_2]_1}, \qquad (3)$$

where V_{HCO_3} is the volume of distribution of administered bicarbonate, ΔCO_2 is the CO_2 balance in millimols (total CO_2 given as bicarbonate less total CO_2 excreted in the urine), $[CO_2]_2$ is the serum CO_2 content in millimols per liter at the end of the experiment and $[CO_2]$ is the serum

CO_2 content in millimols per liter at the beginning of the experiment.

The validity of such calculations of distribution volumes has been discussed by Bourdillon and Lavietes (19).

RESULTS

Overventilation

The carbon dioxide exchange during mild overventilation of 5 to 10 minutes duration was measured in 8 experiments. The results are presented in Table I A and Figure 1. It is apparent that in every experiment the total quantity of CO_2 given up by the organism exceeded the amount lost from the extracellular fluids alone. A portion must therefore have come from the tissue cells.

The observed fall in serum CO_2 content was very small in the first three experiments; consequently, the cells were credited with a large contribution toward total expired CO_2. It appeared that the peripheral circulatory slowing known to occur during overventilation (20) was masking, in blood drawn from the arm veins, the true fall in serum CO_2 content. Since to

TABLE I

Exchanges of CO_2 during change of acid-base balance

Experiment	Duration	Change of serum CO_2 content	Oxygen consumption	CO_2 production	Basal R. Q.	Metabolic CO_2 output	Non-metabolic CO_2 balance		
							Total	of *ECF*	of cells
number	*minutes*	*volumes per cent*	*cc.*	*cc.*		*cc.*	*cc.*	*cc.*	*cc.*
A. LOSS OF CO_2 DURING OVERBREATHING									
1*	6.0	−0.8	1725	2376	0.78	1351	−1025	−150	−875
2*	6.0	−0.2	1800	2298	0.88	1587	− 711	− 33	−678
3*	5.5	−0.7	1800	2555	0.86	1542	−1013	−117	−996
4	5.6	−2.8	1700	2044	0.80	1364	− 677	−466	−211
5	8.5	−2.2	2370	2630	0.81	1932	− 698	−367	−331
6	10.5	−3.1	2790	2836	0.78	2181	− 655	−518	−137
7	8.2	−3.2	2280	2654	0.81	1850	− 804	−534	−270
8	7.0	−3.5	2130	2388	0.73	1562	− 826	−515	−311
B. RETENTION OF CO_2 DURING REBREATHING									
1*	8.0	+1.0	2410	1330	0.78	1871	+ 541	+155	+ 386
2*	8.3	−0.5	2750	1200	0.91	2511	+1311	− 85	+1396
3*	7.5	−0.4	2153	936	0.87	1867	+ 931	− 75	+1006
4	8.3	+3.4	2749	1361	0.81	2230	+ 869	+510	+ 359
5	7.5	+4.4	2210	1050	0.82	1803	+ 753	+660	+ 93
6	10.7	+2.0	2920	1700	0.84	2453	+ 753	+331	+ 422
7	11.5	+3.4	2960	1480	0.82	2427	+ 947	+567	+ 380
C. EXCHANGE OF CO_2 FOLLOWING INGESTION OF NH_4CL									
6b		+0.2	2246	1593	0.73	1642	+49	+ 33	
5a		−0.4	1933	1542	0.75	1453	−89	− 67	
2		−0.5	2200	1790	0.80	1753	−37	− 83	
5b		+0.5	1900	1479	0.75	1428	−51	+ 83	
8a		+0.6	1927	1446	0.77	1489	+43	+100	
7a		+0.7	2234	1578	0.72	1614	+36	+117	
7b		+1.0	1947	1485	0.72	1404	−81	+167	
7c		−1.1	2089	1469	0.72	1506	+37	−184	
6a		−1.1	2149	1560	0.73	1571	+11	−184	
1		−1.1	2300	1845	0.80	1849	+ 4	−184	
8c		+1.3	1929	1487	0.77	1490	+ 3	+217	
8b		−1.6	1954	1538	0.77	1510	−28	−267	
6c		−1.6	1932	1444	0.73	1412	−32	−267	
3		+1.7	2160	1726	0.79	1723	− 3	+283	
4		+2.5	1923	1498	0.77	1497	− 1	+417	

* Peripheral vasodilatation not maintained.

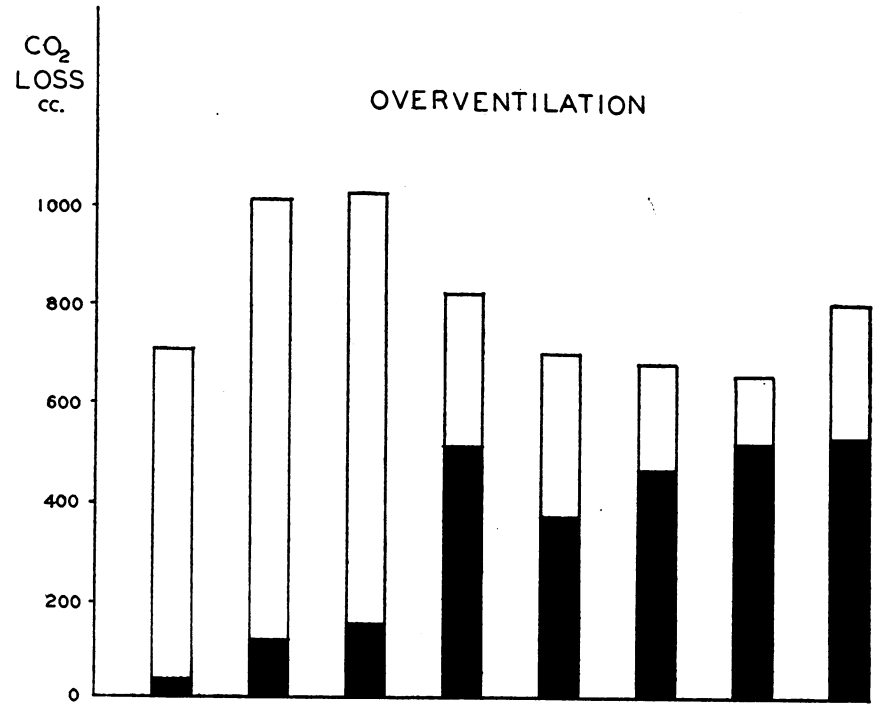

FIG. 1. LOSS OF CO_2 DURING OVERVENTILATION

The total height of each column represents the total loss of nonmetabolic CO_2. The solid portion represents the amount lost from the extracellular fluids. The unshaded portion indicates the amount lost from the cells.

obtain mixed venous blood was hardly feasible, the samples were rendered approximately arterial in subsequent experiments by immersing the arm in hot water (21). Under these conditions, the observed change in serum CO_2 content increased and a more reasonable estimate of intra- and extracellular losses of CO_2 was obtained (Experiments 4 to 8, Table I A).

Rebreathing

Seven experiments were carried out in order to study the effects of rebreathing a CO_2-air mixture for 5 to 10 minutes. The data are presented in Table I B and Figure 2. In none of the experiments did the extracellular fluids accommodate all of the CO_2 retained by the body; hence, the tissue cells must have participated in the storage. In the first 3 experiments, local circulatory effects were even more apparent than in the observations on overventilation. Respiratory CO_2 excess produces local vasodilatation (20) which may completely mask, in peripheral venous blood, the rise of CO_2 content associated with rebreathing. Blood drawn after rebreathing was uniformly brighter red than that obtained before rebreathing, although no mechanical stasis was utilized during either venepuncture. When peripheral circulatory changes were minimized by immersion of the arm in hot water throughout the experiment, a consistently greater rise in venous CO_2 concentration was observed and a more reliable estimate of extracellular retention of CO_2 was obtained (Experiments 4 to 7, Table I B).

Ammonium chloride ingestion

The results of 15 determinations of carbon dioxide exchange following the ingestion of ammonium chloride are presented in Table I C and Figure 3. There was considerable fluctuation in serum CO_2 content in the first 2 hours after ingestion of NH_4Cl, with a general tendency for a fall to occur during this period. The sporadic increases of serum CO_2 content were attributed to erratic absorption of the enteric coated salt, and to probable stimulation of gastric secretion

(suggested by the occurrence of slight nausea in some experiments). No alterations in respiratory rate were observed during the experiments, nor was there significant variation in the volume of expired air collected in the spirometer over 10-minute periods. Alterations in serum CO_2 content could therefore be attributed with confidence to primary alteration in bicarbonate ion concentration.

In all but 2 experimental periods, in which serum CO_2 was practically constant, the alteration in serum CO_2 content indicated that the extracellular fluids had gained or lost quantities of carbon dioxide, much in excess of any change in the CO_2 content of the whole organism as measured by the respiratory output. Indeed, some of the observations indicated that a considerable loss of CO_2 from the extracellular fluid could occur while the respiratory output actually declined. There was, therefore, not even a directional correlation between the carbon dioxide balance of the extracellular fluids and of the subject as a whole under the conditions of these observations. None of the discrepancies in CO_2 exchange could be explained by urinary excretion, since this was negligible. With a very few exceptions, the fluctuations in respiratory output of carbon dioxide were extremely small, exceeding 3 per cent of the total output for 10 minutes in only 3 instances, and never exceeding 6 per cent. If the change in extracellular CO_2 content had been reflected in the respiratory production, the observed changes would have been greater than 3 per cent in all but 1 period, and would have ranged from 6 to 28 per cent in all but 4 periods.

Bicarbonate distribution

Data pertaining to the volume of distribution of bicarbonate ion, administered intravenously as sodium bicarbonate in 7 experiments, are presented in Table II and Figure 4.

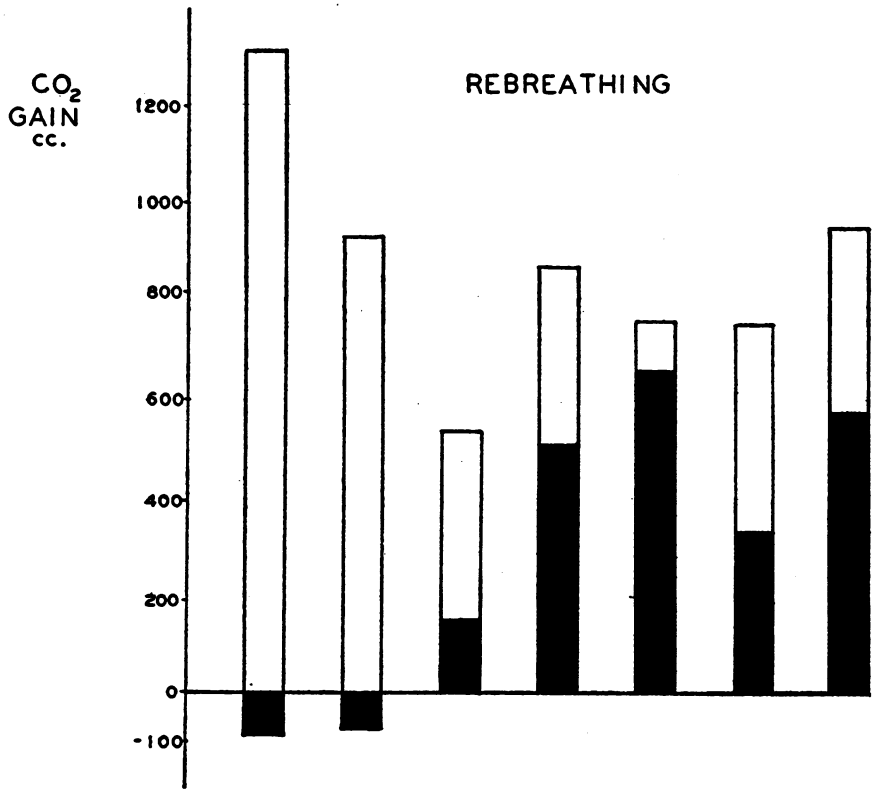

FIG. 2. RETENTION OF CO_2 DURING REBREATHING

Total retention of CO_2 is represented by the total height of each column. The solid portion represents extracellular retention, the unshaded portion, intracellular retention.

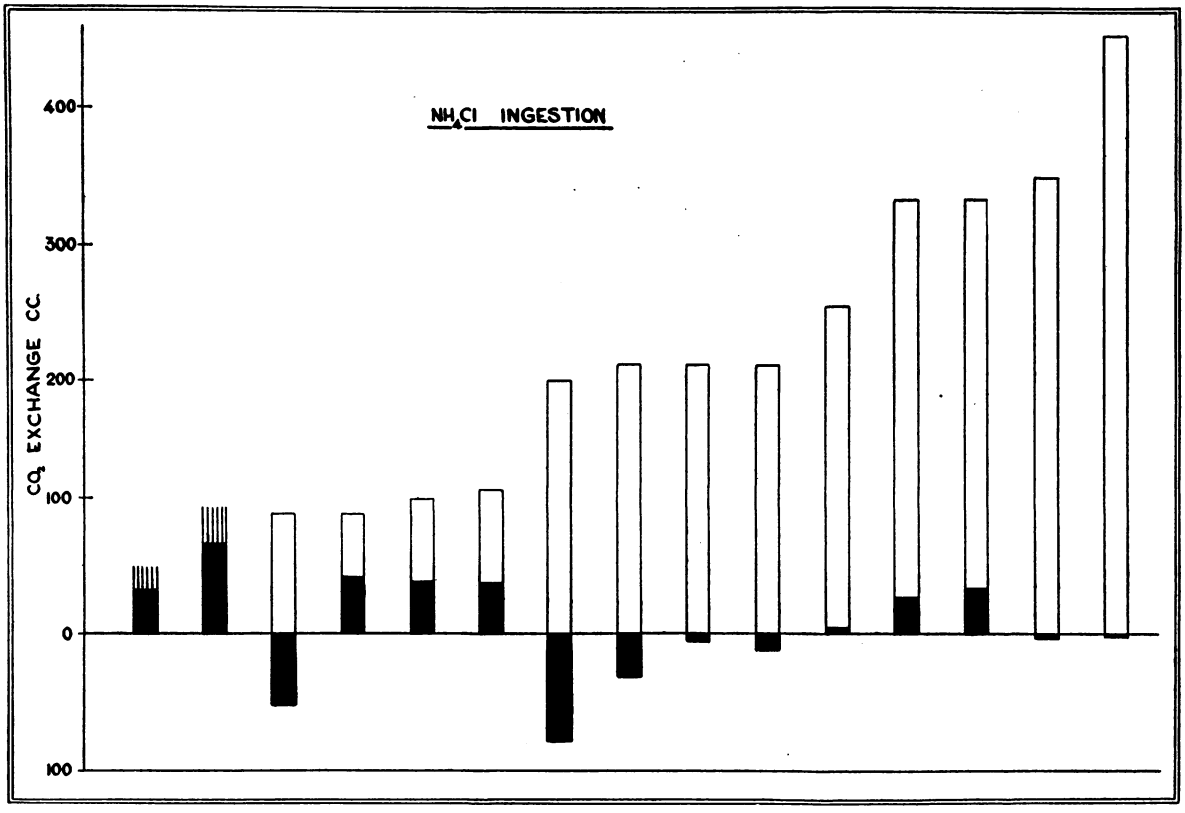

FIG. 3. EXCHANGES OF CO_2 FOLLOWING INGESTION OF NH_4Cl

The CO_2 balance of the extracellular fluids (whether positive or negative) is represented by the total height of each column. The corresponding CO_2 balance of the body as a whole is indicated by the solid portion of the column. The vertical lines above the first 2 columns indicate change in total CO_2 in excess of the extracellular change.

In the majority of experiments, the volume through which the retained bicarbonate ion was distributed approximated the extracellular fluid volume of the subject as measured by the thiocyanate method. In the first experiment, bicarbonate space was apparently less than thiocyanate space. However, in this instance, no estimation of urinary chloride excretion was made. It is clear from the formulae given for calculation of distribution volume that neglecting chloride excretion will, by increasing the value given Δ Cl in formula (*1*), increase the value of ECF_2. This in turn will increase the value of ΔECF (Formula (*2*)). Too high a value for ΔECF will give too low a value for V_{HCO_3} in Formula (*3*). Obviously, therefore, the figures given for bicarbonate space in Experiments 1a and 1b are too low. Furthermore, the value obtained for the same subject in the next experiment was considerably higher and agreed well with the distribution volume of thiocyanate. The volume calculated for bicarbonate distribution in Experiment 4 may also be in error since, because of considerable difficulty with venepuncture, some stasis was employed. Interpretation is further complicated because the patient also received sodium sulfadiazine intravenously in connection with another study. In 4 of the remaining 5 experiments, agreement between bicarbonate space and thiocyanate space was very close. The discrepancy in Experiment 3, carried out on a 37-year-old woman suffering from an agitated depression, is unexplained.

The possible summation of errors involved in the calculation of bicarbonate distribution volume may amount to several liters. For this, there are two chief sources. The first lies in the estimation of change in extracellular fluid volume, which depends upon small alterations in serum chloride, sodium, or SCN concentrations,

TABLE II

The distribution of intravenous HCO_3

Experiment	Subject	Duration	HCO_3 retained	Change of serum concentration of				Change of *ECF* volume in liters calculated from [1]			Volume of distribution of added HCO_3 in liters calculated from [2]			Final extracellular fluid volume
				CO_2	SCN	Cl	Na	SCN	Cl	Na	SCN	Cl	Na	
number		*minutes*	*m. eq.*	*m. eq. per liter*	*mgm. per cent*	*m. eq. per liter*	*m. eq. per liter*							*liters*
1a	JDR	25	109.0	+7.7		−5.0			+0.8			10.6		17.5
1b	JDR	75	87.0	+5.0		−5.1			+0.8			12.3		17.5
2	JDR	68	83.4	+4.4		−1.8			+0.4			16.0		17.1
3a	Ba	40	121.1	+4.9		−1.0			−0.05			25.0		15.9
3b	Ba	120	95.4	+3.9		−2.0			−0.08			25.1		15.8
4	Was	30	148.3	+6.6	−0.45	−1.6		+1.2	+0.5		15.9	19.7		21.0
5	Wat	90	117.8	+3.5	−0.57	−3.0		+0.8	+0.4		26.1	30.0		19.1
6	We	40	121.9	+4.1	−0.47	−2.4	+2.0	+0.9	+0.4	+0.7	22.6	26.6	24.1	19.7
7	Me	60	132.1	+5.7	−0.60	−2.5	+3.2	+0.8	+0.6	+0.8	18.2	19.4	18.2	17.2

[1] By formula (*1*) of text.

[2] By formula (*2*) of text.

during the experimental period. Change in serum chloride concentration rarely exceeded 3 milliequivalents per liter. Since the possible error of each determination is approximately 1 milliequivalent, the error of the difference between 2 estimations may reach 2 milliequivalents per liter. Reference to formula (*1*) shows that the error in estimating *ECF* change may consequently reach 0.4 liter. This error in Δ *ECF* would produce an error of 2 liters in V_{HCO_3} (Formula (*3*)). The importance of this source of error is apparent in the last 4 experiments, where significant differences may be noted among the changes in extracellular fluid volume calculated from thiocyanate, chloride, and sodium concentrations, respectively. When all three ions were determined, the best agreement was obtained between thiocyanate space and sodium space.

The second significant source of error lies in

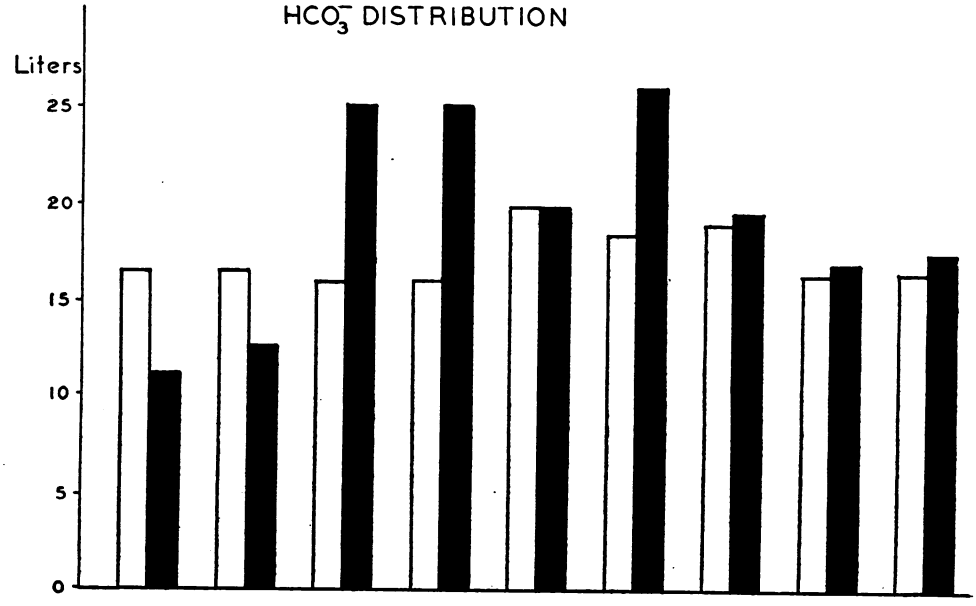

FIG. 4. APPARENT VOLUME OF DISTRIBUTION OF INTRAVENOUS BICARBONATE

The volumes of distribution of bicarbonate ion are represented by the solid columns. The extracellular fluid volumes of the subjects are indicated by the open columns.

estimating change in serum CO_2 content. The error of the chemical determination is not more than 0.15 m.eq. per liter, so that the maximum error of the difference between 2 determinations is 0.3 m.eq. Reference to Formula (*3*) indicates that this possible error of 0.3 m.eq. will produce an error of about one liter in the calculated bicarbonate distribution volume. Moreover, slight changes in peripheral blood flow can lead to change in venous serum CO_2 content, unrelated to bicarbonate administration.

Since the respiratory output of CO_2 could not be measured over the relatively long periods required for determination of the distribution volume of administered bicarbonate, the possibility of alteration in the total CO_2 content of the organism due to changes in ventilation cannot be excluded. The occurrence of such changes would lead to an error in the estimation of the CO_2 balance (ΔCO_2, formula (*3*)), which was taken as the difference between CO_2 administered intravenously as bicarbonate, and total CO_2 excreted in the urine. Retention of CO_2, due to decreased ventilation, would therefore lead to an erroneously low value for the distribution volume of bicarbonate. However, previous studies have shown that sodium bicarbonate infusions either increase ventilation rate (22 to 25) or have no effect on the breathing (23, 26). Consequently, failure to measure respiratory production of carbon dioxide could lead only to erroneously high values for the distribution volume of administered bicarbonate ion, but could not cause low results.

DISCUSSION

Rebreathing and overbreathing, even when of brief duration, change the carbon dioxide content of the body considerably. A significant portion of the total change in CO_2 content is accounted for by altered concentration within the tissue cells. Since the primary effect of overbreathing or rebreathing is to lower or raise, respectively, the CO_2 tension of the blood (14), the presumption is strong that carbon dioxide can enter or leave the tissue cells in the form of dissolved CO_2. Direct demonstration of cellular permeability to dissolved CO_2 has been accomplished by Lowry and Hastings (27), using isolated rat muscle. The existence of similar permeability in the intact animal is also suggested by the observations of Irving and coworkers (9, 10), and of Shaw (8, 13, 28).

Free permeability of cell membranes to dissolved CO_2 implies that, under equilibrium conditions, CO_2 tension in the extracellular fluids and in the cell water shall be equal. If CO_2 tension is lowered in the blood and extracellular fluids by overbreathing, the tissues should give up enough CO_2 to lower the tension in cell water to the same degree. Under these circumstances the amount of CO_2 lost from the *ECF* and cell water will be proportional to the respective volumes of these fluid compartments, except insofar as their CO_2 absorption curves may differ. Irving, Foster and Ferguson (29) have shown that the CO_2 absorption curve of cat muscle is not dissimilar from that of the blood. It seems justifiable, therefore, to assume that under equilibrium conditions the contribution of the tissues in exchanges of dissolved CO_2 should be approximately twice that of the extracellular fluids, since their volumes are in a ratio of about 2 : 1 (30). Failure to observe such proportionality in either the rebreathing or overbreathing experiments suggests that equilibrium was not attained. But a lag in exchange of CO_2 between cells and extracellular fluid may occur because of the absence of carbonic anhydrase from tissue cells (31). The exchanges between alveoli and blood and between blood and extracellular fluid on the other hand are enormously accelerated by the carbonic anhydrase present in red cells. Failure to attain equilibrium was therefore to be expected and the relative amounts of CO_2 lost from or gained by extracellular fluids and intracellular water are of little importance.

The studies of bicarbonate distribution indicate quite clearly that the tissue cells are impermeable to CO_2 bound as bicarbonate. This observation is in keeping with the demonstration by Lowry and Hastings (27) that the cells of isolated rat muscle, although permeable to dissolved CO_2, are impermeable to bicarbonate ion. Previous studies on human subjects by Palmer and Van Slyke (32) and by Hartmann and Senn (33) led to the conclusion that ingested or intravenously administered sodium bicarbonate was distributed through the total volume of body

water. However, in neither investigation was account taken of urinary excretion of bicarbonate, nor was correction for expansion of extracellular fluid volume attempted. Neglect of either of these factors would give an erroneously high value for the calculated volume of distribution of the administered bicarbonate Furthermore, many of the observations were on patients with acidosis and dehydration, whose serum bicarbonate concentrations and extracellular fluid volumes were subject to considerable change independent of bicarbonate administration.

Shaw and Messer (28) have reported the changes of serum CO_2 content in 5 cats following intravenous administration of sodium bicarbonate. Since ureteral ligation was carried out prior to injection of the hypertonic bicarbonate solution, the volume through which the bicarbonate was distributed can be calculated from their data, with the assumptions that no urinary excretion occurred, and that enough water left the tissues to restore osmotic equilibrium between cell water and extracellular fluid. If the volume of extracellular fluid in the cat is estimated to be 30 per cent of the body weight, the calculated values for the volume of distribution of bicarbonate ion range from 31.5 to 42.3 per cent of the body weight. Although these values must be considered approximations, they indicate that the bicarbonate space of the cat is far less than the total volume of body water, but agrees fairly well with the volume of extracellular fluids.

The possibility that cell membranes might exhibit a differential permeability to CO_2 and bicarbonate ion was considered in 1920 when Jacobs (34, 35) reported a group of ingenious experiments on plants, protozoa, and amphibia, which suggested strongly that the cell membranes studied were penetrated much more rapidly by carbon dioxide than by bicarbonate ion. Jacobs also inferred from observations on taste sensation in man that carbon dioxide could enter mammalian cells which were relatively impermeable to bicarbonate ions. Subsequently, Gesell (23, 36) supported Jacobs' views concerning cellular impermeability to bicarbonate ion, but his observations were largely concerned with the permeability of the respiratory center, and the evidence obtained was quite indirect or chiefly inferential. Gesell (37), and other advocates (38, 39) of the theory that the activity of the respiratory center is chiefly determined by local hydrogen ion concentration, have argued on the basis of Jacobs' observations that the apparent specificity of CO_2 as a respiratory stimulant is merely a manifestation of its rapid effect upon the intracellular pH of the center. The observations reported here support Jacobs' hypothesis concerning cellular impermeability to bicarbonate ion. However, recent investigations of respiratory function (40, 41) indicate that the respiratory responses to CO_2 are not merely a manifestation of intracellular pH change.

The effects of ammonium chloride ingestion differed strikingly from those of either rebreathing or overbreathing. Respiratory CO_2 output was not materially altered despite significant losses or gains of CO_2 by the blood and extracellular fluids. Since urinary excretion was negligible, CO_2 leaving the extracellular fluids must have entered the tissue cells. The apparent paradox of an increase in cellular total CO_2 content during fall in CO_2 content of the extracellular fluids is resolved when concentrations of bicarbonate ion and dissolved CO_2 are considered independently. Ammonium chloride acts as would the addition of hydrochloric acid to the blood. That is, it decreases bicarbonate ion concentration, but increases CO_2 tension (14). Depression of bicarbonate concentration in serum does not cause bicarbonate to emerge from the tissues, because of the impermeability of the cell membranes. The increased CO_2 tension, however, results in transfer of dissolved CO_2 from extracellular fluids to the tissues. Consequently, the total CO_2 content of the blood falls, that of the tissues rises, and, by a sort of internal compensation, little or no CO_2 is left for excretion by the lungs. During periods when bicarbonate ion concentration increases, the reverse sequence of events probably occurs. With further depression of serum bicarbonate, and consequently greater increment in CO_2 tension, CO_2 excretion by the lungs would presumably increase, due to stimulation of the respiratory center. Acidosis cannot increase indefinitely without leading to overventilation. It is noteworthy, however, that many attempts to induce overbreathing by ingestion of acid salts (26, 40, 42), and even by

infusion of mineral and organic acids (26, 42 to 44), have produced most undramatic results.

The influence of acidosis upon respiratory output of CO_2 appears therefore to depend upon the type of acid-base change involved. It is well recognized that during the development of or recovery from diabetic acidosis considerable depletion of serum bicarbonate may be unassociated with overventilation (45, 46, 47). Ketone acids may well act like ingested ammonium chloride. They enter the blood stream from the liver (48) and liberate CO_2 from the bicarbonate of the extracellular fluid. But intracellular stores of CO_2 are preserved because bicarbonate cannot leave the cells and ketone acids that enter convert little intracellular bicarbonate to CO_2, because they are so rapidly oxidized (49). Only when acidosis progresses and overventilation supervenes, will the bicarbonate of cells be depleted. An entirely different situation exists when lactic acid is produced intracellularly. Cell bicarbonate is converted to free CO_2 which diffuses out into the extracellular fluids. Moreover, the lactic acid itself diffuses out of the cells to liberate more CO_2 extracellularly and the gas can only escape through the lungs. Because of the preponderant contribution by the tissues, large increments in the respiratory production of CO_2 may be observed, with only relatively small changes in the CO_2 content of serum (50).

By virtue of their different distribution and diffusibility, the determinants of the acid-base system achieve a considerable degree of independence. Changes in the CO_2 content of the blood can be correlated with respiratory carbon-dioxide production and with the acid-base balance of the organism as a whole only if the individual variation of each dimension of the system is taken into account.

Because of the uncertain significance of respiratory quotients determined during alteration in acid-base equilibrium, the possibility of calculating metabolic CO_2 production from total CO_2 output, by correcting for the effects of acid-base change, should be considered. Since alterations of CO_2 tension are manifested throughout the body fluid, they will usually have a greater influence upon the respiratory output of CO_2 than will variations in bicarbonate which are limited to the extracellular compartment. Non-metabolic CO_2 production due to change of bicarbonate ion may be readily calculated as the product of change in serum bicarbonate concentration by the extracellular fluid volume. But estimation of non-metabolic CO_2 related to change in CO_2 tension is subject to several important limitations. Determination of the CO_2 tension of the blood, either by analysis of alveolar air or by calculation from concentrations of bicarbonate and hydrogen ions in serum, involves a minimum possible error of 1.0 mm. of mercury. It can be shown that this error alone would make calculation of metabolic CO_2 production over short periods quite unreliable. Moreover, this calculation requires a knowledge of the CO_2 absorption curve of the tissues, which has not been determined for man, and can only be approximated from the absorption curve for cat muscle determined by Irving, Foster and Ferguson (29). Finally, since the CO_2 tension of the tissues may well fail to attain equilibrium with that of the blood during rapid fluctuations in acid-base equilibrium, estimation of alterations in the carbon dioxide content of the tissues is rendered even more hazardous. Consequently, quantitative correction of overall respiratory CO_2 output for non-metabolic CO_2 does not seem feasible at present.

SUMMARY AND CONCLUSIONS

The influence of changes in acid-base equilibrium upon the output of carbon dioxide by the lungs was studied in human subjects.

Overventilation produced large increments in respiratory output of CO_2. A portion of the CO_2 was given up by the tissues.

Ventilation with a CO_2-enriched air caused a marked diminution in the volume of CO_2 given out by the lungs. Part of the CO_2 retention was intracellular.

Alterations of the CO_2 content of blood, produced by ingestion of ammonium chloride, may be unassociated with any significant change in the output of carbon dioxide by the lungs.

The volume of distribution of bicarbonate ion administered intravenously as sodium bicarbonate was found to approximate the extracellular fluid volume as determined by the thiocyanate method.

The observations indicate that the tissue cells of man are freely permeable to dissolved molecular CO_2, but are impermeable to bicarbonate ion.

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