

# THE INFLUENCE OF ALTERATIONS IN ACID-BASE BALANCE UPON TRANSFERS OF CARBON DIOXIDE AND BICARBONATE IN MAN<sup>1</sup>

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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<sub>2</sub> stored within the body, which occurs during alkalosis or acidosis, may produce alterations in respiratory CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>-rich mixtures, and correlated the CO<sub>2</sub> exchange of the whole animal with variations in blood CO<sub>2</sub> content. From such observations, the amount of CO<sub>2</sub> absorbed by the tissues could be estimated. Irving and his co-workers (9, 10) made direct determinations of the CO<sub>2</sub> content of various tissues of dogs and cats overventilated with air and with CO<sub>2</sub> enriched mixtures. They attempted to account for the net CO<sub>2</sub> exchange of the whole organism in terms of altered CO<sub>2</sub> content of blood, muscle, bone, and viscera. Applications of Shaw's technique in an effort to determine the CO<sub>2</sub> capacity of the human body has been reported by Adolph, Nance, and Shilling (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<sub>2</sub>-rich mixtures, procedures which primarily altered the concentration of dissolved CO<sub>2</sub> in the body (14). No comparable studies have been reported concerning the influence of primary alteration in bicarbonate ion concentration upon respiratory CO<sub>2</sub> production.

The present study deals with the effects of acidosis and alkalosis upon respiratory CO<sub>2</sub> output in the post-absorptive state, when the oxidative metabolic mixture is relatively constant. Concentration of dissolved CO<sub>2</sub> 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<sub>2</sub> was found to be strikingly altered during primary change of the concentration of dissolved CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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  $\text{CO}_2$ -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 re-breathing.

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  $\text{NH}_4\text{Cl}$  in 0.5 gram enteric coated tablets. Blood samples for serum  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{SCN}$  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  $\text{CO}_2$  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  $\text{CO}_2$  output produced by altered acid-base equilibrium was calculated as follows:

1. The respiratory quotient of the basal period was calculated from the  $\text{CO}_2$  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"  $\text{CO}_2$  production during

acid-base change. The metabolic  $\text{CO}_2$  production was then subtracted from the total  $\text{CO}_2$  output during the period of acid-base change to give the non-metabolic  $\text{CO}_2$  production. The value for non-metabolic  $\text{CO}_2$  thus obtained represented the net increase or decrease of  $\text{CO}_2$  in the body as a whole, since urinary excretion during the brief periods of over-ventilation or rebreathing was negligible, and the  $\text{CO}_2$  content of the urine following ingestion of ammonium chloride proved to be insignificant.

Change in the amount of  $\text{CO}_2$  contained within the extracellular fluids was calculated by multiplying change in the concentration of total  $\text{CO}_2$  in the serum by the extracellular fluid volume. In all of the experiments, except those dealing with the effects of  $\text{NH}_4\text{Cl}$  ingestion, blood for estimation of serum  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  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:

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

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,

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

$\Delta\text{Cl}$  is the amount of chloride in milliequivalents retained during the experiment,

and  $[\text{Cl}]_2$  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  $\text{SCN}$  by analogous formulae, substituting  $[\text{Na}]$  or  $[\text{SCN}]$  for  $[\text{Cl}]$ .

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

where  $\Delta ECF$  is the change in liters in the volume of extracellular fluid during the experiment.

$$V_{\text{HCO}_2} = \frac{\Delta\text{CO}_2 - \Delta ECF[\text{CO}_2]_2}{[\text{CO}_2]_2 - [\text{CO}_2]_1}, \quad (3)$$

where  $V_{\text{HCO}_2}$  is the volume of distribution of administered bicarbonate,  $\Delta\text{CO}_2$  is the  $\text{CO}_2$  balance in millimols (total  $\text{CO}_2$  given as bicarbonate less total  $\text{CO}_2$  excreted in the urine),  $[\text{CO}_2]_2$  is the serum  $\text{CO}_2$  content in millimols per liter at the end of the experiment and  $[\text{CO}_2]_1$  is the serum

CO<sub>2</sub> 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 over-ventilation 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<sub>2</sub> 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<sub>2</sub> content was very small in the first three experiments; consequently, the cells were credited with a large contribution toward total expired CO<sub>2</sub>. 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<sub>2</sub> content. Since to

TABLE I  
*Exchanges of CO<sub>2</sub> during change of acid-base balance*

Experiment	Duration	Change of serum CO <sub>2</sub> content	Oxygen consumption	CO <sub>2</sub> production	Basal R. Q.	Metabolic CO <sub>2</sub> output	Non-metabolic CO <sub>2</sub> balance		
							Total	of ECF	of cells
number	minutes	volumes per cent	cc.	cc.		cc.	cc.	cc.	
A. LOSS OF CO <sub>2</sub> 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 <sub>2</sub> 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 <sub>2</sub> FOLLOWING INGESTION OF NH <sub>4</sub> CL									
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.

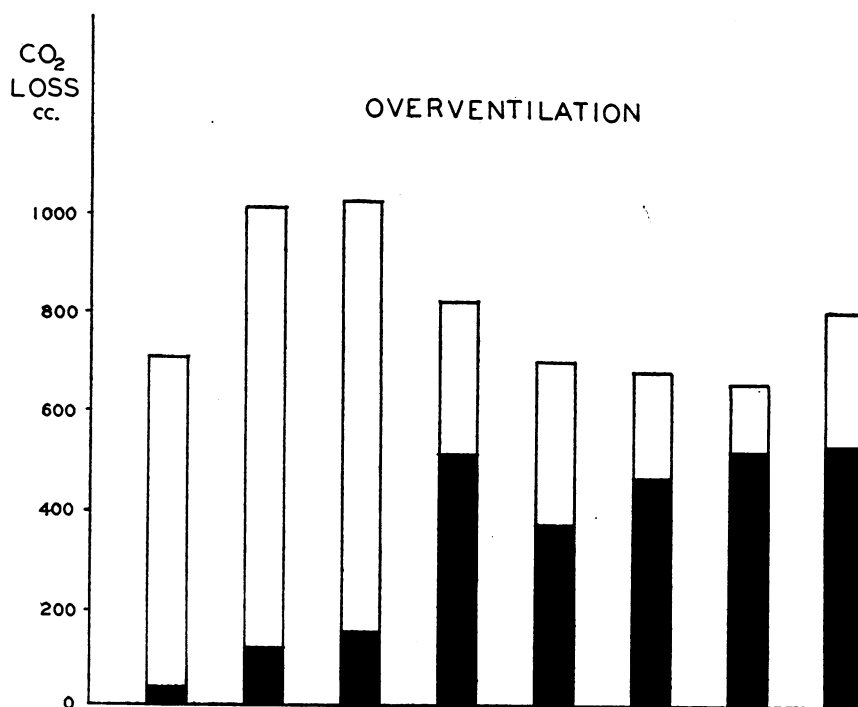


FIG. 1. LOSS OF CO<sub>2</sub> DURING OVERVENTILATION

The total height of each column represents the total loss of nonmetabolic CO<sub>2</sub>. 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<sub>2</sub> content increased and a more reasonable estimate of intra- and extracellular losses of CO<sub>2</sub> 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<sub>2</sub>-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<sub>2</sub> 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<sub>2</sub> excess produces local vasodilatation (20) which may completely mask, in peripheral venous blood, the rise of CO<sub>2</sub> 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<sub>2</sub> concentration was observed and a more reliable estimate of extracellular retention of CO<sub>2</sub> 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<sub>2</sub> content in the first 2 hours after ingestion of NH<sub>4</sub>Cl, with a general tendency for a fall to occur during this period. The sporadic increases of serum CO<sub>2</sub> 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  $\text{CO}_2$  content could therefore be attributed with confidence to primary alteration in bicarbonate ion concentration.

In all but 2 experimental periods, in which serum  $\text{CO}_2$  was practically constant, the alteration in serum  $\text{CO}_2$  content indicated that the extracellular fluids had gained or lost quantities of carbon dioxide, much in excess of any change in the  $\text{CO}_2$  content of the whole organism as measured by the respiratory output. Indeed, some of the observations indicated that a considerable loss of  $\text{CO}_2$  from the extracellular fluid could occur while the respiratory output actually declined. There was, therefore, not even a directional correlation between the carbon diox-

ide balance of the extracellular fluids and of the subject as a whole under the conditions of these observations. None of the discrepancies in  $\text{CO}_2$  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  $\text{CO}_2$  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.

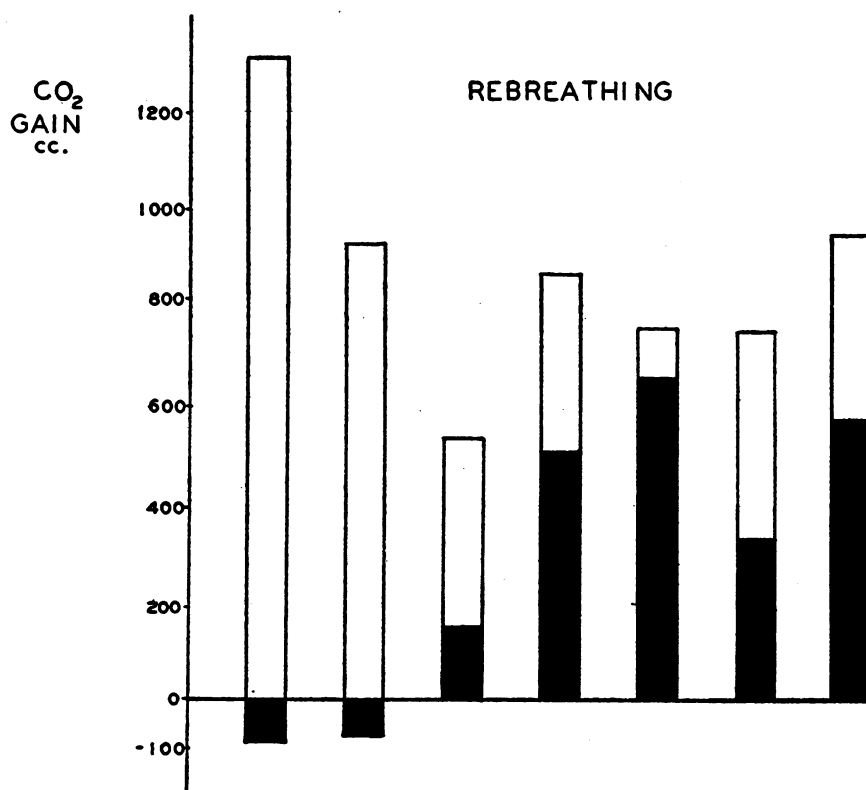


FIG. 2. RETENTION OF  $\text{CO}_2$  DURING REBREATHING

Total retention of  $\text{CO}_2$  is represented by the total height of each column. The solid portion represents extracellular retention, the unshaded portion, intracellular retention.

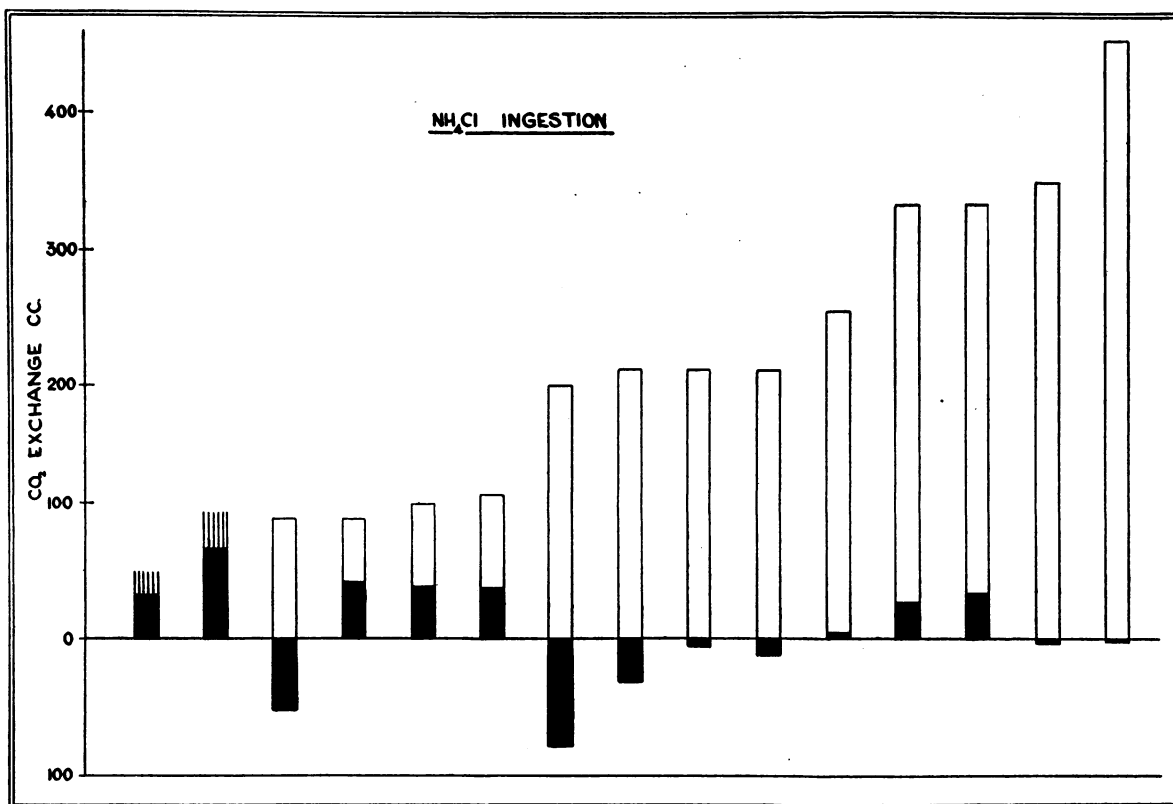


FIG. 3. EXCHANGES OF CO<sub>2</sub> FOLLOWING INGESTION OF NH<sub>4</sub>Cl

The CO<sub>2</sub> balance of the extracellular fluids (whether positive or negative) is represented by the total height of each column. The corresponding CO<sub>2</sub> 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<sub>2</sub> 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  $\Delta \text{Cl}$  in formula (1), increase the value of  $\text{ECF}_2$ . This in turn will increase the value of  $\Delta \text{ECF}$  (Formula (2)). Too high a value for  $\Delta \text{ECF}$  will give too low a value for  $V_{\text{HCO}_3}$  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  $\text{HCO}_3^-$

Experiment number	Subject	Duration minutes	$\text{HCO}_3^-$ retained m. eq.	Change of serum concentration of				Change of ECF volume in liters calculated from <sup>1</sup>			Volume of distribution of added $\text{HCO}_3^-$ in liters calculated from <sup>2</sup>			Final extracellular fluid volume liters
				$\text{CO}_2$ m. eq. per liter	SCN mgm. per cent	Cl m. eq. per liter	Na m. eq. per liter	SCN	Cl	Na	SCN	Cl	Na	
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

<sup>1</sup> By formula (1) of text.<sup>2</sup> 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  $\Delta \text{ECF}$  would produce an error of 2 liters in  $V_{\text{HCO}_3^-}$ .

(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

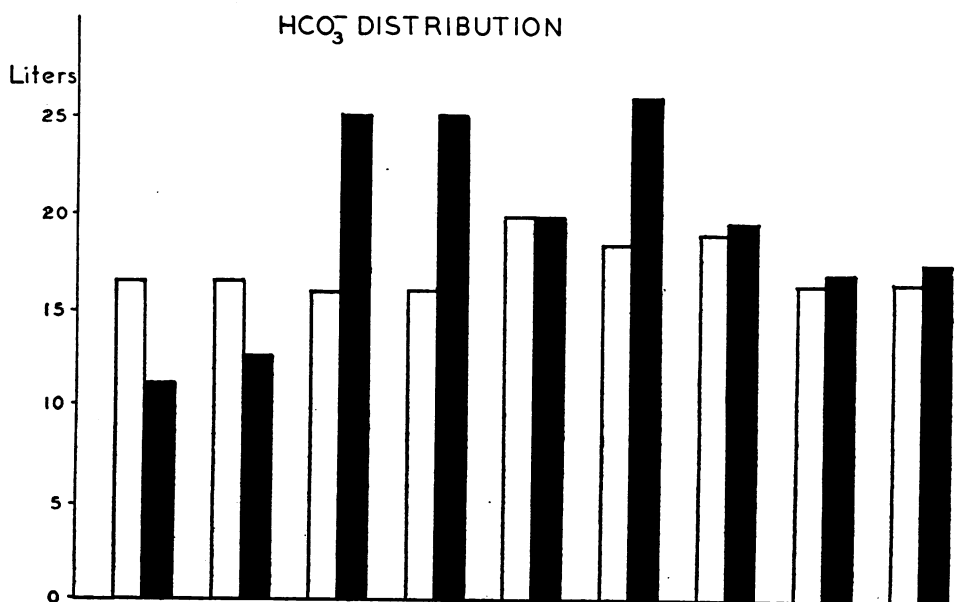


FIG. 4. APPARENT VOLUME OF DISTRIBUTION OF INTRAVENOUS BICARBONATE

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  $\text{CO}_2$  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  $\text{CO}_2$  content, unrelated to bicarbonate administration.

Since the respiratory output of  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  balance ( $\Delta \text{CO}_2$ , formula (3)), which was taken as the difference between  $\text{CO}_2$  administered intravenously as bicarbonate, and total  $\text{CO}_2$  excreted in the urine. Retention of  $\text{CO}_2$ , 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  $\text{CO}_2$  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  $\text{CO}_2$  tension of the blood (14), the presumption is strong that carbon dioxide can enter or leave the tissue cells in the form of dissolved  $\text{CO}_2$ . Direct demonstration of cellular permeability to dissolved  $\text{CO}_2$  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  $\text{CO}_2$  implies that, under equilibrium conditions,  $\text{CO}_2$  tension in the extracellular fluids and in the cell water shall be equal. If  $\text{CO}_2$  tension is lowered in the blood and extracellular fluids by overbreathing, the tissues should give up enough  $\text{CO}_2$  to lower the tension in cell water to the same degree. Under these circumstances the amount of  $\text{CO}_2$  lost from the *ECF* and cell water will be proportional to the respective volumes of these fluid compartments, except insofar as their  $\text{CO}_2$  absorption curves may differ. Irving, Foster and Ferguson (29) have shown that the  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$ , 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  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  output was not materially altered despite significant losses or gains of  $\text{CO}_2$  by the blood and extracellular fluids. Since urinary excretion was negligible,  $\text{CO}_2$  leaving the extracellular fluids must have entered the tissue cells. The apparent paradox of an increase in cellular total  $\text{CO}_2$  content during fall in  $\text{CO}_2$  content of the extracellular fluids is resolved when concentrations of bicarbonate ion and dissolved  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  tension, however, results in transfer of dissolved  $\text{CO}_2$  from extracellular fluids to the tissues. Consequently, the total  $\text{CO}_2$  content of the blood falls, that of the tissues rises, and, by a sort of internal compensation, little or no  $\text{CO}_2$  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  $\text{CO}_2$  tension,  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  from the bicarbonate of the extracellular fluid. But intracellular stores of  $\text{CO}_2$  are preserved because bicarbonate cannot leave the cells and ketone acids that enter convert little intracellular bicarbonate to  $\text{CO}_2$ , 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  $\text{CO}_2$  which diffuses out into the extracellular fluids. Moreover, the lactic acid itself diffuses out of the cells to liberate more  $\text{CO}_2$  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  $\text{CO}_2$  may be observed, with only relatively small changes in the  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  production from total  $\text{CO}_2$  output, by correcting for the effects of acid-base change, should be considered. Since alterations of  $\text{CO}_2$  tension are manifested throughout the body fluid, they will usually have a greater influence upon the respiratory output of  $\text{CO}_2$  than will variations in bicarbonate which are

limited to the extracellular compartment. Non-metabolic  $\text{CO}_2$  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  $\text{CO}_2$  related to change in  $\text{CO}_2$  tension is subject to several important limitations. Determination of the  $\text{CO}_2$  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  $\text{CO}_2$  production over short periods quite unreliable. Moreover, this calculation requires a knowledge of the  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  output for non-metabolic  $\text{CO}_2$  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  $\text{CO}_2$ . A portion of the  $\text{CO}_2$  was given up by the tissues.

Ventilation with a  $\text{CO}_2$ -enriched air caused a marked diminution in the volume of  $\text{CO}_2$  given out by the lungs. Part of the  $\text{CO}_2$  retention was intracellular.

Alterations of the  $\text{CO}_2$  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  $\text{CO}_2$ , but are impermeable to bicarbonate ion.

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