TISSUE AND RENAL RESPONSE TO CHRONIC RESPIRATORY ACIDOSIS *

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The elevation of pCO_2 which occurs during respiratory acidosis is accompanied by a rise in the concentration of bicarbonate and a fall in the concentration of chloride in serum (1). The increased bicarbonate concentration results from both cellular and renal responses. The cellular response is characterized by the exchange of extracellular hydrogen ions for intracellular cations, thereby contributing to the elevation of the extracellular concentration of bicarbonate (2, 3).

The renal factors responsible for the elevated bicarbonate concentration may be divided into two phases: *1*) acid excretion, and *2*) bicarbonate reabsorption. In acute human experiments, augmented acid excretion, as evidenced by increased excretion of ammonia and titratable acid, has been reported (4). Barker, Singer, Elkinton and Clark observed these changes also, but noted that if the urine was acid prior to breathing carbon dioxide, further increase in acid excretion may not occur (5). Perhaps this explains, in part at least, the failure of Longson and Mills to find increased acid excretion during short term human experiments (6).

In acute respiratory acidosis tubular reabsorption of bicarbonate is increased (7). Moreover, in chronic respiratory acidosis in dogs, there is a further augmentation of reabsorption of bicarbonate that varies directly with the duration of the imposed acidosis (8).

The fall in the concentration of serum chloride during respiratory acidosis may also result from both cellular and renal responses. The only cell into which chloride has been clearly demonstrated to enter during respiratory acidosis is the red cell (1, 3, 9). The role of renal losses in the production of hypochloremia is less certain. In short term human experiments, chloruresis has not been observed during respiratory acidosis (5, 10). However, in the rat, Epstein, Branscome and Levitin observed increased chloride excretion during the first 24 hours of an imposed respiratory acidosis (11).

The present study in the rat was undertaken in an attempt to delineate the pattern of renal and extrarenal responses to respiratory acidosis. The excretion of sodium, potassium and chloride was studied during the early and late phases. The contribution of acid loss, as ammonia and titratable acid, to the elevation of plasma bicarbonate concentration was examined. The activities of the renal enzymes concerned with acid excretion, glutaminase and carbonic anhydrase, were determined in order to ascertain whether adaptation of these enzymes occurs as a result of chronic respiratory acidosis. Finally, possible extrarenal contribution to the buffering of carbon dioxide was examined in both muscle and bone.

PROCEDURE

Male Sprague-Dawley rats weighing between 300 and 400 Gm. were used in all experiments. In early experiments, the rats were tube-fed a liquid diet twice daily that provided a total of 2.0 Gm. of protein, 1.5 Gm. of fat and 2.0 Gm. of glucose per day together with essential minerals and vitamins. Later experiments used the same diet; however, the diet was offered in a standard drinking fountain, a measured amount of diet being offered twice daily. Particular care was taken to ascertain that each rat took the entire amount of diet offered. The intake of equal amounts of diet by both control and experimental animals is essential, for, as has been previously pointed out, variations in dietary intake result in altered excretion of acid by the kidneys (12). With respect to sodium, potassium and chloride, two different diets were used. The standard diet (Std. Diet) provided 2.0 mEq. of sodium, 1.0 mEq. of potassium and 3.0 mEq. of chloride per day. The electrolyte deficient diet (E.D.

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Diet) contained only negligible amounts of these ions. All rats were given distilled water *ad libitum*.

A large box was constructed for use as a metabolic cage for those animals subjected to respiratory acidosis. The design was such that daily urines could be collected from individual rats. The proper content of carbon dioxide within the box was maintained by automatic electrical controls which intermittently released carbon dioxide into the box. By this method, the carbon dioxide content of the box could consistently be maintained within ± 1.0 per cent of the desired value. Oxygen content of the atmosphere in the box was frequently checked with an Orsat gas analysis apparatus, and was found to range from 19 to 21 per cent. It was not necessary to remove rats from the box during an experiment; feeding and cage cleaning were carried out through airtight arm ports. By means of air-conditioning, both control and experimental animals were kept in an atmosphere maintained nearly constant at 73° F.

Twenty-four hour urines were collected from individual rats under oil and preserved with phenylmercuric nitrate and toluene. Approximately midway through this study, changes in the urine collection system were made that resulted in different values for urinary constituents. The change was the application of a silicone coating to the surface of all glassware used in the collection system. This gave rise to an immediate flow of urine from the rat into the collection vessels and made it unnecessary to rinse the collection system at the end of each collection period, since there was no significant evaporation of urine on the siliconized surfaces. Using the new urine collection system, it was found that apparent ammonia excretion was approximately 1.66 times greater than that found using the old urine collection system. An additional control study, using the new urine collection system, was made in order to facilitate comparison with experimental groups. The study included eight groups of rats. Groups 1 through 3 were those in which the original urine collection method was used (Table II). The new urine collection method was used in the remaining five groups (Table II).

All groups were observed for a period of 11 days. Group 1 served as a control for Groups 2 and 3. In these three groups, all rats received the Std. Diet, and daily urines were collected for determination of pH, ammonia and titratable acidity. Group 2 was subjected to an atmosphere of 10 per cent carbon dioxide. Group 3 was subjected to 15 per cent carbon dioxide.

Group 4 served as a control for Groups 5 and 6. These three groups received the Std. Diet, and as in previous groups daily urines were collected for the determination of pH and acid excretion. In addition, sodium, potassium and chloride were determined in the urines from Groups 4 and 5. Group 5 was subjected to 10 per cent carbon dioxide. In an attempt to produce a marked additive effect on acid excretion, Group 6 was subjected to 10 per cent carbon dioxide for two days, then 15 per cent for two days, these cyclic changes being maintained throughout the experiment. These rats were sacrificed while breathing 10 per cent carbon dioxide. Groups 7 and 8 were a control group and a group subjected to 10 per cent carbon dioxide, respectively. In an attempt to magnify changes in plasma and muscle electrolytes, as well as in urinary excretion of electrolytes, these two groups received the E.D. Diet. Urines were collected and analyzed as in Groups 4 and 5.

METHODS

Rats subjected to carbon dioxide were killed while breathing an atmosphere having the same carbon dioxide content as was used for the experiment. This was accomplished by supplying an appropriate oxygen-carbon dioxide mixture to a small box into which the anesthetized rat's head was placed by way of a snugly fitting neck port. Both control and experimental animals were anesthetized by an intraperitoneal injection of pentobarbital. Blood was received anaerobically into a previously heparinized syringe from the abdominal aorta. Muscle samples were removed from the hind legs. In some animals, the bones of the hind legs were taken for electrolyte analysis. The kidneys were perfused with chilled isotonic saline in order to wash out all red blood cells, then removed and immediately frozen for enzyme assays.

Methods for the determination of plasma electrolytes, urine ammonia, pH and titratable acidity, muscle electrolytes and kidney glutaminase activity were those previously described from this laboratory (12, 13). Urine sodium and potassium concentrations were determined by flame photometry (14). Urine chloride concentration was determined by a modification of the Volhard-Harvey method (15). Whole blood pH was anaerobically determined at 37° C. by means of a Cambridge water-jacketed glass electrode and a Cambridge pH meter. Plasma carbon dioxide tension was estimated by means of the Henderson-Hasselbalch equation using the blood pH, plasma carbon dioxide content and a pK of 6.11. Buffer anion was estimated by means of a Singer-Hastings nomogram assuming a red cell hematocrit of 50 per cent in each case (16).

Electrolyte composition of marrow-free bone was determined in Groups 4 and 5. The method of analysis was a modification of the ion-exchange column method of Forbes and D'Ambruso (17).

Renal carbonic anhydrase activity was determined in water homogenates of kidney tissue. The method described by Ashby and Chan (18) was modified in the following way. A veronal-sodium veronal buffer (0.03 M) as suggested by Roughton and Booth (19) was substituted for the carbonate-bicarbonate buffer of the original method because the former provides a better pH range for the enzymatic reaction and does not adversely affect the enzyme. Since the reaction was carried out from pH 7.9 to pH 6.3, water soluble brom-thymol-blue was used as the indicator in a final concentration of 1.57 mg. per 100 ml. This concentration was below that demonstrated by Wilbur and Anderson to cause inhibition of the enzyme (20). The reaction was carried out at 3° C. (± 0.2° C.). The exact time for the reaction $CO_2 + H_2O$

TABLE I

Plasma electrolyte concentrations

Group	Procedure	Na	K	Cl	CO_2 content§	Whole blood pH§	pCO_2§	Buffer anion
		mEq./L.	*mEq./L.*	*mEq./L.*	*mEq./L.*		*mm. Hg*	*mEq./L.*
1 and 4*	Std. Diet only	†144.0 (10)‡ ±2.2	5.0 (10) ±.44	108.8 (10) ±2.8	24.7 (10) ±1.8	7.43 (5) ±.02	35.4 (5) ±2.6	48 (5) ±1.4
2 and 5*	Std. Diet + 10% CO_2	151.4 (10) ±3.9	4.73 (10) ±.46	91.8 (10) ±3.0	45.2 (10) ±2.3	7.28 (9) ±.04	102.3 (9) ±6.6	58 (9) ±4.0
3	Std. Diet + 15% CO_2	149.3 (4) ±3.1	4.31 (5) ±.25	85.3 (4) ±4.5	52.4 (4) ±3.2	7.15 (5) ±.07	147.7 (5) ±29.3	60 (5) ±1.3
6	Std. Diet + 10 and 15% CO_2	150.9 (4) ±4.0	3.60 (4) ±.34	93.5 (4) ±3.9	42.3 (4) ±2.9	7.19 (4) ±.01	108.3 (4) ±9.2	55 (4) ±2.2
7	E.D. Diet only	145.6 (5) ±1.8	4.81 (5) ±.42	104.6 (5) ±1.4	22.2 (5) ±1.9	7.39 (5) ±.01	36.7 (5) ±1.8	46 (5) ±1.4
8	E.D. Diet + 10% CO_2	156.4 (9) ±3.1	2.85 (9) ±.47	89.1 (9) ±2.4	46.2 (9) ±1.0	7.24 (9) ±.04	102.5 (9) ±2.7	58 (9) ±2.4

* Groups 1 and 4 both received the same Std. Diet. Methods for urine collection differed in the two groups. The electrolyte composition in blood was identical in both groups, and these data were pooled. Since the same circumstances existed for Groups 2 and 5, their data were also pooled.

† Mean and standard deviations.

‡ Number in parentheses, number of animals used.

§ The mean and standard deviation for pCO_2 was *not* estimated from the *mean* values for CO_2 content and pH. Instead, the pCO_2 was estimated for *each* rat from the CO_2 content and pH; then the mean and standard deviations were determined. As a consequence, there is an apparent disparity between the mean values for CO_2 content and pH on the one hand and pCO_2 on the other as these values appear in this table.

$= H_2CO_3$ to proceed sufficiently far to the right to lower the pH of the buffer to 6.3 without added enzyme was the control time. The amount of enzyme (kidney homogenate) necessary exactly to halve this time was defined as a unit of enzyme activity.

Throughout, results are reported as mean values for respective groups together with the standard deviation of the mean. P values were obtained from appropriate tables after the t value was calculated.

RESULTS

Rats used in these experiments were able to withstand an atmosphere of 10 per cent carbon dioxide for 11 days without obvious untoward effect. Experiments lasting 14 days gave rise to a mortality of approximately 5 per cent. An atmosphere of 15 per cent carbon dioxide resulted in a mortality of approximately 40 per cent within 11 days. A mortality of 100 per cent occurred within 30 hours when rats were subjected to 20 per cent carbon dioxide.

Degree of acidosis

Rats subjected to 10 or 15 per cent carbon dioxide atmosphere developed severe respiratory acidosis as evidenced by increased plasma CO_2 content, decreased blood pH and increased plasma pCO_2 (Table I). Fifteen per cent carbon dioxide gave rise to a more profound acidosis than did 10 per cent carbon dioxide in that the mean blood pH for Group 3 was significantly lower ($p < 0.01$) than those values found in Groups 2 and 5.

The somewhat higher CO_2 content found for Group 3 was, however, of only doubtful significance ($p > 0.02$) when compared with the CO_2 content of Groups 2 and 5. Although the mean blood pH of Group 6 (10 and 15 per cent carbon dioxide) was significantly lower ($p < 0.001$) than that value for Groups 2 and 5, the CO_2 content and pCO_2 were not significantly different. These data suggest that in both Group 3 and Group 6 the acidosis was more severe and the compensation less complete than in Groups 2 and 5.

There was no significant difference found in the degree of acidosis produced with 10 per cent carbon dioxide between groups that received the Std. Diet (Groups 2 and 5) and the E.D. Diet (Group 8) (Table I).

As was expected, respiratory acidosis produced

an elevation of whole blood buffer anion (16). Since the value found for 10 per cent carbon dioxide was not significantly different from that found for 15 per cent carbon dioxide (Table I), it would appear that under these experimental conditions, buffer anion concentration displayed a maximal concentration not entirely proportionately related to the degree of hypercapnea.

Plasma sodium, potassium and chloride

In all groups subjected to respiratory acidosis, the plasma sodium concentration was significantly increased above control values (Table I). Although acute respiratory acidosis has been demonstrated to accelerate the excretion of water (10), no increased urine volumes were observed in rats during chronic respiratory acidosis. The hypernatremia may have been the consequence of the inability of rats who are breathing rapidly to increase the intake of water sufficiently to replenish deficits resulting from enhanced respiratory water loss.

Plasma potassium concentrations were, in all groups with respiratory acidosis, significantly lower than control values (Table I). The only abnormally low potassium concentrations occurred in those rats receiving the potassium deficient E.D. Diet (Group 8). There was no definite relationship between plasma pCO_2 and potassium concentration (Table I). Potassium concentration and blood pH likewise had no definite relationship (Table I).

In all our experimental groups, the expected hypochloremia of respiratory acidosis was observed (Table I). The decrement in chloride concentration was, for the most part, only slightly less than the increment in carbon dioxide content of the plasma.

Acid excretion

Table II summarizes the cumulative acid excretion for experimental and control groups during the 11 days of observation. The actual differences between rats subjected to respiratory acidosis and control animals were small, although significant for the most part. Ammonia excretion was sig-

TABLE II

Cumulative acid excretion during respiratory acidosis for 11 days

Group (No. of rats)	Procedure	NH_3*	TA*	NH_3 + TA*
		mEq.	*mEq.*	*mEq.*
Group 1† (5)	Std. Diet only	14.0 ± 0.8	3.87 ± 0.52	17.71 ± 0.9
Group 2† (10)	Std. Diet + 10% CO_2	18.0 ± 2.4 p = < 0.001	4.05 ± 0.38 p = > 0.5	22.1 ± 2.9 p = 0.01
Group 3† (5)	Std. Diet + 15% CO_2	20.4 ± 2.2 p = < 0.001	4.09 ± 0.69 p = > 0.5	24.1 ± 2.8 p = < 0.01
Group 4‡ (5)	Std. Diet only	24.6 ± 0.9	3.26 ± 1.09	27.7 ± 1.6
Group 5‡ (4)	Std. Diet + 10% CO_2	26.2 ± 0.5 p = < 0.02 > 0.01	5.98 ± 1.04 p = 0.01	31.5 ± 0.8 p = < 0.01
Group 6‡ (5)	Std. Diet + 10 and 15% CO_2	28.9 ± 3.4 p = < 0.05 > 0.02	5.77 ± 0.84 p = < 0.01	32.9 ± 6.8 p = < 0.20 > 0.10
Group 7‡ (6)	E.D. Diet only	23.5 ± 1.0	4.59 ± 0.83	28.1 ± 1.7
Group 8‡ (10)	E.D. Diet + 10% CO_2	25.6 ± 2.5 p = < 0.001	4.40 ± 0.84	29.7 ± 3.0 p = < 0.20

* Cumulative excretion is expressed as mean and standard deviations. Statistical expressions compare experimental groups with their respective controls.

† Groups 1, 2 and 3, original urine collection method.

‡ Groups 4 through 8, later urine collection method.

nificantly increased in most of the groups subjected to either 10 or 15 per cent CO_2 (Groups 2, 3, 5 and 8), although in one instance the significance of the increase was borderline (Group 6). The cumulative excretion of titratable acid (TA) was increased in two groups (Groups 5 and 6). In all experimental groups excepting Group 8, the cumulative excretion of acid (NH_3 plus TA) rose significantly. The daily excretion of total acid

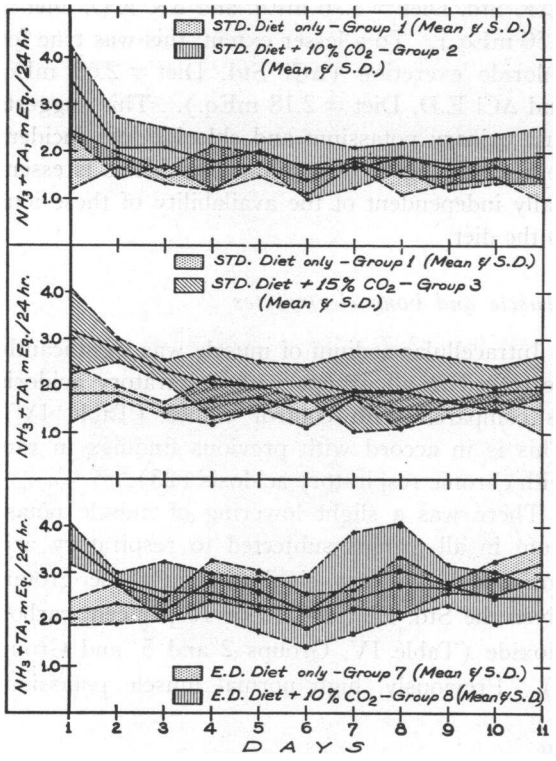

FIG. 1. DAILY TOTAL ACID EXCRETION (NH_3 PLUS TA) DURING RESPIRATORY ACIDOSIS

for three representative groups (2, 3, 8) of rats subjected to respiratory acidosis is plotted in Figure 1. There was a tendency for the highest acid excretion to occur during the first two days of respiratory acidosis, after which acid excretion tended to fall to a level only slightly greater than that observed in control animals.

To examine the daily pattern of urine pH, NH_3 and titratable acid during respiratory acidosis, data from rats subjected to 10 per cent CO_2 are compared with their controls in Figure 2. All other groups behaved in similar fashion. The early rise in total acid excretion was the result of a rise in

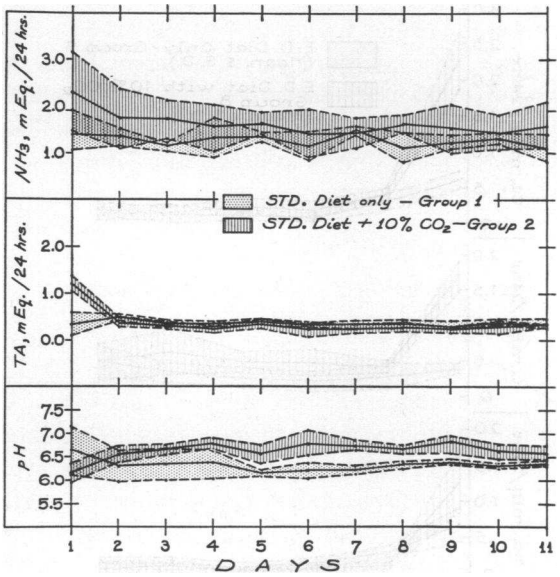

FIG. 2. MEAN DAILY URINARY ACID EXCRETION DURING RESPIRATORY ACIDOSIS

ammonia and titratable acid excretion. Ammonia excretion tended to remain slightly above control values throughout the study. Early in respiratory acidosis, urine pH tended to be lower than in the control groups but rose to control values or above, more or less paralleling the fall in ammonia excretion, as the study was continued.

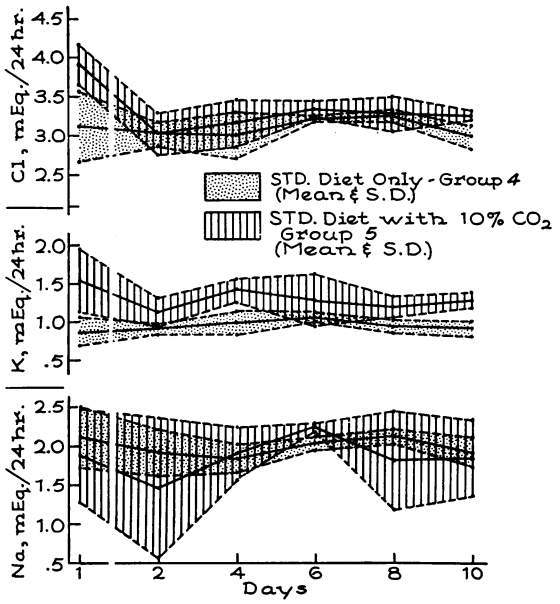

FIG. 3. MEAN DAILY URINARY EXCRETION OF NA, K AND CL DURING RESPIRATORY ACIDOSIS

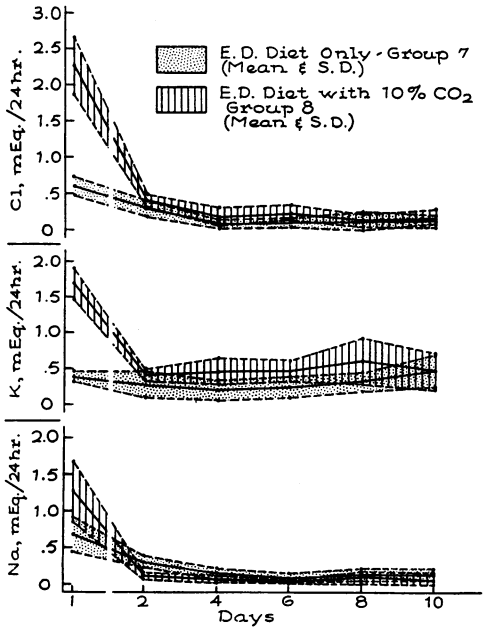

FIG. 4. MEAN DAILY URINARY EXCRETION OF NA, K AND CL DURING RESPIRATORY ACIDOSIS

Sodium, potassium and chloride excretion

The mean daily urinary excretion of sodium, potassium and chloride was compared in rats subjected to 10 per cent carbon dioxide while on Std. Diet (Figure 3) and while on E.D. Diet (Figure 4) with their respective controls. Sodium excretion was not significantly changed by respiratory acidosis. Both potassium and chloride excretion were significantly increased during the first day. Thereafter, chloride excretion fell to control values while potassium excretion remained slightly elevated throughout the study. Similar findings have been obtained by others (11). In Table III, the total cumulative mean excretions of sodium, potassium and chloride are given for the rats during respiratory acidosis on Std. and E.D. Diet. The excess potassium excreted in the groups with respiratory acidosis was strikingly similar for both dietary regimens (ΔK Std. Diet = 3.76 mEq. and ΔK E.D. Diet = 3.50 mEq.). To a lesser extent, this was true for chloride excretion (ΔCl Std. Diet = 2.65 mEq. and ΔCl E.D. Diet = 2.18 mEq.). This suggests that urinary potassium and chloride loss incident to a given degree of respiratory acidosis is essentially independent of the availability of these ions in the diet.

Muscle and bone electrolytes

Intracellular sodium of muscle was significantly decreased in all groups with respiratory acidosis as compared with control values (Table IV). This is in accord with previous findings in rats with chronic respiratory acidosis (23).

There was a slight lowering of muscle potassium in all groups subjected to respiratory acidosis, and this was significant in those groups given the Std. Diet and 10 or 15 per cent carbon dioxide (Table IV, Groups 2 and 5, and Group 3). Previously, high normal muscle potassium

TABLE III

Total cumulative mean excretion of Na, K and Cl

	Procedure	Sodium	Potassium	Chloride
		mEq.	*mEq.*	*mEq.*
Group 4	Std. Diet only (5)*	21.61† ±1.21	10.63 ±0.92	34.28 ±1.59
Group 5	Std. Diet + 10% CO_2 (4)	21.73 ±0.92	14.39 ±0.92 p = > 0.01 < 0.001	36.93 ±0.22 p = > 0.01 < 0.001
Group 7	E.D. Diet only (6)	2.35 ±0.51	3.27 ±1.38	2.28 ±0.27
Group 8	E.D. Diet + 10% CO_2 (5)	2.07 ±0.42 p = > 0.50 < 0.30	6.77 ±1.30 p = > 0.01 < 0.001	4.46 ±0.60 p = > 0.001

* Number in parentheses, number of animals used.
† Mean and standard deviation.

TABLE IV

Muscle and bone electrolytes

Group		1 and 4 (5)*	2 and 5 (5)	3 (5)	6 (5)	7 (5)	8 (5)
Procedure		Std. Diet only	Std. Diet + 10% CO_2	Std. Diet + 15% CO_2	Std. Diet + 10 and 15% CO_2	E.D. Diet only	E.D. Diet + 10% CO_2
Muscle†	Na_i‡	4.45§ ±0.43	3.31 ±0.53	3.16 ±0.78	2.82 ±0.45	4.26 ±0.42	2.86 ±0.54
	K	45.1 ±1.3	42.0 ±1.6	40.3 ±1.7	43.8 ±2.3	46.8 ±1.2	43.2 ±2.9
Bone‖	Na_i¶	254 ±13	258 ±8				
	K	16.6 ±1.6	20.0 ±2.1				
	Mg	578 ±13	480 ±21				
	Ca	13,070 ±100	13,320 ±100				

* Number in parentheses, number of animals used.
† mEq. per 100 Gm. fat-free dry muscle.
‡ Na_i in muscle was calculated from the total muscle sodium by correcting for the sodium in the extracellular fluid of the muscle sample after the method of Yannet and Darrow (21).
§ Group mean value and standard deviation.
‖ mEq. per Kg. fat-free dry bone.
¶ Na_i in bone was calculated from the total bone sodium by correcting for the sodium in the extracellular fluid of the bone sample. Extracellular fluid volume was derived by assuming all bone chloride to be extracellular (22).

values have been reported for rats with chronic respiratory acidosis (23).

In Table IV, electrolyte composition of bone during respiratory acidosis is compared with controls. The most striking finding was a marked fall in bone magnesium.

Renal glutaminase and carbonic anhydrase

Renal carbonic anhydrase activity failed to show any significant change after 11 days of respiratory acidosis (Table V). The increased bicarbonate reabsorption which occurred in the animals subjected to respiratory acidosis was not, therefore, mediated by adaptive increase in renal carbonic anhydrase activity as measured by the *in vitro* method employed.

Despite prolonged and persistent respiratory acidosis, the activity of renal glutaminase did not rise (Table V).

To explore the significance of chloride loads during respiratory acidosis, rats subjected to 10 per cent CO_2 were given in addition 3 mMole NH_4Cl daily and were killed after 11 days. The following results were obtained: plasma CO_2 content, 39.2 ± 2.4 mEq. per L.; plasma pCO_2, 90.9 ± 10.2 mm. Hg; plasma chloride, 93.6 ± 0.8 mEq. per L.; blood pH, 7.25 ± 0.08. Ammonia excretion was elevated by about 3 mEq. per day above those levels found in animals given 10 per

TABLE V

Renal glutaminase and carbonic anhydrase activities

Group	Procedure	Renal glutaminase*	Renal carbonic anhydrase†
1 and 4	Std. Diet only	‡608 (10)§ ±60	370 (5) ±6
2 and 5	Std. Diet + 10% CO_2	657 (13) ±119	365 (5) ±40
3	Std. Diet + 15% CO_2	535 (5) ±79	378 (4) ±4
6	Std. Diet + 10 and 15% CO_2	781 (4) ±113	
7	E.D. Diet only	537 (4) ±77	313 (5) ±4
8	E.D. Diet + 10% CO_2	811 (9) ±176	314 (5) ±3

* μMole NH_3 per 100 mg. dry kidney tissue per hour.
† Units per Gm. wet kidney tissue.
‡ Mean and standard deviation.
§ Number in parentheses, number of animals used.

cent CO_2 alone. Urine pH was lower (mean, 5.8) than that found in respiratory acidosis alone (mean, 6.2 to 6.8). Renal glutaminase activity was 2,228 ± 371 mMole per 100 mg. dry tissue per hour as contrasted to a mean value of 657 for respiratory acidosis alone.

DISCUSSION

The pattern of urinary acid excretion during respiratory acidosis differs markedly from that resulting from other forms of acid loading. In the present study, there was initially a small but significant rise in the excretion of acid (both as ammonia and titratable acid) during the first day or two of acidosis; thereafter, despite continued, severe, systemic acidosis, urinary acid excretion returned virtually to control values. By contrast, the administration of an acid buffer, such as sodium acid phosphate, promptly increases the excretion of titratable acid as long as the acid load is given, even though the amounts of acid cause only a slight lowering of the concentration of serum bicarbonate (24).

The character of acid excretion during ammonium chloride acidosis differs even more strikingly from that encountered during respiratory acidosis. The administration to rats of small amounts of a strong acid, *e.g.,* 2 mMole NH_4Cl daily, results in the following response (13): *1*) Acid excretion (almost entirely as ammonia) rises over a five day period to a maximum level. *2*) This level is maintained throughout the duration of the acid loading. *3*) Despite the absence of changes in the pH or concentrations of bicarbonate in blood (or at most, minor depressions), far more acid is excreted than in control animals. *4*) Indeed, acid excretion is directly proportional to the magnitude of the strong acid load (whereas in respiratory acidosis, 10 and 15 per cent CO_2 elicit the same minor rise in urinary acid). The sharp dichotomy between the profound degree of systemic acidosis (blood pH 7.15 ± 0.07 in 15 per cent CO_2 group) on the one hand and the comparatively minor rise in acid excretion on the other constitutes one of the most striking features of the pattern of acid excretion during respiratory acidosis. The failure of respiratory acidosis to produce a sustained increase in renal acid excretion cannot be attributed to the fact that as plasma pCO_2 increases, carbonic acid can be excreted via the lungs and therefore would not require renal compensation beyond the initial period, since the blood pH was markedly acid throughout the period of study.

Unlike metabolic acidosis, the serum bicarbonate rises during CO_2-rebreathing. This rise begins within the first hour and reaches a maximum value in about one day, after which the elevated serum concentration is maintained throughout the duration of exposure to CO_2 (25). It is probable that the absence of greater augmentation of acid excretion is linked with this elevation of serum bicarbonate. During the early period, before the concentration of serum bicarbonate has reached maximum values, mean urine pH falls below control levels; the excretion of ammonia and titratable acid is therefore accelerated. After the first day, the urine becomes more alkaline. Although the elevated pCO_2 of blood accelerates bicarbonate reabsorption, it is probable that a portion of the greatly increased load of filtered bicarbonate escapes into the urine, especially when the serum bicarbonate concentration is maximal, rendering it more alkaline. In turn, the more alkaline urine may serve to suppress the excretion of ammonia and titratable acid.

The early rise in the concentration of serum bicarbonate is roughly paralleled by a commensurate fall in serum chloride. This fall in filtered chloride and rise in filtered bicarbonate could result in a sharp reduction in the proportion of chloride as compared with bicarbonate reaching the exchange site in the distal tubule. It is especially noteworthy, in this connection, that the administration of 3 mMole NH_4Cl to rats during respiratory acidosis markedly accelerated NH_3 excretion and lowered urine pH, at the same time that renal glutaminase was activated to levels (2,228 ± 371 mMole NH_3 per 100 mg. dry tissue per hour) not significantly different from that found in rats subjected to 3 mMole NH_4Cl daily alone (Figure 5). It is likely that the NH_4Cl load, by reducing the concentration of bicarbonate and raising the concentration of chloride in serum, diminished the amount of bicarbonate reaching the distal tubule, at the same time that the delivery of NaCl to the exchange site was increased. As a result, ammonia excretion was accelerated

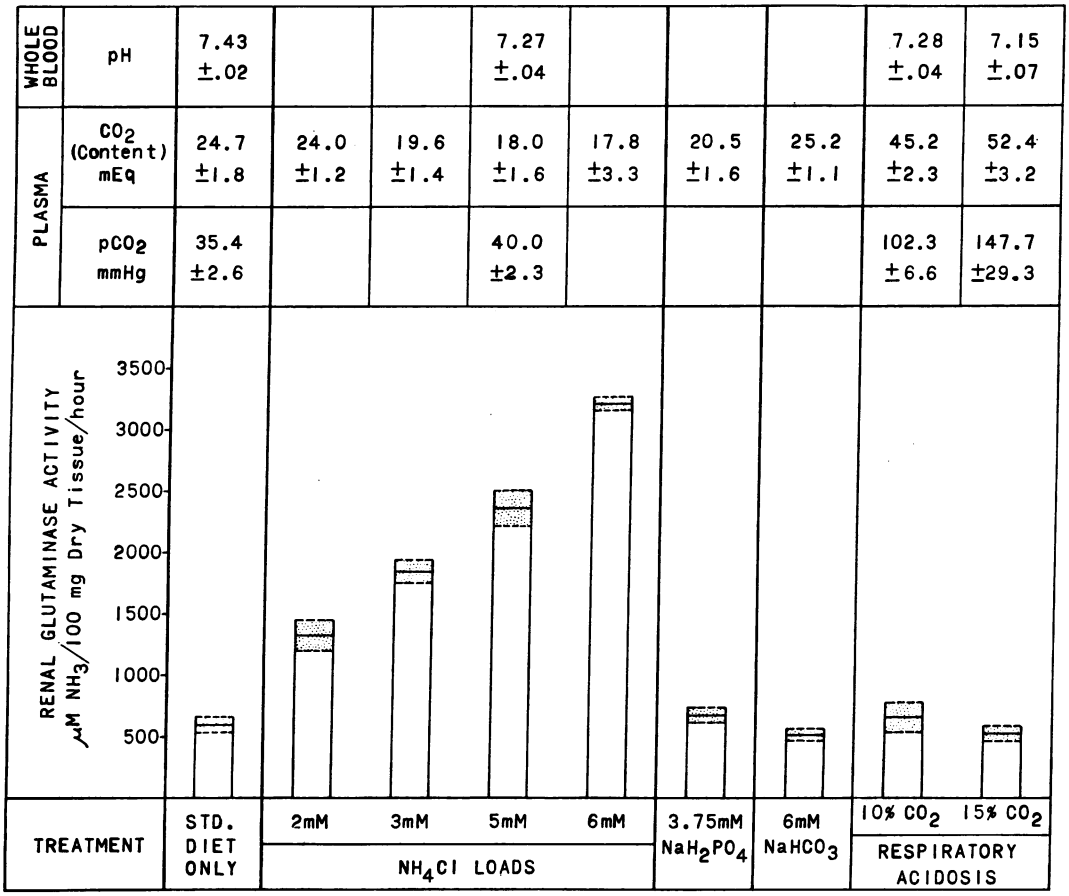

FIG. 5. RESPONSE OF RAT RENAL GLUTAMINASE ACTIVITY TO VARIOUS EXPERIMENTAL CONDITIONS

and this, in turn, could have activated renal glutaminase.

In keeping with the minor and transient rise in ammonia excretion during respiratory acidosis is the finding that renal glutaminase is not activated. Figure 5 summarizes the response of this enzyme system to various acid-base loads, as found in our laboratory. Glutaminase adaptation is observed only when ammonia excretion is persistently increased, a circumstance that prevails during chronic administration of a strong acid (NH_4Cl) load. Since CO_2-rebreathing results in a far more severe systemic acidosis (and intracellular acidosis as well), it seems likely that activation of the glutaminase enzyme system cannot be ascribed either to extracellular or intracellular acidosis alone. It is possible that the activation of glutaminase is attributable to product removal (*i.e.*, ammonia excretion); any circumstance tending to accelerate the excretion of ammonia should then result in an activation of the ammonia-producing enzyme systems of the rat.

Although acid excretion is elevated to only a minor extent in respiratory acidosis, the reabsorption of large amounts of filtered bicarbonate indicates that hydrogen ion secretion is increased (*26, 27*). This augmentation in bicarbonate reabsorption and hydrogen ion secretion is best correlated with the rise in tension of CO_2 in plasma (*5, 7, 28, 29*). In chronic respiratory acidosis in the dog, there is a further increase in bicarbonate reabsorption above that which might be predicted for any particular plasma pCO_2 value (*8*). That this apparent adaptation to prolonged respiratory acidosis might be mediated by means of the specific adaptation of renal carbonic anhydrase would seem a distinct possibility. Killion and Schaefer have reported increased renal carbonic anhydrase activity occurring within one hour in the kidneys of rats and guinea pigs exposed to an atmosphere

containing 30 per cent carbon dioxide (30). However, in our more chronic experiments, in atmospheres with less carbon dioxide content, we were unable to show increased activity of this enzyme. The explanation of the progressively more efficient bicarbonate reabsorption at any given pCO_2 during chronic respiratory acidosis is therefore not forthcoming from these studies.

In vitro studies with whole blood have shown that when exposed to carbon dioxide, chloride in the plasma enters the erythrocyte (31, 32). In acute respiratory acidosis in man (2, 3) and the nephrectomized dog (3), this mechanism has been shown to be important in producing hypochloremia. However, that an added mechanism for hypochloremia is the urinary loss of chloride had not been clearly demonstrated until the studies of Epstein, Branscome and Levitin (11). In our experiments, an early, marked, negative balance of chloride was likewise observed. Although the shift of chloride from the extracellular space into the erythrocyte does promote hypochloremia, it is apparent that the early loss of chloride in urine is also an important factor.

The excretion of potassium is slightly increased throughout the period of study. In acute respiratory acidosis in man of one hour duration, potassium excretion is depressed (5), presumably because increased H^+, generated from CO_2 in the tubular cell, rather than K^+, exchanges for reabsorbed Na^+. The persistently elevated potassium excretion, despite lowered serum concentrations, may be the consequence of accelerated delivery of $NaHCO_3$ to the distal tubule as serum bicarbonate rises, a circumstance which could increase the Na^+-K^+ exchange and thus account for the potassium loss. If increased amounts of $NaHCO_3$ are delivered to the distal tubule, the exchange of Na^+ for H^+, as well as for K^+, is in all likelihood augmented, but the enhanced secretion of H^+ into bicarbonate-containing tubular fluid would not augment urinary acid (NH_3 plus titratable acid) excretion.

The finding of a lowered plasma concentration of potassium in the experimental groups of this study contrasts with the elevated concentrations found in acute respiratory acidosis (2, 3, 33), where potassium has been shown to enter the extracellular space, presumably from cells. Similarly, the relationship of potassium concentration to blood pH observed in human subjects with disturbances in acid-base balance (34) was not apparent in this study. It seems likely that respiratory acidosis does in fact cause a transfer of potassium into the extracellular space, but in our chronic studies renal losses probably prevent the usually observed hyperkalemia.

Our finding of a slightly decreased muscle potassium is in agreement with the observation of Levitin, Jockers and Epstein (35). The previously noted potassium shift into the extracelluar space, together with the observed loss of potassium in the urine, is in keeping with the finding of lowered muscle potassium. The lowering of both muscle potassium and sodium in respiratory acidosis could be a consequence of exchange of extracellular hydrogen ion for intracellular anions. Decreased muscle sodium in metabolic acidosis has been previously reported, whereas with an adequate intake of potassium, muscle potassium is usually not lowered by metabolic acidosis (13, 36, 37). Thus, in both metabolic and respiratory acidoses, the sodium of muscle is decreased, whereas only respiratory acidosis significantly lowers muscle potassium. The apparent restriction of pH change principally to the extracellular fluid in metabolic acidosis (38, 39) may in some way explain the difference in response of muscle potassium in metabolic and respiratory acidoses. As suggested by Fenn, Rogers and Ohr, the lowering of muscle sodium in both metabolic and respiratory acidoses may not represent a simple exchange of intracellular sodium for extracellular hydrogen ion, but rather an increased ability of the sodium pump to deliver sodium into a more acid medium (40).

Bone, as an extracellular repository for sodium and potassium, has been recognized as a potential source from which the urinary excretions of these anions during acidosis can be derived (22). That in metabolic acidosis bone sodium is released, apparently in exchange for hydrogen ion, has been demonstrated (22, 37, 41). Bergstrom and Wallace (22) observed a similar role for bone potassium, but Levitt, Turner, Sweet and Pandiri (37) failed to find changes in bone potassium in rats with metabolic acidosis. In respiratory acidosis, no change was found by us in bone sodium, confirming a previous observation (35). It is possible that the chronicity of our experiments, as

compared with the more acute studies of metabolic acidosis, would explain the differences observed in bone sodium. Bone potassium was slightly elevated in respiratory acidosis. Thus, in chronic respiratory acidosis, participation of bone sodium and potassium in the buffering of carbonic acid is not apparent.

SUMMARY

The pattern of renal and cellular responses of electrolytes to chronic respiratory acidosis was studied in rats.

Urinary acid excretion (NH_3 plus titratable acid) increased transiently for the first day or two and then returned to control values despite continued severe respiratory acidosis. A slight rise in urine pH, presumably due to increased bicarbonate excretion as serum bicarbonate reached maximal values, appeared to be chiefly responsible for the rapid return of urinary ammonia to control values. Renal glutaminase was not activated during respiratory acidosis; this is strong evidence against the role of intracellular or extracellular pH as regulatory factors in the adaptation of renal ammonia-producing enzymes. In spite of increased tubular bicarbonate reabsorption, as evidenced by the stabilization of serum bicarbonate at high levels, adaptation of renal carbonic anhydrase did not occur.

Chloride excretion was greatly elevated the first day of respiratory acidosis but thereafter returned to control values. Potassium excretion was also markedly elevated the first day and continued to be excreted at a slightly increased rate for the remainder of the study.

Muscle sodium and potassium were slightly decreased after 11 days of respiratory acidosis. The most striking change in bone electrolytes was a fall in magnesium content.

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