THE PATHOGENESIS OF HYPOCHLOREMIA IN RESPIRATORY ACIDOSIS [1]

By HOWARD LEVITIN,[2] WILLIAM BRANSCOME,[3] AND FRANKLIN H. EPSTEIN [4]

(From the Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.)

(Submitted for publication July 3, 1958; accepted July 31, 1958)

A decrease in the concentration of chloride and an increase in the content of carbon dioxide are characteristic findings in the serum of patients with chronic respiratory acidosis. The elevated CO_2 content of serum has been demonstrated to be at least in part the result of an increased reabsorption of bicarbonate by the kidneys (1–3). The mechanism by which hypochloremia develops, however, has not been clearly established. Possible mechanisms include: *1*) transfer of chloride to an intracellular position, particularly into red cells, *2*) expansion and dilution of the extracellular space by sodium bicarbonate and water, and *3*) increased renal excretion of chloride.

The present experiments indicate that in the rat, the first two alternatives listed above do not contribute importantly to the hypochloremia. The low serum chloride which develops during respiratory acidosis is associated instead with increased renal excretion of chloride together with potassium and ammonium, resulting in a net loss of chloride from the body.

MATERIALS AND METHODS

White male Sprague-Dawley rats weighing 250 to 350 Gm. were maintained on a high caloric, low-residue diet, the composition of which is given in Table I. The animals were trained to take a single 40 minute feeding each day for three weeks preceding a balance study. This permitted them to ingest all of their food at the beginning of each 24 hour balance period, thus avoiding the diminution in food intake which otherwise was found to accompany initial exposure to high concentrations of carbon dioxide. Rats require six to seven days on this feeding program to resume their normal pattern of weight gain. Water was allowed *ad libitum* during the training and study periods. Control and experimental groups were trained and studied concomitantly.

Individual metabolic cages with screens to deflect feces, glass collecting funnels and graduated collecting tubes made up each metabolic unit. Four such units were housed in a large air-tight lucite chamber. Following control periods of balance study, the chamber was sealed and its atmosphere regulated by the controlled inflow of 8 or 12 per cent CO_2 in air. In all experiments the concentration of O_2 remained at 19 ± 1 per cent. Urine was collected under mineral oil in tubes to which 1.0 ml. of 0.1 normal sulfuric acid had been added in order to assure accurate measurement of ammonium excretion. Voiding was induced at the end of each balance period by making the animals sniff ether. At the end of each 24 hour period the funnels were washed down with 20 ml. of distilled water. This washing was added to the 24 hour urine, and the total volume was used for analysis.

At the completion of an experiment the rats were removed from the chamber, lightly anesthetized with pentobarbital and exsanguinated from the abdominal aorta.

Hematocrit of whole blood was determined by a micro-hematocrit method (4), whole blood and serum

TABLE I

Composition of diet

	%
Sucrose	45.8
Lard	20.0
Vitaminized casein	25.0
Corn oil	5.0
Choline	0.4
Mineral mixture*	4.0

Electrolyte composition (by analysis)

	Regular diet	Low sodium diet†
	mEq./Gm.	
Cl	0.135	0.138
Na	0.115	0.0057
K	0.128	0.160

* NaCl, 15.1%; $CaCO_3$, 27.0%; K_2HPO_4, 29.0%; $CaHPO_4$, 5.6%; $MgSO_4$, 20.4%; $MnSO_4$, 0.33%; $ZnCl_2$, 0.022%; $CuSO_4$, 0.026%; KI, 0.071%; $CoCl_2$, 0.026%; and Fe citrate, 2.5%.

† For the low sodium diet, the mineral mixture was made up with KCl substituted for NaCl. The amount of K_2HPO_4 was then reduced slightly to avoid an inordinately large intake of K^+.

[1] Aided by grants from the American Heart Association; the National Heart Institute, United States Public Health Service; and a contract (MD-116) with the office of the Surgeon General, Department of the Army.

[2] American Heart Association Advanced Research Fellow.

[3] James Hudson Brown student fellow, 1957.

[4] Established Investigator, American Heart Association.

chloride by Volhard titration (5), serum CO_2 content by the method of Van Slyke and Neill (6), sodium and potassium in serum and urine by indirect flame photometry, urine ammonium by the Conway microdiffusion method (7), urine chloride by potentiometric titration (8), phosphorus by the method of Fiske and Subbarow (9) and urinary nitrogen by semimicro-Kjeldahl digestion and distillation. Food was digested with concentrated nitric acid containing silver nitrate following which sodium, potassium and chloride were measured by the above techniques. The nitrogen content of the diet was determined by macro-Kjeldahl digestion. Creatinine was measured by the method of Hare (10). Samples of the air in the chamber were analyzed for carbon dioxide and oxygen in a Scholander gas analysis apparatus (11).

Calculations

1. Red blood cell chloride content per liter of red cells (Cl Rbc).

$$\text{Cl Rbc} = \frac{Cl_B - [Cl_S \times (1 - \text{Hct.})]}{\text{Hct.}},$$

where

Cl_B = mEq. of chloride per liter of blood,
Cl_S = mEq. of chloride per liter of serum, and
Hct. = hematocrit.

2. Change in total red blood cell chloride.

$$\Delta\text{Rbc Cl} = \frac{Cl_{Rbc_1} - Cl_{Rbc_2}}{1{,}000} \times 0.07 \times \text{B.W.} \times \text{Hct.},$$

where

Cl_{Rbc_1} = average chloride concentration in the red cells of control rats breathing room air,
Cl_{Rbc_2} = concentration of chloride in red cells after exposure to CO_2, and
B.W. = body weight prior to entering CO_2. Total blood volume in the rat is assumed to equal 0.07 × body weight (12).

3. Change in extracellular fluid volume (chloride space) = $ECF_1 - ECF_2$.

$$ECF_1 = 0.20 \times \text{B.W.}$$

$$ECF_2 = \frac{\frac{ECF_1 \times Cl_{S_1}}{0.95 \times 0.93} - (\text{Cl balance} + \Delta Rbc_{Cl})}{\frac{Cl_{S_2}}{0.95 \times 0.93}}$$

where

Cl_{S_1} = average serum chloride in control rats breathing room air,
Cl_{S_2} = serum chloride after exposure to CO_2,
Cl balance = chloride balance during exposure to CO_2,
0.95 = Donnan correction, and
0.93 = assumed water content of serum.

4. Changes in intracellular sodium and potassium were calculated as described by Elkinton, Winkler and Danowski (13).

RESULTS

Effect of exposure to 8 per cent CO_2 on electrolytes of serum and red cells (Tables II and IV)

Exposure to 8 per cent CO_2 for 24 hours resulted in an average decrease in serum chloride of 10 mEq. per liter and a rise in the plasma CO_2 content of 9 mEq. per liter. There were no significant changes in the hematocrit, serum sodium and potassium, or the calculated chloride content of red cells. These changes were as marked at the end of one day as after two and three days in 8 per cent CO_2. Serum electrolyte values of rats in room air, eating the special diet employed in these studies, did not differ from those of rats on a Purina Chow diet. The changes in serum electrolytes induced by 8 per cent CO_2 which are summarized in Table II were reproduced in other studies, not included here, on rats maintained on Purina Chow.

Rats fed a low-sodium diet showed similar changes in serum chloride and CO_2 content when exposed to 8 per cent CO_2 for 24 hours. Again,

TABLE II

Effect of 8 per cent CO_2 on the serum electrolytes of rats on regular and low sodium diets

	Regular diet			Low sodium diet		
	Room air (6 rats)	8 per cent CO_2 (10 rats)	p*	Room air (8 rats)	8 per cent CO_2 (6 rats)	p*
Serum Cl, *mEq./L.*	104.4 ± 0.97†	94.1 ± 1.5	<0.001	104.6 ± 1.2	94.9 ± 1.5	<0.001
Serum CO_2 content, *mM/L.*	24.6 ± 2.1	33.7 ± 2.1	<0.001	24.8 ± 1.6	33.0 ± 1.0	<0.001
Serum Na, *mEq./L.*	146.2 ± 2.3	146.5 ± 2.9	>0.80	148.3 ± 3.8	146.7 ± 2.0	>0.30
Serum K, *mEq./L.*	4.13 ± 0.36	4.55 ± 1.1	>0.20	4.13 ± 0.58	4.20 ± 0.22	>0.80
Red cell Cl, *mEq./L. rbc*	46.3 ± 7.6	52.5 ± 3.0	>0.10	49.3 ± 5.4	48.9 ± 6.5	>0.90
Hct., %	43.6 ± 3.4	45.3 ± 4.5	>0.10	46.5 ± 3.2	47.3 ± 3.3	>0.60

* The p values are for rats in room air compared with animals in CO_2. No significant difference is present between the two dietary groups in room air or in their response to CO_2.
† Mean ± standard deviation.

TABLE III

Electrolyte balance data of 10 rats on regular diet exposed to CO_2*

		Room air			8% CO_2
		Day 1	Day 2	Day 3	Day 4
Chloride, *mEq.*	Intake	0.365 ± 0.166†	0.411 ± 0.200	0.565 ± 0.151	0.471 ± 0.178
	Output	0.270 ± 0.124	0.369 ± 0.168	0.475 ± 0.170	0.672 ± 0.201
	Balance	+0.095 ± 0.030	+0.042 ± 0.061	+0.090 ± 0.078	−0.201 ± 0.099‡
Sodium, *mEq.*	Intake	0.305 ± 0.208	0.349 ± 0.181	0.485 ± 0.153	0.402 ± 0.163
	Output	0.238 ± 0.149	0.315 ± 0.139	0.408 ± 0.141	0.405 ± 0.222
	Balance	+0.067 ± 0.145	+0.034 ± 0.071	+0.077 ± 0.069	−0.003 ± 0.112
Potassium, *mEq.*	Intake	0.334 ± 0.147	0.387 ± 0.184	0.539 ± 0.146	0.447 ± 0.161
	Output	0.294 ± 0.073	0.348 ± 0.114	0.398 ± 0.99	0.558 ± 0.106
	Balance	+0.040 ± 0.112	+0.039 ± 0.104	+0.141 ± 0.102	−0.111 ± 0.123‡
	Balance§	+0.007 ± 0.005	−0.022 ± 0.003	−0.002 ± 0.003	−0.213 ± 0.002‡
Ammonia, *mEq.*	Output	0.284 ± 0.088	0.278 ± 0.088	0.293 ± 0.063	0.495± 0.095‡
Phosphorus, *mM*	Output	0.195 ± 0.056	0.221 ± .090	0.219 ± 0.053	0.372 ± 0.052‡
Creatinine, *mg.*	Output	3.34 ± 0.46	3.06 ± 0.42	3.39 ± 0.49	3.39 ± 0.51
Nitrogen, *mg.*	Balance	+10.43 ± 22.63	+25.13 ± 27.69	+38.24 ± 19.46	+24.60 ± 34.99

* All values are expressed as amount per 24 hours per 100 Gm. of rat.
† Mean ± standard deviation.
‡ These values are significantly different from those of the preceding day with p values of less than 0.001.
§ Corrected for simultaneous nitrogen balance, assuming 3 mEq. K per 1 Gm. of nitrogen.

no significant change was observed in the hematocrit, serum sodium and potassium concentrations, or the calculated chloride concentration of red cells.

Effect of 8 per cent CO_2 on electrolyte balance in rats on a regular diet (Table III and Figure 1)

Following three days of control observations, during which time the intake of chloride, sodium and potassium approximated the output of these ions, exposure to 8 per cent CO_2 for 24 hours resulted in increased urinary losses and negative balances of chloride and potassium. The excretion of ammonium increased, as did the excretion of phosphorus. Sodium balance was unchanged. There was a slight positive nitrogen balance throughout the entire study with no significant difference in nitrogen balance discernible from day to day. The daily output of creatinine was essentially constant during the four days of observation.

During control observations in room air, the mean weight gain was 1.5 ± 4.4 Gm. per 100 Gm. of rat, with a subsequent mean weight loss of 2.6 ± 2.4 Gm. following exposure to 8 per cent CO_2. Despite this loss of weight, calculated changes in

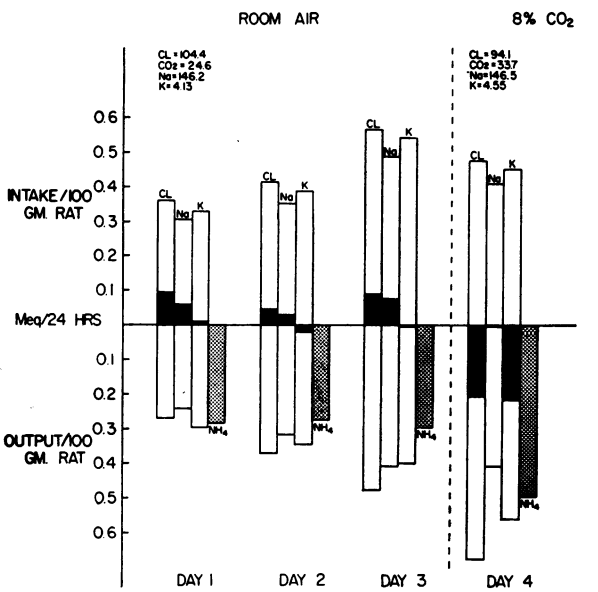

FIG. 1. THE EFFECT OF 8 PER CENT CO_2 FOR 24 HOURS ON THE MEAN ELECTROLYTE BALANCE OF 10 RATS ON A REGULAR DIET

Intake is plotted above the zero line and output below. The net balance is in black. The K balance has been corrected for the simultaneous nitrogen balance (1 Gm. N = 3 mEq. K). Days 1 through 3 represent control observations in room air, and Day 4 the experimental period in 8 per cent CO_2.

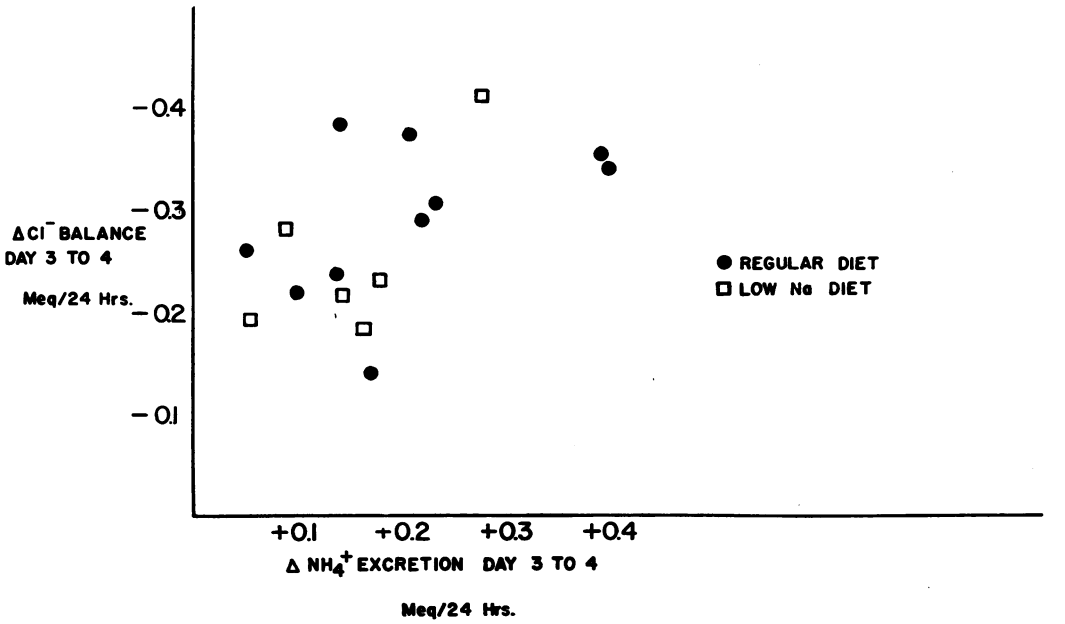

FIG. 2. THE CHANGE IN CHLORIDE BALANCE PLOTTED AGAINST THE CONCOMITANT CHANGE IN AMMONIUM EXCRETION INDUCED BY BREATHING 8 PER CENT CO_2 FOR 24 HOURS

the chloride space were not large, consistent, or significant, the mean value being + 0.15 ± 0.96 ml. per 100 Gm. of rat. No correlation was apparent between the magnitude and duration of individual changes in chloride space and the urinary loss of chloride or the chloride balance.

The mean increase in NH_4^+ excretion induced by 8 per cent CO_2 (0.202 mEq. per 24 hours per 100 Gm. rat) was less than the mean change in chloride balance (− 0.291 mEq. per 24 hours per 100 Gm. rat), and there was no correlation between these two measurements in individual rats

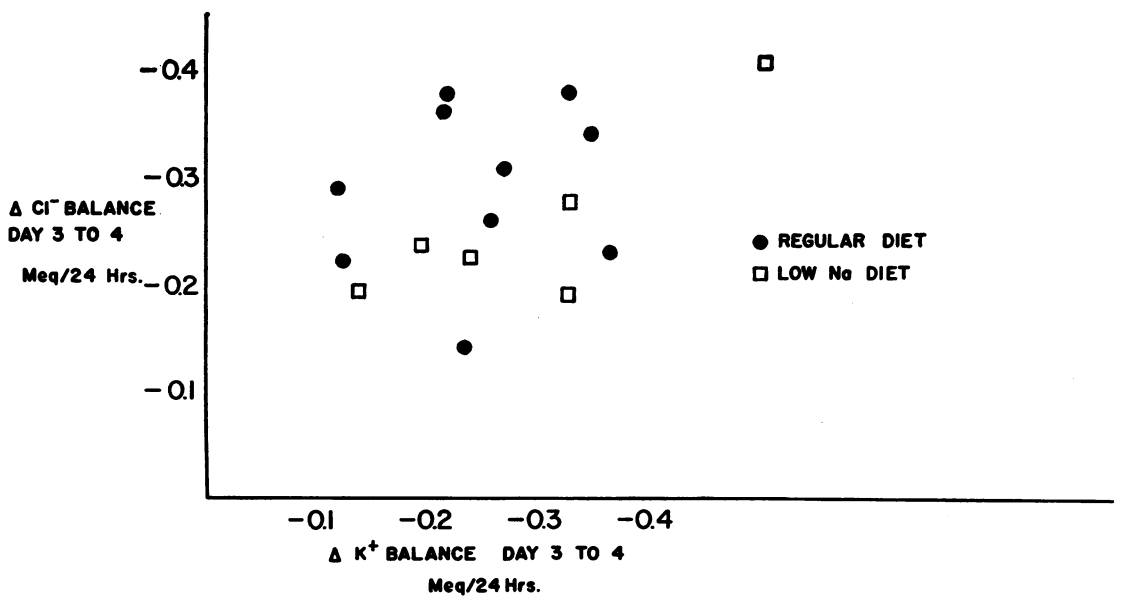

FIG. 3. THE CHANGE IN CHLORIDE BALANCE PLOTTED AGAINST THE CONCOMITANT CHANGE IN POTASSIUM BALANCE INDUCED BY BREATHING 8 PER CENT CO_2 FOR 24 HOURS

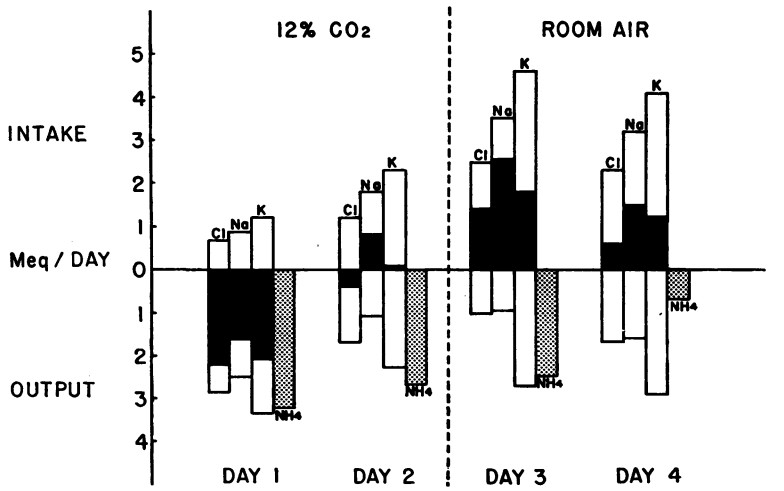

FIG. 4. THE MEAN ELECTROLYTE BALANCE OF FOUR RATS DURING TWO DAYS OF EXPOSURE TO 12 PER CENT CO_2 FOLLOWED BY TWO DAYS OF EXPOSURE TO ROOM AIR

Intake is plotted above and output below the zero line. The net balance is in black. The K balance is not corrected for simultaneous nitrogen balance. Note that values are *not* corrected per 100 Gm. of rat.

(Figure 2). Similarly, the increased loss of chloride correlated poorly in individual rats with the change in balance of potassium which followed exposure to CO_2 (Figure 3), and again, in most instances, exceeded the latter.

Effect of removing rats from an atmosphere high in CO_2 to room air

Four rats (maintained on Purina Chow and not included in Table II) were removed from 12 per cent CO_2 after staying in the chamber for two days

TABLE IV

Electrolyte balance data of six rats on low sodium diet exposed to CO_2*

		Room air			8% CO_2
		Day 1	Day 2	Day 3	Day 4
Chloride, *mEq.*	Intake	0.350 ± 0.105†	0.421 ± 0.064	0.549 ± 0.077	0.471 ± 0.080
	Output	0.321 ± 0.106	0.409 ± 0.058	0.496 ± 0.086	0.674 ± 0.077
	Balance	+0.029 ± 0.120	+0.012 ± 0.068	+0.053 ± 0.043	−0.203 ± 0.054‡
Sodium, *mEq.*	Intake	0.018 ± 0.005	0.022 ± 0.006	0.029 ± 0.007	0.025 ± 0.008
	Output	0.009 ± 0.005	0.006 ± 0.002	0.008 ± 0.007	0.021 ± 0.036
	Balance	+0.008 ± 0.006	+0.016 ± 0.007	+0.021 ± 0.012	+0.004 ± 0.040
Potassium, *mEq.*	Intake	0.405 ± 0.130	0.486 ± 0.068	0.636 ± 0.102	0.544 ± 0.074
	Output	0.347 ± 0.156	0.463 ± 0.088	0.553 ± 0.151	0.756 ± 0.134
	Balance	+0.058 ± 0.158	+0.023 ± 0.092	+0.083 ± 0.112	−0.212 ± 0.124‡
	Balance§	+0.062 ± 0.165	−0.042 ± 0.103	−0.044 ± 0.121	−0.268 ± 0.126‡
Ammonia, *mEq.*	Output	0.274 ± 0.044	0.249 ± 0.076	0.285 ± 0.064	0.437 ± 0.083‡
Phosphorus, *mM*	Output	0.073 ± 0.040	0.067 ± 0.022	0.067 ± 0.023	0.241 ± 0.052‡
Creatinine, *mg.*	Output	3.33 ± 0.61	3.50 ± 0.26	3.68 ± 0.24	3.70 ± 0.18
Nitrogen, *mg.*	Balance	−8.6 ± 39.68	+21.57 ± 13.11	+42.30 ± 19.33	+24.52 ± 22.80

* All values are expressed as amount per 24 hours per 100 Gm. of rat.
† Mean ± standard deviation.
‡ These values are significantly different from those of the preceding day with p values of less than 0.001.
§ Corrected for simultaneous nitrogen balance, assuming 3 mEq. K per 1 Gm. of nitrogen.

(Figure 4). Renal retention of chloride combined with increased ingestion of food to produce a positive balance of chloride on the first day of exposure to room air. Balances of K^+ and Na^+ also became positive, while the excretion of NH_4^+ gradually diminished. These changes were opposite to those which had occurred when the rats were placed in CO_2. After the animals had been in room air for two days they were sacrificed and serum chloride, CO_2 content, sodium and potassium were found to be normal.

Effect of 8 per cent CO_2 on electrolyte balance in rats on a low-sodium diet (Table IV and Figure 5)

In six rats, sodium was eliminated from the diet and replaced by potassium, the chloride content remaining unchanged. While on this diet, rats excreted less than 0.02 mEq. of sodium per day at a urinary concentration ranging from 2 to 5 mEq. per liter. Upon exposure to 8 per cent CO_2, renal excretion of chloride and and potassium increased, producing negative balances of these ions, in association with increases in ammonium and phosphorus excretion. These changes were similar in magnitude to those observed in rats on a diet containing liberal amounts of sodium. Again, breathing CO_2 did not significantly alter net nitrogen balance, the excretion of creatinine or the size of the calculated chloride space.

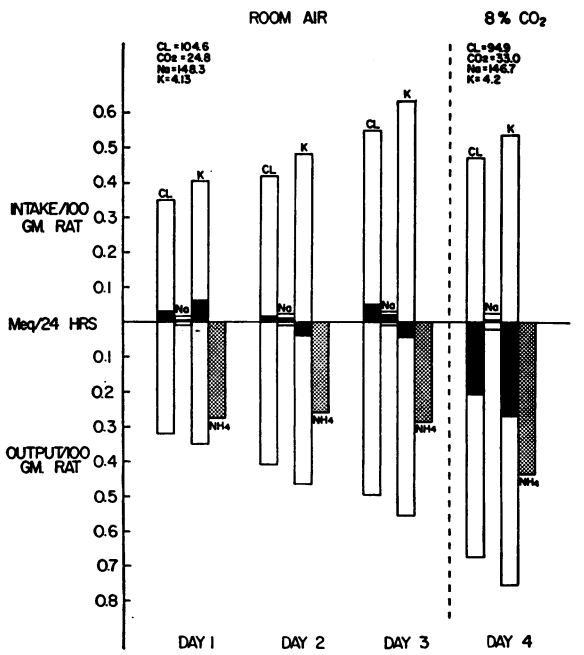

FIG. 5. THE EFFECT OF 8 PER CENT CO_2 FOR 24 HOURS ON THE MEAN ELECTROLYTE BALANCE OF SIX RATS ON A LOW-SODIUM DIET

Intake is plotted above the zero line and output below. The net balance is in black. The potassium balance has been corected for the simultaneous nitrogen balance (1 Gm. N = 3 mEq./K). Days 1 through 3 represent control observations in room air and day 4 the experimental period in 8 per cent CO_2.

TABLE V

Calculated changes in chloride space, "intracellular" sodium, "intracellular" potassium and total red cell chloride in rats exposed to 8 per cent CO_2

	Regular diet	Low sodium diet
Δ Cl space, *ml./100 Gm. rat*	+0.150 ± 0.964	−0.012 ± 0.830
Δ "intracellular" Na, *mEq./100 Gm. rat*	−0.031 ± 0.126	+0.036 ± 0.109
Δ "intracellular" K, *mEq./100 Gm. rat**	−0.224 ± 0.051	−0.270 ± 0.123
Δ total red cell Cl, *mEq./100 Gm. rat*	−0.001 ± 0.034	−0.005 ± 0.016

* The Δ intracellular K for both the regular diet and low sodium diets are significant with p values of <0.001 and <0.01, respectively. Other changes listed are not significant.

Effect of 8 per cent CO_2 on calculated changes in chloride space, "intracellular" Na and K and total red cell Cl (Table V)

Following exposure to 8 per cent CO_2 for 24 hours, calculated "intracellular" potassium decreased significantly. There was no change in chloride space, "intracellular" sodium or total red cell chloride.

DISCUSSION

When blood is exposed to increased concentrations of carbon dioxide, CO_2 diffuses into red cells, and, in a reaction catalyzed by carbonic anhydrase, is hydrated to form carbonic acid. This results in a fall in intracellular pH, reduced ionization of hemoglobin, and a change in the concentration gradient for bicarbonate which favors the outward transfer of this diffusible anion into the plasma. Chloride is then transferred from plasma to erythrocytes, in accordance with the now-modified Gibbs-Donnan effect. This "chloride shift" has been considered to contribute importantly to the hypochloremia of respiratory acidosis

(14, 15). The equilibrium of chloride between red cells and plasma is determined by the equation:

$$r = \frac{[HCO_3^-] \text{ red cell}}{[HCO_3^-] \text{ plasma}} = \frac{[Cl^-] \text{ red cell}}{[Cl^-] \text{ plasma}}.$$

It might be anticipated, therefore, that as the concentration of bicarbonate in the plasma becomes elevated and the concentration of chloride becomes depressed as a result of renal reabsorption of bicarbonate and excretion of chloride, red cell chloride, though initially elevated, would tend to return to the normal value. Normal concentrations of chloride in red blood cells were in fact found by Denton and associates (16) in sheep exposed to CO_2 for up to 72 hours, and by Platts and Greaves (17) in patients with chronic respiratory acidosis, as well as in the rats of the present experiments. It would appear that an increase in the chloride content of red cells is not the explanation for the hypochloremia of chronic respiratory acidosis.

Expansion of the extracellular space might conceivably contribute to the low serum chloride observed when rats are exposed to CO_2. Increases in pCO_2 are known to enhance the reabsorption of $NaHCO_3$ by the renal tubules (1–3). One might expect, therefore, that if the intake of sodium were continued, the volume of extracellular fluid would be expanded and diluted by isotonic reabsorption of $NaHCO_3$ and water. The body might then respond as it usually does to an expansion of the extracellular fluids, *i.e.*, by increasing the excretion of sodium and chloride until the volume of the extracellular fluid returned to normal (18). The result would coincide with that actually observed in rats on a regular diet, namely, a net loss of chloride with an increase in the serum concentration of HCO_3^-, but with no *net* change in sodium balance or change in extracellular fluid volume. This sequence of events appears to be excluded by the results obtained in rats maintained on a diet free of sodium before they were exposed to an atmosphere high in CO_2. These animals were ingesting and excreting minimal amounts of sodium before and during exposure to CO_2; they could not therefore have expanded their extracellular fluid with exogenous sodium. Furthermore, the data of Table V suggest that there was no significant loss of sodium from an intracellular position which might have expanded the extracellular space.[5] Despite this, exposure to 8 per cent CO_2 caused a chloruresis and negative balance of chloride of the same magnitude in rats on a diet free of sodium, as in rats ingesting and excreting normal amounts of sodium. Measurements of extracellular fluid volume during the initial one to three hours of acute respiratory acidosis in dogs (14), humans (15) and rats (20) have also failed to demonstrate significant changes. Expansion and dilution of the extracellular space do not seem, therefore, to play a role in the pathogenesis of the hypochloremia of respiratory acidosis.

Previous studies of acute respiratory acidosis in men (21) and sheep (16) have failed to demonstrate increased excretion of chloride in excess of sodium in the urine. The present experiments, on the other hand, demonstrate prompt renal losses of chloride during initial exposure to increased atmospheric carbon dioxide, and equally prompt renal retention of chloride upon re-exposure to room air. One explanation of the difference between the present studies and previous ones in other animals might conceivably lie with the well-known ability of rats to increase the formation of ammonia rapidly in response to acidosis by increasing the activity of renal glutaminase (22, 23). In this view, renal losses of chloride might be considered secondary to the increased excretion of ammonium which occurred on exposure to CO_2, the chloride remaining in the renal tubule because of the increase in positively charged NH_4^+ ions. Under these circumstances, however, one might anticipate that changes in the excretion of NH_4^+ would parallel and perhaps regularly exceed changes in the excretion of Cl^-, on exposure to CO_2. Figure 2 demonstrates that this was not the case.

What is the mechanism of the chloruresis observed in the present experiments? In nephrons of Necturus (24) and the rat (25), the intratubular lumen has a negative potential when compared with interstitial fluid. This potential is presumably created by the active outward transport of Na^+ ions and is thought to be responsible for the passive reabsorption of chloride and other anions. An increase in the pCO_2 of blood might

[5] This conclusion is buttressed by direct analysis of bone and muscle in more recent experiments (19).

enhance the renal excretion of chloride via the following mechanisms. First, an increase in the secretion of H^+ ions might reduce intraluminal negative potential and thereby decrease the back-diffusion of Cl^- ions. A second possibility is that respiratory acidosis might interfere directly with the passive reabsorption of chloride ions, in the same sense that certain metallic ions are said to interfere with the passive flux of chloride across the frog's skin (26). Finally, "active" transport of chloride by renal tubular cells, if this exists, might be altered by hypercapnia.

Although the excretion of K^+, NH_4^+ and sometimes Na^+ increased in association with Cl^- when rats were placed in CO_2, the change in chloride balance was generally greater than in the balance of potassium or sodium, or in the excretion of NH_4^+. The fact that K^+ excretion increased together with an increase in H^+ excretion in animals exposed to CO_2 is interesting and paradoxical in view of the postulated competition between these cations for secretion by cells of the distal tubule (27). Such simultaneous increases in both K^+ and H^+ excretion are regularly seen, however, in the course of an anion diuresis (28), and might be expected if CO_2 interfered primarily with the reabsorption of chloride and, in addition, stimulated the outpouring of phosphate in the urine that was observed in these experiments. The present experiments are thus compatible with, though they do not prove, the hypothesis that hypercapnia interferes directly with the reabsorption of chloride ions by the renal tubule.

SUMMARY

1. In the rat, the hypochloremia which develops during exposure to 8 per cent CO_2 in air is the result of a net loss of chloride in excess of sodium in the urine.

2. The increased excretion of chloride is associated but not directly correlated with an increased excretion of ammonium, potassium and phosphorus.

3. These changes were observed in rats maintained on a sodium-free diet as well as in rats ingesting and excreting liberal amounts of sodium, suggesting that expansion and dilution of the extracellular fluid with reabsorbed sodium bicarbonate are not responsible for the chloruresis.

4. It is suggested that an increase in pCO_2 may induce the observed renal losses of chloride by directly interfering with the tubular reabsorption of the chloride ion.

ACKNOWLEDGMENTS

The authors are grateful for the technical assistance of Donald K. McKay, Nadia T. Myketey and Eva Taborsky.

REFERENCES

1. Relman, A. S., Etsten, B., and Schwartz, W. B. The regulation of renal bicarbonate reabsorption by plasma carbon dioxide tension. J. clin. Invest. 1953, **32**, 972
2. Dorman, P. J., Sullivan, W. J., and Pitts, R. F. The renal response to acute respiratory acidosis. J. clin. Invest. 1954, **33**, 82.
3. Brazeau, P., and Gilman, A. Effect of plasma CO_2 tension on renal tubular reabsorption of bicarbonate. Amer. J. Physiol. 1953, **175**, 33.
4. Strumia, M. M., Sample, A. B., and Hart, E. D. An improved micro hematocrit method. Amer. J. clin. Path. 1954, **24**, 1016.
5. Van Slyke, D. D. The determination of chlorides in blood and tissues. J. biol. Chem. 1923, **58**, 523.
6. Van Slyke, D. D., and Neill, J. M. The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I. J. biol. Chem. 1924, **61**, 523.
7. Conway, E. J. Microdiffusion Analysis and Volumetric Error, 3rd ed. London, Lockwood, 1950.
8. Sanderson, P. H. Potentiometric determination of chloride in biological fluids. Biochem. J. 1952, **52**, 502.
9. Fiske, C. H., and Subbarow, Y. The colorimetric determination of phosphorus. J. biol. Chem. 1925, **66**, 375.
10. Hare, R. S. Endogenous creatinine in serum and urine. Proc. Soc. exp. Biol. (N. Y.) 1950, **74**, 148.
11. Scholander, P. F. Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. J. biol. Chem. 1947, **167**, 235.
12. Wang, C. F., and Hegsted, D. M. Normal blood volume, plasma volume and thiocyanate space in rats and their relation to body weight. Amer. J. Physiol. 1949, **156**, 218.
13. Elkinton, J. R., Winkler, A. W., and Danowski, T. S. Transfers of cell sodium and potassium in experimental and clinical conditions. J. clin. Invest. 1948, **27**, 74.
14. Giebisch, G., Berger, L., and Pitts, R. F. The extrarenal response to acute acid-base disturbances of respiratory origin. J. clin. Invest. 1955, **34**, 231.
15. Elkinton, J. R., Singer, R. B., Barker, E. S., and Clark, J. K. Effects in man of acute experimental respiratory alkalosis and acidosis on ionic transfers in the total body fluids. J. clin. Invest. 1955, **34**, 1671.

16. Denton, D. A., Maxwell, M., McDonald, I. R., Munro, J., and Williams, W. Renal regulation of the extracellular fluid in acute respiratory acidaemia. Aust. J. exp. Biol. med. Sci. 1952, **30**, 489.
17. Platts, M. M., and Greaves, M. S. The composition of the blood in respiratory acidosis. Clin. Sci. 1957, **16**, 695.
18. Strauss, M. B., Davis, R. K., Rosenbaum, J. D., and Rossmeisl, E. C. Production of increased renal sodium excretion by the hypotonic expansion of extracellular fluid volume in recumbent subjects. J. clin. Invest. 1952, **31**, 80.
19. Levitin, H., Jockers, C. R., and Epstein, F. H. The response of tissue electrolytes to respiratory acidosis. Clin. Res. 1958, **6**, 259.
20. Levitin, H., and Epstein, F. H. Unpublished observations.
21. Barker, E. S., Singer, R. B., Elkinton, J. R., and Clark, J. K. The renal response in man to acute experimental respiratory alkalosis and acidosis. J. clin. Invest. 1957, **36**, 515.
22. Davies, B. M. A., and Yudkin, J. Studies in biochemical adaptation. The origin of urinary ammonia as indicated by the effect of chronic acidosis and alkalosis on some renal enzymes in the rat. Biochem. J. 1952, **52**, 407.
23. Leonard, E., and Orloff, J. Regulation of ammonia excretion in the rat. Amer. J. Physiol. 1955, **182**, 131.
24. Giebisch, G. Electrical potential measurements on single nephrons of Necturus. Fed. Proc. 1958, **17**, 53.
25. Solomon, S. Transtubular electrical potentials of the rat kidney. Fed. Proc. 1956, **15**, 174.
26. Ussing, H. H., and Zerahn, K. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Acta physiol. scand. 1951, **23**, 110.
27. Berliner, R. W., Kennedy, T. J., Jr., and Orloff, J. Relationship between acidification of the urine and potassium metabolism. Amer. J. Med. 1951, **11**, 274.
28. Schwartz, W. B., Jenson, R. L., and Relman, A. S. Acidification of the urine and increased ammonium excretion without change in acid-base equilibrium: Sodium reabsorption as a stimulus to the acidifying process. J. clin. Invest. 1955, **34**, 673.