cells and HA is dependent on divalent cations, and removal of the latter from the environment of agglutinated red cells results in elution of HA.

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Effect of Altered Plasma pCO_2 on Intracellular pH during Potassium Deficiency. (32561)

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Several laboratories have reported a decrease in skeletal muscle cell pH of K-deficient rats(1,2). But the nature of this increased cellular acidity is not known with certainty. Cooke *et al*(3) proposed that muscle cell pH decreases during K-deficiency because hydrogen ion (H^+) is transferred into cells from the extracellular compartment. This hypothesis is based on (i) the fact that K-deficiency in rats leads to a loss of muscle K that is two-thirds compensated by a gain of Na and (ii) the assumption that the difference represents a passage of H^+ into the cell. Recent experiments(2) together with earlier work reported from this laboratory (4,5) produced evidence that the reduced intracellular pH (pH_i) encountered in K-deficient rats is not related to the unequal replacement of muscle K loss by Na gain.

Another explanation for this reduced pH_i may be that CO_2 diffuses from plasma into skeletal muscle during potassium deficiency. This was suggested by Miller *et al*(6) based on the observation that plasma pCO_2 is elevated during K-deficiency. If diffusion of CO_2 into the low-K cell is the major cause of its acidity, the pH_i of control and low-K muscle should be the same at identical pressures of CO_2.

To determine the degree of cellular acidity resulting from our K-depletion regimen, we altered the plasma pCO_2 of normal and K-deficient rats with a respirator and then measured pH_i by 5,5-dimethyl-2,4-oxazolidinedione (DMO) distribution. The data enabled a comparison of normal and low-K muscle pH_i at CO_2 pressures ranging from 20 to 90 mm Hg to be made.

Methods. Young adult male rats (Wistar strain) weighing about 270 g were given the control or the low-K diet used previously(2). The low-K diet contained normal amounts of Na and Cl. The rats were housed in individual cages and given food and water *ad libitum.* Animals receiving control diet were maintained on this diet for 8 days; those to be depleted of K were given low-K diet for 35 days.

About 3½ hours before termination of an experiment, 60 mg of DMO/kg of body wt was injected intraperitoneally. Seventy-five minutes later, the rat was placed under sodium pentobarbital (50 mg ip/kg body wt) anesthesia. The trachea was exposed and connected to a small animal respirator (C. F. Palmer Ltd., London) which controlled respiration during the ensuing 2 hours. A second ip injection of sodium pentobarbital (25 mg/kg) was given after one hour of controlled respiration. At the end of the 2-hour period, the animal was exsanguinated while still connected to the respirator. Exsanguination was accomplished by direct cardiac puncture using a greased, heparinized syringe; the thoracic cavity was not exposed. This procedure made it possible to remove quickly 8-11 ml of blood. The syringe containing fresh blood was sealed and placed on ice. Then the gastrocnemius muscles (essentially blood-free) were removed quickly and placed in sealed weighing bottles on ice. The blood was handled anaerobically and with care to minimize glycolysis during centrifugation and separation of the plasma. The muscle samples were trimmed and minced in a humidor to minimize moisture loss.

Blood pH was determined anaerobically at 38°C with a Radiometer 27 pH meter. The methods for analyses of plasma and muscle samples for moisture, fat, DMO and Cl were the same as in previous studies(2). The calculation procedure outlined by Waddell and Butler(7) was used to obtain pH_i values. Plasma pCO_2 was calculated from the pH and total CO_2 content of plasma; the latter was determined with a manometric Van Slyke apparatus.

Results. Acid-base data for individual rats are listed in Table I. Muscle pH_i as well as plasma pH (pH_p) was lowered when pCO_2 was increased. The concentrations of plasma HCO_3 in the low-K animals were generally higher than the plasma HCO_3 of controls. But the *intracellular* HCO_3 concentrations of low-K muscle were generally *lower* than the values for control muscle at comparable levels of pCO_2.

TABLE I. Acid-Base Data Obtained from Control and Low-K Rats with Altered Plasma pCO_2.

Plasma values				Muscle values		
pCO_2 mm Hg	pH	H^+ 10^{-5} mEq*	HCO_3 mEq†	pH_i	H^+ 10^{-5} mEq‡	HCO_3^- mEq§‖
			Control rats			
18.7	7.54	2.88	15.6	6.97	10.7	4.9
18.8	7.57	2.69	16.7	7.05	8.9	5.9
24.2	7.54	2.88	20.1	6.97	10.7	6.3
35.1	7.38	4.17	23.2	6.94	11.5	8.5
36.5	7.42	3.80	23.0	6.88	13.2	7.8
38.4	7.42	3.80	24.2	6.89	12.9	8.3
39.0	7.43	3.72	22.9	6.88	13.2	8.3
44.1	7.40	3.98	26.5	6.86	13.8	8.9
46.4	7.37	4.27	26.0	6.89	12.9	10.0
47.4	7.38	4.17	27.2	6.80	15.6	8.3
53.8	7.37	4.27	30.2	6.87	13.5	11.0
55.3	7.32	4.79	27.7	6.84	14.4	10.7
58.2	7.32	4.79	29.1	6.79	16.2	10.0
66.2	7.30	5.01	31.6	6.83	14.8	12.3
67.0	7.29	5.13	31.3	6.77	17.0	11.0
80.9	7.22	6.03	32.2	6.73	18.6	12.0
82.5	7.18	6.61	33.6	6.78	16.6	13.8
91.3	7.15	7.08	34.2	6.74	18.2	13.8
98.9	7.14	7.24	33.5	6.64	22.9	12.0
			Low-K rats			
23.0	7.67	2.14	25.7	6.92	12.0	5.3
24.0	7.60	2.51	22.9	6.97	10.2	6.2
24.4	7.67	2.14	27.4	6.91	12.3	5.5
28.6	7.64	2.29	29.8	6.89	12.9	6.2
31.8	7.56	2.75	27.7	6.93	11.8	7.6
39.4	7.55	2.82	33.4	6.75	17.8	6.2
41.3	7.50	3.16	31.3	6.76	17.4	6.6
41.5	7.54	2.88	33.8	6.76	17.4	6.6
41.9	7.51	3.09	32.4	6.81	15.5	7.6
42.6	7.52	3.02	33.8	6.79	16.2	7.2
49.1	7.48	3.31	36.8	6.70	20.0	6.9
49.2	7.53	2.95	39.9	6.82	15.1	9.1
57.6	7.43	3.72	37.1	6.75	17.8	8.9
58.8	7.44	3.63	38.8	6.68	20.9	7.8
60.3	7.45	3.55	40.7	6.73	18.6	8.9
69.5	7.38	4.17	39.9	6.65	22.4	8.7
75.6	7.43	3.72	46.7	6.69	20.4	10.2
85.0	7.25	5.62	39.4	6.58	26.3	8.9
88.8	7.32	4.79	44.4	6.60	25.1	9.8
94.5	7.28	5.25	43.1	6.65	26.3	10.0

* Per liter of plasma fluid.

† Per kg plasma H_2O.

‡ Per liter of intracellular fluid.

§ Per kg intracellular H_2O.

‖ Calculated from the Henderson-Hasselbalch equation by using DMO pH_i and assuming pCO_2 to be equal in intracellular and extracellular fluid. The solubility coefficient used for calculating (H_2CO_3) in intracellular H_2O was 0.035 mEq/liter/mm Hg of plasma CO_2 pressure.

The upper portion of Fig. 1 is an arithmetical plot of plasma H^+ concentration ($[H^+]_p$) as a function of plasma pCO_2; the pH values on the right ordinate correspond to the $[H^+]$ values on the left. A statistical test for linearity indicated that the individual points for control $[H^+]_p$ *vs* plasma pCO_2 follow a straight line relationship ($P<.01$); this was true also for the plasma of low-K rats. Calculation of the pH_p differences between the two regression lines in Fig. 1 indicates the extent to which the plasma of low-K rats became alkaline because of the conditions imposed by the K-depletion regimen. For example, at pCO_2 20, 40 and 80, pH_p

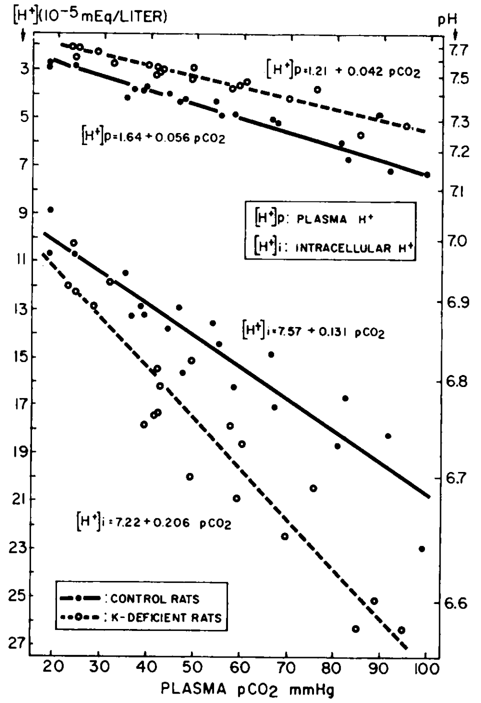

FIG. 1. Effect of altered plasma pCO_2 on plasma and intracellular $[H^+]$. Each point represents one rat.

was 7.56, 7.41, 7.21, respectively, for control rats; for low-K rats the corresponding values were 7.69, 7.54 and 7.34. The increase in pH_p of low-K rats at each of the three pressures of CO_2 was identical (+0.13).

The lower portion of Fig. 1 is a plot of intracellular H^+ concentration ($[H^+]_i$) as a function of plasma pCO_2. Statistically, these data were treated the same as the $[H^+]_p$ data; a linear relation ($P<.01$) was found between $[H^+]_i$ and plasma pCO_2 for both control and low-K rats. Using the regression equations for $[H^+]_i$ shown in Fig. 1, pH_i was calculated to be 6.99, 6.89 and 6.74, respectively, for control rats at pCO_2 20, 40 and 80; for low-K rats the corresponding values were 6.94, 6.81 and 6.62. The pH_i of low-K rats was more acid than the pH_i of the controls at all 3 pressures of CO_2; but the reduction in low-K pH_i became greater (—0.05, —0.08 and —0.12) as plasma pCO_2 was increased from 20 to 40 to 80 mm Hg. A statistical test indicates that the difference between the slopes for control and low-K rats is significant ($P<.01$).

Discussion. In our previous work(2) blood was obtained from control and low-K animals immediately after they were under anesthesia; the rats were breathing without a respirator. The average plasma pCO_2 was 39.6 and 45.9 mm Hg, respectively, for control and low-K rats; the average pH_i values obtained were 6.91 and 6.75, respectively. In the present work, the pH_i value calculated from the regression equation for control rats at pCO_2 39.6 was 6.89. At pCO_2 45.9 the calculated pH_i for low-K rats was 6.78. This pH_i difference (0.11) was only 0.03 greater than the pH_i difference (0.08) between control and low-K animals when the plasma pCO_2 of both was 40 mm Hg. Hence, CO_2 diffusion from the plasma was not the major contributor to the acidity of the low-K muscle cell. The major cause of the cellular acidity has to be attributed to the conditions imposed by the low-K regimen. But it should be recognized that possible diffusion of CO_2 from plasma into low-K cells may contribute somewhat to the intracellular acidity associated with K-deficiency. It also should be recognized that the pCO_2 of skeletal muscle is normally higher than that of plasma.

The values for intracellular HCO_3 concentration ($[HCO_3^-]_i$) in Table I further support our conclusion that conditions imposed by the dietary regimen constituted the major cause of the intracellular acidity. When $[HCO_3^-]_i$ values were plotted as ordinate with plasma pCO_2 as abscissa, the points representing low-K muscle fell below those for controls. If CO_2 diffusion from the plasma were the major cause of low-K cell acidity, the $[HCO_3^-]_i$ of low-K and control muscle would be the same at identical pressures of plasma CO_2.

The values for $[HCO_3^-]_i$ presented here were obtained *indirectly* by calculation based on DMO pH_i and the assumption that plasma and intracellular pCO_2 are the same. This assumption may not be entirely true, particularly at low pressures of CO_2. But since the same procedures were used for both control and low-K animals, any error in these data is probably of the same magnitude and direction for both groups. In a separate series of experiments we have determined $[HCO_3^-]_i$ by direct analysis of tissue for CO_2 content and also have found low-K $[HCO_3^-]_i$ to be less than control $[HCO_3^-]_i$. Others(8,9) using direct analysis for CO_2 have reported similar results.

The fact that an equal increment in plasma pCO_2 acidified low-K muscle cells to a greater degree than control muscle cells (Figure 1) suggests that the buffer capacity* of low-K muscle was less than the buffer capacity of normal muscle. To examine this point, a pH-bicarbonate diagram (Fig. 2) was drawn. Briefly, the rationale for this follows. With increased CO_2 pressure only part of the H^+ formed by dissociation of newly formed H_2CO_3 remains to lower pH_i because a portion of the new H^+ combines with intracellular buffers. For every H^+ that combines with a buffer other than bicarbonate a new HCO_3^- anion is formed. If low-K skeletal muscle buffer capacity is indeed reduced, then the same increment in CO_2 pressure would cause its pH_i to change more and its $[HCO_3^-]_i$ to change less than the pH_i and $[HCO_3^-]_i$ of control muscle.

In Fig. 2, the slope of the regression for control rats is —21.1 [21.1 slykes(12)]. This is essentially the same value obtained by Clancy and Brown(11) for normal dog skeletal muscle (19.5 slykes). But the slope for low-K rats is much less, the difference being statistically significant ($P<.01$). We conclude therefore that the skeletal muscle of low-K animals had less buffer capacity than control skeletal muscle realizing that the Van Slyke definition of buffer capacity applied to muscle *in vivo* includes the capacity of the cell to extrude H^+ or admit HCO_3^- anions(11). It also should be appreciated that this definition excludes the bicarbonate buffering component which we have calculated and found to be much less than the buffering attributable to the weak acid anions beside bicarbonate.

A pH-bicarbonate plot of our (true) plasma data was made. Predictably, there was considerably less scatter than in Fig. 2. The slopes of the regressions for control and low-K rats were essentially the same (—42.2 for controls and —44.5 for low-K rats), but the intercept of the regression for controls (337.2) was considerably lower than the low-K intercept (368.3). These data indicate essentially no difference ($P<.80$) between the non-bicarbonate buffer capacity of plasma from control and low-K rats.

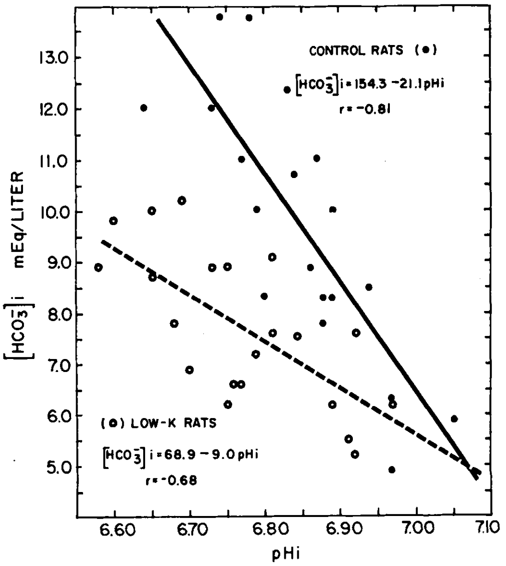

FIG. 2. A pH-bicarbonate diagram showing covariation between the intracellular HCO_8 and pH of control and low-K rat skeletal muscle. The two regression equations were calculated from the individual points, each point representing one rat. The slope of the regression for low-K rat muscle is significantly less ($P<.01$) than the slope for control rat muscle.

Much of the work in which skeletal muscle buffering has been studied as a function of CO_2 pressure was done *in vitro*. Fenn(13) reviewed much of this work. More recently, an *in vitro* study by Adler *et al*(14) showed that rat diaphragms are able to buffer perfectly at CO_2 tensions up to 80 mm Hg. A plot of our values for $[H^+]_i$ *versus* $[H^+]_p$ shows covariation between these two variables. The correlation coefficient (*r*) for the $[H^+]$ values of control rats was + 0.91; for low-K $[H^+]$ values it was + 0.94. A second plot was made omitting all experiments in which pCO_2 was below 30 and above 80. The *r* values were + 0.83 and + 0.80, respectively, for control and low-K $[H^+]$ data. If the *in vitro* buffering observed by Adler *et al* were in operation in our *in vivo* experiments, the

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* The term buffer capacity is used here as originally defined by Van Slyke(10), that is dB/dpH, and as applied recently by Clancy and Brown (11) to compare the *in vivo* buffer capacity of dog cardiac and skeletal muscle.

above *r* values would have been close to zero.

The nature of the cellular acidity associated with K-deficiency remains unknown. But our findings that (i) diffusion of plasma CO_2 is not the major factor and (ii) that the buffer capacity of low-K skeletal muscle is significantly reduced are in harmony with the suggestion of Irvine *et al*(1) that H^+ may be less efficiently extruded during K-deficiency.

Summary. The plasma pCO_2 of control and dietary K-deficient rats was mechanically altered between 20 and 90 mm Hg. The object was to compare the intracellular pH and HCO_3 content of control and low-K skeletal muscle at identical pressures of plasma pCO_2. This made it possible to study the intracellular pH and HCO_3 changes associated with dietary K depletion without complications due to plasma pCO_2 changes. The data indicate that the intracellular acidity of low-K skeletal muscle from dietary depleted rats results mainly from the conditions imposed by the low-K regimen. The results also show that the *in vivo* buffer capacity of low-K skeletal muscle is significantly less ($P<.01$) than the buffer capacity of control skeletal muscle.

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Free Amino Acids in Serum, Cerebrospinal Fluid, and Urine in Renal Disease With and Without Uremia. (32562)

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The cause of uremia as the final stage of renal insufficiency is related to an increase of toxic substances of protein metabolism in the blood and to disturbances in the acid-base balance. In animal experiments, however, it is known that urea, uric acid, and creatinine do not produce symptoms of uremia. Amines and derivatives of phenol and indol have been suspected of being inducing factors of uremic coma(1,2,3,4,5). In this report, the concentration of free amino acids in the serum, cerebrospinal fluid (c.s.f.), and urine in renal diseases, with and without uremia, will be investigated. The relationship between the severity of renal insufficiency and the disturbances of amino acid metabolism have been examined by long-term observations and is reported here. In a previous paper, determinations of free and bound phenolic compounds, indican, and glucuronic acid have been reported(11).

Materials and methods. Two groups of patients with renal diseases and a control group of 50 healthy adults were investigated. The first group consisted of 51 renal patients with severe uremia, which was fatal in 47 cases. The non-protein nitrogen of these patients was between 155 and 412 mg %. The