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Muscle Cell pH in Relation to Chronicity of Potassium Depletion. (31284)

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Rats fed a K-deficient diet and provided dietary sodium chloride undergo a loss of skeletal muscle K which is incompletely replaced by a gain of muscle Na(1,2). Simultaneously, the plasma HCO_3 concentration becomes elevated and the plasma Cl concentration is reduced. Cooke *et al*(3) proposed that an intracellular acidosis in association with an extracellular alkalosis occurs in K deficiency due to transfer of H^+ from the extracellular compartment. This entrance of H^+ into muscle cells was predicted, among other things, on the fact that the sum of Na and K in K-deficient rat muscle is less than in control muscle and the hypothesis that H^+ enters to compensate for the cation deficit. The results of subsequent *in vivo* studies with K-deficient rats(4,5,6,7) have shown an increase, to varying degrees, in the intracellular H^+ concentration of skeletal muscle.

In previous work from this laboratory(8,9) rats placed on low-K diets for periods up to 35 days exhibited an initial rapid loss of muscle K which was quantitatively greater than the gain in Na. Subsequently, during the dietary period 14 through 35 days, K losses were balanced by essentially equivalent gains of Na. Hence, the Na plus K deficit in muscle was constant during the final 21 days of the depletion period. Meanwhile, the plasma HCO_3 content continually increased throughout the entire 35-day period of K deprivation; this was indicative of a continuing decrease in plasma and extracellular H^+ concentration. If extracellular H^+ is transferred to the intracellular compartment of skeletal muscle in compensation for the Na plus K deficit, there should have been no further rise in plasma HCO_3 content during the time the muscle cation deficit was constant. It was therefore of interest to evaluate any changes in intracellular pH (pHi) during the course of K depletion by utilizing the DMO (5,5-dimethyl-2,4-oxazolidinedione) distribution method.

Methods. Young adult male rats of the Wistar strain weighing approximately 270 g were placed either on a control or a low-K, normal Na and Cl diet representing a modification of the diets formulated by Orent-Keiles and McCollum(10). The animals were housed in individual cages and given food

TABLE I. Average* Electrolyte Content of Plasma During Potassium Loss.

Series No.	HCO_3^-	Per kg H_2O Cl	K	Na	H^+†	pH‡	pCO_2
	mEq				10^{-5} mEq		mm Hg
I Controls	26.2 ± 1.8	103.4 ± 1.8	4.8 ± .2	160.4 ± 4.2	3.84 ± .23	7.42	39.7 ± 4.7
II L-K 14 days	‖32.1 ± 2.4	‖94.7 ± 2.6	‖2.5 ± .4	154.0 ± 6.4	‖3.26 ± .11	7.49	41.5 ± 4.1
III L-K 35 days	‖40.2 ± 2.8	‖90.0 ± 1.6	‖2.0 ± .3	158.8 ± 6.4	‖2.90 ± .15	7.54	§45.9 ± 4.1

* The average of 6 experiments ± S.D.
† Per liter of plasma fluid.
‡ Because pH is a logarithmic function, individual values were converted to hydrogen ion concentration. Mean values for hydrogen ion concentration were then reconverted to pH units.
§ Significantly different from control rats, P <.05.
‖ Significantly different from control rats, P <.01.

and distilled water *ad libitum.* Rats receiving the control diet were maintained on the diet for 8 days prior to experimentation while the animals to be depleted of potassium were allowed to remain on the low-K diet for either 14 or 35 days.

At approximately 3½ hours prior to termination of an experiment, a DMO solution representing 60 mg of DMO per kg of body weight was injected intraperitoneally. Prior to being exsanguinated the rat was placed under sodium pentobarbital anesthesia; blood was then collected under oil in a greased syringe by cardiac puncture without exposing the thoracic cavity. Several 60-80 μl aliquots of freshly drawn cardiac blood for pH determination were transferred anaerobically into heparinized capillary tubes. The capillary tubes containing blood were quickly sealed and promptly placed on ice to minimize glycolysis. The remaining blood was transferred under oil into a centrifuge tube containing heparin and centrifuged at once for plasma analyses. The gastrocnemius muscles were removed immediately after exsanguination.

Blood pH was determined at 38° by means of a pH meter (Radiometer 27) equipped with a blood micro-electrode. Plasma water and Cl were determined as in a previous study(11), while the plasma total CO_2 content was obtained by the standard manometric Van Slyke gas apparatus. The DMO content of plasma and muscle was determined as described by Waddell and Butler(12). Muscle Cl, Na, and K were determined on the dry, fat-free tissue remaining after determination of muscle water and fat content. This residue was finely ground and kept in a weighing bottle. Before sampling, the powdered tissue was dried to constant weight at 100-105°. The Cl content of weighed samples of powder was determined as described by Eichelberger and Bibler(13). Other weighed samples of powder were subjected to nitric acid extraction and the extract was used to determine muscle Na and K content by means of internal standard flame photometry. Plasma Na and K values were similarly obtained by flame photometry. The calculation procedure outlined by Waddell and Butler (12) was used to obtain pHi values.

Results. The results of the analyses of plasma from control rats (Series I) and from rats maintained on the low-K diet for 14 and 35 days (Series II and III, respectively) are shown in Table I. Differences between low-K and control animals were statistically analyzed by *t* test. The data in Table I show that the animals given the low-K diet exhibited a typical hypochloremic, hypokalemic alkalosis which became more severe as the period of K deficiency was prolonged. The average decrease in plasma H^+ concentration of Series III animals as compared with Series II animals was found to be significant ($P < 0.01$). It is noteworthy that the plasma pCO_2 was hardly changed after 14 days of K deprivation but significantly ($P < 0.05$) elevated after 35 days of K depletion.

The data in Table II show the changes occurring in skeletal muscle during K depletion. Examination of these data reveals that,

TABLE II. Average* Electrolyte Content and Derived pH_i of Rat Skeletal Muscle During Potassium Loss.

Series No.	Cl	K	Na	Na + K	H_i^+†	pH_i‡
	Per 100 g fat-free solids mEq				10^{-5} mEq	
I Controls	5.9 ± .4	48.5 ± 1.2	8.9 ± .9	57.4 ± 2.0	12.2 ± 1.6	6.91
II L-K 14 days	5.4 ± .6	§33.6 ± 2.5	§15.7 ± .7	§49.3 ± 1.9	13.1 ± 2.5	6.88
III L-K 35 days	6.0 ± .6	§29.1 ± 1.2	§21.6 ± 1.6	§50.6 ± 1.0	§17.6 ± 2.8	6.75

* Average of 6 experiments ± S.D.
† Per liter of intracellular fluid.
‡ See footnote ‡, Table I.
§ Significantly different from control rats, P <.01.

compared with control values, muscle lost on the average 14.9 mEq K and gained 6.8 mEq Na per 100 g fat-free solids during the initial 14 days of the dietary period. During the dietary interval 14 to 35 days the muscle lost, on the average, an additional 4.5 mEq K and gained an additional 5.9 mEq Na per 100 g fat-free solids.

Of immediate interest are the pHi values (and the equivalent concentrations of intracellular H^+) obtained by the DMO method. There was no significant increase in intracellular H^+ concentration during the first 14 days of K deprivation when muscle K loss was greater than Na gain. However, a significant increase did occur later during the time there was essentially no further change in the sum of muscle Na plus K.

Discussion. The results reported here indicate that the increased intracellular H^+ concentration found in skeletal muscle from K-depleted rats is unrelated to the sum of tissue Na plus K. Furthermore, it appears that the increase in plasma pH and in plasma HCO_3 content is also unrelated to the reduced tissue content of Na plus K during K deficiency. If the observed increase in plasma pH and plasma HCO_3 content after 14 days on the low-K diet was due to a transfer of H^+ in response to the cellular Na plus K deficit, no further increase in plasma pH and HCO_3 content would be anticipated during the dietary period when muscle K loss was essentially balanced by Na gain.

A clear explanation for the increased acidity in the intracellular phase of rat skeletal muscle from K-deficient rats is not evident. However, if it is assumed that the pCO_2 is essentially the same in the extracellular and intracellular fluids, the pCO_2 increment observed in Series III may have contributed to the significantly reduced muscle cell pH found in that group. This possibility has been suggested previously by Hudson and Relman(6). The present data allow no definite conclusion regarding this point.

Summary. Intracellular hydrogen ion changes in skeletal muscle from rats on a low-K regimen for 14 or 35 days were determined by DMO distribution. The objective was to determine whether changes in intracellular pH, plasma pH and plasma bicarbonate concentration would parallel the Na plus K deficit in K-deficient muscle. The results of this study were interpreted as indicating that the increased acidity in skeletal muscle from K-deficient rats is apparently related to the chronicity of K restriction but totally unrelated to the unequal replacement of muscle K loss by Na gain.

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Serum Proteins in NZB/Bl and Related Strains of Mice.* (31285)

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Since autoimmune hemolytic anemia was first demonstrated in NZB/Bl mice, these animals have been investigated in detail by many workers(1,2,3), and the role of heredity has been demonstrated clearly(4,5,6,7). In patients with Systemic Lupus Erythematosus (SLE) serum gamma globulin levels are significantly elevated(8), and agammaglobulinemia may occur in association with connective tissue diseases including SLE(9,10). Some authors(11) reported elevation of serum gamma globulin levels in relatives of patients with SLE; others did not(12,13).

In view of these observations in human SLE, we studied the serum protein levels of NZB/Bl mice and related pure and hybrid strains in order to determine whether or not hypergammaglobulinemia occurred in these mice and its possible relationship to heredity and to disease activity.

Material and methods. The inbred strains used in this study were NZB, NZW, NZC and the F_1 hybrids NZB-NZW and NZB-NZC. Animals were grouped at 3, 9, and about 18-20 months of age. Where possible, equal numbers of each sex were studied, but breeding difficulties with the colony inevitably resulted in unequal numbers in the various groups.

All 3- and 9-month mice were clinically healthy when blood was drawn. Many of the 18-month-old mice of the NZB and NZB-NZW strains were ill. In our colony of NZB mice the Coombs test was positive in none of the 3-month animals, in 55% of the 9-month animals, and 100% of the 18-month animals (14). In the NZB-NZW colony the test for antinuclear antibodies was positive in about 12% at 3 months, 75% at 9 months, and 100% at 18 months; the test for LE cells was positive in none at 3 months; 35% at 9 months; and 100% at 18 months(15).

Controls used were 3- and 9-month-old mice of the C_3H, NHA, Cb, and Strong A strains. All were clinically healthy, were housed in the same laboratory and received the same diet and care.

Blood samples were drawn in capillary tubes from the retro-orbital venous plexus under light ether anesthesia. Sera were frozen if not used immediately, and were thoroughly mixed in siliconized wells prior to analysis. Total protein was determined in duplicate by a minor modification of the method of Lowry and his colleagues(16). Freshly dissolved crystalline bovine albumin‡ was used as the standard, and was checked by micro-Kjeldahl, using a conversion factor of 6.54(17). Electrophoresis was done in duplicate on cellulose acetate strips(18).

Results. The results are summarized in Tables I and II. There were no significant differences among the 4 control strains and the results therefore have been pooled in Ta-

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