# EFFECT OF POTASSIUM DEFICIENCY ON CARBON DIOXIDE, CATION, AND PHOSPHATE CONTENT OF MUSCLE*

## WITH A NOTE ON THE CARBON DIOXIDE CONTENT OF HUMAN MUSCLE

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It has previously been demonstrated that dietary potassium deficiency in animals is associated with low serum chloride and elevated serum bicarbonate concentration (1–5). In 1950 the present authors described a decrease of intracellular carbon dioxide in the skeletal muscle of potassium-deficient rats (6), an observation which has since been confirmed by others (7). The present communication provides data concerning the relationships existing between the concentrations of carbon dioxide, chloride, phosphate, sodium, potassium, calcium, and magnesium in the skeletal muscle of normal and potassium-deficient rats. In addition the concentration of carbon dioxide in normal human skeletal muscle has been determined.

### *Methods*

Chronic potassium deficiency was produced in 160 to 175 gm. male, albino rats of the Sprague-Dawley strain by restriction to a diet containing 0.003 per cent potassium and 0.7 per cent sodium for 40 days. The composition of the diet was as follows: (gm. per 100 gm. diet) (1) Devitaminized casein (Sheffield Farms), 25; (2) corn oil (Mazola), 4; (3) U. S. P. dextrose, 66; (4) salt mixture: $CaCO_3$, 1.34; $CaHPO_4 \cdot 2H_2O$, 0.238; NaCl, 0.468; $Na_2HPO_4$, 1.59; $MgSO_4 \cdot 7H_2O$, 1.016; NaI, 0.0003; $CuSO_4 \cdot 5H_2O$, 0.004; $ZnCl_2$, 0.0008; $MnSO_4 \cdot 4H_2O$, 0.0324; iron and ammonium citrate, 0.296. The control rats received the same diet plus $K_2HPO_4$, 0.335 gm. per 100 gm. diet. Growth factors were added as follows (per 100 gm. diet): thiamine chloride, 400 μg.; riboflavin, 800 μg.; pyridoxine hydrochloride, 400 μg.; calcium pantothenate, 2.5 mg.; choline chloride, 100 mg.; vitamin A, 25 I.U.; vitamin D, 25 I.U.; vitamin E, 1 mg.;

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cystine *l*-(—), 200 mg.; nicotinic acid, 10 mg.; methyl naphthaquinone, 10 mg.; inositol, 100 mg.; *p*-aminobenzoic acid, 30 mg.

The rats were kept in separate cages at 78°F. At the time of sacrifice each animal was anesthetized with 25 mg. per kg. amytal intraperitoneally. The abdominal aorta was exposed and the rat was exsanguinated by withdrawing blood into a large syringe under oil. Muscle samples for carbon dioxide analysis were taken from the right and left lateral thigh and transferred to tared analysis tubes within 30 seconds. Muscle samples for other analyses were taken from the same areas and transferred to weighed, stoppered tubes. Muscle samples were taken from the sacrospinalis region in adult human subjects undergoing exploration for ruptured intervertebral disc.

Analyses of serum for water and chloride concentration and of muscle tissue for concentrations of blood, water, fat, chloride, sodium, potassium, calcium, and magnesium were done as described in a previous publication (5).

Serum carbon dioxide was determined by the method of Van Slyke and Neill (8) and serum pH by the method of Hastings and Sendroy (9). The amount of whole blood found in the skeletal muscle of the exsanguinated rats in this experiment was negligible. The total carbon dioxide of skeletal muscle was determined by the method of Danielson and Hastings (10). The average difference between duplicate determinations (done on muscle from the right and left thigh of each rat) was 1.52 mM per kg. Standard sodium carbonate solutions analyzed both by this method and by the method of Van Slyke and Neill (8) gave results which were in close agreement. Aliquots of the muscle powder were analyzed for total phosphorus, inorganic phosphorus, and lipid phosphorus by the method of Fiske and Subbarow as described elsewhere (11).

## RESULTS

*Data from Rat Serum Analyses.*—Information obtained from analyses of the serum of potassium-deficient and control rats is shown in Table I. There was a significant drop in the concentration of serum chloride and increase in serum pH and carbon dioxide in the potassium-deficient animals in confirmation of previous observations (1–4).

*Data from Analyses of Rat Skeletal Muscle.*—Table II provides data from analyses of rat muscle for carbon dioxide, chloride, water, and the four principal cations. There was found to be no significant difference between the concentrations of carbon dioxide and chloride in the muscle of the control and experimental animals. There were significant increases of sodium, magnesium, and calcium, and a marked decrease of potassium in the muscle of the potassium-deficient animals as previously observed (5). The carbon dioxide content of the thigh muscle in the control rats showed a mean value of 11.96 ± 0.68 mM per kg. wet tissue, which is in good agreement with Wallace and Hastings' (12) value of 11.0 ± 1.3 mM per kg. for the leg muscle of the cat, and Conway and Fearon's (13) value of 10.6 ± 1.2 for the leg muscle of the rabbit.

Table III provides data on analyses of skeletal muscle for total, inorganic, and lipid phosphorus in normal and potassium-deficient rats. There was no

significant difference in the total phosphorus content of muscle between the control and experimental animals. There was nearly twice as much inorganic

TABLE I

*Mean Values for Serum in Control and Potassium-Deficient Rats*

The values are reported per kilogram of serum.

| No. of rats | | Weight | pH | $CO_2$ | Cl | $H_2O$ |
|---|---|---|---|---|---|---|
| | | *gm.* | | *m.eq.* | *m.eq.* | *gm.* |
| Control animals | | | | | | |
| 6 | | 388 | 7.42 | 27.4 | 94.0 | 940 |
| | S.E. | | 0.022 | 1.12 | 1.90 | 2 |
| Potassium-deficient animals | | | | | | |
| 8 | | 233 | 7.49* | 42.08* | 78.5* | 941 |
| | S.E. | | 0.004 | 1.44 | 1.74 | 1 |

S.E. = standard error of the mean = $\sqrt{\frac{\Sigma\Delta^2}{N(N-1)}}$

Figures marked with an asterisk (*) differ significantly ($P \leqq 0.02$) from values of corresponding control group.

TABLE II

*Mean Values for Skeletal Muscle in Control and Potassium-Deficient Rats*

The data are reported per kg. blood-free, fat-free, wet tissue for tissue water, and per 100 gm. blood-free, fat-free dry solids for all other constituents.

| Group | No. of rats | $CO_2$ | Cl | $H_2O$ | Na | K | Ca | Mg | Total cations |
|---|---|---|---|---|---|---|---|---|---|
| | | *m.eq.* | *m.eq.* | *gm.* | *m. eq.* | *m. eq.* | *m. eq.* | *m. eq.* | *m. eq.* |
| Control | 6 | 4.82 | 5.91 | 751 | 9.64 | 38.2 | 1.42 | 7.86 | 57.0 |
| S.E. | | 0.27 | 0.53 | 3 | 0.67 | 0.49 | 0.02 | 0.86 | 0.5 |
| K-deficient | 8 | 4.15 | 5.23 | 740 | 15.05* | 26.3* | 2.47* | 16.06* | 59.8 |
| S.E. | | 0.17 | 0.44 | 4 | 0.98 | 1.2 | 0.30 | 1.1 | 1.8 |

S.E. = standard error of the mean.

Figures marked with an asterisk (*) differ significantly ($P \leqq 0.02$) from values of corresponding control group.

phosphorus in the potassium-deficient muscle than was found in the control muscle. There was less lipid phosphorus in the potassium-deficient muscle when compared with the controls.

*Derived Data from Rat Serum and Skeletal Muscle Analyses.*—The Wallace-Hastings calculations (12) for intracellular pH have been applied to the original

TABLE III

*Mean Values for Phosphorus Fractions in Skeletal Muscle of Control and Potassium-Deficient Rats*

The data expressed per 100 Gm. dry solids.

| Group | No. of rats | Total P | Inorganic P | Lipid P |
|---|---|---|---|---|
| | | mM | mM | mM |
| Controls | 5 | 28.5 | 8.9 | 5.7 |
| S.E. | | 3.6 | 0.8 | 0.7 |
| K-Deficient | 6 | 27.4 | 15.0* | 3.5* |
| S.E. | | 1.3 | 0.3 | 0.07 |

S.E. = Standard error of the mean.

Figures marked with an asterisk (*) differ significantly ($P \leqq 0.02$) from values of corresponding control group.

TABLE IV

*Derived Data from Rat Serum and Skeletal Muscle*

Data were derived by the Wallace-Hastings calculation (12). Parentheses designate concentration per kilogram of serum, extracellular or intracellular phase, and whole tissue. Brackets designate concentration per kilogram of water of the particular compartment. Serum, extracellular and intracellular phases are referred to by the subscripts *s*, *e*, and *c* respectively.

| Group | No. of rats | $pCO_2$ | $[Cl]_s$ | $[Cl]_e$ | $(H_2O)_e$ | $[H_2CO_3]_e$ | $[HCO_3]_s$ |
|---|---|---|---|---|---|---|---|
| | | mm. Hg | M.eq. per kg. $H_2O$ | M.eq. per kg. $H_2O$ | gm. per kg. tissue | mm. per kg. $H_2O$ | M.eq. per kg. $H_2O$ |
| Control | 5 | 42.4 | 96.9 | 102.0 | 144.6 | 1.28 | 26.8 |
| S.E. | | 2.3 | 1.4 | 1.5 | 12.5 | 0.11 | 0.9 |
| K-deficient | 6 | 52.4** | 83.2** | 87.4*** | 147.8 | 1.67* | 42.06*** |
| S.E. | | 1.9 | 1.9 | 1.7 | 15.6 | 0.05 | 1.7 |

| | | $[HCO_3]_e$ | $(CO_2)_c$ | $[CO_2]_c$ | $[H_2CO_3]_c$ | $[HCO_3]_c$ | $pH_c$ |
|---|---|---|---|---|---|---|---|
| | | M.eq. per kg. $H_2O$ | Mm. per kg. tissue | Mm per kg. $H_2O$ | Mm. per kg. $H_2O$ | M.eq per kg. $H_2O$ | |
| Control | 5 | 28.2 | 7.7 | 12.66 | 1.41 | 11.24 | 6.98 |
| S.E. | | 1.0 | 1.02 | 1.50 | 0.15 | 1.49 | 0.08 |
| K-deficient | 6 | 44.2*** | 4.13* | 6.90* | 1.83* | 5.05** | 6.42*** |
| S.E. | | 1.8 | 0.80 | 1.52 | 0.10 | 1.31 | 0.05 |

S.E. = standard error of the mean.

Figures marked with asterisks differ significantly from values of corresponding control groups: $P = 0.05$-$0.02$ (*); $P = 0.02$-$0.001$ (**); $P \leqq 0.001$ (***).

data from serum and muscle analyses, as shown in Table IV. The nomenclature of the various extracellular and intracellular phases as used by these authors has been retained. Their paper should be consulted for details of the calcula-

tions. Mean values were not used in the calculations; the data for each animal were calculated all the way through, after which means were then determined.

The intracellular pH of the skeletal muscle of the normal rat was calculated to be 6.98 ± 0.08. Wallace and Hastings obtained a value of 6.93 ± 0.12 for the normal cat (12). The derived data further indicated that in the potassium-deficient rats the serum partial pressure of carbon dioxide ($pCO_2$) was greater than observed in the controls, as were the concentrations of $[H_2CO_3]$ and $[HCO_3^-]$ per kg. extracellular water and $[H_2CO_3]$ per kg. cell water. Conversely, there was observed in the potassium-deficient group a diminution in values for $[CO_2]$ and $[HCO_3^-]$ per kg. intracellular water. There was a fall of intracellular pH to 6.42 ± 0.05.

TABLE V

*Mean Values for Carbon Dioxide Content of Sacrospinalis Muscle Obtained at Operation for Ruptured Intervertebral Disc in Otherwise Normal Adult Males*

The data are reported per kilogram of wet tissue.

| No. of patients | $CO_2$ | Blood in muscle | $CO_2$ (blood-free) |
|---|---|---|---|
| | mM | gm. | mM |
| 5 | 12.16 ± 1.02 | 123.1 ± 17.0 | 9.46 ± 1.31 |

Mean ± standard error of the mean.

*Data from Analyses of Human Skeletal Muscle for Carbon Dioxide.*—Table V records information on muscle samples obtained at operation from five metabolically normal men. The values for muscle carbon dioxide content were found to be within the range which has been recorded by other investigators for carbon dioxide in the skeletal muscle of various animals (10, 12–17). As shown in Table V, the blood content of skeletal muscle introduces an appreciable correction which it is necessary to make in order to obtain the true carbon dioxide content.

## DISCUSSION

It must be emphasized that much of the derived data in Table IV depend upon the validity of two assumptions: (*a*) that the chloride ion does not enter muscle cells, and (*b*) that most of the total muscle carbon dioxide exists as the $HCO_3^-$ ion. Conway and Fearon (13) have criticized the Wallace-Hastings calculations on both counts, and have brought forward evidence suggesting that only a small quantity of total carbon dioxide in mammalian muscle exists as ionized $HCO_3^-$. By the calculations of the latter authors, the pH of mammalian muscle approximates 6.0, rather than the higher figure obtained by the Wallace-Hastings calculation. Since in the present experiment total muscle carbon dioxide was not fractionated by the technique of Conway and Fearon, it was

not possible to apply their calculations to these data. Several other workers have calculated the pH of skeletal muscle to be close to 7.0 (14–17).

The observations (18, 4) that potassium-deficient animals are constantly in negative chloride balance lend support to the hypothesis that there is not a massive movement of chloride into the muscle cell during potassium deficiency. Possibly the increase of inorganic phosphate in potassium-deficient muscle as measured in the present study serves to compensate for the diminution of intracellular $HCO_3^-$.

Tabulation of total cation concentration in the muscle of the control and potassium-deficient animals on a dry weight basis revealed close agreement between the two groups. In this experiment it would appear that total intracellular cation concentration was maintained in spite of reciprocal changes in the concentrations of potassium *vs.* sodium, magnesium, and calcium. These findings are consistent with the observations of Fenn and Haege (19) that excised frog muscles lose potassium in the presence of excess magnesium. Buell and Turner (20) found that the skeletal muscle of adrenalectomized rats lost magnesium in a fixed ratio to its gain in potassium. The balance studies of Orent-Keiles and McCollum (18) showed that rats on a potassium-deficient diet consistently retained more than twice as much magnesium as did the control rats. The same findings have been obtained in tomato plants grown in nutrient media containing varying concentrations of potassium (21). However, Cotlove and colleagues (22) found no increase of calcium and magnesium concentrations in the muscle of potassium-deficient rats. Whether these discrepancies are due to differences in the diets used or to analytic methods is not apparent.

## SUMMARY

Albino rats weighing 160 to 175 gm. were fed a complete synthetic diet containing 0.003 per cent potassium and 0.7 per cent sodium for 40 days. Controls were given the same diet plus adequate added potassium.

1. Data from analyses of serum and skeletal muscle showed (*a*) a fall in serum chloride concentration and an increase in serum carbon dioxide concentration and pH in the potassium-deficient rats; (*b*) increases of sodium, magnesium, and calcium and a decrease of potassium in the muscle of the potassium-deficient rats; (*c*) no change of muscle chloride or carbon dioxide concentrations in the potassium-deficient rats.

(2) Application of the Wallace-Hastings calculations to these data revealed (*a*) intracellular pH of the skeletal muscle of the normal rat to be $6.98 \pm 0.08$; (*b*) an increase in serum partial pressure of carbon dioxide ($pCO_2$) in potassium deficiency, together with increases in concentrations of $[H_2CO_3]$ and $[HCO_3^-]$ per kg. extracellular water and $[H_2CO_3]$ per kg. cell water; (*c*) a decrease in values for $[CO_2]$ and $[HCO_3^-]$ per kg. intracellular water; (*d*) a fall of intracellular pH in potassium deficiency to $6.42 \pm 0.05$.

(3) Analyses of sacrospinalis muscle from five men undergoing operation for ruptured intervertebral disc showed a mean value of 9.46 ± 1.31 mM carbon dioxide per kg. blood-free tissue.

Some problems of interpretation of data are briefly discussed.

## ADDENDUM

In a study of experimental potassium deficiency just published by Cooke and colleagues (23) it was also concluded that there is a transport of hydrogen into the cell in the alkalosis of potassium deficiency.

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