Magnesium, Potassium, and Sodium in "Red" and "White" Muscle in the Rat (37337)

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Skeletal muscles may be classified as "red" (dark) and "white" (pale) depending on their gross color. The difference in color is due largely to the content of myoglobin in the muscle fibers. Mammalian muscles contain varying mixtures of dark and pale fibers and, therefore, are best described as "predominantly red" or "predominantly white." In the rat, the soleus is a good example of the former type of muscle; the superficial portion of the medial head of the gastrocnemius is an example of the latter type.

Red and white fibers are morphologically and chemically dissimilar (1–3). The chemical dissimilarities include differences in the concentrations of potassium (K) and sodium (Na); predominantly white muscle contains more K and less Na than red (4, 5).

Potassium and also magnesium (Mg) are the most abundant intracellular inorganic cations in the soft tissues. The studies reported herein were undertaken to determine whether the distribution of Mg parallels that of K in the soleus and superficial portions of the gastrocnemius in the rat.

Materials and Methods. Two groups of male albino rats (Sprague–Dawley strain) (weights of younger rats, 100–120 g, and of older rats, 250–300 g) were used. The animals were anesthetized with ether and then exsanguinated by means of aortic puncture. The soleus, trimmed of all visible tendinous tissue, and an equivalent amount of the medial head of the gastrocnemius were excised from each leg within 10 min of the animal's death. When measured, the total water content and extractable solids were determined by drying the muscles to constant weight at 110° for 24 hr and then performing chloroform–methanol (2:1, vol/vol) extractions in Soxhlet apparatuses for 24 hr, using borosilicate thimbles. Both gastrocnemius muscles from one young animal were combined in one thimble and the soleus muscles in another. The soleus and gastrocnemius taken from one leg of an older rat were sufficient for both the water content and extractable solid determinations. Extracted and unextracted samples were transferred quantitatively to 5.0-ml volumetric flasks. To each flask, 2 ml of concentrated HNO_3 (reagent grade) was added, and the tissues were digested at 80 to 85°. After dilution to 5.0 ml with distilled-deionized water, the digests were stored in polyethylene tubes until analyzed.

Magnesium was determined by atomic absorption spectrophotometry; Na and K were determined by flame photometry (6). Appropriate quantities of HNO_3 were added to all standards and blanks. Recoveries of known amounts of Mg and K added to the digestion mixtures were 100.4 ± 1.8% and 99.3 ± 0.4%, respectively; this indicated that there was no interfering effect from matrix.

Unextracted muscles from one group of young rats served as controls for extracted muscles taken at the same time from a second group. The soleus and gastrocnemius from one leg of a large rat were used as unextracted controls for muscles excised from the other leg.

For analysis of the significance of differences in the results, either a two-tailed t test or a one-tailed Mann-Whitney test (if the variance of one group was greater than that of the other by four times or more) was used.

Results. The distribution of Mg, K, Na, and water in soleus and gastrocnemius muscles is shown in Table I. The total water content of the gastrocnemius and of the soleus decreased slightly with age ($p < 0.005$ for soleus; $p < 0.001$ for gastrocnemius). The amount of extractable solids was the same in red and pale muscle; therefore, none of the disparities in composition of inorganic substances between the two can be attributed to variations in the content of lipid or other extractable substances. Combining the data for the soleus and gastrocnemius in rats of each group, the quantity of extractable solids was 6.8 ± 1.5% in the younger rats compared to 4.4 ± 1.6% in the older rats (for significance of the difference, $p < 0.01$).

Regardless of age, the concentrations of Mg and K in predominantly red muscle were less than those in white muscle in every rat studied. The reverse was true for Na. Chloroform–methanol extraction had no significant effect on the concentration of Mg; however, 20 to 35% of the total K and 20 to 29% of the total Na were lost from the soleus and gastrocnemius. Extraction of large amounts of Na and K was most likely due to methanol, a polar solvent, since no such loss was observed in preliminary studies using petroleum ether. On the other hand, the ether extractions were quantitatively erratic. Presumably, Mg was not lost from the samples because it is much more tightly bound than the monovalent cations.

Discussion. The distribution of Mg in rat skeletal muscle parallels that of K and constitutes yet another chemical difference between predominantly red and pale muscle. The concentrations reported herein span the range of the more reliable determinations on rat muscle (of undesignated type) that have been reported previously by others (7).

The quantitative aspects and the more important physiochemical functions of Mg in somatic muscles have been discussed in a recent review article (8). In the rat, about 98% of the total muscle Mg is intracellular; this is based on the data in Table I, a plasma level of 0.8 μmole/liter, an ultrafilterable proportion of Mg of about 75% (9), and an extracellular fluid space amounting to 7–12% of the wet weight of muscle (4). Mitochondria are reported to contain relatively large amounts of Mg (10, 11), though significant contamination from sucrose solutions used as density gradients may occur. According to one estimate (12), most of the intracellular Mg in muscle is bound to creatine phosphate, ATP, and myosin. The biochemical importance of Mg in the transmembranous trans-

TABLE I. Distribution of Mg, K, and Na in Soleus and Superficial Fibers of Gastrocnemius in Rats.

	Muscle	Rat groups	
		100–120 g	250–300 g
Total water (% wet wt)[a]	Soleus	77.8 ± 0.9 (8)	76.5 ± 0.4 (6)
	Gastrocnemius	77.2 ± 0.3 (8)	75.8 ± 0.2 (6)
Extractable solids (% dry wt)[a]	Soleus	6.9 ± 1.6 (4)	4.4 ± 2.4 (3)
	Gastrocnemius	6.6 ± 1.5 (4)	4.4 ± 0.8 (3)
Magnesium (μmoles/g dry wt)[a]	Soleus	42.7 ± 1.5 (16)	42.2 ± 1.4 (10)
	Gastrocnemius	54.6 ± 1.8 (16)[b]	53.1 ± 1.6 (10)[b]
	Soleus/gastrocnemius	0.782 ± 0.018 (16)	0.795 ± 0.028 (10)
Potassium (μmoles/g dry wt)[a]	Soleus	444 ± 20 (16)	426 ± 23 (10)
	Gastrocnemius	506 ± 19 (16)[b]	506 ± 9 (10)[b]
	Soleus/gastrocnemius	0.880 ± 0.032 (16)	0.842 ± 0.042 (10)
Sodium (μmoles/g dry wt)[a]	Soleus	136 ± 16 (15)	138 ± 18 (10)
	Gastrocnemius	84.4 ± 10.1 (15)[b]	80.9 ± 9.0 (10)[b]
	Soleus/gastrocnemius	1.61 ± 0.15 (15)	1.73 ± 0.33 (10)

[a] Mean ± SD (no. of rats).

[b] For significance of difference between soleus and gastrocnemius, $p < 0.001$.

port of Na and K, in the activity of actomyosin ATPase, in the "Ca pump" of the sarcoplasmic reticulum, and as an activator of numerous enzyme systems *in vitro*—particularly those involved in transphosphorylation—is well-established.

It is reasonable to assume that the disparity in the concentration of Mg between predominantly red and white fibers is related to their other morphologic or chemical differences or both.[1] Compared to pale muscle, red muscle has more mitochondria and a high content or activity of those enzymes that participate in lipid and oxidative metabolism (1–3). Based on these observations, one might expect to find higher concentrations of Mg in red muscle. On the other hand, myosin ATPase and muscle phosphorylase activities are greater in white fibers; and, while ATP, ADP, and AMP levels are about the same in both types of muscle, the creatine phosphate content of white fibers is almost twice that of red fibers (3). These data, particularly the figures on creatine phosphate, may explain the difference in the distribution of Mg.

The results for total water, Na, and K in older rats are in general agreement with previous reports (4, 5, 13). Despite the similarity in total water content, the inulin space of predominantly red muscle is larger than that of white muscle (4, 13), partly as the result of the greater vascularity of red fibers (1, 3). This variation in extracellular fluid space, however, does not account for the concentrations of Na and K. The estimated content of intracellular K is less and that of Na is greater in red than in white fibers (4), presumably because of differences in the relative permeability of the sarcolemma to these ions in the two types of muscle (5). Predictably, the resting membrane potential and amplitude of the action potential are greater in white fibers (5).

Many of the physiologic responses and enzymatic patterns of predominantly red and, particularly, of white muscle can be reversed by exchanging their nerve supplies (14). Whether cross innervation has a similar effect on the distribution of Mg, Na, and K has not been determined.

Summary. The concentrations of Mg, K, and Na were measured in the soleus ("red muscle") and superficial fibers of the medial head of the gastrocnemius ("white muscle") in rats weighing 100–120 g and 250–300 g. The concentrations of Mg and K were significantly lower and the concentration of Na was significantly greater in the soleus than in the gastrocnemius.

Addendum. Recent data on the unequal distribution of calcium (μmole/g, dry wt) (atomic absorption spectrophotometry) between red and white muscle are as follows (mean ± SD): for eight younger rats, soleus = 4.24 ± 0.32 and gastrocnemius = 5.65 ± 0.42 ($p < 0.001$); for eight older rats, soleus = 2.92 ± 0.29 and gastrocnemius = 5.02 ± 0.27 ($p < 0.001$). With aging, the calcium concentration decreased in both red ($p < 0.001$) and white ($p < 0.005$) muscle.

The technical assistance of Mr. Rudy D. Hanson is acknowledged.

[1] The third major group of fibers histochemically "intermediate" between red and white fibers will not be considered here, since there is not sufficient quantitative information available for comparisons (3).

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Received Feb. 8, 1973. P.S.E.B.M., 1973, Vol. 143.