Sodium and Potassium Contents of Frog Muscle after Extraction in 50% Glycerol.* (23607)

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It is well known that extraction of muscle in 50% glycerol leaves the contractile mechanism of the muscle intact but destroys the electrical excitability(1). The myosin itself, however, will still contract when exposed to ATP. It was of interest to discover to what extent this treatment also destroyed the ability of the muscle to concentrate potassium.

Method. Experiments were carried out with the assistance of Mrs. Sidsel Aksnes of Oslo, Norway. For this purpose a variety of small muscles were dissected from frogs (*Rana pipiens*). All the different muscles appeared to give the same results and they were used interchangeably. At first each muscle was tied to a glass frame to keep it at constant length during the glycerol extraction but this proved to be an unnecessary refinement. The duration of the extraction period was varied from 12 hours to several days without causing any change in the results. The muscles were weighed on dissection, after extraction and after subsequent incubation in the desired solutions. Changes in weight did not alter the significance of the results which are presented in terms of final weights. For analysis the muscles were either dry-ashed in platinum crucibles at 500°C or wet-ashed in nitric acid. The resulting solutions after suitable dilution were analyzed for Na and K on a Coleman flame photometer.

Results. The results, some of which are summarized in Table I, showed to our surprise that these muscles not only failed to concentrate potassium but they actually contained more Na than K even when the concentrations of both of these ions in the solution were practically zero. The ratio of Na/K varied from 1.32 to 3.9. Since this result might not represent a true equilibrium and might depend, therefore, upon the rates of extraction, it seemed worthwhile to re-equilibrate these glycerol extracted muscles in solutions containing equal concentrations of Na and K and to determine again the amounts retained. This has the added advantage that the glycerol is removed from the muscle in the process of equilibration so that the result cannot be influenced in any way by its presence. These results are shown in Table II. In all cases it is found that more Na than K is retained in the muscles. The smaller the concentrations of Na and K in the solution the greater, in general, was the Na/K ratio in the muscle. The muscles lost 20-35% of their initial weight during the process of extraction and subsequent equilibration in the KCl-NaCl solution. The total concentration inside the muscle is higher than that in the solution except in the higher concentrations, where the reverse is true. While the detailed and quantitative interpretation of these fig-

TABLE I. Sodium and Potassium Contents of Muscles Extracted in 50% Glycerol.

Time, days	No. of muscles	K, meq/kg	Na, meq/kg	Na/K
1	3	4.32	6.57	1.52
2	1	4.63	10.04	2.18
4	2	2.90	3.82	1.32
1	2	3.15	12.3	3.90

Calculations based on final weights after equilibration.

TABLE II. Average Na and K Contents of 50% Glycerol-Extracted Muscles when Equilibrated with NaCl + KCl Solutions of Equal Concentration.

Solution, conc. of KCl and of NaCl, meq/l	No. of analyses	Time, hr	K, meq/kg	Na, meq/kg	Na/K
55	8	.16-5.0	50.5	57.3	1.14
33	5	1-5	34.0	54.6	1.60
11	5	1-5	23.5	48.2	2.05
5.5	7	.25-2.0	16.5	26.2	1.59
2.75	3	.25-1	8.18	23.6	2.89

All calculations based on final weights after equilibration.

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TABLE III. Na/K Ratios in Glycerol-Extracted Muscles under Different Conditions.

Condition	No. of muscles	Conc. in solution, meq/l Na_o	Conc. in solution, meq/l K_o	Time, hr	Ratio in muscle $\frac{Na_i/Na_o}{K_i/K_o}$ (S.E.)
10% muscle homogenate	7	15.3	18.2	.01–.18	1.49 ± .14
20 mg % ATP	4	11	11	.1 –.25	1.28 ± .07
pH 7	2	12	11	2	1.25
pH 6	2	12	11	2	1.50
pH 7	2	6	5.5	2	1.50
pH 6	2	6	5.5	2	1.59
2 mM $CaCl_2$	2	11	11	1 – 2.5	1.62
3 mM $MgCl_2$	2	11	11	1 – 2.5	1.18
Control	4	11	11	2	1.52

ures must await further experiments, it is abundantly clear now that under the conditions of these experiments at least there is no tendency for the myosin to prefer K to Na as a cation. To support the theory of preferential combination with K it will be necessary to show how the extraction with glycerol has altered the binding sites on the protein so that Na is preferred to K.

Some additional data are shown in Table III. It was thought that possibly the inability to concentrate K after glycerol extraction depended upon the loss of some normal muscle constituent. In an effort to replace this loss the muscles after glycerol extraction were incubated in a 10% muscle homogenate which had been cleared by centrifugation and to which equal amounts of KCl and NaCl had been added. After varying periods up to 18 hours the muscles were analyzed as well as the solutions. On the average the ratio of (Na_i/Na_o) (K_i/K_o) was found to be 1.44 ± 0.11. Evidently the ability to concentrate K was not appreciably restored by this treatment. Similarly no appreciable effect was obtained by a brief equilibration in a KCl-NaCl solution to which 20 mg % ATP had been added. Here the Na/K ratio in the muscle was 1.23 ± 0.08. Longer periods of equilibration might have proved more effective but were not tried because of the ATP-ase activity of the muscle.

In the last experiments of Table III it was found that the Na/K ratio was greater at pH 6 than at pH 7 and greater at lower concentrations of NaCl-KCl. Phosphate buffers were used for the pH control. None of these variations seemed to destroy the preference of the myosin for Na. $CaCl_2$ and $MgCl_2$ were also ineffective.

In an earlier preliminary study from this Laboratory an abstract was published(2) reporting a preferential combination of myosin with potassium. Later the same year in endeavoring to repeat this finding 3 experiments were tried in which a well washed preparation of myosin was equilibrated with a solution containing .095M KCl and .096M NaCl. After equilibration the protein was centrifuged out and the supernatant was analyzed. The ratio of Na/K in the protein was found to be 1.09. The result is not conclusive since a small excess of Na over K might have been present in the original protein in spite of careful washing although a few analyses of the final protein residue revealed nearly the calculated amounts. Some dialysis experiments were also tried in which the myosin was contained in and was dialyzed against the same KCl-NaCl solution used above. Analyses of the inside and outside solutions after several days revealed an inside/outside concentration ratio of 1.0 for K and 1.03 for sodium. Here again there was perhaps a slight tendency to concentrate sodium rather than potassium.

Some similar experiments were tried (in 1942) with a preparation of a structural protein, renosin, extracted from kidney as described by Szent-Györgyi(3). After careful washing of this protein in H_2O to remove electrolytes it was added to 4 vols. of a solution containing .095M KCl and 0.096M NaCl and allowed to equilibrate for 3 hours. Some of the protein dissolved but the remainder was

TABLE IV. Kidney Protein (Renosin) Suspended in a Solution of .095 M KCl and 0.096 M NaCl Showing Preferential Absorption of Na Relative to K.

Calculated in original protein		In original supernatant		In final protein		In final supernatant		Na_p/Na_s
K	Na	K	Na	K	Na	K	Na	K_p/K_s
2	8	760	768	67	88	695	688	1.22
34	43	760	768	40	64	754	747	1.62
(−10)	30	380	384	38	56	332	358	1.38
10	53	380	384	42	64	348	373	1.43
Mean Na/K 3.8		1.02		1.46		1.01		1.41

Figures for Na and K represent amounts in meq, not concentrations.

then centrifuged out and analyses were made on both the supernatant and the protein residue. The differences between the amount of Na or of K added in the solutions and the sum of the amounts found in the supernatant and residue were taken to be the amounts present in the original washed protein solution. All four experiments gave the same result (Table IV). On the average the Na/K ratio in the original protein was 3.8, the amounts of Na and K being 5.8 and 1.8%, respectively, of the amounts subsequently added. After equilibration 11.8% of the Na and 8.2% of the K were in the protein residue, the Na/K ratio being 1.46. This result confirms even more clearly the conclusion that this protein, like myosin, prefers Na to K. It is concluded that the published abstract was in error for unknown reasons.

Evidence for a preference of myosin for Na as compared to K is not new. Steinbach (4) found data of this sort in muscle homogenates. Szent-Györgyi(5) on the other hand emphasized the general similarity of K and Na in reactions with myosin although his study of the effects of KCl and NaCl on phosphatase activity of myosin might be taken to indicate slightly greater binding of Na at lower concentrations. (See Fig. 35 p. 54 in first edition 1947). Lewis and Saroff(6) however measured association constants of myosin for Na greater than those for K. J. J. Blum and J. Duke (personal communication) have shown that Na is bound to myosin more strongly than K and when so bound it inhibits the ATP-ase activity of the myosin. Studies of electrophoretic mobility of myosin by Miller *et al.*(7) support the same conclusion. All of these results were obtained with extracted myosin or homogenized muscle. The data reported in this paper are the first in a more or less intact muscle which show the same result.

It is difficult to reconcile all these findings with the theory of Ling(8) which predicts fixed anion sites on myosin with preference for K on account of its smaller effective diameter. Certainly if this is true the myosin must be drastically modified by the process of glycerol extraction or by isolation from the muscle. It seems more likely that destruction of the muscle membrane destroys also the ability to concentrate potassium or to "pump out" the sodium.

Summary. After extraction in 50% glycerol frog muscles retain more sodium than potassium. If they are then immersed in solutions containing equal concentrations of KCl and NaCl the muscles are found to contain again more sodium than potassium. The ability of the normal muscle to concentrate potassium seems therefore to depend upon the intactness of the membrane or the presence of some specific transport mechanism.

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