THE ALLEGED PRESENCE OF "BOUND POTASSIUM"
IN MUSCLE

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The importance of potassium in maintaining muscle tone was considered in a publication by Neuschloss in 1923 (1). Muscles of toads and rabbits, after remaining for a short period of time in potassium-free Ringer's solution, lost their physiological tone and their ability to contract. In 1924 Neuschloss (2), published a method for determining the amount and condition of the potassium in the muscle. He related the potassium held by the muscle, when it was placed in potassium-free Ringer's solution, to muscle tone. The greater part of the potassium was found in the solution and only a small part, designated as "bound potassium," was held by the muscle. In later publications (3) Neuschloss showed variations in the amount of bound potassium in red and white muscle of rabbits, the relation of varying quantities of NaCl, KCl, and CaCl₂ to bound potassium, the effect of electrical and chemical stimulation, etc.

In 1927 Raab (4) attempted to repeat Neuschloss' method of determining bound potassium. He modified Neuschloss' method by using a smaller volume of salt solution, calculating his results on a wet weight basis, and coagulating the protein during the determination. With this procedure Raab was unable to confirm Neuschloss' results and concluded (1) that the loss of potassium by muscle placed in isotonic salt solution followed the laws of diffusion; (2) that loss of function of muscle was not related to bound potassium as determined by Neuschloss' method. Neuschloss (5) criticizing Raab's work, contended that the accumulation of acids, autolytic action, and the small volume of salt solution used, were the causes of failure to confirm his results.
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Raab's work was upheld by Höber (6) who showed that the small volume of salt solution did not explain the variation. The presence of acid hindered rather than aided the loss of potassium by the muscle. Stirring the solution, and varying the size of muscle particles changed the amount of potassium found in the solution. Höber confirmed Raab's contention that muscle tone and bound potassium are unrelated.

In connection with studies of inorganic ion changes in muscle, our attention was called to Neuschloss' work. Attempts to

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Conditions</th>
<th>Initial value</th>
<th>Mg. K per 100 gm. dry tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 hr.</td>
</tr>
<tr>
<td>1</td>
<td>Small amount of saline</td>
<td>18.2</td>
<td>7.0</td>
</tr>
<tr>
<td>2</td>
<td>30 cc. saline per gm. wet tissue</td>
<td>14.9</td>
<td>3.0</td>
</tr>
<tr>
<td>3</td>
<td>60 cc. saline per gm. wet tissue</td>
<td>15.5</td>
<td>2.2</td>
</tr>
<tr>
<td>4</td>
<td>Muscle cut as usual. 300 cc. saline changed frequently</td>
<td>18.7</td>
<td>4.5</td>
</tr>
<tr>
<td>5</td>
<td>Muscle cut in very small pieces. 300 cc. saline changed frequently</td>
<td>19.0</td>
<td>2.4</td>
</tr>
<tr>
<td>6</td>
<td>30 cc. saline per gm. wet tissue at 5°</td>
<td>15.7</td>
<td>4.9</td>
</tr>
<tr>
<td>7</td>
<td>30 cc. saline per gm. wet tissue at 20°</td>
<td>12.0</td>
<td>3.4</td>
</tr>
</tbody>
</table>
determine bound potassium by Neuschloss' procedure were unsuccessful and the following results support the contention of Raab and Höber.

EXPERIMENTAL

The gastrocnemii muscles of rabbits were cut into small pieces and each muscle placed in isotonic salt solution (0.9 per cent). Samples of the muscles were taken at the beginning of the experiment and usually at the end of 1, 3, 6, 12, 18, and 24 hours. The samples were placed in vitreosil crucibles, and dried at 100° to constant weight. The weighed, dried tissue was treated with a small amount of 4 N sulfuric acid and ashed in a muffle furnace at a temperature of approximately 600°. Shohl and Bennett's method (7) of determining the amount of potassium was used. Potassium values were expressed as mg. of potassium per 100 gm. of dried tissue.

The values obtained (Table I, Experiment 1) for the amount of bound potassium were higher than those given by Neuschloss (1.2 as against 0.36). On increasing the quantity of saline to 30 cc. and 60 cc. per gm. of wet muscle, the values for the bound potassium were much lower (Table I, Experiments 2 and 3) and the values varied considerably. Having secured a change in bound potassium by increasing the amount of saline, large quantities (300 cc.) with frequent changes of saline were tried (Table I, Experiment 4). On cutting the muscle into very small pieces (Table I, Experiment 5) the bound potassium values decreased more rapidly and only a trace remained in the muscle at the end of 12 hours. Such a change in the amount of potassium was not expected as Neuschloss found that regardless of the size of the particles and the amount of saline used, the values for the bound potassium remained the same after the 6th hour until autolysis began.

To decide the importance of autolysis, the gastrocnemii were cut into small pieces and each muscle placed in saline, the proportion 30 cc. of saline to 1 gm. of muscle being used. One solution containing the small pieces of one gastrocnemius was placed in the ice box and kept at a temperature of 5°. The solution containing the opposite muscle was kept at room temperature (20°). From the results (Table I, Experiments 6 and 7) it would appear
that autolysis plays little part for the first 24 hours in the potassium leaving the muscle under the experimental conditions.

SUMMARY

The results obtained by using Neuschloss' method for determining bound potassium were unsatisfactory. The decrease in bound potassium in muscle tissue placed in isotonic saline is related to the amount and frequency of change of isotonic salt solution and to the size of the pieces of muscle tissue. Autolysis for a period of 24 hours did not apparently affect the diffusion of potassium into the solution. The present experiments furnish no evidence for the existence of bound potassium in muscle tissue.

BIBLIOGRAPHY

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