THE SELECTIVE ABSORPTION OF POTASSIUM BY ANIMAL CELLS.

III. The Effect of Hydrogen Ion Concentration upon the Retention of Potassium.

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The amphoteric reaction of inactive muscle is altered during contraction to an acid reaction. Fletcher and Hopkins (1) showed that in dying muscle there is an increase in acidity up to the point of death, final loss of irritability, after which the lactic acid content remains nearly constant. Salkowski (2), as early as 1890, stated that muscle produces lactic acid only as long as it is alive.

In resting muscle the per cent of lactic acid is very small, probably close to zero (1). After a few preliminary observations, Ritchie (3) stated that resting muscle is more alkaline than pH 7.5, probably between 7.6 and 7.8. Porcelli-Titone (4), quoted by Ritchie, found a pH of 7.8 for resting skeletal muscle, and a pH of 7.2 for fatigued muscle. In 1914, Pechstein (5) found the juice of muscles stimulated to fatigue to be almost neutral, pH 6.8 to 7.0.

Marcuse (6) found an increase in lactic acid as a result of stimulating to fatigue the excised muscle of the frog. It was pointed out in the first paper of this series (7) that in such a condition of fatigue, potassium is lost in large amounts when the cells are bathed in a potassium-free Ringer solution. As much as half of the cell potassium may be lost in about 5 hours. The work by Marcuse, done in 1886, was confirmed in 1907 by Fletcher and Hopkins (1). Later work on this subject has been done recently by Ritchie, who has again shown that if muscle is stimulated to fatigue there is a slow rise in acidity.

The lactic acid produced by muscle contraction may alter the equilibrium of acids and bases within the cell. Ritchie states that the lactic acid involved in muscle contraction is not present as such within the cell, except for a moment. It is only when an impaired condition of the muscle is brought about that an increase in acidity becomes detectable.

Gray (8) has shown that living cells, trout eggs, react to acids by taking up the hydrogen ion and releasing an equivalent amount of cation. If such cells are exposed to a KCl solution of toxic strength, a recovery may be brought about by treating the cells with an acid solution. Loeb (9) has shown that before potassium can pass through the membrane of a *Fundulus* egg, a trace of salt or acid must be present.

Inasmuch as a condition of acidity develops in a working or fatigued muscle, it was thought that hydrogen ion concentration might be the causative agent of the processes by which potassium is retained or set free in cells. The following experiments were devised to test out this possibility.

EXPERIMENTAL.

The bull frogs used in the following experiments were caught in a swampy brook not far from the laboratory. Only healthy specimens were retained for the work. At the laboratory the frogs were well cared for, as evidenced by the fact that they remained active and in good condition. No case of red-leg developed on any of the frogs.

The frogs were caught at various times during the summer, and no frog was used that had been kept at the laboratory for over 2 or 3 months. The frogs used were practically all of the same species, *Rana catesbiana* Shaw (10). Measurements from nose to anus varied from 4 to 6 inches; from nose to toes, with legs stretched out, from 9 to 12 inches. The weight varied from about 100 to 260 gm., averaging 150 gm.

Perfusion solutions of the following composition were employed:

NaCl	6.70 gm.
$CaCl_2$	0.25 "
Water	to 1 liter.

Perfusions were made through a small glass cannula in the dorsal aorta, the frogs having been previously killed by pithing.

At first some difficulty was experienced in adjusting the perfusion solutions with $NaHCO_3$ to the desired constant pH. It was found that after short intervals, 1 hour or so, a noticeable change in the pH had taken place. For example, one solution adjusted to a pH of 7.0 changed in 1 hour to a pH of 7.6, while another changed from a pH of 7.0 to a pH of nearly 8.0 in the same time. The explanation is doubtless to be found in the fact that CO_2 is liberated according to the equation

$$NaHCO_3 = CO_2 + NaOH$$

The CO_2 escapes, leaving the solution more alkaline. In an attempt to overcome this difficulty a lot of perfusion solution was made up and adjusted to a pH of 7.0 with a 1 per cent solution of Na_2CO_3, in place of the customary 1 per cent $NaHCO_3$. After standing at room temperature for nearly 3 hours, the pH of the solution was still at 7.0. Consequently all perfusion solutions used in this work were adjusted by means of a 1 per cent solution of Na_2CO_3. After one of the 16 hour perfusions, it was found that the pH of the perfusion solution had changed only a very little, from pH 6.5 to pH 6.6.

In every case the right branch of the dorsal aorta was tied off, after the right gastrocnemius had been washed free from blood. This muscle was then removed and used as the control muscle. Perfusion was continued through the left branch of the dorsal aorta for the designated period of time. The left muscle was then removed and used as the experimental muscle. These excised muscles were dried to constant weight in ether vacuum over sulfuric acid, then digested with strong acid, and were subsequently analyzed for potassium in accord with the method of Clausen (11). All the results herein presented are based on the analyses of frog gastrocnemius muscle.

Perfusions were carried out using solutions the pH of which varied from 6.0 to 8.0. The duration of the perfusions varied from 30 minutes to 16 hours. These limits of time and of pH seem sufficient to warrant the conclusions drawn in the subsequent pages. All perfusion solutions were adjusted to the desired pH with 1 per cent solution of Na_2CO_3 by use of the indicators of Clark and Lubs (12). After some practice one becomes quite proficient in comparing the colors of solutions with colors shown in the chart of Clark's book.

The following six tables show, in condensed form, the results obtained in this work.

TABLE I.

Loss of Potassium from Muscles Perfused for 30 Minutes with a Potassium-Free Ringer's Solution.

Sex.	pH of solution.	K in moist perfused muscle.	K in moist control muscle.	K in solids of the control muscle.	K in solids of the perfused muscle.	Apparent loss of potassium.*
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
F.	6.0	0.291	0.315	1.685	1.626	3.50
M.	6.5	0.248	0.321	1.649	1.582	4.06
"	7.0	0.214	0.325	2.000	1.940	3.00
"	7.4	0.324	0.381	1.897	1.830	3.53
"	8.0	0.271	0.330	1.655	1.602	3.20

* Figures in this column are obtained by computing the difference between potassium in solids of control muscle and in those of perfused muscle as per cent of the potassium in the solids of the control muscle.

TABLE II.

Loss of Potassium from Muscles Perfused for 1 Hour with a Potassium-Free Ringer's Solution.

Sex.	pH of solution.	K in moist perfused muscle.	K in moist control muscle.	K in solids of the control muscle.	K in solids of the perfused muscle.	Apparent loss of potassium.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
F.	6.0	0.256	0.313	1.850	1.737	6.11
"	6.5	0.285	0.378	1.968	1.858	5.59
M.	7.0	0.229	0.361	2.310	2.180	5.62
"	7.4	0.264	0.381	1.986	1.874	5.64
F.	8.0	0.279	0.333	2.025	1.909	5.72

TABLE III.

Loss of Potassium from Muscles Perfused for 2 Hours with a Potassium-Free Ringer's Solution.

Sex.	pH of solution.	K in moist perfused muscle.	K in moist control muscle.	K in solids of the control muscle.	K in solids of the perfused muscle.	Apparent loss of potassium.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
F.	6.0	0.268	0.333	1.700	1.600	5.88
"	6.5	0.330	0.361	1.863	1.719	7.72
"	7.0	0.268	0.365	1.823	1.705	6.47
"	7.4	0.267	0.355	2.180	2.060	5.50
"	8.0	0.285	0.331	1.652	1.581	4.30*
M.	8.0	0.228	0.336	1.918	1.813	5.47

TABLE IV.

Loss of Potassium from Muscles Perfused for 4 Hours with a Potassium-Free Ringer's Solution.

Sex.	pH of solution.	K in moist perfused muscle.	K in moist control muscle.	K in solids of the control muscle.	K in solids of the perfused muscle.	Apparent loss of potassium.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
F.	6.0	0.285	0.346	1.784	1.628	8.74
"	6.5	0.272	0.366	1.899	1.713	9.79
M.	7.0	0.234	0.345	1.910	1.721	9.90
F.	7.4	0.276	0.365	1.839	1.703	7.39
"	8.0	0.261	0.348	1.725	1.572	8.87

TABLE V.

Loss of Potassium from Muscles Perfused for 8 Hours with a Potassium-Free Ringer's Solution.

Sex.	pH of solution.	K in moist perfused muscle.	K in moist control muscle.	K in solids of the control muscle.	K in solids of the perfused muscle.	Apparent loss of potassium.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
F.	6.0	0.241	0.331	1.792	1.510	15.74
"	6.5	0.239	0.362	1.941	1.617	16.60
"	7.0	0.245	0.337	1.669	1.411	15.40
"	7.4	0.229	0.309	1.521	1.363	10.40*
"	8.0	0.238	0.343	1.700	1.430	15.90
M.	7.4	0.235	0.329	1.660	1.400	15.66

TABLE VI.

Loss of Potassium from Muscles Perfused for 16 Hours with a Potassium-Free Ringer's Solution.

Sex.	pH of solution.	K in moist perfused muscle.	K in moist control muscle.	K in solids of the control muscle.	K in solids of the perfused muscle.	Apparent loss of potassium.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
M.	6.0	0.181	0.347	1.803	1.484	17.70
"	6.5	0.235	0.343	1.821	1.525	16.25
—	7.0	—	—	—	—	—
F.	7.4	0.210	0.317	1.675	1.387	17.19
"	8.0	0.190	0.327	1.588	1.333	16.05

A careful record was kept in regard to the sex of each frog in order to determine, if possible, whether or not the potassium content differed in the two sexes. The analytical results show that the amount of potassium in frog muscle apparently has no definite relation to the sex of the animal. The figures showing the per cent of potassium in the solids of fresh muscle overlap so much that no definite statement can be made. There is a tendency, however, for the figures of the male frogs to be higher than those of the female frogs.

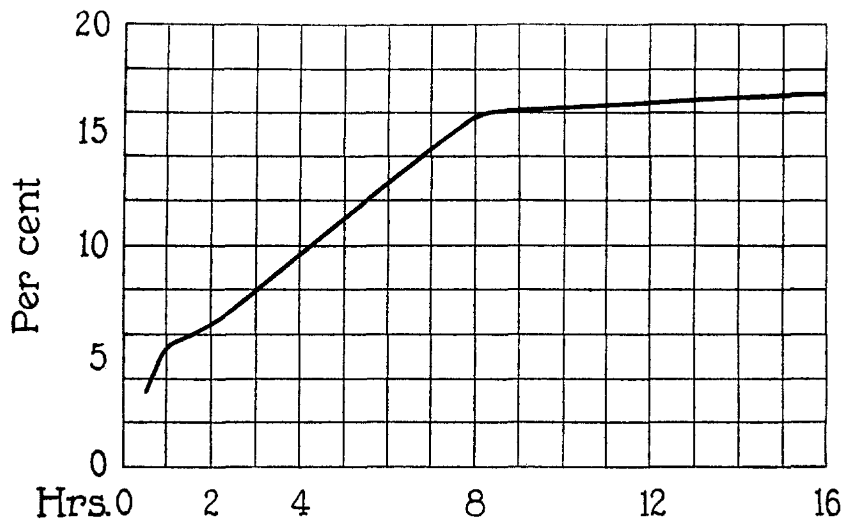

FIG. 1. The chart is a composite of five charts (not shown here) and is presented simply to show the average loss of potassium in chart form. The separate curves, showing the loss of potassium at differing degrees of pH, are so nearly identical that this composite curve will suffice.

It will be noticed that there is a steady loss of potassium up to 8 hours, after which there is little or no loss. The remainder of the potassium is tenaciously held until the muscle becomes fatigued.

The lowest figure was found among the female frogs, 1.521 per cent; the highest among the male frogs, 2.310 per cent. The average for the females was 1.794 per cent; for the males, 1.874 per cent. According to these two figures the muscles of the male frogs are 4.26 per cent richer in potassium than those of the female frogs.

The average amount of potassium in frog muscle is 0.34 per cent, according to Fahr (13). For the species used in this work, *Rana* *catesbiana*, the average is 0.343 per cent. This figure is based on the per cent of potassium in the fresh moist muscle, as is the figure of Fahr. The average amount of potassium in the solids of frog muscle was found to be 1.822 per cent. The minimum per cent of potassium in the fresh moist muscle was found in this work to be 0.309 per cent; the maximum was found to be 0.381 per cent. This is a difference of 18.9 per cent, and shows the necessity of expressing the per cent loss of potassium, due to perfusion, on a basis of dried solids. This is true because the per cent of water in the muscles varies even for the corresponding muscles of the same frog. As pointed out in the first paper of this series, the analytical error is small, in this work averaging 1.25 per cent.

In the two cases, starred in Tables III and V, it will be noticed that the per cent loss of potassium falls somewhat lower than the other figures in the respective columns. But it should be further noticed that the actual amount of potassium in the muscle was lower in each case than is usually found. It is also interesting to note that in each case the frog was a female. Perhaps during the production of eggs the mobile reserve of potassium in the muscle is somewhat depleted. Further work on this phase of the subject would be interesting.

At the end of each experiment the muscles showed good irritability, both to mechanical pinching of the nerves supplying the muscles and to direct and indirect electrical stimuli. After being excised the muscles were seen to quiver, thus indicating that they were far from being dead.

SUMMARY.

By perfusing frogs for varying periods with potassium-free Ringer solutions having a pH ranging from 6.0 to 8.0, it has been determined that such solutions have little or no effect upon the retention of potassium by muscle cells.

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