THE SELECTIVE ABSORPTION OF POTASSIUM BY ANIMAL CELLS.

I. Conditions Controlling Absorption and Retention of Potassium.

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The accumulation of potassium with little or no sodium in the cell living in a medium richer in sodium than in potassium is so widespread a phenomenon as to be considered a general attribute of living cells. In the few exceptions on record the distribution of sodium between cells and tissue fluids has not been satisfactorily investigated. The mechanism governing potassium selection has not been explained. Loeb (1) as early as 1906, stated, "we may take it for granted that, at least, potassium forms a non-dissociable constituent of the protoplasm of a number of tissues of animals and plants." The view that much of the potassium of the cell is not dissociated seems generally accepted. Robertson (2), for example, uses the selective action of cells for potassium as one of the proofs of the dissociation of protein salts so as to hold inorganic constituents as a part of protein ions without the formation of free inorganic ions. Such a theory explains very well the retention of accumulations of potassium in the cells but it gives no clew as to why potassium is "selected" instead of sodium nor, in the face of the apparent impermeability of normal cells to electrolytes, does it explain how potassium makes its way into growing cells. Our investigations bear on such aspects of the problem.

It seemed of first importance to find some of the limiting conditions for the retention of potassium. The work of Loeb (3), showing the so called salt effect as necessary for both the inward and outward diffusion of potassium from the fertilized eggs of *Fundulus*, does not apply to such a structure as a muscle cell. It does show that the presence of a certain amount of salt or of acidity or both is necessary

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for the passage of potassium through the egg membrane and that sufficiently prolonged action of pure water makes this membrane very impermeable to potassium. But, as Loeb points out, the behavior of the hatched embryo is very different from that of the egg. In the egg we are dealing merely with the passage of potassium through a comparatively distinct membrane but in the case of most cells we must consider the taking up of potassium, whatever that means physicochemically, by the cell acting as a whole. Salts are always present and acidity absent in the medium bathing the great majority of living cells. Hamburger (4) has shown that blood corpuscles, washed with glucose solution, yield potassium to a potassium-free Ringer solution, and Jannink (5) has reported that potassium is given off by heart muscle during perfusion with a potassium-free solution. Howell and Duke (6) recorded a liberation of potassium from the heart perfused with Ringer solution during prolonged stimulation of the vagus. These and other experiments, though they point out interesting possibilities, do not reach the explanation of the conditions limiting the entrance or exit of potassium, considering cells in general.

Muscle seemed a favorable material to use because it gives an easily controlled range of physiological activity. The work of Lillie and of McClendon indicates that, under some circumstances at least, contracted muscles are more permeable than resting ones. Is this true for potassium? We sought the answer to the question by making determinations of the amount of potassium in frog muscles after perfusion with various solutions either with or without simultaneous excitation. The results have shown the great tenacity with which the cells hold potassium while bathed in a potassium-free Ringer solution irrespective of whether or not the muscles are made to contract. As soon, however, as the muscles are fatigued beyond physiological limits potassium diffuses out of the cells and as much as half of their store may be lost in about 5 hours. Since potassium is known to diffuse slowly from dead muscle and since extreme fatigue in excised muscle is an irreversible process this was to be expected. A study of the intermediate stage between mild activity and exhaustion, showed that a part, from 8 to 15 per cent, of the potassium may be removed by perfusion, either with or without stimulation, without

marked loss of irritability in the muscle substance. Our experiments though not including any quantitative measurements of irritability have shown to rough observation a progressive loss of excitability accompanying potassium depletion. This is to be expected from the well known contrast between the physiological effects of a potassium-free physiological saline and those of Ringer solution.

We have further undertaken to test the possibility that muscular contraction is favorable to the process by which potassium is absorbed. We have found that only a contracting muscle, in sharp contrast to a resting one, can take up rubidium and cesium, substances whose chemical properties are more like those of potassium than sodium, so as to retain them in the same sense that potassium is retained.

EXPERIMENTAL.

Large bull frogs weighing from 125 to 400 gm. were used. Some of them were collected in Louisiana in February, shipped in March and used for the experiments in April, May and June. Others were taken in Rhode Island or Massachusetts in summer and used shortly after collection. Perfusions were made through a glass canula in the dorsal aorta of the pithed animals. The muscles used for analysis were lightly drained on paper after removal from the animal and immediately weighed. After digesting the muscle in a mixture of nitric and sulphuric acids potassium determination was made by the method described by Clausen (7) for blood analysis. Muscles removed from frogs without experimentation, merely killing and bleeding the animal, gave results as shown in Table I.

The averages of these results, whether computed for the same muscles of different frogs or for the various muscles of the same frog, are fairly uniform and are in agreement with averages obtained by Fahr (8) and others, placing the normal content of potassium in frog muscle at 0.34 per cent. The considerable variation in the results is partly due to errors in sampling. The sartorius is too small in many frogs for a satisfactory analysis. The vastus cannot be removed intact in its sheath so as to yield clean cut prisms of muscle but is apt to suffer loss of some of its fluids during sampling. For this reason most of the experiments reported below were done with the gastrocnemius muscles which can be more satisfactorily sampled. Another

and more important cause of variation is due to fluctuations in the water content of the muscles. In a series of nineteen determinations of the water in fresh (not perfused) frog muscles the minimum was 79.10 per cent and the maximum 82.22 per cent. Comparison of these results with the previous history of the animals, season when collected, duration of captivity, etc., showed no consistent correlation. The variation is sufficient, however, to make it advisable, when comparing the potassium content of different muscles, to determine potassium as per cent of the dried weight of the muscle. The average for the series was 80.78 per cent which is within the range of the averages commonly given for water content of muscles of cold-blooded animals. A third, and chief reason, is the actual variation in the

TABLE I.

Potassium Content of Fresh Normal Frog Muscle.
Results Are in Per Cent of Potassium in Moist Muscle as Weighed.

Muscle.	First frog.		Second frog.		Third frog.		Aver-age.
	Left leg.	Right leg.	Left leg.	Right leg.	Left leg.	Right leg.	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Gastrocnemius....................	0.330	0.322	0.355	0.362	0.331		0.340
Sartorius........................	0.370	0.342	0.337	0.378	0.328	0.305	0.343
Vastus..........................	0.328		0.300	0.327	0.348	0.349	0.330
Average......................	0.338		0.343		0.332		0.338

potassium content of the muscles of different frogs. For example the average of twelve analyses on eight frogs taken from localities in Rhode Island and Massachusetts during the summer and analyzed soon after collection was 1.807 per cent of potassium in the dried muscle while the average of seven analyses on muscles of six frogs brought from Louisiana in winter and used some months later was 1.591 per cent of potassium in dried muscle. The relation of the potassium content of frog muscle to reproductive activities, to sex, to the food supply, and to species would be interesting. Such studies have not been attempted in this work. The results indicate that a prolonged stay in an aquarium without food causes a diminution of potassium. The individual variations are greater than the analyti-

cal error and are due to something more than a mere seasonal variation since in a group of twelve analyses on eight summer frogs the maximum variation was 17 per cent of the average potassium content of the dried muscle while the analytical error as indicated by duplicate analyses of the same muscle or by analyses of the two fresh gastrocnemii, of the same frog did not exceed a maximum of 2.3 per cent and averaged 1.37 per cent.

The effect of perfusion with a potassium-free Ringer solution on the potassium content of muscle was contrasted with that of similar perfusion with Ringer solution. In each case both legs were perfused during 15 minutes with about 300 cc. of solution until the venous outflow appeared bloodless; the artery of one leg was then tied

TABLE II.

The Potassium Content of Perfused Muscles.
Results Are in Per Cent of Potassium in the Moist Muscle, as Weighed.

Muscle.	Perfused with potassium free solution.		Perfused with Ringer solution.	
	Slightly perfused. 300 cc.	Much perfused. 1800 cc.	Slightly perfused. 300 cc.	Much perfused. 1800 cc.
	per cent	*per cent*	*per cent*	*per cent*
Sartorius	0.263	0.240		
Vastus	0.275	0.222	0.292	0.248

off and 1500 cc. of solution perfused in 1¾ hours through the other leg. Results are given in Table II.

The potassium content of the moist muscle was decreased in both experiments. Fahr (8) has shown that frog muscle immersed in Ringer solution during 20 hours contained a smaller percentage of potassium than fresh muscle, though after a similar immersion in isotonic sugar solution a greater potassium loss was shown. The lower per cent of potassium obtained after perfusion seemed attributable in part, at least, to the higher water content. This possibility was indicated by the obvious distention and edematous appearance of the much perfused leg. Edema as a result of perfusion with a number of physiological salt solutions has been observed by Guntzberg (9), Abel (10), and others. Another experiment was therefore

tried with determinations of water as well as potassium in the muscles. After perfusing 200 cc. of a potassium-free Ringer solution through both legs, one leg, was tied off and 800 cc. were then perfused in the course of 8 hours through the other leg. The gastrocnemius of the slightly perfused leg yielded 0.304 per cent of potassium and the same muscle of the other leg 0.267 per cent; but the first of these muscles contained 18.85 per cent of solids while the second had only 16.7 per cent, so that the slightly perfused muscle contained 1.61 per cent of potassium in its solids and the more extensively perfused one had 1.60 per cent of the solid matter. The average content of potassium in the solids of the fresh muscle is 1.75 per cent, so that muscles of both legs had doubtless lost some potassium. These experiments were done before the extent of variability in water and potassium content of muscles of different frogs was fully appreciated. In a further series samples of the gastrocnemius of one leg were taken after a brief perfusion, lasting less than 10 minutes, sufficient to remove all visible blood. The muscles of the other leg were then perfused during a suitable period and samples of both gastrocnemii, after vacuum desiccation to constant weight, were used entire for wet ashing and potassium titration. The effect on the perfused muscle was then computed as the percentage loss of the original potassium content as shown by the muscle that was not perfused. Such experiments gave consistent results and showed, as set forth in Table III, a loss of potassium during early stages of perfusion with the limit of potassium loss approached after 5 hours. Subsequently little or no loss of potassium occurs. The time of perfusion showed more consistent relation to potassium loss than did the amount of perfusion solution used. This indicates diffusion of potassium from the muscle cells into the fluid of the lymph spaces irrespective of considerable change in the rate at which the latter is replaced. Such a result may be explained by the fact that in any case the perfusion solution, present in such quantities as to greatly distend the lymph spaces, would be notably lower in potassium content than the intracellular fluid.

The relation of muscular activity to loss of potassium from the muscle cells was sought in the following experiment. Potassium-free Ringer solution was perfused through both legs during 2 hours.

500 cc. of solution were used. During the last 1½ hours the muscles of one leg were stimulated intermittently with strong induction shocks applied to the distal end of the severed lumbar plexus. The average potassium content of the muscles of the rested leg was then found to be 0.350 and of the stimulated muscle 0.306 per cent. In another experiment 2½ liters of solution were perfused and stimulation with intermittent rest periods was applied during 2½ hours to one leg. The average potassium content of the muscles of the rested leg

TABLE' III.

Loss of Potassium from Muscles Perfused with Potassium-free Ringer Solution.

Total time of perfusion.	Total amount of solution used.	Average rate of perfusion.	Solids in control muscle.	Potassium in moist control muscle.	Potassium in solids of control muscle.	Solids in perfused muscle.	Potassium in moist perfused muscle.	Potassium in solids of perfused muscle.	Apparent loss of potassium.*
hours	*cc.*	*cc. per minute*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
7	800	1.9	18.69	0.376	2.016	15.65	0.276	1.765	12.5
8	900	1.9	19.51	0.336	1.879	14.73	0.243	1.648	12.3
12	1800	2.5	18.87	0.335	1.775	16.20	0.245	1.511	14.8
13¾	1500	1.8	18.96	0.335	1.765	14.25	0.214	1.499	15.1
1¼	100	1.3	19.20	0.281	1.465	16.97	0.237	1.399	4.5
5⅓	800	2.4	20.60	0.292	1.417	17.97	0.233	1.298	8.3
14¼	1000	1.2	18.75	0.313	1.670	14.64	0.225	1.537	8.5
18¾	1600	1.4	18.56	0.309	1.667	15.48	0.237	1.532	8.1

*Figures in this column are obtained, as explained in the text, 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.

The frogs used in the first four experiments reported in this table were collected in summer and used shortly after they were brought to the laboratory. Those used in the last four experiments were collected in winter in Louisiana and used some months later.

was 0.299 per cent and of the stimulated leg 0.237 per cent. The stimulated muscles show lower potassium content than the resting ones. Siebeck's (11) observation, confirmed by Meigs and Atwood (12), that a muscle in isotonic potassium chloride solution takes on more weight if active than if at rest should be recalled in this connection. The apparent losses of potassium in the stimulated as contrasted with the rested muscles are not, indeed, greater than could be accounted for as percentage changes due to absorption of

water. We hoped to avoid this effect and produce more nearly
physiological conditions by the use of gum arabic in the perfusion
solution, employing, in short, a modified Bayliss solution. Gum
arabic, purified by six alcohol precipitations from dilute hydrochloric
acid solution, and showing by analysis an amount of potassium less
than 0.01 per cent was used. The solution contained the following:

NaCl	6.5 gm.
CaCl₂	0.25 "
NaHCO₃	0.20 "
Gum arabic	45 "
Phenol-sulphonephthalein	a few drops.
Water	1 liter.

It had a pH of approximately 7.3; 700 cc. of the solution were per-
fused in $6\frac{1}{2}$ hours through both legs. The muscles of the left leg were
stimulated through the lumbar plexus with maximal tetanizing induc-
tion shocks lasting 1 second each, given by an automatic stimulator at
30 second intervals during the first half of each hour with half hour
rest periods intervening. These muscles, then, made 360 contractions
lasting 1 second each. The muscles of both legs showed, at the end
of the experiment, good though not equal irritability. The average
potassium content of the muscles of the stimulated leg was 0.229 per
cent, of the muscles of the other leg, 0.228 per cent. Both of these
figures are lower than the percentages of potassium found in normal
frog muscle. Both legs showed, however, some swelling in spite
of the gum arabic. To determine the effect of contraction on the
potassium content it seemed necessary to compute it in relation
to the solids of the muscle. A further experiment for this purpose
was undertaken. 900 cc. of potassium-free Ringer solution were
perfused through both legs in $8\frac{1}{2}$ hours. Gum arabic because of the
labor involved in making it potassium-free was not used. The muscles
of one leg, stimulated by the method described for the preceding
experiment, gave 480 contractions, of 1 second each. The muscles of
both legs showed good irritability at the end of the experiment. The
average potassium content of the muscles of the stimulated leg was
0.203 per cent, of the muscles of the other leg, 0.230 per cent, but
computed as percentages of the solids: 1.86 per cent for the former
and 1.85 per cent for the latter. No especial loss of potassium due to
stimulation was shown.

In addition, the experiment reported on the second line in Table III included stimulation by the same method so that the muscles in the course of 8 hours of perfusion gave 330 contractions of 1 second each. The results of this experiment do not fall out of line with those including no stimulation. Results therefore give no indication of loss of potassium while the muscle is contracting, other than the loss attributable to the presence of a potassium-free medium.

Such results are in marked contrast to those obtained when muscle is stimulated enough to cause loss of irritability. The considerable difference between the potassium content of the rested and the exhausted muscle as found in the following experiment seemed too great to be explained on the basis of change in muscle weight. Both legs were perfused with potassium-free Ringer solution during 2 hours. The muscles of one leg were given prolonged tetanizing stimuli at first through the nerves until they failed to respond and then by direct stimulation until exhausted. The average potassium content of the muscles of this leg was 0.141 per cent and that of the rested muscles, 0.266 per cent. A similar experiment to determine the potassium content of the muscle *solids* after exhaustive stimulation was made. 1600 cc. of potassium-free Ringer solution were perfused through both legs in $6\frac{1}{4}$ hours. The muscles of one leg were given direct tetanizing stimuli for varying periods with intervening rest. The total time of stimulation was 98 minutes. The average potassium content of the muscles of this leg was 0.102 per cent; of the others, 0.221 per cent. Computed as percentages of the solids there was 0.89 per cent in the fatigued and 1.70 per cent in the rested muscles. The stimulated muscles lost about half of their potassium.

The development of acidity in a fatigued muscle suggests that hydrogen ion concentration is a factor in the control of the processes by which potassium is retained or set free in the cells. Experiments to test this possibility will be the subject of a later communication. For the experiments reported in this paper perfusion solutions were in general adjusted with sodium bicarbonate, using Clark and Lubs (13) indicators, to a pH of approximately 7.3.

That muscular contraction does enable the cells to *take in* potassium is indicated by experiments on the absorption by muscles of rubidium and cesium, substances very similar to potassium in chemical and

physiological behavior. This work is reported in another paper (14), but may be briefly summarized in so far as it applies to the subject under discussion. It included experiments in which frog's legs were perfused with a Ringer solution whose potassium chloride was replaced by equivalent amounts of rubidium or cesium chloride. The muscles of one leg were meanwhile stimulated to contract without complete fatigue. Subsequently both legs were perfused with a potassium-free Ringer solution until the outgoing fluid gave no spectroscopic test for rubidium or cesium. The muscles of both legs were then analyzed for rubidium or cesium. Only those of the leg which had been stimulated during the first perfusion showed the presence of these metals. To take them, and therefore their close homologue, potassium, into the muscle in such manner as to be retained, in a non-diffusible form would seem to require contractile activity of the muscle.

DISCUSSION.

These experiments indicate that, as Loeb (1) has suggested, the potassium of muscle cells can be considered as existing in two different states. The portion constituting one of them is comparatively mobile, consists of about 15 per cent of the muscle potassium in summer frogs and a smaller proportion in winter frogs, and can be removed from frog muscle, under the conditions described, by mere perfusion with a potassium-free Ringer solution. The rate of its loss seems to be unaffected by contractile activity of the muscle. The remainder of the potassium, amounting to approximately 15 per cent of the dry solids of a perfused muscle, markedly resists outward diffusion but is liberated and rapidly lost when the muscle is stimulated to an extreme stage of fatigue. That some deep-seated change in the chemical composition of a muscle occurs during unusually prolonged exertion is evident from the long duration of the after effects. That the change causes an alteration of the conditions governing the partition of inorganic constituents between dissociable and non-dissociable forms is suggested by these observations. The greater osmotic pressure of fatigued muscle as compared with non-fatigued was shown by Moore (15) and taken to indicate an increased concentration of electrolytes in the cell during contraction unless they are removed by the circulation. The work of Loeb (16) showing the absolute

dependence of the formation of metallic salts of proteins on hydrogen ion concentration is helpful in forming a conception of the behavior of potassium. If one believes with Robertson (2) that salts of the proteins may be so built that the inorganic constituent is, partly at least, in non-dissociable form then one can readily conceive of how an intracellular change of hydrogen ion concentration such as occurs in a fatigued muscle would markedly alter the distribution of potassium, and doubtless other inorganic constituents, between the dissociable and the non-dissociable forms.

As to the absorption of potassium a conception of how contraction might be essential to the mechanism involved is difficult. One notes the generally accepted idea that ion movements having definite relation to membranes are concerned in excitation. This idea is, of course, the basis of the Nernst theory of stimulation and is assumed in the conception of the mode of propagation of excitation as developed by Lillie (17). Because ions move during excitation and perhaps penetrate membranes, are we to assume that the potassium ion can penetrate the cell only at that time? In the light of our present knowledge it seems necessary to believe that cell permeability is not merely a matter of passage through an external membrane but that the physiological behavior of the protoplasm as a whole must be considered. The work of Wishart (18) on the distribution of glucose between plasma and corpuscles may be cited as one example of such observations. If contraction, involving migration of ions, causes a sort of fixation of rubidium or cesium within the cell it is fair to suppose that potassium would be similarly affected. That potassium favors excitability by its presence at cell surfaces has been amply demonstrated for a variety of tissues, so that some significant movement of potassium ions is involved, among other things, in a response such as muscular contraction. Our results suggest that movement at the cell surface is not the only factor concerned but that some transformation of potassium into a non-dissociable form occurs, presumably within the protoplasm. In this connection the work of Crozier (19) on sensory activation of cells by acids is of interest since it led him to the view that stimulation does not merely involve an increase in permeability but that the change of condition of materials at the surface of cells is instrumental in determining diffusion of ions within the cell.

SUMMARY.

1. Individual variations in the potassium content of the fresh muscles of frogs are notable even when computed as percentages of the dry solids. The potassium content averaged higher in freshly collected summer frogs than in winter frogs after a period of captivity.

2. Muscles show a loss of from 8 to 15 per cent of their potassium during perfusion with potassium-free Ringer solution but tenaciously hold the remainder.

3. Muscles, stimulated to contract under conditions that do not produce irreversible stages of fatigue, show losses of potassium no greater than those attributable to the presence of a potassium-free medium.

4. A condition favorable to the taking up of potassium probably occurs in a contracting muscle because rubidium and cesium, substances very similar to potassium in chemical and physiological behavior, are absorbed in retainable form by a contracting muscle but not by a resting one.

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