THE SELECTIVE ABSORPTION OF POTASSIUM BY ANIMAL CELLS.

II. THE CAUSE OF POTASSIUM SELECTION AS INDICATED BY THE ABSORPTION OF RUBIDIUM AND CESIUM.

By PHILIP H. MITCHELL, J. WALTER WILSON, and RALPH E. STANTON.

(*From the Biological Laboratory of Brown University.*)

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That the peculiarities of the physiological behavior of potassium may be related to its ionic mobility has been suggested by Bayliss (1). Loeb (2) has referred its peculiarities to its electronic structure; *i.e.*, to the outside ring of electrons and its atomic number. These, in accord with modern views, would determine hydration. Hydration, according to Jones (3), is responsible for the difference in ionic velocities of members of the group of alkali metals. The arrangement of outer electrons and the atomic number then may be regarded as determining the relative ionic mobility. This is the general explanation as set forth by Mines (4) and others as to why ionic mobilities have a complex relationship to ionic volumes. The fact that potassium is generally selected in preference to sodium by a great variety of living cells is especially notable among its several peculiarities. Is there any evidence to show that the difference in the migration velocities of potassium and sodium are concerned in this so called "selective action"?

Among the comparative ionic conductances of the univalent metallic ions, together with the ammonium ion which has similar behavior, we note certain relations. The figures below are taken from Landolt (5) and refer to ionic conductances at 18°C.

Li	Na	NH_4	K	Rb	Cs
33.4	43.5	64	64.6	67.5	68

The interval between Na and NH_4 is much greater than intervals between the others. Lithium and sodium may be regarded as constituting one group, while ammonium, potassium, rubidium, and cesium fall into another. These are all univalent ions so that their absolute velocities of migration show the same relations as their conductances. If, then, the absorption of potassium by cells in preference to sodium is related to comparative ionic mobilities, lithium should behave like sodium, but rubidium and cesium like potassium. In a number of other physiological processes, just such relationships have been shown. Loeb (2) has pointed out how lithium and sodium stand in one group, physiologically speaking, while ammonium, potassium, rubidium, and cesium clearly constitute a distinctly different group. The members of each group show among themselves a number of similarities in physiological behavior, while the effects of the two groups may be regarded, in some ways, as physiologically divergent. Because of the manner in which hydration affects ionic mobilities the migration velocities of the members of this series seem to provide a basis for grouping them in accordance with their physiological behavior more satisfactorily than do other periodic functions, as for example, atomic volumes. We have sought to show whether or not the contrast of physiological properties among this series holds for their intracellular incorporation. We have not yet made a study of lithium. There is much in the literature on the subject to indicate that lithium is capable of penetrating into cells with no greater facility than sodium. The behavior of ammonium ions is obviously without much bearing on the question of potassium selection, since they freely pass in and out of all cells and are to be regarded, in general, as a waste product and not as constituents of cellular architecture. The experiments herein reported go to prove that rubidium and cesium behave like potassium in processes involving incorporation into the physicochemical structure of muscle and other animal cells.

Mendel and Closson (6) found that rubidium injected into cats and dogs was largely stored in muscle tissue. Zwaardemaker (7) and his coworkers in researches on aequiradio activity have shown that rubidium and in some degree cesium can replace potassium in maintaining the heart beat, but whether this means penetration into cells or action at their surfaces it is difficult to say. In the light of

our observations we would conclude that it shows the former to be true even though the effect upon irritability may be at the cell surface. So far as we have been able to read Zwaardemaker's reports in the original we have found no evidence on this aspect of the question and in this connection our results seem of some significance.

EXPERIMENTAL.

We perfused frog muscles with a Ringer solution modified by the replacement of potassium chloride with an aequimolar concentration of rubidium chloride. While both legs were perfused the muscles of one were made to contract by stimulation of the lumbar plexus with maximal tetanizing induction shocks lasting 1 second at 30 second intervals during one-half hour periods with alternating one-half hour periods of complete rest. In one experiment this procedure was continued during 5 hours and was followed by perfusion with an isotonic cane sugar solution during $1\frac{1}{2}$ hours. All the muscles of both legs showed irritability at the end of the experiment. Samples of the gastrocnemius and sartorius of each leg were then decomposed in a mixture of nitric and sulphuric acids. The resulting solutions enabled us to detect rubidium spectroscopically in the muscles of the stimulated leg but not in those of the other. In another similar experiment the muscles of one leg were given nine half-hour work periods, that is, 540 contractions of 1 second each, and were then while resting, perfused during 2 hours with a potassium-free Ringer solution. The muscles of both legs showed good irritability at the end of the experiment. The wet-ashed muscle samples, taken from the gastrocnemius and vastus muscles of each leg, were made up to 10 cc. and examined spectroscopically by Gooch's method for quantitative estimation of rubidium. No trace of rubidium could be detected in the muscles perfused without stimulation. The samples used were 1.27 gm. from the gastrocnemius and 2.37 gm. from the vastus. In the muscles of the stimulated legs, however, we found approximately 0.011 per cent of rubidium. The standard solution for spectroscopic comparison with the muscle material contained 3 per cent of H_2SO_4, 0.075 per cent of KCl, 0.025 per cent of NaCl, and 0.0025 per cent of rubidium added in the form of the carbonate. It was designed to imitate, approximately, the acid, potassium and

sodium content of the solutions of wet-ashed muscle. The samples taken from the stimulated muscles were 1.86 gm. from the gastrocnemius, and 2.03 gm. from the vastus. The standard solution diluted 3.5 times gave the faintest rubidium spectrum possible to detect. The solution from the gastrocnemius behaved similarly when diluted 3 times and the one from the vastus when diluted 3.5 times. A careful uniformity of technique was used in making all the flame tests. This estimation shows 0.011 per cent of rubidium in the gastrocnemius and 0.012 per cent in the vastus. As a matter of fact a proportion of rubidium larger than these figures indicate was present because the muscles were obviously edematous. Rubidium content, in proportion to the dry solids, was not estimated. An amount of rubidium, equivalent to more than 3 per cent of the normal potassium content

TABLE I.

The Absorption and Retention of Cesium by Frog Muscle

Volume of cesium solution used.	Duration of first perfusion.	Volume of potassium-free Ringer solution used.	Duration of second perfusion.	Weights of samples of muscle used for analysis.				Cesium found.	
				Right gastrocnemius.	Right vastus.	Left gastrocnemius.	Left vastus.	Left gastrocnemius.	Left vastus.
cc.	*hrs.*	*cc.*	*hrs.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
625	7	530	4½	2.80	2.78	2.57	2.37	0.008	0.006
475	6⅓	500	3⅓	3.01	2.39	2.55	2.97	0.006	0.006

of frog muscle, was taken up when the muscles made 540 contractions of 1 second each. The retention of rubidium in the muscles during perfusion with a rubidium-free solution and the absence of rubidium from the unstimulated muscles, points to its actual entrance into and incorporation with the cell substance in the same sense that potassium is normally held there. Whether or not it actually replaces potassium has not yet been determined.

Similar experiments were made with cesium chloride, replacing, in equimolar concentration, the potassium chloride of Ringer solution. The data of two such experiments are presented in Table I. The muscles of the left leg of the frog used in each experiment made 420 contractions of 1 second each, while the cesium-containing solution was perfusing through both legs. The muscles of the right leg were meanwhile at rest. At the end of the experiment the muscles of

both legs showed good response to both direct and nerve stimuli; though, to the same stimulus, muscles of the right leg responded, of course, more vigorously than those of the left. Adequate samples of muscles of the right legs (amounts of samples are recorded in the table) showed no trace of cesium in a careful spectrum analysis. Muscles of the left legs showed brilliant cesium spectra, permitting a quantitative estimation. Cesium, then, like rubidium was taken into the muscle substance so as to be retained, in part, during the subsequent perfusion with potassium-free Ringer solution.

To further test the replaceability of potassium by rubidium and cesium young white rats were fed on purified synthetic diets in which salts of rubidium or cesium were substituted for those of potassium as ordinarily used in such diets. The basal diet had the composition shown below.

	per cent		*per cent*
Casein	18	Dried yeast	5
Starch	54	Salts	5
Butter fat	18		

In the experiments with rubidium each 5 gm. of salt mixture contained approximately:

	gm.		*gm.*
$MgSO_4 \cdot 7H_2O$	0.55	$CaH_4(PO_4)_2 \cdot H_2O$	0.54
$NaH_2PO_4 \cdot 4H_2O$	1.10	Calcium lactate	1.40
RbCl	1.29	Iron lactate	0.12

In the cesium experiments each 5 gm. of the salt mixture contained approximately:

	gm.		*gm.*
$MgSO_4 \cdot 7H_2O$	0.46	$CaH_4(PO_4)_2 \cdot H_2O$	0.45
$NaH_2PO_4 \cdot 4H_2O$	1.27	Calcium lactate	1.12
CsCl	1.59	Iron lactate	0.11

Water was given freely and to it a few drops of iodine solution were added once a week. The animals were put in separate cages, weighed frequently and kept under observation. After varying periods (10 to 15 days) the rats showed marked symptoms of derangement. We noted a sluggishness and general inactivity, followed by a period of marked irritability, with trembling and intermittent twitching which later amounted to tetanic spasms. Within 48 hours after the first tremblings were noted

the rats died in violent tetanic spasms. As a control, the above diet with potassium chloride in amounts equivalent to the rubidium or cesium chlorides was fed to rats with satisfactory results although the same diet plus rubidium or cesium chloride was fatal with the usual symptoms. Moreover a diet in which only one-half of the potassium was replaced by an equivalent amount of cesium or rubidium was lethal. In other words the rubidium and cesium in quantities used were toxic, irrespective of the presence or absence of potassium. The toxicity of rubidium and cesium was more pronounced than we had expected from the literature dealing with a variety of biological experiments in which these salts have been employed. We have found that as little as 2 cc. of 1.44 per cent solution of rubidium

TABLE II.

The Absorption of Rubidium and Cesium by Rat Tissue.

Weight of rat at beginning of diet.	Duration of feeding.	Kind of diet.	Weight at end of experiment.	Rubidium or cesium content of tissues weighed fresh.					
				Muscle.	Heart.	Liver.	Kidney.	Spleen.	Lungs.
gm.	*days*		*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
55	17	Rb	50	0.33	0.19	0.22	0.21		
40	17	Rb	39	0.49	0.23	0.34	0.21		
53	13	Cs	57	0.120	0.042		0.060	0.055	0.035
56	10	Cs	46	0.043	0.034	0.039	0.038	0.036	0.017
70	10	Cs	82	0.538	*	0.430	0.555	*	0.219

*Richly present as shown by spectrum. Amount not measured.

chloride (a concentration isotonic with 0.7 per cent sodium chloride), injected into the dorsal lymph sack of frogs weighing from 30 to 40 gm., killed within 24 hours, while 1 cc. on two successive days was also lethal. Autopsy of the rats revealed nothing of note except in one case showing an infection of the lungs. Various tissues of some of the rats which had been on diets containing rubidium or cesium were decomposed in a mixture of sulphuric and nitric acids. The resulting clear solution was made up to 10 cc. and subjected to spectrum analysis for estimation of rubidium or cesium. Results are summarized in Table II. We satisfied ourselves that these metals were actually contained in the cells by a thorough perfusion through a cannula in the left ventricle of a rat, chloroformed after 10 days on the cesium-containing diet. Potassium-free Ringer solution was

employed. The tissues of this animal showed a high content of cesium.

The considerable quantity of rubidium or cesium in the rat muscle, as shown in some of the experiments, is striking. Computed in actual concentration, it amounts in three of the experiments to about half the concentration of potassium as given for normal mammalian muscle (0.32 to 0.42 per cent). This looks like an actual replacement of potassium by rubidium or cesium. We have not yet undertaken the difficult and somewhat uncertain methods for determination of potassium in the presence of such quantities of the interfering substances, rubidium and cesium. The spectroscope, beyond revealing qualitatively that some potassium was present, would not serve the purpose. We cannot, therefore, draw any conclusions as to whether rubidium and cesium are taken into the cell in the place of potassium or in addition to it. It is interesting to note that rubidium and cesium can replace potassium with considerable success in furthering excitability as studied by Zwaardemaker (7) and his coworkers, in antagonistic salt reactions as shown by Loeb (2), and in permitting the development of *Arbacia* eggs as shown by Loeb (8). All of these effects may be due, although proof is not entirely adequate, to action at cell surfaces. In contrast to this, a prolonged action of rubidium and cesium, under circumstances permitting their incorporation into the cell would seem from our observations to constitute physiologically a less successful substitution for potassium.

SUMMARY.

1. Frog muscles perfused with Ringer solution in which potassium chloride has been replaced by an equivalent amount of rubidium or cesium chloride take up rubidium or cesium and incorporate them into the tissue substance in such form as to be retained during a subsequent perfusion with potassium-free Ringer solution, provided the muscles contract during the first perfusion. Retention of rubidium or cesium by a resting muscle does not occur.

2. Rats on synthetic diets, adequate in all respects except that potassium was replaced by an equivalent amount of rubidium or cesium, died after a period varying from 10 to 17 days with characteristic symptoms including tetanic spasms. Muscle, heart, liver,

kidney, spleen, and lung tissues were then found to contain significant amounts of rubidium or cesium. The concentration of these metals in the muscle amounted, in some cases, as shown by a spectroscopic estimation, to about half the concentration of potassium normally found in mammallian muscle.

3. The results are regarded as tending to confirm the theory that the peculiarities in the physiological effects of potassium, including the facility with which it is "selected" by living cells in preference to sodium, are related to the electronic structure of the potassium ion as compared with that of similar ions. The possible relationship of the comparative migration velocity, a function of the electronic structure, to physiological effects is suggested.

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