CXC. DO THE POTASSIUM IONS INSIDE THE MUSCLE CELLS AND BLOOD CORPUSCLES EXCHANGE WITH THOSE PRESENT IN THE PLASMA?

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In order to find out if and to what extent the potassium ions within the muscle cells and blood corpuscles exchange with those present in the plasma we have administered labelled potassium as potassium chloride (^{42}KCl) to rabbits and frogs and followed its path in the body.

Experimental procedure

Metallic K was bombarded by deuterium ions of 5·5 million volts energy supplied by a cyclotron. We are most grateful to Prof. Niels Bohr for putting at our disposal the cyclotron and to Dr Jacobsen and Mr Lassen for preparing the active samples. The K was converted into KCl and administered to rabbits by subcutaneous injection or to frogs by injecting it into the lymph sac; it has also been used in experiments *in vitro* [Hahn *et al.* 1939].

The blood samples, after centrifuging to separate the plasma and the corpuscles, and the tissue samples were ashed below 500°, and their activity determined by making use of a Geiger counter. The labelled K (^{42}K) has a half-lifetime of 12·8 hr. No corrections for this decay had to be applied, since all samples were measured relatively to an aliquot of the ^{42}KCl preparation used in the experiments and the results were calculated in percentage of this "standard" preparation. As K has a "natural" radioactivity, due to the presence of ^{40}K in all K samples, we had to apply a correction for the presence of the latter. This was done by measuring the remaining activity of the tissue samples, which was then solely due to the presence of ^{40}K, after the lapse of several days, and subtracting this value from the activity measured at an early date. The maximum extent of the correction to be applied was 10 % of the value obtained for the activity of the sample at an early date and amounted in many cases to only 1 % or less. The corrected activity of the sample is a measure of its labelled K content.

Experiments on frogs

To *Rana esculenta* var. *red.*, weighing 60, 65 and 58 g. respectively, kept at 22°, 0·7 ml. of a solution containing 3·7 mg. labelled K as KCl was injected. 1 hr. after the start of the experiment one leg was removed and a small plasma sample secured; after the lapse of 24 hr. the animal was killed. The comparison of the activities of the gastrocnemius of the left and right legs, removed after 1 and 24 hr. respectively, is seen in the tables below, which contain also data on the activity of the plasma, the femur epiphysis and diaphysis. The values are expressed relatively to the activity of plasma of the same fresh weight secured 1 hr. after the start of the experiment.

To facilitate the removal of the extracellular labelled K by the Ringer solution several incisions were made in the muscle during the last-mentioned experiment. By this procedure possibly some of the cell walls were destroyed, and a part of the labelled K content of the Ringer solution is of cellular origin.

As seen from the figures below, 1 g. muscle tissue contains more labelled K than 1 g. plasma and, therefore, the labelled K present in the muscle tissue cannot be interpreted as being located in the extracellular volume since this amounts to only about 12 % of the total muscle volume [Fenn & Cobb, 1935; Manery & Hastings, 1939]. At first sight, this result seems to prove the exchangeability of the cellular K. This is not the case, for 1 g. muscle contains about 30 times more K than 1 g. plasma [Fenn, 1936], and, therefore, in the case of an easy exchangeability we should expect 1 g. muscle to contain 30 times as much ^{42}K

Table I

Frog I. Relative activities of fresh samples of equal weight

Sample	Activity after 1 hr. left leg	Activity after 24 hr. right leg
Plasma	100	95
Gastrocnemius	135	146
Epiphysis	152	161
Diaphysis	63	138

Urine secreted during the experiment contains 4·2 % of the labelled K injected. Haemotocrite value 21·6.

Table II

Frog II

Sample	Activity after 1 hr. left leg	Activity after 24 hr. right leg
Plasma	100	124
Gastrocnemius	116	148
Epiphysis	135	179
Diaphysis	70	118

Urine contains 4 % of the labelled K injected.

Table III

Frog III. In this experiment the gastrocnemius was, before ashing, immersed into 15 ml. Ringer solution for 2 min.

Sample	Activity after 1 hr. left leg	Activity after 24 hr. right leg
Plasma	100	99
Muscle after immersion	93	113
15 ml. Ringer solution	32	51

as 1 g. plasma. We find, however, the ratio to be only 1·25 in the 1 hr. experiment, and 1·34 in that of 24 hr. duration. Thus about 96 % of the cellular K content does not take part in an exchange process in the course of 1 hr. More significant than the above figure itself is the fact that, when the time of the experiment is increased from 1 to 24 hr., the above figure decreases only to 95·7 %. We thus find an uptake of some labelled K by the muscle tissue: this cannot be interpreted as due to the invasion of the extracellular liquid by labelled K, since the labelled K involved is about 10 times larger than can be taken up by the

extracellular liquid, though only about 1/22 of the amount which should have been taken up in the course of the experiment had the K ions incorporated into the cells come into exchange equilibrium with those present in the plasma in the course of the experiment.

One could argue that, though the exchange equilibrium stage is not reached after the lapse of 1 hr., it may be obtained after the lapse of several hours. But, as seen above, in the course of 24 hr. only a very modest increase is reached, showing that we are not faced with a progressive process of reasonable speed, but mainly with a rapid influx of labelled K into the muscle tissue in the early stages of the experiment. Possibly only a fraction of the cell K migrates easily through the cell walls or only certain cells present in muscle tissue are easily permeable by K ions. The result of the above experiment shows clearly, however, that the greater part of the cellular K is certainly not exchangeable under the conditions which prevailed in our experiments. The sudden influx mentioned above could also be explained by assuming that an active secretion of K into the muscle cells is engendered by the increase (amounting to a maximum of 50 %) in the KCl content of the plasma. If this were the correct explanation, the sudden influx of ^{42}K into the muscle tissue should cease if an increase in the K content of the plasma were avoided by injecting a negligible quantity of labelled K. We intend to carry out this experiment, though the results of experiments with rabbits, given below, in which a K content of the plasma was almost normal throughout the experiment, were very similar to those obtained with frogs.

Experiments on rabbits

The result of an experiment on a rabbit weighing 2·1 kg. is seen in Table IV.

Table IV. *Labelled K content of the organs of a rabbit 24 hr. after subcutaneous injection of 15·5 mg. labelled K as chloride**

Time	Organ	Relative labelled K content
16 min.	Plasma	69
35 ,,	,,	83
61 ,,	,,	93
93 ,,	,,	110
3·0 hr.	Plasma	103
15·5 ,,	,,	103
19·5 ,,	,,	106
24·5 ,,	,,	100
24·5 hr.	Corpuscles	65
24·5 ,,	Muscles (gastrocnemius)	145
24·5 ,,	Liver	141
24·5 ,,	Kidneys	162
24·5 ,,	Brain	96
24·5 ,,	Tibia epiphysis	107
24·5 ,,	Tibia diaphysis	65
24·5 ,,	Marrow	79
24·5 ,,	Spleen	110

The ratio found for the labelled K content of 1 g. fresh muscle tissue and of 1 g. plasma after the lapse of 24 hr. is found to be 1·45, while a similar figure (1·35) was obtained in the experiments on frogs.

* The rate of resorption of labelled K in human subjects was studied by Hamilton [1938], in rats by Greenberg *et al.* [1938].

Taking 18 mg. per 100 ml. as the K content of the plasma and 450 as that of the muscle cells, on the assumption of a free exchange of the K ions between cell tissue and plasma, an individual K ion should have $\frac{450}{18}=25$ times greater chance of being located in the cells than in the plasma of the same weight. Thus we should find about 25 times more ^{42}K in 1 g. muscle tissue than in 1 g. plasma.

It is of interest to remark that a progressive influx of ^{42}K on a larger scale into the muscle cells in the course of 24 hr. is incompatible with the comparatively constant ^{42}K level of the plasma recorded in Table IV. The K content of the total extracellular fluid including plasma of a rabbit amounts to only about 1/30 of the total K content of the intracellular fluid of the muscles and therefore an influx of ^{42}K into the muscle cells would soon rob the plasma of most of its ^{42}K content.

Apparent extracellular volume in experiments with K

Numerous investigations have been carried out by different workers on the change of the K content of muscle tissue during muscular action, recovery tremor etc. [Fenn, 1936]. The conditions prevailing in these experiments were different from those in ours. It is however of interest to compare the results obtained by Bourdillon [1937], and Winkler & Smith [1938], respectively, with those obtained in our experiments. These authors administered K salts by mouth or by intraveneous injection to resting human subjects and animals respectively, and determined the apparent extracellular volume from the increase in concentration of the K content of the serum water. The volume of the fluid of the body through which any substance is distributed after injection may be calculated by the following formula.

$$\text{Apparent volume of distribution}=\frac{\text{amount injected minus amount excreted}}{\text{increase in concentration in serum water}}.$$

When calculating the apparent volume of distribution of Cl or Na ions, for example, one arrives at the result that water amounting to about 27 % of the body weight takes part in the dilution of the substances introduced into the plasma, the diluting liquid being considered to be solely extracellular. When carrying out such experiments with K salts, Bourdillon, and also Winkler & Smith, arrived at a value 2 to 3 times as high for the apparent volume of distribution, and this result was interpreted by them as due to the influx of a part of the K into the cells of the body. From their figures it follows that the cell water present in the tissues took up about twice as much K as the extracellular water. The ratio of the K content of the cell water and the extracellular water of the muscles amounts, however, to more than 100. Should the muscle cells be permeable to K ions, the K administered should have been distributed in a ratio larger than 100 : 1 between the cell water and the extracellular water of the muscles. Winkler & Smith determined the volume of K distribution at different times after the start of the experiment. After the lapse of an hour or two no further increase was found, such as we should expect in the case of progressive intrusion of the excess of K into the cells. In fact, in many cases a decrease with time after the lapse of about $1\frac{1}{2}$ hr. was observed by them.

While in the above experiments the large values obtained for the volume of K distribution were interpreted as due to the influx into the cells, it is quite possible that the mineral constituents of the bone tissue take up a part of the excess K introduced into the circulation. It is quite conceivable that in part the circulation is getting rid of its excess K by incorporating K ions into the bones

in place of some of the Ca or Na atoms of the latter. We are led to this possibility by the results obtained when investigating the ^{42}K uptake by the bones of frogs and rabbits.

Uptake of labelled K by the bones

As seen in Tables I, II and IV, 1 g. of fresh femur epiphysis takes up more labelled K than found in the plasma of equal weight. The water content of the epiphysis amounts to about 25 % of its weight. This water can be considered to be mostly of extracellular origin. Therefore we can assume about 1/4 of the ^{42}K found in the epiphysis to be extracellular K, while the rest will presumably be mainly that which is incorporated into mineral constituents of the bone.[1] As we must suppose that other individual K ions present simultaneously in the plasma will show the same behaviour as ^{42}K, we can conclude that in the above experiments on frogs 1 g. fresh epiphysis tissue took up about 0·1 mg. of the K ions which were located in the plasma after the start of the experiment. These K ions presumably exchanged with those present in the bone. Such an exchange process presumably goes on in such a way that some of the K ions of the bone are released into the lymph and an equal number during the same time in the opposite direction. If we disturb this equilibrium by introducing into the plasma a K excess, it is probable that more K ions than before will enter into the bones during a given time, their uptake being compensated by a release of other cations present in the bone.

The diaphysis takes up markedly less labelled K than the epiphysis. Similar results were obtained by us in numerous cases when investigating the labelled phosphate uptake by bones of diverse animals [Hevesy *et al.* 1937], and also by Dolls *et al.* [1939]. The difference is probably due to the less effective lymph circulation in the diaphysis. An exchange or uptake of ions into the mineral constituents of the bone can only take place if these constituents are in contact with the lymph from which the uptake takes place. The diaphysis contains less water and correspondingly less K in the water phase, which can, however, account for only a minor part of the difference in the uptake of labelled K.

Rate of penetration of the phosphate ions into the muscle cells

It is of interest to compare the rate of penetration of K ions with that of phosphate ions into the tissue cells. When working with labelled phosphate, due regard must be taken of the rapid exchange of the plasma phosphate with that of the bones and other tissues, which leads to a rapid decrease of the labelled phosphate content of the plasma. To avoid the latter we inject the labelled Na phosphate drop by drop subcutaneously into rabbits during the experiment. After the lapse of 4 hr. we find that 1 g. fresh gastrocnemius tissue contains 0·6 times as much labelled P as does 1 g. plasma. The labelled P content of the muscle tissue is partly located in the extracellular space. Making use of the data discussed on p. 1555, we reach the result that the extracellular P content of 1 g. fresh gastrocnemius tissue amounts to 1/12 of that of an equal weight of plasma. It follows that the cellular labelled phosphate present in 1 g. gastrocnemius tissue amounts to 0·51 times that in 1 g. plasma. As we find the inorganic P content of the plasma of the rabbits to be 4·2 mg. per 100 ml., it follows that in the course of 4 hr. 0·02 mg. P originally located in the plasma migrated into

[1] As the epiphysis contains about the same amount of ^{42}K as muscle tissue of the same weight, and the cellular part of the bone makes up about 1/10 of the bone weight, the bone cells should take up much more ^{42}K than do the muscle cells to account for the non-extracellular ^{42}K present in the epiphysis.

the cells of each g. of the gastrocnemius, while an equal amount of non-labelled P migrated in the opposite direction.

The rate of penetration of the phosphate ions into the muscle cells depends not only upon the properties of the cell wall but also upon the rate at which the labelled inorganic P is incorporated into the organic compounds inside the cell, and thus is prevented, for the time being, from diffusing back into the plasma. Simultaneously with the formation of these new labelled organic molecules the decomposition of an equal number of "old" organic molecules takes place. This produces non-labelled phosphate ions, which "dilute" the active inorganic P content of the cells. When evaluating the number of labelled P atoms which pass the cell wall during the experiment we must consider the labelled inorganic and organic P molecules present in the cells as well. We then arrive at the result that the number of phosphate ions migrating from the plasma into the cells during the course of the experiment is equivalent to about 1/80 of the total acid-soluble P present in the cells.

Exchangeability of the K of the corpuscles

As seen in Table IV, in blood samples removed from a rabbit 24·5 hr. after the start of an experiment the labelled K is distributed between equal weights of plasma and corpuscles in the ratio 100 : 65. We also determined this ratio after the lapse of 2 hr., and found it to be 100 : 20. An appreciable part of ^{42}K ions, and thus a corresponding part of all the K ions originally present in the plasma, was found in the corpuscles. We must, however, remember that the corpuscles contain about 20 times as much K as does the same weight of plasma. In case of a complete exchangeability of the K of the corpuscles we should have found 20 times as much ^{42}K in the corpuscles as in the plasma or an effect about 30 times larger than found in the above-mentioned experiment after the lapse of 1 day.

Experiments in vitro

In experiments *in vitro* rabbit's blood was shaken, after addition of labelled KCl, in a CO_2-O_2 atmosphere at 37° for 2 hr. After centrifuging and rapidly washing the corpuscles twice with non-active plasma of another rabbit (2 g. plasma for 1 g. corpuscles in each case), we found the ratio of ^{42}K in equal weights plasma and corpuscles to be 100 : 35. Adding the ^{42}K found in the washing plasma to the amount found in the corpuscles, the above ratio becomes 100 : 38.

In other experiments labelled KCl was added to the blood sample and, after shaking for 1 hr., the corpuscles were separated. The latter were not quickly washed with non-active plasma, as described above, but shaken with non-active plasma for half an hour at 37°. This operation was repeated twice more, the corpuscles being separated each time by centrifuging. The results obtained are seen in Table V.

Table V. *Distribution of labelled K between plasma and corpuscles in blood samples (ratio of volume of plasma and corpuscles 2 : 1)*

Plasma	Corpuscles before treatment with non-active plasma	First washing plasma	Second washing plasma	Third washing plasma	Corpuscles after treatment with non-active plasma
88%	12%	3·1%	1·2%	0·47%	7·2%

The ratio of the ^{42}K content of equal weight of plasma and untreated corpuscles equals 100 : 21. It is seen from the above figures that, when corpuscles into which ^{42}K had been previously incorporated during an experiment lasting 1 hr. were shaken for 1½ hr. with non-active plasma again, only 40 % of the ^{42}K taken up by the corpuscles phase could be removed. This suggests that besides adsorption of ^{42}K on the surface of the corpuscles and penetration into the wall of the latter a leak into the interior of the corpuscles took place as well. The corpuscles present in the blood are a mixture of individual corpuscles differing in age and resistance and it is possible that only particular ones exhibit such a leakage. Henriques & Órskov [1939] have shown in a recent paper, which is of interest in this connexion, that the blood of animals in which strong anaemia was produced, and which thus contains a large percentage of young corpuscles, contains corpuscles with a very high K content. Experiments on such animals may throw light on the point raised above.

From the above considerations it follows that the permeability of an average corpuscle of a fully grown rabbit to K ions is certainly very minute, or, more correctly, the bulk of the K ions present in such corpuscles is not replaced in the lifetime of the latter.

Experiments with labelled Na

We also subjected rabbits to subcutaneous injections with labelled Na as $^{24}NaCl$. In blood samples taken after the lapse of about 1 day, we found, on an average, that ^{24}Na was distributed between equal weights of plasma and corpuscles in the ratio of 100 : 6 when the corpuscles were washed twice with non-active plasma and recovered each time by centrifuging. The ratio was 100 : 14 when the activity of the unwashed thoroughly centrifuged corpuscles was compared with the activity of the plasma of the same weight. An equipartition of ^{24}Na between equal weights of corpuscles and plasma would correspond to a ratio of about 100 : 25.

A large amount of ^{24}Na was found in these experiments in the plasma employed in washing the active corpuscles after their separation being in some cases even larger than the remaining ^{24}Na content in the corpuscles. In view of the large Na content of the plasma and the comparatively low Na content of the corpuscles of rabbit's blood, Na is less suitable than K for studying the interpenetration of alkaline ions. The results obtained suffice, however, to show the great difference in the behaviours of Na and K in the corpuscles.

Distribution of Na in the organs

As is to be expected, 1 g. of muscle tissue takes up much less labelled Na than K, for the Na uptake is known to be confined to a very large extent to the extracellular fluid of the muscle tissue. From the extent to which labelled Na injected into the veins of a rabbit became diluted Griffiths & Maegraith [1939] calculated that the volume of fluid in which the ^{24}Na was dissolved must have been about 840 ml., amounting to 36 % of the body weight in water. In other animals they found values between 30 and 35 %. Owing to the high activities at our disposal we could compare the activity of 1 g. plasma with that of 1 g. muscle tissue, which is the most direct method of determining the extracellular volume of the tissue in question. We find the ratio of the ^{24}Na content of 1 g. plasma to that of 1 g. gastrocnemius to be 100 : 8·5. Manery & Hastings [1939] found an almost identical figure, 8·6, for the ratio of the Na content of 1 g. plasma and of 1 g. fresh gastrocnemius tissue of a fully grown rabbit. Our

experiment leads thus to the same value for the volume of the extracellular space of the gastrocnemius muscle as stated by them, namely 11, expressed as g. per 100 g. blood-free, fat-free tissue. We determined also the extracellular space of the total body, as we will discuss below.

The distribution of ^{24}Na between 1 g. plasma and 1 g. fresh tissue of different organs, 67 hr. after subcutaneous injection of 23 mg. labelled Na to a rabbit weighing 2·2 kg., is seen in Table VI.

Table VI. *Distribution of labelled Na between plasma and fresh tissue of equal weight*

Organ	Relative ^{24}Na content
Plasma	100
Gastrocnemius	8·5
Heart	30·4
Liver	33·7
Kidneys	53·4
Spleen	29·6
Lungs	34·5
Marrow	35·3
Brain	32·0
Tibia epiphysis	59·1
Tibia diaphysis	50·9

In these experiments of long duration (67 hr.), a part of the ^{24}Na found in some of the tissues is possibly not of extracellular origin. This is certainly the case for the bones. As only 1/4 of the latter is water, the extracellular ^{24}Na content of the bone tissue cannot be more than 1/4 of that of an equal weight of plasma. However, about twice this much was found. Since the non-labelled Na ions also present in the plasma must show the same behaviour as ^{24}Na, we can conclude that, of the 3 mg. Na present in 1 g. plasma, about 0·8 mg. exchanged in the course of 67 hr. with Na atoms present in 1 g. epiphysis at the start of the experiment. From entirely different considerations Harrison [1937] (cf. also Harrison *et al.* [1936]) came to the conclusion that 42 % of the total bone Na is not extracellular, which compares well with our results.

To determine the variation of the ^{24}Na content of the plasma with time we carried out the following experiment. 23 mg. ^{24}Na, as NaCl, were injected into rabbit A. After the lapse of 3 days 35 ml. of blood were removed from rabbit B, the corpuscles were separated and mixed with 29·6 ml. of plasma from rabbit A and the blood thus obtained was introduced into rabbit B, the injection requiring 2 min. The ^{24}Na content of the 29·6 ml. plasma injected into rabbit B was found, after the lapse of 3 min. (excluding the time of injection), to have been brought

Table VII. *Change of the labelled Na content of the plasma with time, after intraveneous injection*

Time	% of ^{24}Na injected present in 1 g. plasma	% of ^{24}Na injected present in total plasma (assumed to be 80 ml.)
0 min.	—	100 (extrapolated)
2 ,,	0·164	13·1
5 ,,	0·158	12·7
15 ,,	0·155	12·4
33 ,,	0·150	12·0
56 ,,	0·110	8·8
203 ,,	0·104	8·3
47 hr.	0·069	5·5

down, by dilution by the body fluids, to 1/20·6 of its initial value. The volume of the diluting fluid must have amounted to 610 ml. or 28 % of the body weight. In calculating the above figure no regard was taken of the possible uptake of some ^{24}Na by the mineral constituents of the bones. This figure compares well with that found by entirely different methods for the value for the extracellular volume of the total body of a rabbit. The rapid fall in the ^{24}Na content of the plasma witnessed immediately after the start of the experiments is followed by a moderate decrease with time as seen in Table VII. This decrease is possibly due to some extent besides excretion to penetration of ^{24}Na into the non-extracellular part of the tissues.

Summary

Labelled K as ^{42}KCl was administered to rabbits and frogs and its penetration into the corpuscles, the muscles and other organs was investigated.

It was found that, after the lapse of 1 day, corpuscles contain about 60 % as much ^{42}K as the same weight of plasma. Thus some uptake of ^{42}K by the corpuscles takes place. The amount of ^{42}K found in the corpuscles, however, is about 30 times less than is to be expected in the case of an equipartition of ^{42}K between the K ions of the corpuscles and those of the plasma. The bulk of the K ions present in the corpuscles of a fully grown rabbit is thus not replaced in the lifetime of the corpuscles.

The gastrocnemius of frogs contains, after the lapse of 1 hr., 1·25 times, and after the lapse of 24 hr., 1·35 times as much ^{42}K as does the same amount of blood plasma at the same time. Its labelled K content is thus about 10 times larger than expected on the assumption that all the ^{42}K taken up by the muscles is present in the extracellular space. The amount of ^{42}K found, however, is about 20 times less than expected assuming an exchangeability of all K present in the muscle cells. A similar result was obtained with rabbits.

The ^{42}K content of the skeleton was found to be about double that expected if all ^{42}K is confined to the extracellular space.

The rate of penetration of phosphate ions into the muscle cells is discussed.

After the lapse of a day about 1/2 of the Na in the corpuscles was replaced by ^{24}Na.

Gastrocnemius tissue contained 8·5 % of the ^{24}Na content of the same weight of plasma, the corresponding figures for the tissues of all other organs were found to be much larger.

The extracellular space of the total rabbit was calculated to be 28 % of the body weight.

Note added 28 September 1939. In a recent paper Joseph *et al.* [1939] record a low permeability of rat's muscle to labelled K. A remarkably high permeability of the corpuscles of the dog to labelled sodium was found by Cohn & Cohn [1939].

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