15. A STUDY OF THE ISOTOPIC CONSTITUTION OF POTASSIUM IN VARIOUS RAT TISSUES

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PRELIMINARY investigations [Brewer, 1936; 1937; Lasnitzki & Brewer, 1938] have disclosed the somewhat surprising fact that the abundance ratio of the isotopes of potassium is not a constant figure for all organic tissues. Since the observed isotope effect is necessarily associated with processes involved in K metabolism, it was considered advisable to make a comprehensive study of the isotope ratio in various animal tissues in hope that it would contribute to our knowledge of the mechanism of K assimilation. Various tissues from the rat, including red blood corpuscles and blood plasma, have been chosen for investigation.

Potassium is known to have three natural isotopes, designated as: $^{39}_{19}\text{K}$, $^{40}_{19}\text{K}$ and $^{41}_{19}\text{K}$, the indices '39', '40' and '41' indicating the mass number and the common index '19' the nuclear charge. The 39-isotope represents the bulk of the element, the 41-isotope a relatively small fraction, while the 40-isotope (which is radioactive) exists only in a very minute quantity. The present study is concerned only with the 39- and 41-isotopes.

Methods

Preparation of ashes. Adult or preadult rats, taken from mixed strains, were studied first. The animals were anaesthetized with chloroform, and, after opening the chest, blood was drawn from the heart, usually by puncture of the left ventricle. The blood was cooled on ice, mixed with a small amount of 5% sodium citrate solution to prevent clotting, and then centrifuged at high speed. The citrate plasma was separated, centrifuged again and filtered through an ash-free filter. The red blood corpuscles were freed from residual plasma and white blood cells by washing them several times with 0.9% NaCl. Plasma and blood corpuscles were then dried at about 100°, part in glass and part in silica vessels.

The solid tissues were taken immediately after the recovery of the blood. The soft organs were freed as far as possible from adhering connective tissue, etc., and cut into small pieces; these were rapidly rinsed in distilled water and dried on Petri dishes at the above temperature. The bones were treated similarly. By the cardiac puncture and subsequent section of the venae cavae the blood content of the tissues was lowered appreciably. When no heart puncture was made, the animal was bled in a similar way after being chloroformed.

Some solid tissues were taken also from new-born rats. The animals were killed by decapitation and freed as far as possible from blood. The tissues were treated as above.

After drying, the corresponding material obtained from different animals was mixed and then ashed, wholly or in part. The ashing was carried out in a Pt dish, which had been previously cleansed and ignited to bright redness in order to remove any K possibly absorbed by the metal. The number of animals employed in the preparation of one ash sample varied considerably. Among the adult and preadult animals an equal number of males and females was as a rule utilized.

Mass-spectrographic measurement. The ash prepared in this way was used, without further treatment, for the determination of the isotopic ratio $^{39}\text{K}/^{41}\text{K}$ by means of a mass-spectrograph. The special type of apparatus employed was previously described in detail [Brewer, 1938]. The essential parts are shown schematically in Fig. 1. A Pt disk, 1.5 mm. in diameter, was the source of the K atoms. The disk was spot-welded to a tungsten filament which was heated by a heavy duty storage cell. Before coating, the disk was ignited at white heat until all residual K was removed. A small quantity of ash was then placed on the disk and heated in a vacuum to about 700° for 30 min. During this heating process the Pt absorbed sufficient K to become an excellent emitter of K ions after the ash had been carefully scraped away. The impregnated disk was mounted at a distance of 5–8 mm. directly above the entrance slit to the analysing chamber. This arrangement ensured that all the potassium ions leaving the disk and impinging on the slit had attained the same velocity. Between the disk and the slit a potential drop of 540 v. was maintained, the disk being positive. By surrounding the disk with a washer type shield, maintained positive to the disk by about 60 v., it was possible to bring all the emitted ions to a focus on the slit, and by a special arrangement (not illustrated) the slit could be placed directly in the centre of the path of the focussed ion beam. When the disk was brought to a temperature of about 600° , the positive ion current from the disk to the slit was of the order of 10^{-9} to 10^{-10} amp.

Fig. 1. Diagram of mass-spectrograph.

The ion current entering the analysing chamber was resolved by means of a magnetic field produced by an electromagnet with poles directed against the side walls of the chamber, which were made of Swedish iron. By adjusting the current to the magnet it was possible to bring all the ions of the same mass number to a focus at the collector slit. Since the latter was wide with respect to the entrance

slit, the entire resolved ion beam was caught by the collector. The intensity of each resolved ion current was recorded by means of an FP 54 Plotron. The ratio of the galvanometer deflexions at the 39 and 41 mass positions was equal to the isotopic ratio $^{39}\text{K}/^{41}\text{K}$, since under the given conditions the deflexions were linear over a large scale. From the isotopic ratio the percentage of the heavy isotope ^{41}K was calculated, neglecting the very minute amount of ^{40}K .

The most common error occurring in these measurements was associated with the 'background' resulting from the scattering in the resolved ion beams. Since accurate measurements could not be performed in the presence of background, all measurements were excluded in which the galvanometer deflexion at the 40 mass position exceeded 0.1 mm. Another error might have arisen from the platinum disk failing to take up the two isotopes exactly in the same ratio in which they were present in the ash; careful tests showed no evidence for such a possibility. In practice about thirty estimations of the isotopic ratio were always made in succession on each ash sample. While individual estimations showed some variation, the average of any ten estimations usually agreed to within ± 0.01 , corresponding to $\sim \pm 0.005\%$ ^{41}K .

Results

Ordinary commercial KCl (A.R.) served as a normal standard for the comparison with the K contained in tissues. For the mass-spectrographic analysis the chloride was converted into the perchlorate, which could be used in the same manner as the ash. The isotopic ratio $^{39}\text{K}/^{41}\text{K}$ determined with this preparation was 14.20, the ^{41}K content being 6.579%. Results obtained with ashes from various tissues are shown in Tables 1-5, each corresponding to one particular group of tissues.

In Table 1 the results from liver, kidney, lung and salivary gland are given. It will be seen that the isotopic ratios are, in the majority of cases, very close to that

Table 1. *Analysis of K in liver, kidney, lung and salivary gland*

Sample no.	Tissue	No. of animals used	Average animal wt. in g. (approx.)	Isotopic ratio $^{39}\text{K}/^{41}\text{K}$	% of ^{41}K
1	Liver	8	210	14.20	6.579
2	"	4	220	14.27	6.549
3	"	4	150	14.20	6.579
4	Liver (perfused)	2	235	14.23	6.566
5	Kidney	12	210	14.28	6.545
6	"	6	215	14.22	6.570
7	"	4	150	14.20	6.579
8	Lung	12	210	14.24	6.562
9	"	4	150	14.20	6.579
10	Salivary gl.	16	215	14.31	6.532
11	" "	10	210	14.21	6.575
12	" "	6	145	14.20	6.579

of mineral K. On the other hand, there are three values—one each with liver, kidney and salivary gland—which are very slightly increased, more than can be accounted for by the experimental error. The content of ^{41}K was thus decreased (by 0.5–0.7%). Ash sample no. 4 is of special interest. The livers used for the preparation of this sample were perfused *in situ* through the portal vein by K-free Ringer's solution with an increased content of Ca. This perfusion

removed almost completely the blood present in the tissue. The effect on the isotopic ratio was, however, negligible or zero.

Table 2 contains the results from skeletal muscle, heart muscle and brain. The values of the isotopic ratio do not show any noticeable deviation from that of mineral K, except one sample from skeletal muscle. Here again a minute increase of the isotopic ratio was observed, and a corresponding decrease of the $^{41}_{19}\text{K}$ content (by about 0.4 %).¹

The tissues dealt with in Table 3, i.e. spleen, lymph glands, testis, and bone including marrow, are distinguished from the above tissues by the greater rate of

Table 2. *Analysis of K in skeletal muscle, heart muscle and brain (cortex and medulla)*

Sample no.	Tissue	No. of animals used	Average animal wt. in g. (approx.)	Isotopic ratio $^{39}_{19}\text{K}/^{41}_{19}\text{K}$	% of $^{41}_{19}\text{K}$
1	Skeletal muscle	8	210	14.26	6.553
2	" "	4	220	14.20	6.579
3	" "	4	150	14.20	6.579
4	Heart muscle	12	210	14.20	6.579
5	Brain	12	210	14.22	6.570
6	"	4	155	14.19	6.583

Table 3. *Analysis of K in spleen, lymph glands, testis and bone including marrow*

Sample no.	Tissue	No. of animals used	Average animal wt. in g. (approx.)	Isotopic ratio $^{39}_{19}\text{K}/^{41}_{19}\text{K}$	% of $^{41}_{19}\text{K}$
1	Spleen	12	210	14.22	6.570
2	Lymph glands	16	225	14.20	6.579
3	Testis	12	250	14.20	6.579
4	Bone including marrow*	8	220	13.95	6.689
5	Do.*	2§	250	13.97	6.680
6	Do.*	2	195	13.97	6.680
7	Do.†	4	150	13.79	6.761
8	Do.‡	1§	225	13.88	6.721

* Diaphyses of humerus, femur and tibia of both sides.

† Diaphyses of femurs only.

‡ Diaphyses of left bones only.

§ Males only. || Females only.

proliferation of their cellular elements. It will be seen that with ashes from spleen, lymph glands and testis no noticeable deviation of the isotopic ratio was found. A marked difference, however, was obtained with ashes from bone including marrow. In each of the five samples analysed the isotopic ratio was clearly decreased, and the content of $^{41}_{19}\text{K}$ thus increased. The value of this increase showed some variation. In three of the samples it was 1.5–1.7 %, in one sample about 2.2 %, and in one sample about 2.8 %. The average was 1.9 %.

¹ Another analysis not included in the table was carried out with a sample of KClO_4 , prepared by HClO_4 precipitation from an extract of rabbit muscle. No deviation of the isotopic ratio was found. This result agreed with previous findings by Lasnitzki & Oeser [1937] concerning the radioactivity of K in rabbit muscle, a similar preparation (after its conversion into the chloride) having been used.

Further, it was found that ash prepared from a humerus, femur and tibia, after complete removal of the marrow from the cavity, showed an isotopic ratio of 14.03, corresponding to a $^{41}_{19}\text{K}$ content of 6.653 %. The bones were taken from the right side of the same animal from which the corresponding bones of the left side constituted ash sample no. 8. Thus K contained in the bone substance appears to show a smaller deviation of its isotopic ratio than K derived from both bone substance and marrow. K from marrow alone would show, therefore, rather a greater deviation of its isotopic ratio than that which was actually found.

The conclusion that the K present in bone marrow of the rat has a higher content of $^{41}_{19}\text{K}$ than ordinary mineral K agrees with similar findings previously obtained with marrow from horse and beef [Brewer, 1937].

The results obtained with red blood corpuscles and blood plasma are shown in Table 4. It is evident that the isotopic ratio of K in the ash of red blood corpuscles was not different from normal. On the other hand, a marked decrease was observed in the ash of blood plasma. The deviation was clearly indicated with each of the five plasma samples, yet with two of them more pronouncedly than with the others. The corresponding increases in the content of $^{41}_{19}\text{K}$ were 3.0-3.2 and 1.8-2.3 % respectively; the average increase was 2.5 %.

Table 4. *Analysis of K in red blood corpuscles and blood plasma*

Sample no.	Source	No. of animals used	Average animal wt. in g. (approx.)	Isotopic ratio $^{39}\text{K}/^{41}_{19}\text{K}$	% of $^{41}_{19}\text{K}$
1	Red blood corpuscles	12	220	14.20	6.579
2	" " "	6	220	14.19	6.583
3	" " "	4	155	14.20	6.579
4	Blood plasma	12	220	13.74	6.784
5	" " "	6	220	13.92	6.702
6	" " "	6	215	13.72	6.793
7	" " "	2	200	13.87	6.725
8	" " "	4	155	13.89	6.716

A special test was carried out to determine whether the very high Na content of the plasma ash, due to the mixture of blood and sodium citrate solution, might have interfered with the mass-spectrographic measurement; although there was no theoretical reason for such a possibility. Dry liver and muscle tissue were thoroughly mixed with sodium citrate in a ratio approximating plasma. The ashes of these mixtures and those of the plain dry tissues were compared respectively, but no difference in the isotopic ratio was found. Thus the high preponderance of Na in the plasma ash did not interfere with the measurements. (The same can be said with regard to Ca in bone ash, since the addition of a large amount of calcium phosphate to dry tissue did not affect the isotopic ratio.)

In Table 5 are given the results for liver, kidney and skeletal muscle, taken from new-born animals. While K contained in the ash of liver and muscle tissue showed about the same isotopic ratio as mineral K, that present in kidney tissue showed a minute increase of this value, and thus a slight decrease in the $^{41}_{19}\text{K}$ content (by about 0.7 %). This deviation is comparable with that obtained with one of the ash samples from adult kidney, but does not agree with the absence of any noticeable deviation in the two other samples. On the whole, no difference seems to exist in the isotopic ratios of K animals in these three tissues in relation to the age of the animal.

Table 5. *Analysis of K in liver, kidney and skeletal muscle of new-born animals*

Sample no.	Tissue	No. of animals used	Average animal wt. in g. (approx.)	Isotopic ratio $^{39}\text{K}/^{41}\text{K}$	% of ^{41}K
1	Liver	111	5.5	14.19	6.583
2	Kidney	127	5.5	14.30	6.536
3	Skeletal muscle	7	5.0	14.21	6.575

A representation of the whole of our results is shown in Fig. 2. The height of each column indicates the % of ^{41}K found in K contained in one particular ash sample, while the dotted line indicates the % of ^{41}K in mineral K. The columns are interrupted between 0.2 and 6.4 % ^{41}K .

Fig. 2. Content of ^{41}K in potassium present in various rat tissues.

DISCUSSION

Considering the results as a whole, it appears that a constant deviation from the isotopic ratio of mineral K was observed only for K present in bone marrow and blood plasma. In both cases the deviation consisted in a slight but definite increase in the content of the heavy isotope. The K contained in other tissues, i.e. liver, kidney, salivary gland and skeletal muscle, has occasionally shown minute deviations in an opposite direction.

In the living animal a continuous K exchange takes place between the various tissue cells and the blood plasma, the latter being probably in direct communication with the intercellular spaces. The exchange occurs in both directions, and generally at a slow rate. Further, the actual K content of the majority of tissues is very much (on an average perhaps twenty times) higher than that of the blood plasma, and this difference is maintained independently of the K exchange between cell and plasma. Although no definite picture of the mechanism of this exchange is available, it is certain that while K is specifically absorbed by the cell body, it remains in a state of dynamic equilibrium with the K in the surrounding medium. Under these conditions the appearance of an isotope effect will be possible.

A simple kinetic mechanism can be devised to account for the difference in the isotopic ratios of K in tissue (excluding bone marrow) and plasma. This mechanism depends on the difference in velocity of movement of the K atoms in the two media concerned. As far as we are aware, K atoms in the plasma and the medium directly surrounding the cell exist as free ions. The ratio of the numbers of ^{39}K and ^{41}K atoms striking the outer surface of the cell membrane, and entering thereby the cell, is then given by

$$n_{39}/n_{41} = \frac{C_{39}}{\sqrt{39}} / \frac{C_{41}}{\sqrt{41}}, \quad \text{.....(1)}$$

where C_{39} and C_{41} denote molar concentrations of ^{39}K and ^{41}K in the plasma. Since a state of equilibrium exists, K atoms are also moving in the reverse direction. In the cell, in contrast to the plasma, K is associated with heavy molecular groups of mass M (possibly proteins); in consequence the ratio of the numbers of ^{39}K and ^{41}K atoms striking the inner surface of the cell membrane, and thereby leaving the cell, is expressed by

$$n'_{39}/n'_{41} = \frac{C'_{39}}{\sqrt{(M+39)}} / \frac{C'_{41}}{\sqrt{(M+41)}}, \quad \text{.....(2)}$$

where C'_{39} and C'_{41} denote molar concentrations of ^{39}K and ^{41}K in the tissue. Since M is a mass large compared with that of the K atom, equation (2) may be written

$$n'_{39}/n'_{41} = C'_{39}/C'_{41}. \quad \text{.....(2a)}$$

At equilibrium not only the total number of K atoms but also the ratio of the numbers of ^{39}K and ^{41}K atoms striking the cell membrane in the two directions must be the same. In consequence

$$\frac{C_{39}}{\sqrt{39}} / \frac{C_{41}}{\sqrt{41}} = C'_{39}/C'_{41}. \quad \text{.....(3)}$$

From equation (3) the isotopic ratio of plasma K can be calculated if that of tissue K is given, and vice versa. If the value 14.20 is considered as the isotopic ratio of tissue K, the isotopic ratio of plasma K is found to be 13.85. This represents a 2.4 % increase in the ^{41}K content of plasma K compared with that of tissue K (and mineral K), a figure which agrees well with the observed average increase of 2.5 %. The value 14.30 for tissue K would correspond to the value 13.95 for plasma K, representing a 2.3 % enrichment in ^{41}K .

The mechanism described is proposed simply as a working hypothesis. Its claim to consideration rests on the fact that it accounts quantitatively for the observed isotope effect; while its validity depends on the condition that the intracellular K is completely associated with heavy molecules, and on the further condition that the hydration of the extracellular K ions can be neglected. If these conditions do not hold, the difference in the isotopic ratios calculable from equation (3) will be more or less reduced. Since this possibility exists, it is of interest to note that in addition to the kinetic mechanism a chemical mechanism may be in operation.

An appreciable fractionation of isotopes, due to slight differences in their chemical properties, can be obtained by means of 'exchange reactions' involving one or two phases, and a number of methods for isotope separation have been developed on this basis. A two-phase exchange reaction takes place, for instance, if the solution of an NH_4 salt is brought into contact with NH_3 gas. At equilibrium the ratios of the two N isotopes ($^{14}\text{N}/^{15}\text{N}$) in the liquid and gaseous phases were found to be as $\sim 1 : 1.023$, thus indicating a slight enrichment of the heavy isotope in the liquid compared with the gaseous phase [Urey, 1940]. Another kind

of two-phase exchange reaction takes place if certain aluminosilicates known as zeolites, which normally contain Na as a basic constituent, are brought into contact with solutions of Li or K salts. The Na of the silicate is exchanged for Li or K, but the light isotope of Li and the heavy isotope of K are taken up preferentially. The deviations in the isotopic ratios obtained in this way were similar to the above [Taylor & Urey, 1938]. Correspondingly, the K normally present in another kind of zeolite can be exchanged for Na, the heavy K isotope being more difficult to replace than the light one [Brewer, 1939]. A certain fractionation of Li isotopes was further obtained by means of an exchange reaction between Li in alcoholic solution and Li present in Li-amalgam [Lewis & Macdonald, 1936]. Exchange reactions of these types, involving a liquid and a solid phase or two liquid phases (possibly water and lipoid), may occur in the process of K assimilation by the cell. Although the magnitude and direction of the isotope effect cannot be computed, differences approaching those observed may appear.

It remains to say that the actual values of the isotopic ratios of tissue K and plasma K do not depend solely on the mechanism responsible for their difference, but also on the isotopic ratio of the K which enters the blood stream by way of the intestine, and on the ratio of the total amount of K in tissue and plasma. Assuming that K when passing into the blood has an isotopic ratio close to 14:20, and considering the fact that the total amount of tissue K is several hundred times greater than the total amount of plasma K, the observed constancy or very slight increase in the isotopic ratio of the former and the marked decrease in the isotopic ratio of the latter are to be expected.

SUMMARY

The isotopic constitution of K present in various rat tissues, including red blood corpuscles and blood plasma, was studied as far as the two main isotopes ^{39}K and ^{41}K are concerned. It was found that the isotopic ratio $^{39}\text{K}/^{41}\text{K}$ in the ashes from bone marrow and blood plasma was distinctly lower than that of ordinary mineral K as contained in KCl (A.R.), and the content of the heavy isotope ^{41}K was accordingly increased. K present in ash samples from other tissues showed, on the whole, the same isotopic ratio as mineral K, although a minute increase was found in exceptional cases. A kinetic mechanism has been proposed, which accounts for the difference in the isotopic ratios of tissue K and plasma K.

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