TABLE II

SYSTEM $NaNO_3$–NaI–H_2O AT 25°

Original complex, wt. %		Satd. solution, wt. %		Solid phase
$NaNO_3$	NaI	$NaNO_3$	NaI	
...	0.00	47.87	0.00	$NaNO_3$
35.90	25.27	22.57	30.49	$NaNO_3$
26.84	36.37	14.38	42.48	$NaNO_3$
26.07	40.95	10.64	49.22	$NaNO_3$
20.07	46.19	8.73	52.70	$NaNO_3$
16.01	52.02	6.77	57.64	$NaNO_3$
15.61	54.45	5.63	60.88	$NaNO_3$
9.96	60.88	5.47	61.11	$NaNO_3$ + $NaI \cdot 2H_2O$
8.02	62.92	5.48	61.13	$NaNO_3$ + $NaI \cdot 2H_2O$
5.43	64.73	5.50	61.14	$NaNO_3$ + $NaI \cdot 2H_2O$
Average (of 3)		5.48	61.13	$NaNO_3$ + $NaI \cdot 2H_2O$
2.97	66.35	3.93	62.06	$NaI \cdot 2H_2O$
2.05	67.08	2.57	63.12	$NaI \cdot 2H_2O$
0.00	...	0.00	64.71	$NaI \cdot 2H_2O$

The experimental results are presented in Tables I and II, and are shown graphically also in Figs. 1 and 2, which are self-explanatory. The solubility isotherms are seen to be of the simple two-branched type, with no evidence of any complex formation. The compositions of the isothermally invariant solutions for the two systems, are, respectively, as follows: for saturation with respect to both $NaNO_3$ and $NaBr \cdot 2H_2O$, 13.62% $NaNO_3$, 41.05% NaBr, 45.33% H_2O; for saturation with respect to $NaNO_3$ and $NaI \cdot 2H_2O$ simultaneously, 5.48% $NaNO_3$, 61.13% NaI and 33.39% H_2O. These values are in each case the averages of at least three determinations.

Summary

Solubility measurements are given for the two ternary systems $NaNO_3$–NaBr–H_2O and $NaNO_3$–NaI–H_2O at 25°; no complex formation is found, the solid phases being $NaNO_3$ and, respectively, $NaBr \cdot 2H_2O$ and $NaI \cdot 2H_2O$.

NEW YORK, N. Y. RECEIVED MARCH 15, 1937

[CONTRIBUTION FROM THE BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE]

Abundance Ratio of the Isotopes of Potassium in Animal Tissues

BY A. KEITH BREWER

The utilization of the mass spectrograph in determining the isotope abundance ratio and the atomic weight of potassium in minerals, plants and ocean water has been discussed in recent publications.[1-3] The results indicate that the isotope ratio is not necessarily constant in nature, but in specific instances may deviate appreciably from the normal. In the present paper this investigation has been extended to a study of the potassium isotope ratio in various animal tissues.

Experimental Technique

The apparatus was essentially the same as that described previously,[1] although several improvements were incorporated. The position of the filament slit was made adjustable by means of a screw operated through a sylphon tube; this permitted a better focusing of the maximum ion beam on the filament slit. The evacuation channel was extended completely along the bottom and both sides of the pole pieces; the channel not only facilitated evacuation but served as a trap to prevent ions not in focus from being reflected into the collector slit. The entire spectrograph was heavily chromium plated and the pole pieces highly burnished; it was found that when the iron pole pieces were exposed a deposit of rust collected on the surface which, because of its poor conductivity, became charged and in consequence distorted the resolved ion beam.

The samples of tissue to be tested were washed in distilled water and then ashed in a platinum crucible. The ion source used was a small platinum disk of the type described previously. The platinum was impregnated with potassium by placing a piece of ash about the size of a pin head on the disk and moistening with distilled water. The entire filament assembly was placed in a separate evacuation system and the filament heated to just redness for about thirty minutes; during the heating process an appreciable quantity of alkali dissolves in the platinum. After heating the disk was scraped free of all visible deposit; in cases where the ash fused on heating it was necessary to remove the disk from the filament to free it from ash. The disk was then smoothed by lightly tapping between plates and rewelded in position.

Results

The abundance ratios for the two principal isotopes of potassium found in various animal sources are presented in Table I.

In the abundance ratio column limits are expressed for the apparent uncertainty for each sample. The ratios given are the averages of determinations made on several samples while the uncertainty factor represents the limits to

(1) A. Keith Brewer, THIS JOURNAL, **58**, 365 (1936).
(2) A. Keith Brewer, *ibid.*, **58**, 370 (1936).
(3) A. Keith Brewer, *J. Chem. Phys.*, **4**, 350 (1936).

which the various readings deviated from the average. The atomic weights given are computed by assuming 1.00027 for the conversion factor from the physical to the chemical scale, and −7.0 for the packing fraction.

Discussion of the Method

The advantages of the mass spectrographic method in studying comparative atomic weights are: (1) the sensitivity is high, (2) no chemical purifications are necessary, (3) small quantities of material are required; the amount of potassium actually involved probably does not exceed 10^{-8} to 10^{-9} g. while the presence of far smaller quantities can be detected.

The possibility of error in mass spectrographic measurements may enter from two types of sources, general and functional. The general errors involve uncertainties in the isotope effect at the source, in the conversion factor from the physical to the chemical scale, and the packing fraction. None of these errors affects the relative values of the abundance ratios or the atomic weights.

Functional errors enter from an improperly operating apparatus and are not likely to affect all observations similarly. These errors have their source in some factor which gives rise to (1) a resolution of the primary ion beam between the source and the filament slit, (2) a general background which makes it difficult to measure the exact peak height, or (3) a broadening or scattering of the resolved ion beam. It is necessary to test for the presence of each of these factors in every sample to obtain comparable results. Ordinarily these difficulties are overcome by washing the filament slit free of any deposit, and by scraping all adhering ash from the disk source.

An accurate determination of the abundance ratio from measurements of the resolved ion currents necessitates that all the ions of the isotope in question which pass through the filament slit reach the collector. This was made possible in the present set-up by using filament and collector slits 0.22 mm. and 0.77 mm. in width, respectively; the width of the collector slit is thus appreciably greater than that of the resolved ion beam provided no scattering of the beam occurs. Under proper operating conditions the flat top of the K^{39} peak was one-fifth the width of the separation between the center of the K^{39} and K^{41} peaks. Since scattering tended to broaden the beam, it was discernible by a sharpening of the resolved peaks. It was found in practice that particles of ash which had not been removed from the disk and irregularities in the disk itself were responsible for most of the scattering.

Considering the various sources of error and nonuniformity in the results that are involved, it seems probable that the sixth significant figure in the comparative atomic weight calculations is accurate to within ±1 to ±5 depending on the sample. No such accuracy, however, can be claimed for the absolute value of the atomic weight. In this respect it should be pointed out that an abundance ratio of 14.20 gives 39.094 for the atomic weight using the most probable values for the packing fraction and the conversion factor, but since either of these quantities may be in error by as much as one part in 10,000, the atomic weight as computed may be inaccurate by as much as ±4 in the fifth significant figure. The calculated values in Table I, are included for comparative purposes only.

Significance

There are very few references in the literature relative to an isotope effect for potassium in the animal organisms. Potassium, nevertheless, is without doubt the most interesting of all the elements in this connection, since it is the only element necessary to the vital process that is radioactive, one of its isotopes emitting two hard β rays. Ernst[4] making use of this radioactivity reports that potassium from human and animal sources is more radioactive than normal. A. and M. Lasnitzki[5] observe some differentiation between mineral and biological potassium in the feeding of mice. A general review of the effect of natural processes on the isotope ratio has been given by Vernadsky.[6]

A survey of the data presented in Table I shows that the isotope abundance ratio for potassium in most animal tissues is close to 14.20; this is the same as that for sea water and the majority of plants and minerals. In only a few instances are any marked deviations from the normal to be found. The heart muscles and especially the lining of the right auricle as well as the membranes supporting the valves are inclined to be low in K^{41}. The heart was tested in parti-

(4) E. Ernst., *Naturwissenschaften*, **22**, 479 (1934).
(5) A. and M. Lasnitzki, *Nature*, **138**, 800 (1936).
(6) W. I. Vernadsky, *Compt. rend. acad. sci. U. R. S. S.*, **3**, 129 (1936).

TABLE I

ABUNDANCE RATIO AND COMPUTED ATOMIC WEIGHT OF POTASSIUM IN VARIOUS ANIMAL TISSUES

Source of sample	Part	K^{39}/K^{41}	% K^{41}	Calcd. at. wt.
Horse 30 years	Marrow	13.90 ± 0.03	6.71	39.0967
Horse 26 years	Marrow	13.92 ± .04	6.70	39.0965
Beef Bull (old)	Marrow	13.80 ± .02	6.76	39.0975
Beef Bull (old)	Marrow oil	13.90 ± .05	6.71	39.0967
Beef Bull (old)	Marrow hard	13.68 ± .03	6.81	39.0986
Beef Veal	Marrow	13.70 ± .03	6.80	39.0985
Beef Mature	Marrow	13.83 ± .02	6.74	39.0973
Beef Mature	Bone	14.03 ± .02	6.65	39.0955
Beef Mature	Red meat	14.21 ± .01	6.58	39.0939
Beef Mature	Thyroid	14.21 ± .02	6.58	39.0939
Beef Mature A	Kidney	14.22 ± .03	6.57	39.0938
Beef Mature B	Kidney (Leached)	14.21 ± .03	6.58	39.0939
Beef Mature	Suet	14.21 ± .01	6.58	39.0939
Beef Mature	Liver	14.20 ± .03	6.58	39.0940
Beef Mature	Liver extract	14.23 ± .03	6.57	39.0938
Pork	Liver	14.21 ± .03	6.58	39.0939
Pork	Pancreas	14.24 ± .02	6.56	39.0937
Pork	Lung	14.16 ± .01	6.60	39.0934
Pork	Adrenal	14.21 ± .01	6.58	39.0939
Pork	Kidney	14.19 ± .02	6.58	39.0941
Pork	Stomach	14.22 ± .01	6.57	39.0938
Pork	Pituitary	14.17 ± .02	6.59	39.0943
Pork	Ventricle muscle	14.20 ± .01	6.58	39.0940
Pork	Auricle muscle	14.24 ± .02	6.56	39.0937
Pork	Auricle membrane	14.28 ± .03	6.55	39.0933
Pork	Blood	14.23 ± .03	6.57	39.0938
Pork	Intestines	14.25 ± .02	6.56	39.0935
Mutton	Cartilage	14.13 ± .02	6.62	39.0946
Mutton	Suet	14.20 ± .02	6.58	39.0940
Oyster		14.19 ± .02	6.58	39.0941

cular since it has long been known that potassium in the blood is a factor in regulating heart beat, and since Zwaardemaker[7] and Loeb[8] have raised the question of the effect of the radioactive potassium isotope on beat stimulation. The results indicate that the lining of the auricle may contain less than the normal amount of the radioactive isotope. Some uncertainty still remains regarding which potassium isotopes are responsible for the radioactivity although the abundant isotope K^{39} is known to be non-radioactive. Recently Smyth and Hemmendinger[9] have shown that the exceedingly rare isotope K^{40} is responsible for part and possibly all of the radioactivity. Any process, however, that will concentrate K^{41} will doubtless concentrate K^{40}; in consequence an abundance ratio below 14.20 should represent a radioactivity above normal. The facts that this radioactivity is feeble and that the deviations in the abundance ratio recorded in Table I are small make it seem very doubtful whether the potassium from various tissues is distinguishable by radioactive measurements. The relative abundance of K^{40} has been measured by A. O. Nier[10] and by the writer[11] but measurements of the K^{39}/K^{40} ratio involve too much uncertainty for a comparison of various sources.

The most conspicuous concentration of K^{41} was observed in bone marrow. The data, while insufficient, indicate a possible dependence of the abundance ratio on the age of the animal; in all the cases examined the ratio was higher for young than for old animals. It should be mentioned that bone marrow was investigated in some detail, since Hoffmann[12] had shown that the potassium content appears to be associated with embryonic cell development, diminishing markedly at the conclusion of growth activity, and increasing again on the development of carcinoma. Unfortunately, it was not possible to make abundance ratio tests on any cancerous animals.

(7) H. Zwaardemaker, *Geneesk Blader*, **20**, 253 (1918); *J. Physiol.*, **55**, 33 (1921).

(8) J. Loeb, *J. Gen. Physiol.*, **3**, 229 (1920).

(9) W. R. Smyth and A. Hemmendinger, *Phys. Rev.*, **51**, 146 (1937).

(10) A. O. Nier, *ibid.*, **50**, 1041 (1936).

(11) A. Keith Brewer, *ibid.*, **48**, 640 (1935).

(12) T. Hoffmann, *Biochem. Z.*, **243**, 145 (1931).

In conclusion it should be mentioned that while the deviations which have been reported for animal and plant tissues are small, they are nevertheless appreciable. Deviations of this magnitude are exceedingly difficult to produce in the laboratory; the writer has tried several times himself and has examined many samples prepared by eminent scientists but in no instance has a definite isotope shift been observed. While it is possible to conjecture concerning the mechanism of the isotope effect in nature, the data are still too meager to permit any definite conclusion to be drawn.

The writer is indebted to Mr. N. G. Barbella, of the Bureau of Animal Industry for furnishing many of the samples tested.

Summary

The abundance ratio of the two principal isotopes of potassium present in animal tissues have been measured with a mass spectrograph. The K^{39}/K^{41} ratio for most organs is close to 14.20, which is the same as that previously obtained for most plants and minerals and for ocean water. A few tissues, such as the lining of the auricle and the lining of the small intestine, appear to possess an abnormally high concentration of K^{39}, while bone marrow is high in K^{41}. The results with bone marrow are significant in that they indicate a possible relationship between the abundance ratio and the age of the animal, and hence with the development of embryonic cells within the organism.

The atomic weight of potassium has been calculated using the most probable values for the packing fraction and the conversion factor; the value thus obtained for most tissues is 39.094. Since all deviations from this normal value are small, it does not seem probable that the potassium in animal tissue could be distinguished from mineral potassium by radioactive measurements as has been suggested by some investigators.

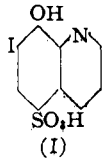
WASHINGTON, D. C. RECEIVED MARCH 13, 1937

[CONTRIBUTION FROM THE COBB CHEMICAL LABORATORY, UNIVERSITY OF VIRGINIA]

A Study of 7-Iodo-8-hydroxyquinoline-5-sulfonic Acid as a Reagent for the Colorimetric Determination of Ferric Iron[1]

BY JOHN H. YOE AND ROBERT T. HALL[2]

In 1932 Yoe[3] reported a method for the colorimetric determination of iron by means of 7-iodo-8-hydroxyquinoline-5-sulfonic acid (I).[4] It was found that this compound reacts instantaneously with ferric ions yielding green colored solutions, the color intensity varying with the iron concentration. The reaction is very sensitive and may be used to determine ferric iron in the presence of ferrous, since the latter produces no color with the reagent. Also, the color does not fade on standing, which is an advantage over the thiocyanate method as frequently carried out in acid solution.



(I)

In view of the advantages offered by this method, namely, the stability of the colored compound, the high sensitivity of the reaction, and its ability to distinguish between ferric and ferrous ions, it seemed desirable to investigate it in detail. In fact, the reagent appears to be specific for ferric ions, since no other ion has been found to yield a color reaction with Ferron.[3]

The following studies have been made on the reagent and its reaction with ferric ions: solubility in various solvents; nature of the color reaction; physical and chemical properties of Ferron; influence of various ions; sensitivity; Lambert–Beer law; effect of hydrogen-ion concentration; aging effect and the effect of temperature.

Reagents and Solutions.—The general technique and method of investigation has been described in a previous paper by Yoe and Wirsing.[5] 7-Iodo-8-hydroxyquinoline-5-sulfonic acid (Ferron) was first prepared by Claus.[6] The compound used in this investigation was a very pure product obtained from G. D. Searle and Co., Chicago, Ill. A saturated aqueous solution (approximately 0.2%) of Ferron makes a satisfactory reagent solution. For convenience in obtaining various molecular ratios between the Ferron and ferric iron, several other Ferron solutions of known concentration were prepared.

(1) Original manuscript received April 11, 1936.

(2) Present address, Swann and Company, Birmingham, Ala.

(3) Yoe, THIS JOURNAL, **54**, 4139 (1932).

(4) On account of the lengthy name of this compound we are proposing that it be called "Ferron."

(5) Yoe and Wirsing, THIS JOURNAL, **54**, 1866 (1932).

(6) Claus, *Friedl.*, **3**, 964 (1892).