a mild, non-progressive anemia which morphologically, biochemically and kinetically resembled the anemia associated with chronic disorders in man. It is suggested that "adjuvant disease" in the rat is an appropriate experimental model in which to study the complex pathogenesis of this distinctive but not uncommon form of anemia in man.

We wish to express our appreciation to Dr. R. Sidney Cloud for advice and assistance in the production of adjuvant disease and to Mrs. Alice Tustison and Mrs. Barbara Halliday for technical assistance.

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Received July 5, 1967. P.S.E.B.M., 1967, v126.

Isotope Abundances for Potassium of Biological Origin. (32442)

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The isotopic composition of potassium from a wide variety of sources is now generally accepted as constant. A rare example of a departure from the normal isotopic composition is provided by the unusual concentration of potassium-40 in iron meteorites (1,2). Here the increase in concentration of this isotope is attributed to the action of cosmic radiation.

The earlier reports of fractionation of the isotope of potassium by biological system (3-9) must be accepted with reserve. Much of this work was done on samples that had not been purified. Later work on carefully purified samples yielded "constant" ratios(10-16) although the values of the ratios varied from author to author by 1-2%. The most recent measurements by Reutersward(12) (who had reviewed the earlier work) and Kendall(15) show that for samples which have not been purified, the isotope ratios are influenced by the impurities present.

It was mainly because of the earlier work of Lasnitski and Brewer(4,7,8) who found an increase in the $K^{39}:K^{41}$ isotope ratio for cancer tissues of human and animal origin and the more recent findings of Kendall(15) that impure samples of potassium from cancer tissue have a different isotope ratio from impure samples of potassium from normal tissue, that the present investigation was undertaken.

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Materials and methods. All potassium-39/potassium-41 ratio determinations were made on a Metropolitan-Vickers, M.S.2, S-G mass spectrometer, ion current measurements being made from recorder tracings. Although results are given for isotope ratios, this does not mean that these are absolute abundance ratios. The mean given for potassium-39/potassium-41 ratios on A.R. potassium nitrate (14.09) is some 4% higher than the usually accepted value of Nier(10) (13.48 ± 0.07). Subsequent per cent abundance measurements, using the stable isotope of calcium, indicate that the instrument preferentially measures lighter isotopes, but the extent of the preference is not known. Consequently, no attempt has been made to give corrected potassium-39/potassium-41 ratios. Nevertheless the ratios quoted do allow a comparison of relative differences to be made.

Most of the normal and malignant tissues were obtained in operations performed at the Special Unit, Prince of Wales Hospital. Some tissues, both normal and malignant, were obtained from post-mortems immediately after death. Crown St. Women's Hospital supplied the human foetal specimens from terminated pregnancies and the State Abbatoirs, Homebush, supplied the pig foetal material. Kelp samples *(Macrocystis pyrifera)* came from

TABLE I. List of Tissue Samples Collected for Isotope Analysis.

Cancer tissue I	Normal tissue from cancer patients II	Normal tissue from non-cancer patients III	Foetal tissue IV	Plant tissue V
Rhabdomyosarcoma of thigh	Sternum† Liver†	Breast Skin from axilla (2)	*Human:* Brain	Kelp bladders Kelp fronds
Wilm's Tumour				
	Whole kidney†	Granulation tissue from burn (3)	Voluntary muscle	Kelp stipes
Subcutaneous metastases from Ca. Colon	Thigh muscle†		Liver	Whole plants†
		Rectal wall		
Reticulosarcoma of rib	Tail of pancreas (2)		Kidney	Tobacco
		Marjolin's ulcer of thigh		
Lymphoma-axillary node	Bronchus		Heart	
		Edge of ulcer		
			Lung	
Lymphosarcoma-axillary node	Cervix			
		Skin around ulcer	Long bones	
Ca. Rectum-extending into the sub-mucosa	Spleen			
			Intestine	
Ca. tail of Pancreas (3)	Ovary			
Ca. Bronchus (3)	Uterine body		*Pig:*	
Ca. Breast*	Red blood cell (5)	Granulation tissue from burn*	Liver (8)	
Wilm's Tumour*	Saliva	Red blood cells (5)	Brain (8) Muscle (8)	
Secondary ca. ovary from breast		Saliva		
Fibrosarcoma, hip				
Medulloblastoma				

Unless otherwise stated, all samples were subjected to the chemical treatment outlined in text.
† Samples for which both ashed and digested results are available.
* Samples ashed, not digested.

Southport, Tasmania, by courtesy of the School of Biological Sciences, University of New South Wales.

When selecting the tumor tissue for analysis, only the lesion itself was taken. Normal tissue from either cancer patients or patients free of diagnosible cancer was selected to be representative of the organ concerned and not from any specific layer or region. All samples were freed of excess blood, oven dried at 110°C and fat-extracted with chloroform for 18-24 hours. The dried residues were ground in a small hammer mill and then either ashed (for "impure" samples) or chemically treated.

Samples from which the potassium was chemically isolated were digested in a nitric-perchloric acid mixture, the potassium being precipitated as the tetraphenyl boron salt and converted to the nitrate. (Preliminary trials had shown that potassium nitrate gave the most satisfactory results for isotope ratio determination on potassium salts in our apparatus.) A sample of A.R. potassium nitrate which was subjected to this chemical purification, showed no alteration in its isotope ratio. For impure samples the tissues were ashed at 400°C and extracted with nitric acid. The sample was ionized from a triple filamented source, by vapour ionization from a centre (tantalum) filament, the sample being placed on a side (tungsten) filament. Average sample size was 1 $\mu g/mm^2$. Currents of 2.5-2.9 amps on the centre filament were required to produce a potassium-39 ion current of around 3×10^{-10} amps. The ion current was detected by a single plate collector using a slit width of .025" and a grid leak resistor of 20,000 megohms (Welwyn 1%).

Results. Some considerable time was first devoted to improving techniques of sampling and measuring, so that the method error could be kept as low as possible. With this in mind, 40 separate sub-samples of the standard A.R. potassium nitrate§ (triple crystallized ex Stassfurt) were subjected to potassium-39/potassium-41 ratio analysis. On each sub-sample, 3 separate analyses, each of 11 isotope ratio determinations, were made. The mean value for A.R. potassium nitrate,

TABLE II. Mean for Potassium-39/Potassium-41 Ratios for Purified Samples.

Sample	Potassium-39/ Potassium-41 Ratio
A.R. Potassium Nitrate (40)*	14.09 ± .01 (s.e.)
Cancer Tissues (14)	14.11 ± .02 (s.e.)
Normal Tissues	
From patients with cancer (22)	14.08 ± .03 (s.e.)
From patients free from cancer (18)	14.12 ± .02 (s.e.)
Foetal Tissues	
Human (8)	14.13 ± .02 (s.e.)
Pig (24)	14.12 ± .01 (s.e.)
Plant Tissues (13)	14.08 ± .02 (s.e.)

* Numbers in brackets refer to number of samples analysed for each group.

(Table II), is the potassium-39/potassium-41 ratio obtained from the 40 sub-samples. In other words the figure of 14.09 represents the mean of 1320 separate ratio determinations on the same A.R. potassium nitrate. The standard error quoted reflects the ratio variation from sub-sample to sub-sample (*i.e.*, it gives a measure of the precision of the determination), reflecting variations due to changes in source alignment, source pressure, filament assembly, filament current, etc. It gives a measure of the reproducibility of the method. The same procedure has been adopted for all mean potassium-39/potassium-41 ratio determinations listed in Table II. From the error quoted for the potassium-39/potassium-41 ratio determinations on A.R. potassium nitrate, a method error of 0.14% of the mean should be expected for isotope ratio determinations.

The mean potassium-39/potassium-41 ratio determination on the cancer tissues listed in Table II (*i.e.*, the mean of 14 separate samples) was 14.11 ± .02 (s.e.), a value not significantly different from that found for A.R. potassium nitrate. These results are for the purified samples only, the results for all ashed samples being given in Table III.

The ratio found for normal tissue from persons with cancer was 14.08 ± .03 (s.e.), again a value which does not differ significantly from the ratios obtained for the standard. However, there was a great variation from sample to sample for isotope ratio determi-

§ Assumed to be isotopically homogenous.

TABLE III. Potassium-39/Potassium-41 Ratios for Impure (Ashed) Samples.

Sample	Potassium-39/ Potassium-41 Ratio
Cancer tissues (4)	14.04 ± .02 (s.e.)
Normal tissues (5)	14.08 ± .02 (s.e.)
Plant tissues (2)	14.10 ± .04 (s.e.)
Mean potassium-39/ potassium-41	14.07 ± .02 (s.e.)

Numbers in brackets refer to number of samples analysed for each group.

nations on these samples, a deviation of up to 3.7% of the group mean occurring. Repetition of the analysis removed the anomaly and gave values which did not differ from the mean. Cook(17) observed variations in the isotope ratio of up to 2.5% for a sample left overnight in the mass spectrometer. However, all ratios reported in this study were made on samples which were analyzed within a short time of loading onto the filament. Therefore, this effect would not be an explanation for the variations in the isotope ratio observed for these samples.

For the analysis of normal tissues from persons without cancer, a significantly higher potassium-39/potassium-41 ratio was consistently found for one sample of staphylococcal pus. A mean of 14.37 for potassium-39/ potassium-41 ratio was obtained, compared to the group mean of 14.12 ± .02 (s.e.). An elevated ratio was found even after repurification. Ratio determinations made on a different sample of pus however, gave values within the normal mean potassium-39/potassium-41 ratio of range of this survey, a 14.03 being obtained.

Samples of human foetal tissues analyzed were from pooled organs of 2-3 month old foetuses. A mean ratio of 14.13 ± .02 (s.e.) was found. The pig foetal samples consisted of only 3 different tissues—liver, brain and muscle—from litters of varying foetal age. A mean potassium-39/potassium-41 ratio of 14.12 ± .01 was obtained. No correlation was found between foetal age and isotope ratio.

Plant samples consisted of different parts of the Kelp plant *(Macrocystis pyrifera)* and cigarette tobacco. A mean ratio of 14.08 ± .02 was found, a value which again is not significantly different from the standard.

In an attempt to find whether impure samples of potassium gave anomalous ratios, the samples indicated in Table I as having been ashed only, were subjected to mass spectrometric analysis. A mean ratio of 14.07 ± .02 (s.e.) was found (Table III) a value which does not differ significantly from that obtained from A.R. potassium nitrate. This finding differs from those of Reutersward (12) and Kendall(15) (and perhaps also the earlier studies of Brewer and co-workers) who observed alterations in the potassium-39/ potassium-41 ratios for impure potassium salts. Failure to observe differences in isotope ratios in the present study was not due to inability of the mass spectrometer to detect variations in ratios, since samples which had their isotope abundances deliberately altered, were found to give anomalous isotope ratios. Perhaps the levels or nature of the impurities present in the samples used in the present work were such that they exercised no influence on the isotope ratio. The deliberate addition of salts (such as those of sodium, iron, calcium, etc.) to A.R. potassium nitrate did result in alteration of the isotope ratio obtained; hence the inability to detect alterations in the ratios for ashed samples is not due to the mass spectrometer being "impurity insensitive." It must be concluded therefore, that if these impurities were present in the samples of biological origin used in this work, the levels were too low to affect the ratio.

Discussion. The findings herein reported do not support the earlier work of Lasnitski and Brewer(4,7,8) who claim to have observed an alteration in the potassium-39/ potassium-41 ratio for cancer tissue. The reason for the difference reported by these workers has been shown by Reutersward(12) and Kendall(15) to be due to the presence of impurities in the sample. The same reason may be responsible for the high potassium-39/ potassium-41 ratio found for staphylococcal pus sample in this survey, although repurification of the sample did not lower the ratio obtained. However, "impurities" such as rubidium and caesium would not be excluded by this repurification being precipitated with the potassium. These elements, although they would not produce nuclides in the mass 39-41 region, could result in isotope difference during vaporization of the sample prior to ionization (cf. Reutersward(12)). Again, the presence of trace amounts of impurities in some of the normal tissues may have been responsible for the higher variation in ratios obtained for these samples when compared to cancer tissues. The variety of normal tissues from both cancerous and non-cancerous patients was similar to that of the cancer samples and therefore should have a similar variation in mineral content. However, due to the high concentration of potassium in actively growing cancer, the potassium to "impurities" ratio in cancer tissues would be high and perhaps less variable than in normal tissues. This, however, is contrary to the findings of Kendall(15) who reported an alteration in isotope ratios for impure samples from cancer tissues compared with impure samples from normal tissues. In this survey impure (ashed) samples, which probably had a relatively high level of impurity, were found to give potassium-39/potassium-41 ratios which did not differ from the ratio found for a standard A.R. potassium nitrate. The levels must have been lower than those of the salts deliberately added.

Summary. The results of measurements of potassium-39/potassium-41 isotope ratios on biological samples presented in this paper do not support earlier claims(4,7,8) that the isotopes of potassium are fractionated by biological systems. Ratios obtained for potassium nitrate isolated from normal and malignant human tissues, pig and human foetal tissue and plant tissue did not differ significantly from the mean value found for A.R. potassium nitrate 14.09 $\pm$ .01 (s.e.). The ratios obtained for impure (ashed) tissue samples similarly were not significantly different from the mean found for the A.R. potassium salt. This differs from the results presented by Reutersward(12) and Kendall(15) who found a higher ratio for samples from which the potassium was not chemically isolated.

The authors wish to acknowledge the support of the New South Wales State Cancer Council, enabling this survey to be made and to Dr. K. W. Starr, Honorary Director, Special Unit, Prince of Wales Hospital, for giving permission to publish these results. The cooperation of the Department of Nuclear and Radiation Chemistry, School of Chemistry, University of New South Wales, is acknowledged and, in particular, Commander J. Mason and Mr. J. Bell are thanked for assistance with problems associated with the Mass Spectrometer.

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Received July 5, 1967. P.S.E.B.M., 1967, v126.