JOURNAL OF NUCLEAR MEDICINE 7:917-927, 1966

Neutron Activation Analysis of Magnesium, Calcium, Strontium, Barium, Manganese, Cobalt, Copper, Zinc, Sodium, and Potassium in Human Erythrocytes and Plasma[1]

D. A. Olehy,[2] R. A. Schmitt,[3] and W. F. Bethard

San Diego, California

Except for strontium and barium, the elements considered in this study are of known biological importance. Strontium and barium are of toxicologic interest. The method presented was developed in conjunction with studies on the changes in concentrations of selected cations in human erythrocytes during storage (1).

Samsahl, Brune, and Wester (2, 3, 4, 5) have developed excellent ion exchange procedures for separating many radioactivated elements in biological media. These procedures involve group separations, using more than one type of resin, i.e., anion, cation, and mixed bed. It was desired that a rapid and simplified ion exchange method be devised for selected cations which would have good decontamination factors with a minimum of chemistry. Parr and Taylor (6) developed an ion exchange method for Co, Cu, Fe, and Zn, using an anion resin equilibrated with hydrochloric acid. Co, Cu, and Fe were separated as a group from Zn after decontaminating for sodium-24.

Using Kraus and Moore's data (7) on the adsorption coefficients of elements from hydrochloric acid as a guide, the selective elemental elution method was formulated.

It is conceded that atomic absorption, emission spectrometry, and other methods of analysis (8, 9, 10) may be just as accurate and more convenient for certain elements, such as sodium, potassium, and group II A metals; however, for some elements radioactivation has certain advantages, depending on the matrix and trace element concentrations.

---

[1]These studies were supported in part by the Office of Naval Research, Department of the Navy, under Contract Nonr-4397(00).

[2]Present address: National Reactor Test Site, Idaho Falls, Idaho.

[3]Present address: Department of Chemistry, Oregon State University, Corvallis, Oregon.

APPARATUS AND REAGENTS

Columns used were 1 cm^2 in cross sectional area by 10 cm with a coarse sintered-glass filter, stopcock. Resin used was Bio-Rads Dowex 1 × 10, 200 to 400 mesh in Chloride form. Mallinckrodt TransistAR grade hydrochloric and nitric acids were used together with Mallinckrodt reagent-grade sodium hydroxide, calcium carbonate, magnesium metal, zinc, cobalt nitrate, strontium carbonate, barium nitrate, and manganese dioxide. Copper was from 99.999% pure copper foil. These reagents were used to make up the combined carrier solution.

Resin was prepared for use by rinsing with a 2:1 ratio, by volume, of deionized water to resin allowing the resin to settle, then decanting the liquid. This procedure was repeated three to four times until the liquid was clear above the resin.

Elemental standards were made from Johnson, Matthey and Co., Ltd., Specpure reagents.

EXPERIMENTAL

Blood samples were collected intravenously with polyethylene syringes or from blood-bank stores. Sample volumes averaged 15 ml of whole blood, from which 5 ml aliquots of plasma were taken, and 5.0 to 6.0 ml of red blood cells were used for the analyses. Specimens were transferred to clean Vycor crucibles and placed in an oven at 110° to 120°C to evaporate the water. After the specimens were thoroughly dry, they were placed in a muffle furnace at 200° to 250°C and the temperature was gradually increased to a maximum of 550°C over an

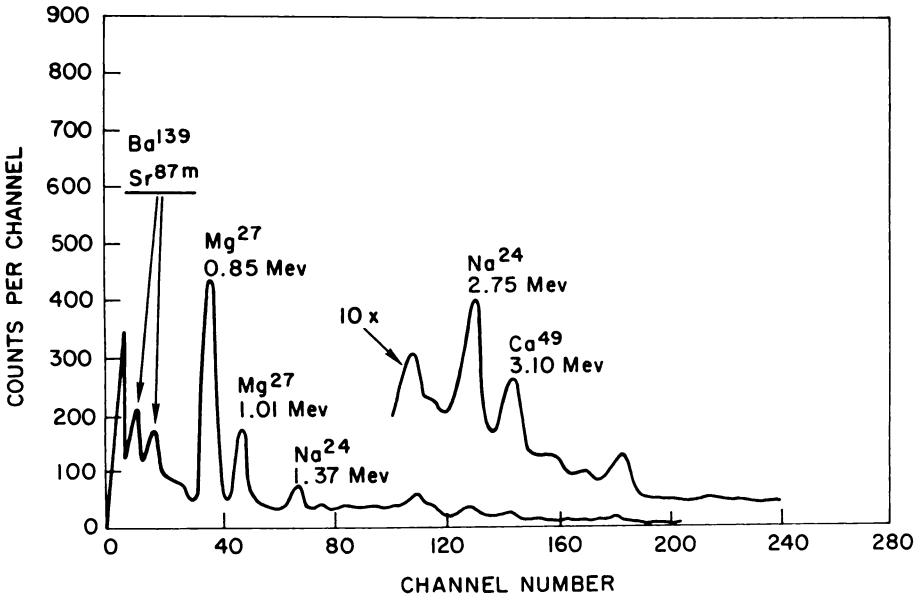

Fig. 1.

eight-hour period; then they were allowed to stand overnight for complete ashing. However, the plasma samples may require a much longer period for ashing in order to avoid fusing the samples to the Vycor crucibles.

Vycor crucibles were chosen because some of the cations absorb to platinum crucibles while in the furnace, apparently attributable to the reducing atmosphere created by carbon monoxide and dioxide from the specimens. This condition was confirmed by using radioactive tracers in specimens during ashing in order to check on volatility losses. After cooling, the specimens were quantitatively transferred to clean, weighed, 10 ml polyethylene vials for irradiation. The average weight of ash per unit volume for red blood cells and plasma was 8.9 mg/ml.

Specimen ash and elemental standards were irradiated for 30 minutes at a flux of 2×10^{12} neutrons cm^{-2} sec^{-1} in the General Atomic TRIGA reactor. At the end of irradiation, the specimens were removed and transported to the laboratory as quickly as possible. It should be noted that prior to irradiation the ion exchange columns had been equilibrated with concentrated hydrochloric acid and all necessary equipment, carrier solutions, and other apparatus were prepared for immediate use.

CHEMICAL SEPARATION OF ELEMENTS

The irradiated specimen ash was transferred to a test tube containing 1.00 ml of mixed carriers having approximate concentrations of magnesium (10.00 mg), calcium (5.00 mg), manganese (0.2-0.3 mg), cobalt (5.00 mg), copper (5.00 mg), zinc (5.00 mg), strontium (5.00 mg), and barium (5.00 mg). The polyethylene vial was rinsed with concentrated hydrochloric acid to dissolve any remaining ash on the walls of the vial, and this residue was added to the

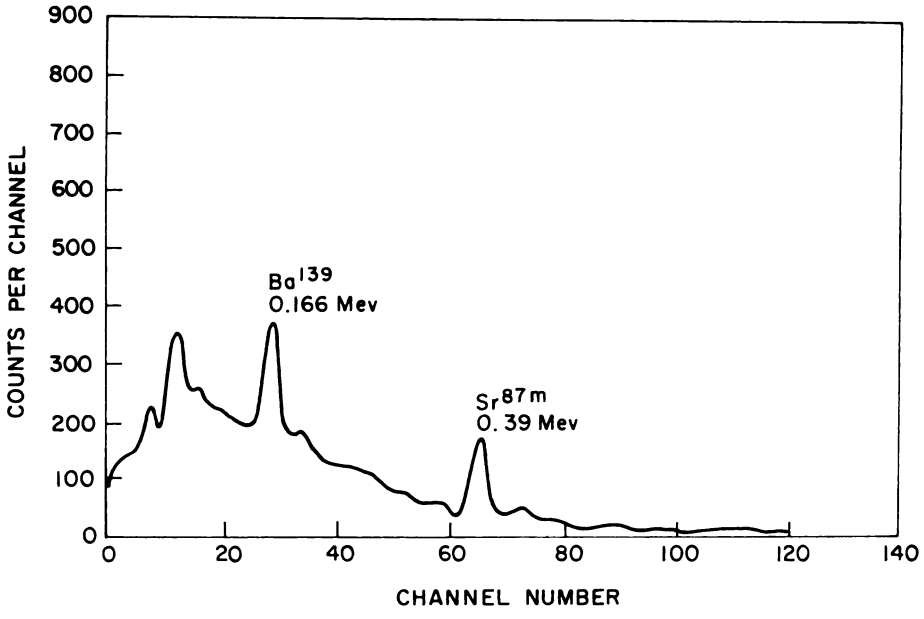

Fig. 2.

carrier solution. Eight to ten drops of 30% hydrogen peroxide were added to the carrier solution with enough concentrated hydrochloric acid to make the total volume of solution about 10 milliliters. The solution was heated to boiling to dissolve the ash and to reduce manganese (IV) to manganese (II), as well as to destroy excess hydrogen peroxide. More hydrochloric acid was added until the volume was approximately 13 ml, then the solution was put through the ion exchange column. Initial elution was performed under less than 1 psi nitrogen pressure with a maximum rate of 1 cm^3 per min.

Because of the short half lives of 9.5-min ^{27}Mg and 8.8-min ^{49}Ca, pressure is necessary to speed the elution. The initial effluent, which contained approximately 50% Mg plus Ca, Sr, and Ba activities, was collected. This effluent was diluted with about 10 ml of water and a few drops of phenolphthalein indicator and 19 N sodium hydroxide was added until it was neutral with slight excess. Sodium carbonate was added at 0°C to precipitate the carbonates of Ba, Ca, Mg, and Sr. After centrifuging and decanting the supernate, the precipitate was dissolved in a few drops of 6 N nitric acid, diluted and reprecipitated with 6 N sodium hydroxide, adding 7 to 8 drops in excess of the end point and again adding 1 to 2 ml of sodium carbonate during cooling in an ice bath. After centrifuging and decanting, the precipitate was washed once or twice with deionized water, dissolved in a few drops of 6 N nitric acid, and transferred to a clean polyethylene vial. The volume was normalized with the standards of Mg, Sr, Ca, and Ba and counted.

Gamma-ray spectra were taken of all elements analyzed, using a multichannel analyzer with 3 in. × 3 in. sodium iodide thallium-activated crystals, both well and solid types. Since the ^{27}Mg, ^{49}Ca, ^{87m}Sr and ^{139}Ba activities are all com-

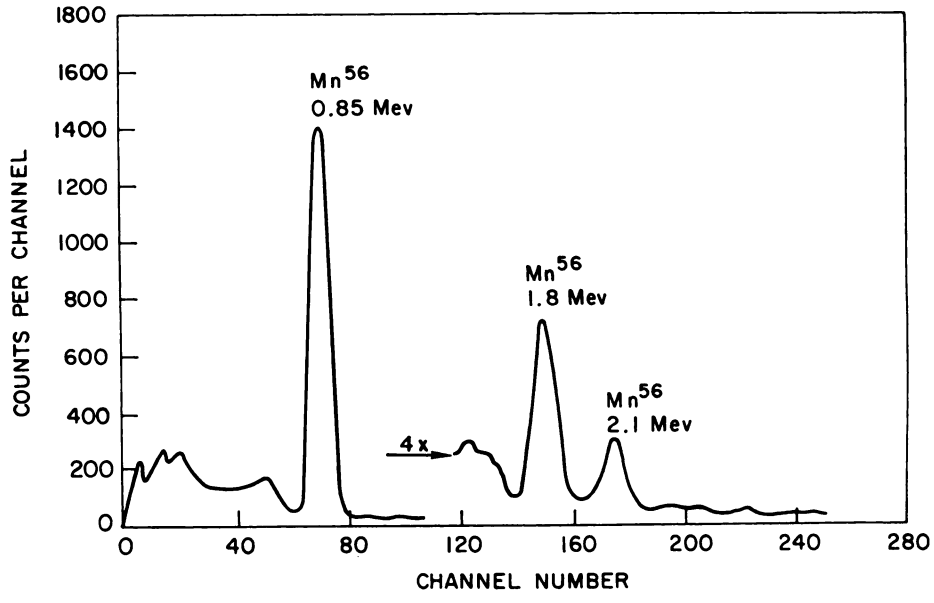

Fig. 3.

bined, two spectra are necessary. To obtain ^{49}Ca and ^{27}Mg, spectra were taken at 20 to 30 keV per channel in a well crystal (see Fig. 1), then recounted at 5 to 6 keV per channel for a longer period to obtain good spectra of ^{87m}Sr and ^{139}Ba activities (see Fig. 2). The gamma rays of these particular isotopes (^{139}Ba, 0.16 MeV; ^{87m}Sr, 0.39 MeV; ^{27}Mg, 0.84-1.0MeV; and ^{49}Ca, 3.1 MeV) have sufficient energy differences so as not to interfere appreciably with each other. Spectrum stripping of the Compton contribution due to high energy gamma rays would delineate more sharply the ^{139}Ba and ^{87m}Sr photopeaks and reduce considerably the statistical error in Ba and Sr analysis.

After the initial effluent was collected, an additional 10 ml of concentrated hydrochloric acid was put through the column and the eluent set aside. Manganese was eluted with 10 to 13 ml of 6 N hydrochloric acid and two hydroxide precipitations were performed after addition of 2 to 3 mg of iron (III) carrier. After a water wash, the precipitate was dissolved with a few drops of 6 N nitric acid plus 1 to 2 drops of 30% hydrogen peroxide and transferred to a polyethylene vial for counting (see Fig. 3).

Another 8 to 10 ml of 6 N hydrochloric acid was eluted and set aside before eluting the ^{60}Co activity. Cobalt was eluted with 10 to 13 ml of 4 N hydrochloric acid directly into a 4-dram polyethylene vial for counting. This step is optional and can be used when high cobalt content is suspected.[1]

[1]The Co sensitivity via counting 5.3-yr ^{60}Ca and with the irradiation time and neutron flux specified above is about 2 micrograms, which is about 20 to 40 times above the Co content of whole blood. Extension of the irradiation time to 10 hours or increasing the neutron flux by a factor of 20 to 40 will yield the necessary sensitivity. On the other hand, the Co sensitivity via 10.5-min ^{60m}Co under the above irradiation conditions and to the exclusion of the other elements is estimated at about 0.4 micrograms.

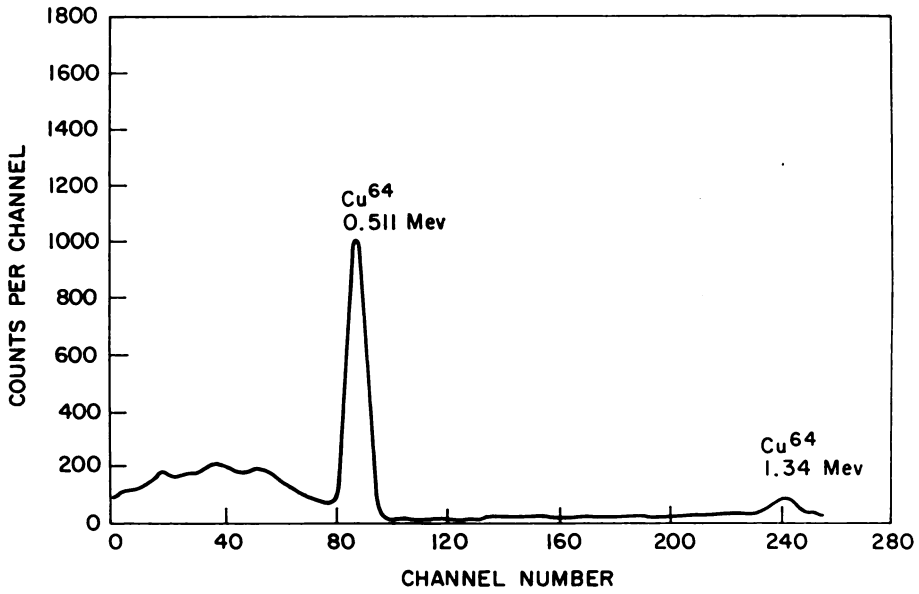

Fig. 4.

Copper was next eluted with 10 ml of 2 N hydrochloric acid directly into a 4-dram polyethylene vial, and spectra were taken (see Fig. 4). After the copper elution, 10 ml of 0.01 N hydrochloric acid was put through the column and discarded to eliminate any iron activity.

Zinc was eluted with 10 to 15 ml of deionized water. If zinc content is high, the eluent can be collected in the same manner as cobalt or copper and the spectra taken directly. However, if the zinc content is low, as it is in blood plasma, then it is best to collect the eluent in a test tube, precipitate zinc sulphide from a warm ammoniacal solution with hydrogen sulphide, dissolve the precipitate with a few drops of 6 N nitric acid, transfer it to a 2-dram polyethylene vial and make the count in a well crystal (see Fig. 5).

Sodium and potassium can be determined instrumentally by collecting all the discarded eluents and supernates up to the cobalt elution in beakers and evaporating the combined solutions to about 50 ml. In separate beakers dilute the ^{24}Na and ^{42}K standards to the same volume as the samples, normalizing all to one volume. Beakers should be covered with Parafilm to prevent splashing or spilling. Spectra should be taken at the appropriate geometry after allowing for one to two days decay (see Fig. 6) and subtracting out ^{24}Na to determine the ^{42}K content (see Fig. 7).

CHEMICAL YIELDS

Chemical yields were determined by neutron activation of the recovered solutions and comparing them with the appropriate standards, using a flux of 7.6×10^{10} neutrons cm^{-2} sec^{-1} for 10 minutes.

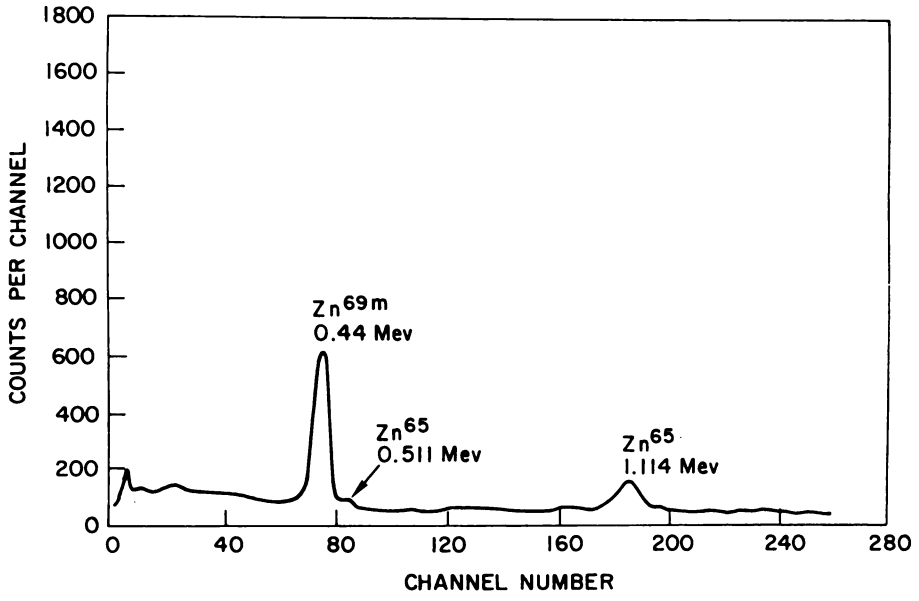

Fig. 5.

Because of the low abundances and cross sections of ^{46}Ca and ^{48}Ca, the chemical yields for calcium could not be determined with sufficient accuracy by this method. Therefore, an EDTA titration procedure was used, with calcein as an indicator: (10)

$$\underset{\text{(fluorescent)}}{\text{Ca-calcein}} + \text{EDTA} \xrightarrow{\text{pH 12}} \text{Ca}^{++} + \underset{\text{(brown nonfluorescent)}}{\text{calcein}}$$

Under these conditions magnesium does not interfere, but strontium and barium will be titrated together with calcium. However, strontium and barium can be subtracted after determination of their yields.

RESULTS AND DISCUSSION

The present ashing procedure has been tested for volatility losses at the temperatures used with traces of the elements being analyzed, and less than 1% loss was the maximum with the exception of potassium. A comparison experiment was made by taking aliquots of red blood cells and determining their sodium and potassium contents by instrumental neutron-activation analysis. After allowing the sodium and potassium activities to decay away, these same aliquots were ashed and put through the radiochemical procedure. The concentrations determined in this manner differed by 16% for potassium, but no change within experimental error was noted for sodium. Sodium and potassium recovery was performed, using the radiochemical separation procedure by adding known amounts of ^{24}Na and ^{42}K activities and 98% to 99% recovery was achieved.

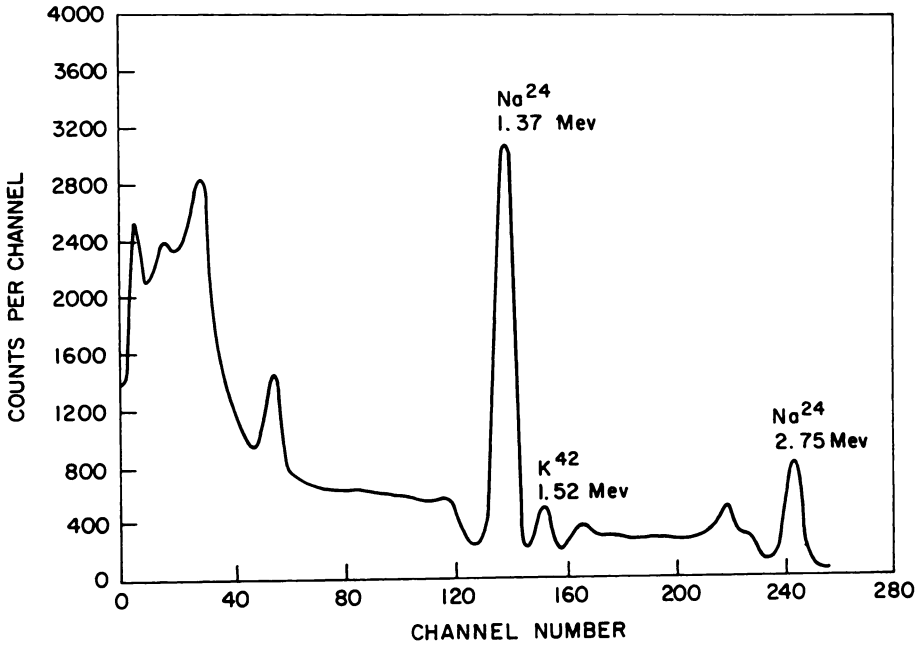

Fig. 6.

A quartz liner was made for the muffle furnace to prevent contamination of the samples by flaking from the furnace walls. Error due to neutron flux changes was averaged by rotation of samples and standards in the rotary rack of the TRIGA reactor at approximately 1 rpm.

When ^{56}Mn is being determined in red blood cells, a correction is necessary because of the ^{56}Fe (n,p) ^{56}Mn reaction. This reaction has been shown (1) to contribute 30% to the total ^{56}Mn activity.

Figures one through seven show typical spectra obtainable with this procedure. For spectra of individual isotopes, see Heath's Catalog (11).

Table I compares the average normal erythrocyte and plasma values obtained with the ranges published in the literature. The average values shown for magnesium, manganese, copper and zinc in red blood cells were from 15 individuals, and the plasma values were from 12 individuals. Calcium in red blood cells was from duplicate samples from one individual and calcium in plasma was from four individuals. Strontium and barium in the plasma were determined from five individuals. Sodium and potassium in red blood cells were from six individuals.

Correction for the trapped plasma contribution to red cell values was not made because, for most of the elements analyzed, the amounts are negligible in relation to the technical errors. The exception would be for sodium and calcium from plasma, according to Valberg, *et al.*, (8). They have shown by two different correction methods that trapped intracellular plasma does contribute substantially to sodium and calcium values of erythrocytes. One method was accomplished by using a composite calibration curve prepared with Evans blue dye for trapped plasma correction; the second and more accurate method involved the use of ^{131}I, labeled serum albumin (RISA), on each individual blood sample. Both the mean values and ranges of erythrocyte sodium and calcium were smaller using RISA measurements than those obtained with a composite calibration curve. Therefore, an average of 40% to 50% correction is necessary; e.g., the corrected Na value in erythrocytes is 0.20 mg/ml.

All samples analyzed had ACD preservative added. This preservative solution and heparin, another commonly used anticoagulant, were analyzed. It was found that the samples with the ACD solution contained negligible amounts of the elements concerned, except for sodium, and that heparin contained substantial amounts of manganese, copper and zinc (1). The statistical errors in counting magnesium, manganese, copper, zinc and sodium in red blood cells averaged 0.5% to 1.0%; in potassium, 2% to 4%; in strontium, 12% to 15%; in barium, 20% to 50%; and in calcium, 40% to 50%. The statistical counting errors for the elements determined in blood plasma were 3% to 4% for magnesium, 20% to 30% for calcium, 5% to 15% for strontium, 30% to 50% for barium, and 1% to 2% for manganese, copper, and zinc.

Weighing errors and transferring losses can be limited to 1.0% if sufficient care is taken in handling. Chemical yield errors involve only the statistical counting errors, since the recovered carrier is irradiated and counted without other chemistry or transfer of solution. Since the elements in the solutions are in

TABLE I

AVERAGE VALUES OF SELECTED CATIONS IN NORMAL HUMAN ERYTHROCYTES AND PLASMA*

		Mg μg/ml	*Ca* μg/ml	*Sr* μg/ml	*Ba* μg/ml	*Mn*[a] μg/ml	*Cu* μg/ml	*Zn* μg/ml	*Na* mg/ml	*K* mg/ml
Erythrocytes	General Atomic	46 ± 2	2.4 ± 1.2	.0072 ± .0009	0.007 ± 0.002	0.016 ± 0.001	0.80 ± 0.04	11.5 ± 0.5	0.36 ± 0.01	2.8 ± 0.8
	Range in Literature	30 – 68	0 – 18	*b*	*b*	0.022 – 0.25	0.74 – 1.32	6.9 – 14.4	0.2 – 0.5	2.1 – 5.6
Plasma	General Atomic	13 ± 2	39.0 ± 4.0	0.044 ± 0.005	0.066 ± 0.005	0.0043 ± 0.0005	0.85 ± 0.05	0.93 ± 0.05	—	—
	Range in Literature	18 – 27	20 – 40	0.004 – 1.0	0.02 – 0.87	0.0027 – 0.07	0.49 – 1.47	0.8 – 3.9	3.3	0.2

*(±) Standard deviation

a All manganese values for erythrocytes have been corrected for ^{56}Fe (n,p) ^{56}Mn reaction

b No values found in literature

macro amounts, the errors are less than 2.0%. The titrimetric error for calcium is also less than 2.0%. The percentage range of chemical yields are given in Table II.

Sodium and potassium values for plasma have not been indicated in Table I because they have been well established by other methods. Strontium and barium in erythrocytes have apparently never been determined. Since values in the literature are only for serum or whole blood, no literature values are listed in Table I.

TABLE II

PERCENTAGE RANGE OF CHEMICAL YIELDS

Elements	*Range* (%)
Na	98–100
Mg	40–55
K	98–100
Ca	30–50
Mn	80–90
Cu	80–90
Zn	70–90
Sr	8–40
Ba	8–40

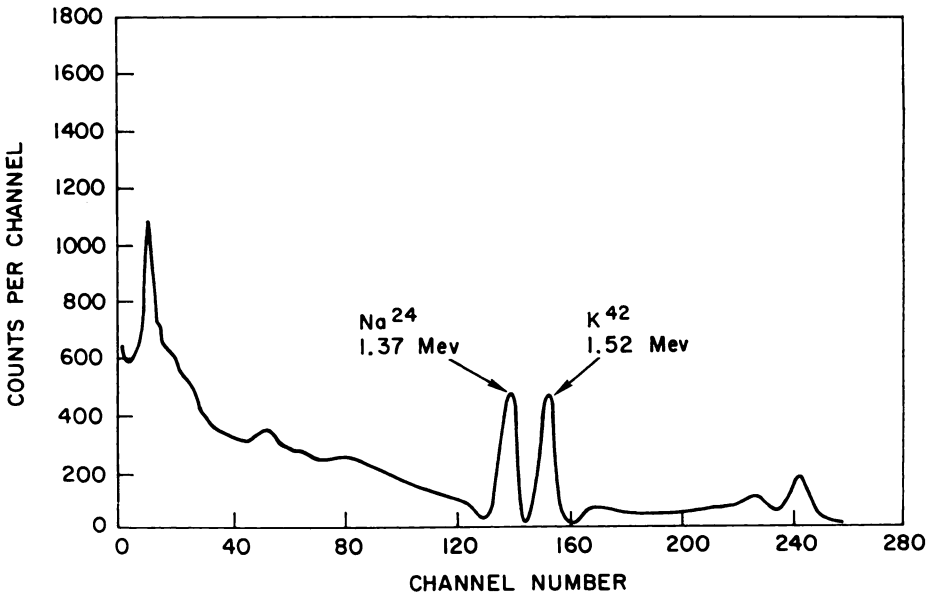

Fig. 7.

SUMMARY

A method has been described for quantitation of Mg, Ca, Sr, Ba, Mn, Co, Cu, Zn, Na and K in human erythrocytes and plasma by thermal neutron activation and rapid ion exchange radiochemical separation. Representative values have been presented and compared with previously published ranges, where available in the literature. Limits of error inherent in the method have been given.

REFERENCES

1. BETHARD, W. F., D. A. OLEHY, AND R. A. SCHMITT: The Use of Neutron Activation Analysis for the Quantitation of Selected Cations in Human Blood, *L'Analyse Par Radioactivation et ses Applications aux Sciences Biologiques*. University Press of France, Paris, France (1964).

2. SAMSAHL, K.: A Fast Radiochemical Method for the Determination of Some Essential Trace Elements in Biology and Medicine, *Aktiebolaget Atomenergie Report AE-168* (December 1964).

3. WESTER, P. O., D. BRUNE, AND K. SAMSAHL: Radiochemical Recovery Studies of a Separation Scheme for 23 Elements in Biological Material, *Intern. J. Appl. Radiation and Isotopes* **15**:59-67 (1964).

4. BRUNE, D., K. SAMSAHL, AND P. O. WESTER: Determination of Elements in Milli-, Micro-, and Submicrogram Quantities in Human Whole Blood by Neutron Activation Analysis, *Atompraxis* **9**:368 (1963).

5. BRUNE, D., K. SAMSAHL, AND P. O. WESTER: "The Amounts of As, Au, Br, Cu, Fe, Mo, Se, and Zn in Normal and Uraemic Human Whole Blood. A Comparison by Means of Neutron Activation Analysis. *Aktiebolaget Atomenergie Report AE-134* (1964).

6. PARR, R. M., AND D. M. TAYLOR: The Concentrations of Cobalt, Copper, Iron and Zinc in Some Normal Human Tissues as Determined by Neutron Activation Analysis, *Biochem. J.* **94**:424 (1964).

7. KRAUS, K. A., AND G. E. MOORE: Anion Exchange Studies, VI. Divalent Transition Elements Manganese to Zinc in Hydrochloric Acid, *J. Am. Chem. Soc.* **75**:1460-1466 (1953).

8. VALBERG, L. S., J. M. HOLT, E. PAULSAN, AND J. SZIVEK: Spectrochemical Analysis of Sodium, Potassium, Calcium, Magnesium, Copper, and Zinc in Normal Human Erythrocytes, *J. Clin. Invest.* **44**, No. 3 (1965).

9. WACKER, W. E. C., C. IDA, AND K. FUWA: Accuracy of Determinations of Serum Magnesium by Flame Emission and Atomic Adsorption Spectrometry, *Nature*, **202**:659 (1964).

10. DIEHL, HARVEY: *Calcein, Calmagite and o,o'-Dihydroxyazobenzene Titrimetric, Colorimetric, and Fluorometric Reagents for Calcium and Magnesium*. G. Frederick Smith Chemical Company (1964).

11. HEATH, R. L.: Scintillation Spectrometry Gamma-ray Spectrum Catalogue, 2nd ed., USAEC Report TID-4500 (1964).

12. WINTROBE, N. M.: *Clinical Hematology*, 5th ed., Lea and Febiger (1961).