Elimination of Sodium-24 and Potassium-42 Interferences in Activation Analysis of Biological Samples

Abstract. *We have developed a simple novel column technique for the removal of sodium-24 and potassium-42 based on a heterogeneous isotopic exchange reaction between an organic eluting solution (acetone-hydrochloric acid) and fine crystals of sodium chloride or potassium chloride or their analogs. The technique is fast, efficient, and highly selective; it has been successfully applied to irradiated human tissues prior to gamma-ray spectrometry with lithium-drifted germanium detectors.*

Because of their excellent resolving power for gamma rays, lithium-drifted germanium [Ge(Li)] detectors have been used in instrumental activation analysis for the simultaneous measurement of the concentrations of many elements in rocks (*1*), pottery, paintings, coins, atmospheric pollutants (*2*), and, more recently, lunar samples (*3*). The technique has been successfully applied in the determination of a few trace elements in biological tissues (*2*); however, tissues are quite different from other types of samples because of their high content of sodium and potassium relative to the elements normally studied by activation analysis. Sodium and potassium are strongly activated under neutron irradiation to produce very large amounts of ^{24}Na (half-life, 15 hours) and ^{42}K (half-life, 12.8 hours). The intense radioactivity produced by these species in most tissue samples makes it impossible to observe other radioactive species with half-lives between approximately 1 hour and a few days.

In order to observe the photopeaks of trace elements in Ge(Li) spectra of irradiated tissues, it is necessary to reduce the activity of ^{24}Na and ^{42}K by at least a factor of 10^6 without losing elements of interest. Such a requirement in decontamination is beyond the capability of most conventional procedures for chemical separation.

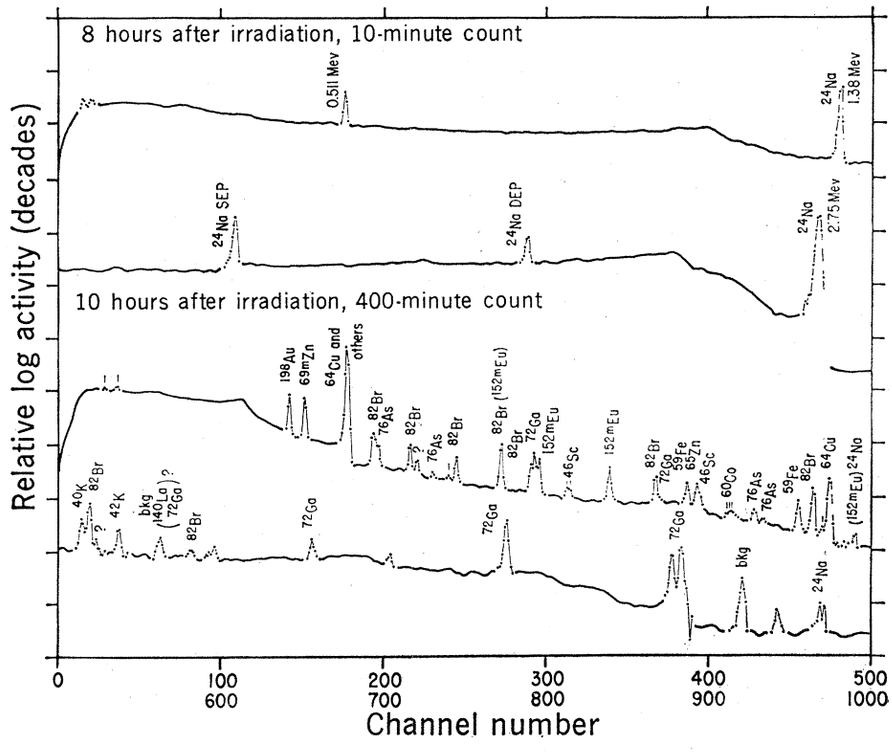

Fig. 1. Gamma-ray spectra of human lung tissue before and after removal of ^{24}Na and ^{42}K. About 100 mg of the lyophilized tissue was irradiated for 3 hours at a thermal neutron flux of 2.2×10^{13} cm^{-2} sec^{-1}. Spectra were measured with a Ge(Li) detector (volume, 20 cm^3) and are presented as overlapping halves. Note the small residues of ^{24}Na and ^{42}K, even with the large decontamination ($\geqq 10^6$) among the many peaks in the separated spectrum (*SEP*, single escape peak of annihilation radiation; *DEP*, double escape peak).

Previous techniques for the removal of sodium have included, for example, ion exchange (*4*), precipitation with reagents like α-methoxyphenylacetic acid (*5*) or 6-benzaminoanthraquinone-2-sulfonic acid (*6*), or crystallization as sodium chloride in a hydrochloric acid–butanol medium (*7*). All these procedures suffer from poor specificity and low decontamination factors. Because of the lack of success in efforts to remove sodium, little has been done to alleviate the concomitant problem of potassium interference.

Girardi and Sabbioni (*8*) described a technique for the selective removal of radiosodium by retention on specially prepared proprietary (*9*) hydrated antimony pentoxide (HAP) in highly acid systems. At a lower acid concentration, it is claimed that potassium is removed as well. Various laboratories are presently evaluating this method for use with activation analysis.

We have developed a column technique for the removal of radiosodium which is simple, economical, versatile, and rapid and avoids the necessity of handling high concentrations of corrosive acids as with the HAP method. It allows us to remove either ^{24}Na or ^{42}K separately or both radioelements simultaneously. Furthermore, the principle of the method can also be adapted for the isolation of other radioelements.

Our procedure combines the selectivity and speed of isotopic exchange with the efficiency of column elution. Isotopic exchange, with its atom-for-atom transfer of the same element, is inherently a more selective technique than others. Since the reaction occurs mainly on surfaces, interferences due to occlusion and coprecipitation of other ions are minimized. The organic eluate permits the use of water-soluble compounds.

Briefly, the procedure consists of combining a small volume of the aqueous sample with a much larger volume of organic solvent and passing the mixture through a column of an inorganic salt containing an ion in common with the radioelement to be removed. The radioelement is retained on the column while practically all other components are eluted.

For the removal of ^{24}Na, the steps are as follows: In order to prepare the column, dried, powdered NaCl (reagent grade, granular) (*10*), sifted to obtain the desired crystal sizes, is slurried with acetone, and an appropriate amount of the suspension is introduced into the

column as in the packing of a conventional column of ion-exchange resin (*11*). The crystals to be used for the columns are dried in an oven at 110°C for at least 3 hours before being ground. After sifting, the desired fractions are stored in desiccators until ready for use. Powdered NaCl that has been exposed to the atmosphere for many days gives lower decontamination than freshly dried material. The eluate is prepared by mixing a 1-ml aqueous solution of the sample with 9 ml of acetone and five to ten drops of concentrated HCl (*12*). The HCl helps to prevent other elements from being retained in the column. The eluate (10 ml) is introduced into the column with a capillary Pasteur pipette. Approximately 20 ml of acetone are used for washing the column (*13*).

The separation of ^{42}K is similarly achieved with KCl used in place of NaCl. By packing KCl on top of NaCl (or the reverse) in the same column, both ^{42}K and ^{24}Na can be removed from the sample in one single elution. No detectable amounts of ^{42}K or ^{24}Na are picked up from the column by further washing with acetone.

Isotopic exchange of ^{38}Cl (half-life, 37 minutes) also occurs in the column, although to a lesser extent than for either ^{42}K or ^{24}Na, probably because of the holdback effect of the HCl. Since most samples containing sodium also contain chlorine at concentrations that interfere seriously with the detection of trace elements when samples are counted shortly after irradiation, the capability of the technique to remove ^{38}Cl is an added advantage and can be exploited further.

We determined whether elements other than sodium would be retained on the column by eluting through the column a mixed tracer solution of 18 radioelements: ^{46}Sc, ^{51}Cr, ^{54}Mn, ^{59}Fe, ^{60}Co, ^{65}Zn, ^{75}Se, ^{85}Sr, ^{109}Cd, ^{110m}Ag, ^{113}Sn, ^{124}Sb, ^{133}Ba, ^{134}Cs, ^{144}Ce, ^{155}Eu, ^{182}Ta, and ^{203}Hg. The eluting solution was prepared by mixing 1 ml of the mixed tracer (digested with unirradiated, lyophilized tissues in HNO_3 and $HClO_4$ as described below), 9 ml of acetone, and five drops of concentrated HCl. The Ge (Li) spectra of both the column and eluant were compared with the spectrum of the mixed tracer solution. The only elements of the mixed tracer solution that were retained on the NaCl column are Sc, Mn, Sr, Ag, Ba, and Ta. Except for tantalum, which appeared to be quantitatively retained (as it is also on HAP), the other five elements are fractionated between the column and the eluant to varying extents.

As an example of the effectiveness of our procedure, Fig. 1 shows Ge(Li) spectra of an irradiated sample of human lung tissue before and after the removal of ^{24}Na and ^{42}K. The top spectrum represents a 0.5-ml portion of the 10-ml eluate before separation, and the lower spectrum represents the remainder of the sample after the separation. The irradiated sample of lyophilized tissue was digested by boiling it in a mixture of 7 ml of concentrated HNO_3 and 3 ml of concentrated $HClO_4$ until the volume of the solution was reduced to approximately 1 ml. For this sample, the eluate was prepared by mixing the concentrated tissue solution with 7 ml of acetone, 2 ml of hexone (methyl isobutyl ketone), and ten drops of concentrated HCl (*14*). The eluate was passed once through a glass column in which 10 cm of KCl was packed on top of 10 cm of NaCl (230- to 325-mesh crystals) and then through another 10 cm of NaCl. The decontamination factor for ^{24}Na is estimated as $\geqq 10^8$. Over 40 photopeaks are visible in the spectrum, and of these we have identified 18 nuclides, excluding background activities of ^{40}K and ^{208}Tl.

The distribution of activities on the salt column after the sample had been eluted was determined from autoradiographs made by wrapping film around the column and exposing it to the radiation for an appropriate period. Figure 2 shows a typical autoradiograph and its densitometric tracing of a tandem column of KCl on NaCl through which a sample prepared from irradiated human lung tissue had been eluted. It is apparent from the autoradiographs that ^{42}K and ^{24}Na are well separated on the column, the former being retained in the KCl section and the latter in the NaCl section. Furthermore, the activities are preferentially accumulated in the top portion of the corresponding salt sections. However, ^{42}K is removed completely with a sharp trailing edge, whereas ^{24}Na is distributed farther down the column, resulting in some breakthrough. This discrepancy is pres-

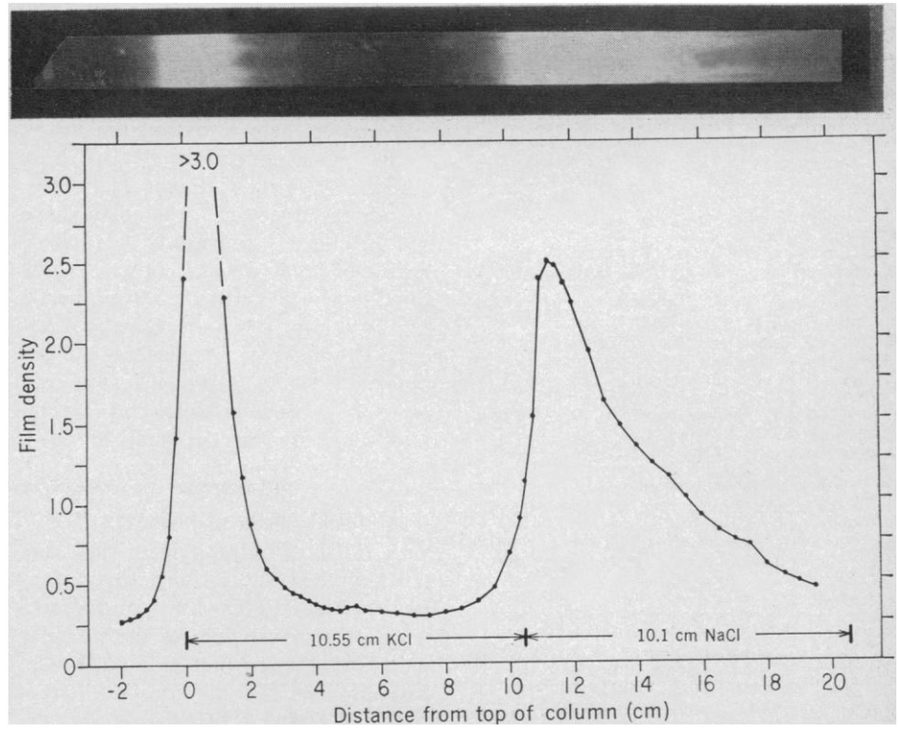

Fig. 2. Autoradiograph and its densitometric tracing of activities removed from the eluate by a tandem column of KCl packed on top of NaCl. The eluate was obtained from ~ 100 mg of dry lung tissue (different from the sample for Fig. 1) irradiated at a thermal neutron flux of 2.2×10^{13} cm^{-2} sec^{-1} for 3 hours. No Screen Medical x-ray film (Eastman Kodak) covered with aluminum foil was wrapped around the column with tape, aligned so that the bottom of film and the column coincided, and exposed for 1.5 hours. The arrows indicate the physical boundaries of the salts in the column and do not coincide with the exposed edges because of resolution effects. Even though the interaction with the film is caused mainly by beta rays for ^{42}K and by beta and gamma rays for ^{24}Na, the resolution for the two is nearly the same as shown by the similar leading edges.

ently attributed to surface effects rather than to the total K and Na or ^{42}K and ^{24}Na contents of the sample.

A good elution flow rate through the column is about 1 ml per minute. If we include the time for both digestion and elution, the fastest separation we have achieved thus far is approximately 1 hour, and it is possible to reduce this time further.

We are applying this procedure in our studies of trace elements in normal and diseased human tissues. We have also demonstrated its usefulness in the study of contaminants in reactor cooling water and in investigations of cyclotron-produced radionuclides, in both of which cases copious quantities of interfering ^{24}Na are produced when aluminum components or target wrapping foils are subjected to fast neutron bombardment.

CHUNG-WAI TANG
CONSTANTINE J. MALETSKOS
Cancer Research Institute,
New England Deaconess Hospital,
and *Department of Pathology,*
Harvard Medical School,
Boston, Massachusetts 02215

References and Notes

1. G. E. Gordon, K. Randall, G. G. Goles, J. B. Corliss, M. H. Beeson, S. S. Oxley, *Geochim. Cosmochim. Acta* **32**, 369 (1968).
2. J. R. DeVoe, Ed., *Modern Trends in Activation Analysis, Proceedings of the 1968 International Conference, Gaithersburg, Maryland, 7–11 October 1968* [*Nat. Bur. Stand. U.S. Spec. Publ. 312* (1969), vols. 1 and 2.]
3. See List of Investigators and Investigations, Lunar Sample Program, *Ind. Res.* **11**, 26 (July 1969).
4. N. Spronk, *Nucl. Appl.* **2**, 259 (1966).
5. C. V. Banks and J. J. Richard, *Talanta* 2, 235 (1959).
6. H. Sanke Gowda and W. I. Stephen, *Anal. Clin. Acta* **25**, 153 (1961).
7. M. P. Menon and R. E. Wainerdi, in *Proceedings of the 1965 Conference Modern Trends in Activaiton Analysis* (Texas A & M Univ., College Station, 1965), p. 152.
8. F. Girardi and E. Sabbioni, *J. Radioanal. Chem.* **1**, 169 (1968).
9. Available from Carlo Erba, Milan, Italy.
10. Preliminary experiments show that NaBr and Na_2SO_4 are effective in removing radiosodium. For obvious reasons we are presently concentrating on the NaCl system.
11. Our early experiments were conducted with columns made from glass tubes (5 mm in inner diameter, 30 to 60 cm long) drawn to a nozzle on one end and plugged with a wad of glass wool. Later we used wider-diameter glass tubes fitted with glass frits (1 cm in inner diameter); at present we use polypropylene chromatographic tubes (1.5 cm in inner diameter, 15 cm long), each sealed at one end with a perforated polypropylene disk and fitted with a removable nozzle. To prevent the fine crystals from passing through the perforations, a filter paper disk (1.4 cm in diameter) is placed at the bottom of the polypropylene tube. These commercially available plastic columns are easier to pack, give more uniform and faster flow rates, and are convenient to handle. More NaCl is required, but, as compared to passage through three or more NaCl columns 10 cm long in glass tubes 5 mm in inner diameter, one pass through a column of 230- to 325-mesh NaCl 5 to 7 cm long in the plastic tube is usually sufficient to remove ^{24}Na from 100 mg of irradiated lyophilized muscle tissue.
12. The physics and chemistry of the water-acetone-HCl system and the mechanism of exchange are not clearly understood at present. The small amount of water presumably plays an important role in recrystallization. Columns packed with NaCl equilibrated with a mixture of acetone and HCl for over 2 weeks appear to give lower decontamination factors than columns packed with fresh materials.
13. The low boiling point (56°C) of acetone allows one to rapidly concentrate the eluant and the washings by evaporation at relatively low temperature.
14. For reasons not understood, the addition of a little hexone in the eluate appears to improve the decontamination; however, it is not essential, since an acetone-H_2O mixture alone can remove ^{24}Na or ^{42}K, respectively, with NaCl or KCl. The H_2O-acetone-HCl system described here may not be optimum, but it is the most effective system found thus far.
15. Supported by AEC contract AT(30-1)-3778 with the New England Deaconess Hospital. We thank Professors C. D. Coryell and G. E. Gordon, Department of Chemistry, Massachusetts Institute of Technology and University of Maryland, respectively, for discussions and review of the manuscript.

9 July 1969; revised 10 November 1969

Maxillary Mycangium in the Mountain Pine Beetle

Abstract. *A mycangium containing blue-stain fungi and yeasts is located in the cardo of the maxilla of the mountain pine beetle,* Dendroctonus ponderosae *Hopk. Symbiosis between one or more of the microorganisms and the insect is indicated.*

We report here a mycangium in the maxilla of a scolytid beetle; we have cultured blue-stain fungi from this specialized structure. Mycangia have been reported previously. Nunberg speculated that special organs (now called mycangia) in the ambrosia beetle *Trypodendron lineatum* Oliv. = *Xyloterus* Oliv., were involved in fungus transmission (*1*). Mycangia were subsequently found in this and several other ambrosia beetles and in some bark beetles. The occurrence of mycangia, their distribution, and function have recently been reviewed (*1*). Blue-stain fungi colonize phloem and sapwood of trees successfully attacked by bark beetles. Farris (*2*) reported evidence of a mycangium in the pregular region of the mountain pine beetle. We now describe the location, structure, and contents of this mycangium.

We obtained newly emerged beetles from infested lodgepole pine *Pinus contorta* Dougl. var. *latifolia* Engelm., in the East Kootenay region of British Columbia. Insects not used immediately were stored individually up to 1 week at 4°C with pieces of lodgepole pine phloem. Sagittal and transverse sections and whole mounts of maxillae were stained (*3*) for examination for fungal material. To culture microorganisms from mycangia, we separated aseptically the cardines from the maxillae that were extracted from recently removed heads. The cardines were placed in either sterile water on microslides or in a petri plate of 2 percent water agar or potato dextrose agar with 0.02 percent yeast extract. Cultures were kept in a moist chamber on the laboratory bench and examined at ×450 at 12 hour intervals. Pure cultures originating from mycangial openings were obtained from hyphal tips or spores. For scanning electron microscopy (*4*), unfixed whole maxillae were mounted with egg albumen on a cover glass affixed to a specimen stub with high conductivity paint, coated with approximately 100 Å of carbon and 300 Å of gold paladium at an angle of 45° from the point of evaporation, and examined at a beam voltage of 3 kv.

Cultures from aseptic dissections of the pregular region consistently showed fungal growth arising toward the proximal end of the cardines. The mycangium was located during detailed examination by light and scanning electron microscopy (Fig. 1). It was present in both cardines of both sexes of this beetle and opened on the inner surface slightly above the fossa between the muscle processes (*5*). The position of the mycangium on the contorted cardo was readily seen under the scanning electron microscope (Fig. 1). This greatly facilitated examination and comprehension of whole mounts and sections of cardines viewed by light microscopy (×100). A column of hyphae and spores was seen frequently extending from the mycangial opening. One column was approximately as long as the cardo (about 175 μm). Stained sections (Fig. 2) and whole mounts of maxillae revealed a reticulate tube containing fungus material in one of the two muscle processes of the cardo. The structure of this mycangium, and the peglike protrusions on its inner surface as seen in Stereoscan preparations, resemble those of several species of *Trypodendron* (*6*).

The blue-stain fungi and yeasts that occur in lodgepole pine attacked by the mountain pine beetle (*7*) were cultured from mycangia. Isolations were made from one or the other mycangium of 32 unsexed insects. The fungi obtained and the number of mycangia that produced them were: *Ceratocystis montia* Rhumb., 5; *Europhium clavigerum* Robinson and Davidson, 16; yeasts, 18