NEUTRON ACTIVATION ANALYSIS OF CALCIUM IN BIOLOGICAL SAMPLES

David A. Weber and Howard L. Andrews

The University of Rochester School of Medicine and Dentistry, Rochester, New York

Reports in the literature have described the use of whole-body neutron activation analyses in man for estimating the content of certain elements (*1–7*). Thermal neutron reactions can be used to identify and assay such elements as sodium, chlorine, and calcium through n,γ reactions. Irradiations have been made with partially moderated fast neutrons in order to approach uniform thermal neutron flux distributions throughout a body cross section. The additional reactions dependent on the primary energy spectrum of the incident neutron energy, however, have been shown both to complicate dosimetry and interfere with the analysis of the n,γ activation products (*8*). Precise calibration of the neutron spectrum and extensive phantom studies are required to minimize these problems. The recent work of Cohn, et al (*6,7*) was the first to show that these coupled reaction products could be used to identify and assay the content of additional elements in man.

The majority of thermal neutron activation analysis studies at selected anatomic sites in man have been limited to the study of the thyroid gland. The work of Boddy, et al (*9*) and Lenihan, et al (*10*) indicates that results from the $^{127}I(n,\gamma)^{128}I$ reaction can be used to determine the iodine content of this gland. Radiation dose levels delivered to the thyroid were estimated to be comparable to those associated with conventional ^{131}I tracer uptake studies.

This communication reports on the use of thermal neutron activation for determining the calcium content at selected anatomic sites in man. Changes in calcium content of the fingers or calcaneous have been studied by x-ray densitometry and photon absorption techniques to evaluate various pathologic or physiologic states (*11,12*). Although these determinations do not necessarily reflect whole-body changes in calcium, they have been found to provide useful correlation with bone mineralization changes. The relatively small volumes involved and the possibility of shielding contiguous areas suggest that neutron activation might serve as a preferable method of analysis. Direct assays of calcium content should provide a more sensitive determination.

In this study, relatively low thermal neutron fluences have been used to assay calcium content in mice and human cadaver fingers. Activation products were detected and identified by gamma-ray counting, using NaI(Tl) and Ge(Li) detectors. The weights of the elements which yielded detectable daughter products were determined from linear least-squares analyses of the NaI(Tl) spectra. The resulting estimations of calcium content in selected specimens were compared with atomic absorption determinations.

MATERIALS AND METHODS

Irradiation facility. All irradiations were made in the central region of a hohlraum within the thermal column of a Triga Mark II Reactor*. The irradiation runs were all made with the reactor operating at 100 kw steady power level. Thermal neutron flux density determinations were made on sample irradiations by gold foil activation measurements of the reaction $^{197}Au(n,\gamma)^{198}Au$. Cadmium shielded foils were used to determine the contamination of the thermal flux by fast neutrons. Constant geometric relations were maintained between the reactor core and the specimens undergoing neutron irradiation.

Specimen irradiation and activity measurements. Standard solutions, phantoms, sacrificed mice, and disarticulated fingers were irradiated in polyethylene containers with snap-on covers. The inside dimensions of these containers were 8 cm long $\times$ 2.6 cm in diameter. The wall thickness was 2.6 mm. The

Received July 14, 1971; revision accepted Nov. 15, 1971.

For reprints contact: D. A. Weber, Div. of Nuclear Radiology, The University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd., Rochester, N.Y. 14642.

* The Triga Mark II Reactor at the Ward Reactor Laboratory, Cornell University, Ithaca, N.Y., was used by cooperative arrangement with the Department of Radiation Biology and Biophysics, University of Rochester, Rochester, N.Y.

irradiated samples were transferred to polyethylene counting vials immediately following activation and prior to counting.

Biological specimens undergoing irradiation included:

1. 17 female Swiss Webster ICR mice, 12–29 weeks of age, and
2. the second and third digits, disarticulated from an adult human cadaver.

Solutions prepared from atomic absorption reference standards and high-purity reagents were used to standardize activation and counting procedures. Solutions of each element identified by Ge(Li) and NaI(Tl) spectral analyses of preliminary specimen irradiations were studied individually. The effects of irradiation volume and concentration of each element on flux density and activation product counting efficiency were evaluated by:

1. NaI(Tl) detector measurements of the activity resulting from standard solution irradiations at several different volumes and concentrations and
2. ^{198}Au activation measurements of foils irradiated at various positions along the incident skin surface, within the abdomen, and in a sagittal cut of the vertebrae in mice.

Mixtures of the same elements detected in the mouse and finger activations were also studied in phantoms. An irradiation phantom was designed to approximate the geometry of the mouse and the human finger. It consisted of two coaxial hollow cylinders. A standard activation vial was used as the outer cylinder to serve as a "soft-tissue compartment". A capped Lucite tube 1 cm in diam with 1.4-mm walls centered in the irradiation vial was used for the inside cylinder or "bone compartment". The outer annulus contained solutions of stable isotopes of all of the elements detected, with the exception of calcium. Calcium solutions were irradiated in the inner cylinder.

Individual radioactive activation products were initially identified by evaluating several irradiated specimens with a nominal 30-cm^3 volume Ge(Li) detector. The specimens were positioned directly on the vacuum cryostat for all measurements. Measurements of the induced radioactivity levels were carried out with a 7 × 5-in. NaI(Tl) scintillation crystal. The cross-sectional view of the lead-shielded scintillation crystal is shown in Fig. 1.

DATA RECORDING AND CALCULATIONS

Pulse-height distribution data were recorded with a 1,024 multichannel analyzer. The spectra were pre-

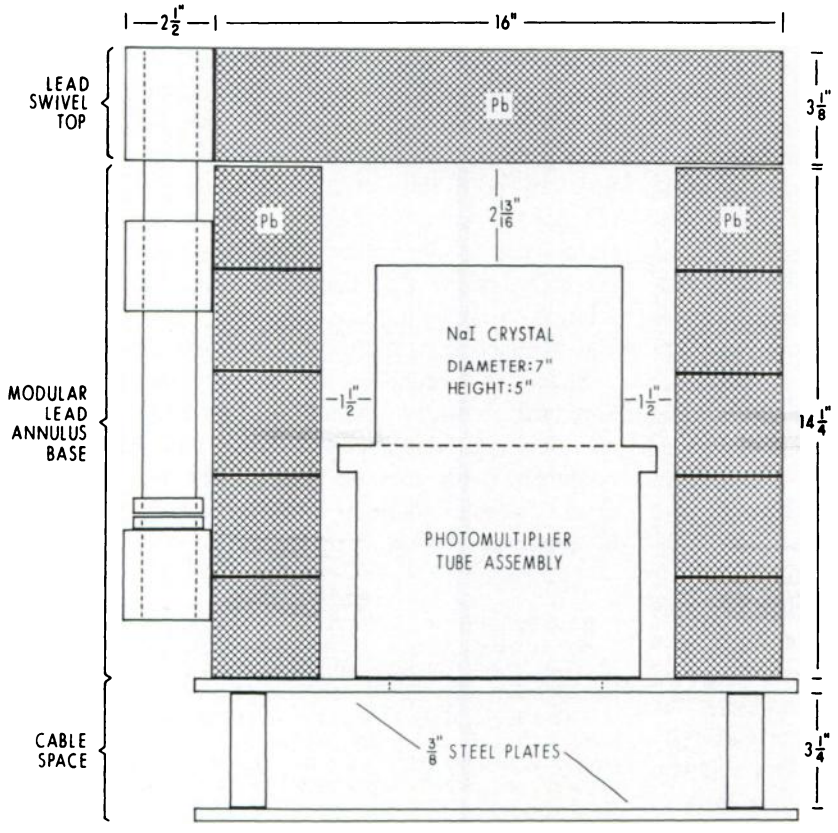

FIG. 1. Cross-sectional view of NaI(Tl) detector assembly. Irradiated specimens are transferred to counting vials and positioned on sample holder; sample holder attaches to top of NaI(Tl) crystal for constant geometry measurements.

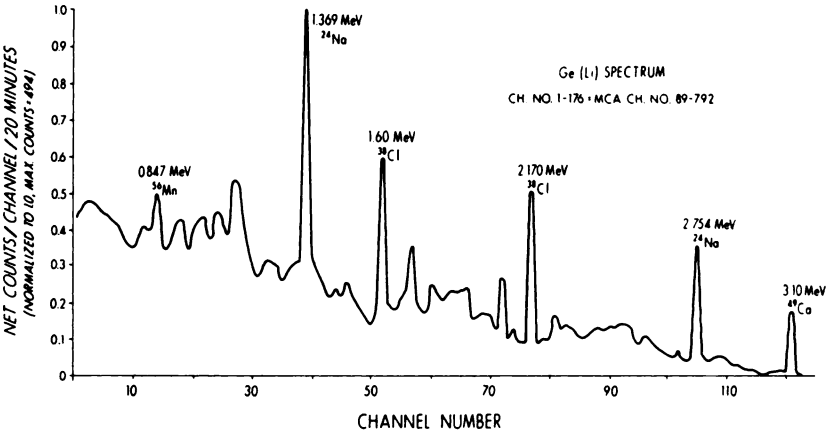

FIG. 2. Gamma-ray spectrum of irradiated mouse recorded with Ge(Li) detector. Mouse was irradiated for 6 min at thermal neutron flux density of 1.8×10^8 n/cm²/sec; counting was initiated 3.3 min after termination of irradiation and spectrum was recorded for 20 min. (Spectrum illustrated in figure and spectra in Figs. 3 and 4 show results from summing every four adjacent multichannel analyzer data channels. This was carried out to achieve good photoreproduction of spectral data.)

sented for analyses in three forms: a digital listing, a punched paper tape record, and a Polaroid print of an oscilloscope trace. All data reduction was carried out on an IBM System 360/44 computer following transfer of punched paper tape data to magnetic tape.

RESULTS

Flux density. A thermal neutron flux density of 1.8×10^8 n/cm²/sec incident on the surface of the irradiation vials was used throughout the study. The average cadmium ratio was 480. Gold-foil activation measurements indicated a relatively constant neutron distribution throughout the irradiated mice. Flux density measurements at the skin surface, within the abdomen, and in a sagittal cut of the vertebrae showed a random variation ranging from −6.0% to +4.5% of the incident flux density.

Ge(Li) and NaI(Tl) measurements. Ge(Li) detector measurements were made immediately after 2–6-min irradiations of sacrificed mice. The spectra recorded from each animal were plotted and analyzed for spectral components. Figure 2 illustrates a typical spectrum recorded for 20-min live-time following activation. Half-life and photopeak analyses of spectra serially recorded on each specimen showed ^{49}Ca, ^{24}Na, ^{38}Cl, and ^{56}Mn to be present. These were confirmed by standard and phantom measurements.

Similar measurements were made with a NaI(Tl) detector on both sacrificed mice and finger specimens. Samples were transferred to counting vials, and counting was initiated within 1.9–6 min after termination of irradiation. Irradiation times ranged from 1 to 4 min for the sacrificed mice; the time was 2 min for each of the finger specimens. Spectra were recorded in live-time for 20-min intervals. Multichannel analyzer measurements were repeated several times on each sample during the first 24 hr following irradiation. The NaI(Tl) detector primary-energy photopeak distributions for the mice and fingers were observed to duplicate the Ge(Li) detector's distributions observed on mice immediately following irradiation. Figures 3A and 4A show examples of the spectra. The radioisotopes of Na, Ca, and Cl were identified in both the mice and fingers. Serial spectra, however, revealed that the half-life of the photopeak, positively identified as due to 2.6-hr ^{56}Mn in the mice, was considerably different in the finger specimens. In the latter the activation product was identified as the 9.5-min radioisotope of Mg, ^{27}Mg. The similar characteristic decay energies of ^{56}Mn and ^{27}Mg, 847 keV for ^{56}Mn and 840 keV for ^{27}Mg, prevented identification in the spectra recorded immediately after irradiation.

Figures 3B and 4B show a second set of spectra recorded on the specimens at approximately 40 min after termination of irradiation. These illustrate the disappearance of the ^{27}Mg characteristic energy photopeak for the finger specimen; the longer lived ^{56}Mn is still observed in the mouse.

NaI(Tl) detector. Measurements recorded on irradiated standard solutions indicated that the induced activity was directly proportional to element weight for the range of element weights and volumes observed in biologic samples. This was observed for solutions irradiated in the standard irradiation vials and in the specially prepared phantoms.

Analysis. The activity of each of the radioactive components in the NaI(Tl) spectra from irradiated phantom solutions, mice, and fingers was calculated by a linear least-squares method (*13–16*). Each spectrum was assumed equal to a linear combination of the individual reference spectra obtained from standard solution irradiations.

The calculations were programmed in Fortran IV language for execution on the IBM 360/44 computer*. The results are shown in Table 1. The weights

* A copy of the Fortran IV computer program used to carry out these calculations is available on request from the authors.

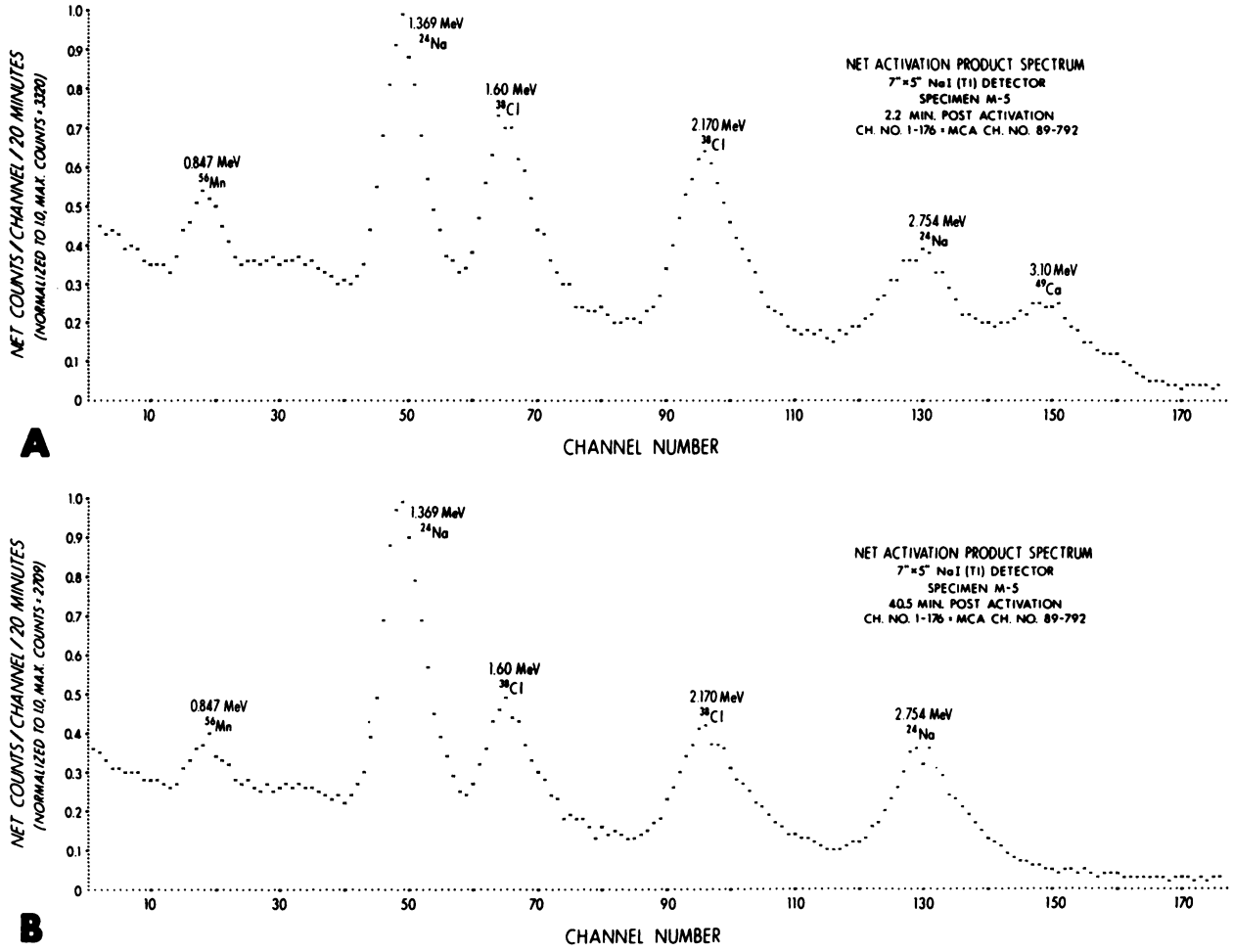

FIG. 3. A shows gamma-ray spectrum of irradiated mouse (M-5) recorded with NaI(Tl) detector. Mouse was irradiated for 4 min at flux density of 1.8×10^8 n/cm²/sec; counting was initiated 2.2 min after termination of irradiation. Each spectrum in Figs. 3 and 4 was recorded for 20 min in live-time. B is repeat spectrum on M-5. Counting was initiated 40.5 min after completion of irradiation.

of the mice ranged from 23.80 to 35.61 gm. The two finger specimens, F-1 and F-2, weighed 21.65 and 26.41 gm. Two mice, M-6 and M-11, and one finger, F-1, were also ashed at 600°C for calcium determinations by atomic absorption. These results and those observed for the same specimens by neutron activation are shown in Table 2.

Radiation dosimetry. The radiation dose absorbed by the mice and fingers was determined from: (A) measurements of background gamma radiation level in the hohlraum, (B) calculation of the energy absorbed from capture radiation resulting from interaction of the thermal neutrons with tissue elements, and (C) the calculation of energy absorbed from activation product radioactivity. The fast neutron dose was considered negligible on the basis of the high cadmium ratio and the lack of any significant activation products from fast neutron reactions. Table 3 shows the estimated dose to a 35-gm specimen irradiated for 2 min at a thermal neutron flux density of 1.8×10^8 n/cm²/sec. The hohlraum gamma dose was measured with an ionization chamber. The significant capture radiation dose was assumed to be from $^{14}_{7}N(n,p)^{14}_{6}C$ and $^{1}_{1}H(n,\gamma)^{2}_{1}H$ reactions; calculations assumed the element distribution found in standard man (*17*). Two examples of the absorbed dose from the decay of activation product radioactivity are shown for ^{24}Na and ^{32}P. The radiation dose expressed in rem was calculated using a quality factor of 8.28 for the recoil protons and 1.0 for the gamma radiation (*18*).

DISCUSSION

The standard solution and phantom activity measurements showed that there was no significant flux depression in the irradiated reference solutions for the range of element concentration and volume presented by the mice and finger specimens. This held true for Ca, Na, Cl, Mg, and Mn.

The mouse provided a readily accessible source with an element content and volume similar to the finger for studying flux density homogeneity and

neutron fluence level effects on the activation products. Gold foils placed at various regions in the mouse indicated a small random flux density perturbation. It is felt that these variations were due to the combined effects of additional elements present in the tissue matrix and the irregular geometry of the specimens. As there was no significant detectable gamma emission from these elements, their effect on flux depression was not evaluated in this study. Since the finger presents a similar heterogeneous subject for irradiation, the limitations on the overall technique were found to be difficult to assess. The flux density perturbations appear to be unavoidable in this technique. The observed flux density depression appears compatible with the depth dose curves published by Snyder (*19*), provided a uniform incident field is assumed.

Since the least-squares method of data analysis assumes the specimen activation products to correlate directly with the standard solutions and phantom measurements, the effectiveness of the technique to measure calcium was checked by atomic absorption determinations. The comparative weight determinations, shown in Table 2, confirm the proposed method of neutron activation for calcium assay. The flux density perturbations observed in the mice do not appear to affect the accuracy of the technique.

For the 2-min irradiation at a thermal neutron flux density of 1.8×10^8 n/cm²/sec, the absorbed radiation dose to a 35-gm specimen was calculated to be 14.6 rem for the irradiation configuration in this study. No attempts were made to reduce the dose level since this did not interfere with the in vitro studies made to test the technical feasibility of the proposed application of thermal neutron activation analysis. To consider the technique for in vivo studies, one must minimize this dose. The primary dose contribution from the hohlraum gamma-ray background level could probably be reduced by at least a factor of ten by adding several centimeters of bis-

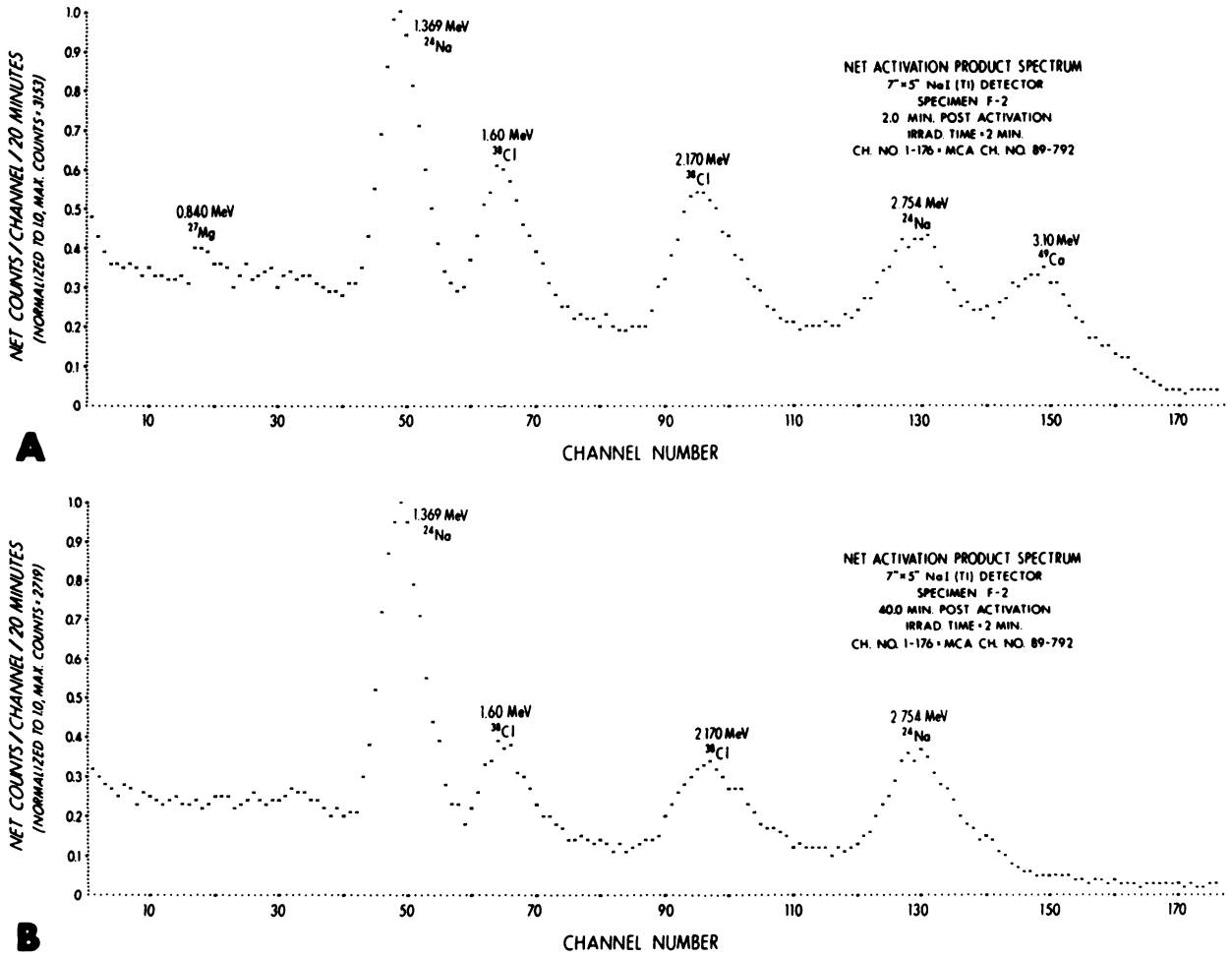

FIG. 4. A shows gamma-ray spectrum of irradiated human finger (F-2) recorded with NaI(Tl) detector. Finger was irradiated for 2 min at flux density of 1.8×10^8 n/cm²/sec; counting was initiated 2 min after termination of irradiation. B is repeat spectrum on F-2. In this case, counting was initiated 40 min after completion of irradiation.

TABLE 1. ELEMENT WEIGHTS IN MICE AND FINGER SPECIMENS DETERMINED BY NEUTRON ACTIVATION ANALYSIS

Specimen	Total weights of elements in irradiated specimens (mg)				
	Ca	Na	Cl	Mn	Mg
M-1*	230 ± 10	36.0 ± 0.4	39.6 ± 0.4	0.048 ± 0.002	—
M-2	247 ± 8	32.5 ± 0.4	36.8 ± 0.4	0.051 ± 0.002	—
M-3	259 ± 7	29.9 ± 0.4	33.4 ± 0.3	0.029 ± 0.002	—
M-4	240 ± 6	27.0 ± 0.4	30.2 ± 0.3	0.052 ± 0.002	—
M-5	247 ± 7	32.0 ± 0.4	33.8 ± 0.3	0.042 ± 0.002	—
M-6	273 ± 5	34.2 ± 0.3	41.7 ± 0.5	0.068 ± 0.002	—
M-7	269 ± 8	32.7 ± 0.5	36.2 ± 0.4	0.050 ± 0.003	—
M-8	288 ± 9	34.7 ± 0.5	40.6 ± 0.4	0.056 ± 0.003	—
M-9	271 ± 10	34.3 ± 0.4	38.2 ± 0.4	0.050 ± 0.002	—
M-10	291 ± 8	34.4 ± 0.5	36.9 ± 0.4	0.051 ± 0.002	—
M-11	289 ± 8	33.6 ± 0.4	38.8 ± 0.4	0.055 ± 0.002	—
M-12	305 ± 10	37.2 ± 0.6	43.0 ± 0.5	0.065 ± 0.003	—
M-13	259 ± 9	31.5 ± 0.6	34.1 ± 0.5	0.039 ± 0.003	—
F-1*	592 ± 8	52.7 ± 0.5	38.4 ± 0.4	—	5 ± 1.0
F-2	578 ± 10	62.1 ± 0.6	47.1 ± 0.5	—	8 ± 1.2

* M and F refer to mouse and finger specimens, respectively.

TABLE 2. COMPARISON OF CALCIUM DETERMINATIONS BY THERMAL NEUTRON ACTIVATION ANALYSIS AND ATOMIC ABSORPTION

Specimen	Calcium in specimen (mg)		Difference (mg)	Difference (%)
	N.A.A.*	A.A.†		
M-6	273	279	+ 6	+2.2
M-11	289	301	+12	+4.2
F-1	592	575	−17	−2.9

* Neutron activation analysis determinations.
† Atomic absorption determinations.

TABLE 3. RADIATION DOSE TO 35-GM SPECIMEN RESULTING FROM 2-MIN EXPOSURE TO A THERMAL NEUTRON FLUX OF 1.8×10^8 n/cm²/sec

Source of radiation	Dose (rem)
1. Gamma-ray background in hohlraum	10.
2. Capture radiation: $^{14}_{7}N(n,p)^{14}_{6}C$	3.9
$^{1}_{1}H(n,\gamma)^{2}_{1}H$	0.7
3. Decay of induced activity: $^{24}Na(\gamma,\beta^-)$	0.007
(examples) $^{32}P(\beta^-)$	0.009

muth between the reactor core and the central thermal column (*20*). The data also suggest that calcium content in the finger could be determined with an estimated standard deviation of ±4.5% with irradiation times as short as 1 min. The addition of bismuth to the irradiation configuration and such a reduction in irradiation time should provide the means for carrying out such studies in vivo. The absorbed dose level would be well below recommended maximum permissible dose levels to the hands for occupational hazards (*20*).

The thermal beam port modifications reported for in vivo thyroid studies (*9,10*) would provide a configuration suitable to carry out this procedure. A uniform thermal neutron field and adequate body shielding could be obtained by extending a lithium-shielded moderator from the beam port to accommodate finger positioning. An alternative configuration might make use of a properly moderated and shielded ^{238}PuBe or a ^{252}Cf neutron source. The former has been shown to be a potentially useful source for whole-body activation analysis studies (*6*).

Provided that only calcium determinations are required, the spectral data collected in this study suggest that the mathematical treatment of data could be considerably simplified. The calculations could be reduced to the evaluation of ^{49}Ca in the presence of ^{24}Na since the latter provides the only spectral interference at the 3.10-MeV decay peak of ^{49}Ca. This determination could be made without requiring computer processing.

CONCLUSIONS

The results of this investigation demonstrate the technical feasibility of determining calcium content in mice and in human cadaver specimens by thermal neutron activation analysis. In addition to calcium, several other elements can be simultaneously assayed.

The proposed irradiation configuration and activation fluence should allow calcium determinations of

the fingers in vivo. The relatively homogeneous thermal neutron flux density observed in animal and phantom calibrations suggests that it might also be feasible to do similar determinations on the os calcis. The data suggest that calcium content of the finger could be estimated with ±4.5% accuracy using a neutron fluence yielding a radiation dose of the order of 2.8 rem. Compared with current techniques of calcium determination at selected body sites (*12*), this procedure has the unique capability to directly assay calcium. Direct assay of calcium content could provide a more sensitive indicator for such metabolic changes as decreased bone density in osteoporosis or demineralization observed in weightlessness or chronic bed rest. Although whole-body activation analysis can provide the same information for the entire body, the procedure and instrumentation requirements can be much more extensive and costly. The additional possibility of obtaining in vivo calcium assays at selected anatomic sites for differential diagnosis in certain pathologic states and for therapeutic followup strongly suggest that this technique could be used in conjunction with or as a substitute for whole-body activation analysis in certain cases.

ACKNOWLEDGMENT

This work is from a Dissertation for the Degree of Doctor of Philosophy in the University of Rochester School of Medicine and Dentistry. The paper is based on work performed partially under contract with the U.S. Atomic Energy Commission at the University of Rochester Atomic Energy Project and has been assigned Report No. U.R.-49-1400.

REFERENCES

1. ANDERSON J, OSBORN SB, TOMLINSON RWS, et al: Neutron activation analysis in man in vivo: a new technique in medical investigation. *Lancet* ii: 1201–1205, 1964

2. ANDERSON J, TOMLINSON RWS, BATTYE CK, et al: Total body sodium, calcium, and chlorine by whole-body neutron activation. In *Compartments, Pools and Spaces in Medical Physiology*. Bergner PE, Lushbaugh CC, eds, USAEC CONF-661010, 1967, pp 112–122

3. PALMER HE, NELP WP, MURANO R, et al: The feasibility of in vivo neutron activation analysis of total body calcium and other elements of body composition. *Phys Med Biol* 13: 269–279, 1968

4. CHAMBERLAIN MJ, FREMLIN JF, PETERS DK, et al: Total-body calcium by whole-body neutron activation: new technique for study of bone disease. *Brit Med J* 2: 581–583, 1968

5. CHAMBERLAIN MJ, FREMLIN JH, PETERS DK, et al: Total-body sodium by whole-body neutron activation in the living subject: further evidence for non-exchangeable sodium pool. *Brit Med J* 2: 583–585, 1968

6. COHN SH, DOMBROWSKI CS, FAIRCHILD RG: In vivo neutron activation analysis of calcium content in man. *Int J Appl Radiat* 21: 127–137, 1970

7. COHN SH, DOMBROWSKI CS: Measurement of total-body calcium, sodium, chlorine, nitrogen, and phosphorus in man by in vivo neutron activation analysis. *J Nucl Med* 12: 499–505, 1971

8. BATTYE CK, TOMLINSON RWS, ANDERSON J, et al: Experiments relating to whole-body activation analysis in man in vivo using 14-MeV incident neutrons. In *Nuclear Activation Techniques in the Life Sciences*. Vienna, IAEA, 1967, pp 573–581

9. BODDY K, ALEXANDER WD: Clinical experience of in vivo activation analysis of iodine in the thyroid gland. In *Nuclear Activation Techniques in the Life Sciences*. Vienna, IAEA, 1967, pp 583–594

10. LENIHAN JMA, COMAR D, RIVIERE R, et al: Estimating thyroid iodine by activation analysis in vivo. *J Nucl Med* 9: 110–115, 1968

11. MACK PB, LACHANCE DA, VOSE GP, et al: Bone demineralization of foot and hand of Gemini-Titan IV, V and VII astronauts during orbital flight. *Amer J Roentgen* 100: 503–511, 1967

12. WHEDON GD, NEUMAN WF, JENKINS DW, eds: *Progress in Development of Methods in Bone Densitometry*. NASA SP-64, U.S. Government Printing Office, 1966

13. HELMER RG, HEATH RL, METCALF DD, et al: *A Linear Least-Squares Fitting Program for the Analysis of Gamma-Ray Spectra Including a Gain-Shift Routine*. IDO-17015, USAEC, 1964

14. MOORE RH, ZEIGLER RK: *The Solution of the General Least-Squares Program with Special Reference to High Speed Computers*. LA-2367, USAEC, 1960

15. NATRELLA MG: *Experimental Statistics*. NBS Handbook 91, US Government Printing Office, 1963, Chap VI

16. SCHONFELD E, KIBBEY AH, DAVIS W: Determination of nuclide concentrations in solutions containing low levels of radioactivity by least-squares resolution of the gamma-ray spectra. *Nucl Inst Meth* 45: 1–21, 1966

17. Report of Committee II on Permissible Dose for Internal Radiation. ICRP Publications 2, New York, Pergamon Press, 1959

18. AUXIER JA, SNYDER WS, JONES TD: Neutron interactions and penetration in tissue. In *Radiation Dosimetry*, vol I, Attix FH, Roesch WC, eds, New York, Academic Press, 1968, pp 275–316

19. Protection Against Neutron Radiation Up to 30 Million Electron Volts. NBS Handbook 63, US Government Printing Office, 1957

20. TOCHILIN E, SHUMWAY BW: Dosimetry of neutrons and mixed n + γ fields. In *Radiation Dosimetry*, vol III, Attix FH, Tochilin E, eds, New York, Academic Press, pp 247–308