Trace Elements in Human Tissue

2. ESTIMATION OF THE CONCENTRATIONS OF STABLE STRONTIUM AND BARIUM IN HUMAN BONE

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The increasing development of nuclear reactors and weapons has necessitated some knowledge of the human metabolism of fission products. Strontium and barium have a particular significance in this respect, since they are among the most abundant of these products, have long half-lives, and are known to be deposited in bone.

The presence of barium and strontium in human tissues has been investigated by several workers with spectrographic techniques. Kunowski (1932) stated that there was no barium in normal human bones, and this was confirmed by Gerlach & Muller (1934), who could not detect barium in human bone although their method was sufficiently sensitive for its detection in several gallstones and faecal specimens. Arnolt (1941), by injecting stable barium chloride into experimental animals, showed that barium was retained chiefly in the liver, with some deposition in the lungs and spleen, but it was not found in the muscles, bones or kidneys. Timm (1932), however, using similar techniques, could detect traces of barium in animal bone, although he was not able to give quantitative details. Forbes, Cooper & Mitchell (1954) found that strontium was not present in the skeleton or soft tissues of the one human individual examined, at the limit of sensitivity of their spectrographic method (1 p.p.m. of ash), or by flame photometry (0.1 p.p.m.). Sheldon & Ramage (1931), investigating various human tissues spectrographically, detected strontium in some tissues but not in bone (limit of sensitivity 0.008 %). However, Gerlach & Muller (1934) maintained that strontium was present in normal and pathological bone in concentrations from 0.01 to 0.1 $\mu\text{g./g.}$ of wet tissue, and Alexander, Nusbaum & MacDonald (1955) stated that the natural levels of stable strontium in the bones of mice, rats and guinea pigs are within the range 0.015–0.19 % of ash.

Asari (1950) estimated the strontium oxide content of 'protohistoric' and recent human bones and found the range to be 0.07–0.25 %. The average strontium concentration of 0.016 % for fetuses

10–18 cm. long and 0.24 % for male and female adults was given in a survey of the strontium content of human bone ash carried out by Hodges *et al.* (1950).

Tipton, Foland, Bobb & McCorkle (1953) stated that their spectrographic method was not suitable for the detection of strontium. Barium was not detected at the limit of sensitivity (200 p.p.m.). However, separate estimations of strontium in human bone by neutron-activation analysis, gave results ranging from 6 to 255 p.p.m. of ashed bone.

Results published for the spectrographic estimation of barium and strontium in bone show some discrepancy, and the reliability of this method for precise quantitative determinations is open to question. Standard analytical procedures for the estimation of these elements have been hampered by the difficulty of their separation from calcium. The advantages of neutron-activation analysis have been outlined previously (Smales, 1953, 1955; Brooksbank, Leddicote & Mahlmann, 1953; Jenkins & Smales, 1956). The sensitivity and precision of this technique render it particularly suitable for the determination of strontium and barium in bone. The errors and limitations inherent in the method have been described by several authors (e.g. Smales, 1953; Bowen & Dymond, 1955; Plumb & Lewis, 1955; Jenkins & Smales, 1956).

METHODS

The technique adopted for the present work was based on the method described by Harrison & Raymond (1955). It is dependent on the irradiation of ashed samples together with standards of strontium and barium salts under similar conditions. After irradiation the strontium and barium were separated from the ash and their concentrations estimated by a comparison of radioactivity with that of the standards. The addition of stable isotopes as carriers, subsequent to irradiation, facilitated the extraction of strontium and barium free from contamination by the radioactive isotopes of these elements. Preliminary investigations to determine the minimum weight for satisfactory recovery of inactive barium and strontium carrier showed that 50 mg. of each was sufficient to ensure an adequate chemical yield.

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It has been stated that the method has a high degree of sensitivity. Within the conditions of the experiment, the limit of detection was found to be $0.75 \mu\text{g.}$ for strontium and $1.0 \mu\text{g.}$ for barium. This assumes the minimal activity required for determination to be twice that of the background, allowing 2.25 hr. for the chemical separation. The limits of $0.04 \mu\text{g.}$ for strontium and $0.1 \mu\text{g.}$ for barium quoted by Bowen & Dymond (1955) could not be achieved under the conditions described.

Reagents

Standards. Johnson, Matthey and Co. Ltd. 'specpure' carbonates were used, the specified impurity being negligible.

Carriers. Standard solution of strontium and barium (50 mg./ml.) carbonates, A.R. grade, was used. Nitrates of potassium, copper, sodium, cadmium, bismuth, manganese, silver and lead in 10% (w/v) aq. solution were used. $(\text{NH}_4)_3\text{PO}_4$ was an aq. solution saturated at room temp.; BaCl_2 was (10% (w/v) aq. solution.

Other reagents. Na_2CO_3 , A.R. grade, aq. solution saturated at room temp. K_2CrO_4 , A.R. grade, aq. solution saturated at room temp. Ammonium acetate, A.R. grade, aq. solution saturated at room temp. Acetic acid, A.R. grade. FeCl_3 , A.R. grade, 5% (w/v) aq. solution. HNO_3 , Hopkin and Williams, C.P.R.; d 1.5 (fuming). HCl (2N), A.R. grade.

Sample form and preparation

Specimens of various bones were collected from autopsies held in different parts of Britain. Material from 'normal' bodies was selected whenever possible (Stitch, 1957). Samples of bone were generally taken from the diaphyses of long bones or the shafts of ribs close to the costal angle. Strict precautions against contamination were observed; the material was transported in polythene bags, and adhering tissues were separated with a quartz knife. No chemical manipulation was permitted before activation.

Specimens were dried in silica crucibles at 100° for 24 hr. and ashed in a muffle furnace at 500° for 24 hr. The bone ash was ground to a fine powder in an agate mortar and stored in polystyrene containers. This was carried out in a 'dry box' (Stitch, 1957), under pressure, so that the fine particles of dust present in the atmosphere were excluded. Instruments, after cleaning with acid, were rinsed with water redistilled from all-glass apparatus.

Activation of samples and standards

Two standard samples, each consisting of a weighed mixture of SrCO_3 (approx. 50 mg.) and BaCO_3 (approx. 100 mg.), and six samples of bone ash (approx. 150 mg.) each in small (1 cm. $\times$ 2.5 cm.) polystyrene containers, were irradiated simultaneously.

The samples were irradiated in a $3\frac{1}{2}$ in. $\times$ 1 in. Al can placed in the 'belt stringer' of the Harwell reactor, B.E.P.O. The can was subjected to a neutron flux of 10^{12} neutrons/cm.²/sec. for 3 hr., the reactions being represented as follows:

The thermal neutron cross-sections for ^{138}Ba and for ^{86}Sr are 0.55 and 1.3 respectively. An activity of 40 mc/g. of barium and 12 mc/g. of strontium is obtained after 3 hr. irradiation in a neutron flux of 10^{12} neutrons/cm.²/sec.

After irradiation, the specimens were left for 30 min. before handling to allow the decay of intense radioactivity from isotopes of short half-life.

Radiochemical purification

The radiochemical purification scheme is shown in Fig. 1.

All chemical manipulations were carried out behind lead shielding of 2 in. thickness. Discarded radioactive solutions were stored behind concrete of 6 in. thickness during the analysis.

Fig. 1. Scheme of radiochemical purification.

After activation the bone ash was weighed into numbered 40 ml. graduated centrifuge tubes. The standard samples were emptied into two 500 ml. volumetric flasks, the containers being washed four times with 2*N*-HCl to ensure the complete transference of the radioactive strontium and barium salts. The volumes were made up to 500 ml. with water. To obtain a level of activity suitable for counting, 1 ml. portions of the activated barium and strontium solutions were pipetted from each volumetric flask into two 40 ml. centrifuge tubes. Approx. 0.5 ml. of the solution of carriers containing potassium, copper, sodium, cadmium, bismuth, manganese, silver and lead were then added to each tube, and 3–4 ml. of 6*N*-HNO₃ to dissolve the ash. A volume (1 ml.) of the solution of stable barium and strontium carrier was added, and the active and stable barium and strontium were precipitated by the addition of excess of saturated aq. Na₂CO₃, then separated by centrifuging (approx. 1000 *g*; MSE major centrifuge), the supernatant liquid being discarded. The insoluble carbonates were dissolved in the minimum volume of conc. HNO₃, reprecipitated and the supernatant discarded as before. The precipitate was redissolved and the volume made up to 5 ml. with water; approx. 1 ml. of (NH₄)₃PO₄ carrier and 14 ml. of fuming HNO₃ were added (Willard & Goodspeed, 1936). After cooling in iced-water for 5 min., the tubes were centrifuged and the supernatants discarded. The nitrates were dissolved in 5 ml. of water and (NH₄)₃PO₄ and a further 14 ml. of fuming HNO₃ was added to reprecipitate them. Six precipitations were carried out in order to remove the calcium from the ashed samples. Ammonium phosphate, which was added to the first three precipitations, acted as a carrier for radioactive phosphorus. Calcium phosphate was not used since calcium would affect the chemical yield, but not the assay of radioactivity owing to the characteristics of the calcium isotopes produced during irradiation. After the final HNO₃ separation, the nitrates were dissolved in a minimum quantity of water, and FeCl₃ solution (approx. 0.1 ml.) and conc. HCl (approx. 0.1 ml.) were added. Ferric hydroxide was precipitated after adding aq. NH₃ soln., thereby scavenging traces of other metals. The precipitate was centrifuged and the supernatant decanted into a further series of centrifuge tubes. The remaining precipitate was dissolved in the minimum of 2*N*-HCl, and the iron scavenge repeated.

To the combined supernatants acetic acid and ammonium acetate were added until acid (pH approx. 5), together with 1 ml. of K₂CrO₄ to precipitate the barium. After heating in a boiling-water bath (2 min.) and centrifuging, the solutions of SrCrO₄ were decanted into numbered centrifuge tubes. The precipitated BaCrO₄ was dissolved in the minimum quantity of 2*N*-HCl and reprecipitated by excess of saturated ammonium acetate. This was repeated a third time, the final precipitate of BaCrO₄ being dissolved in 2*N*-HCl and the three supernatant strontium fractions being combined. In order to remove residual traces of ¹³⁸Ba from the strontium solutions, 0.5 ml. of 10% BaCl₂ was added as carrier and the precipitated BaCrO₄ was separated by centrifuging and discarded. Both strontium and barium fractions were made up to 25 ml. and transferred to separate polystyrene counting pots (9 cm. × 5 cm.).

Estimation of activity

The barium and strontium fractions were counted consecutively over a period (4 hr.) in a thallium-activated, NaI

crystal-type γ -scintillation counter. A decay curve was plotted for each sample in order to check its purity (Fig. 2). Contaminated fractions which did not give a decay curve parallel to that of the standard were discarded.

After assay of radioactivity the solutions of barium and strontium were quantitatively transferred to weighed and numbered centrifuge tubes for estimation of the chemical yield. The barium was precipitated as BaCrO₄ with excess of ammonium acetate, and the strontium as SrCO₃ with excess of Na₂CO₃. The precipitates were centrifuged, washed twice with dilute aq. NH₃ soln. and once with acetone, and dried to constant weight.

The activity of each sample at a specific time was found from the decay curves, and was corrected for the paralysis time, background of the counter and for the overall chemical yield after radiochemical purification. The concentration of barium and strontium originally present in the bone ash was calculated by the equation:

$$M_x = \frac{R_s \times W_s \times Y_s \times f \times 10^6}{R_x \times W_x \times Y_x}$$

where M_x represents Ba (Sr) in bone ash ($\mu\text{g./g.}$); R_x , counts of Ba (Sr)/min., unknown fraction at time t ; R_s , counts of Ba (Sr)/min., standard fraction at time t ; Y_s , wt. (mg.) of Ba (Sr), recovered from standard fraction; Y_x , wt. (mg.) of Ba (Sr), recovered from unknown fraction; f , conversion factor for wt. of Ba (Sr), in carbonate irradiated as standard (Ba 0.696; Sr 0.594); W_s , wt. (mg.) of standard salt irradiated; W_x , wt. (mg.) of bone ash irradiated.

The average measurement of activity of two standard samples was used for comparison with the unknown samples. If the discrepancy between the two standard samples was greater than 5% the analysis was abandoned.

Reproducibility

Repeated determinations of the barium and strontium concentrations of five bone samples were made in order to estimate the reproducibility of results. Results given in Table 1 do not generally vary by more than 5% of the mean. For purposes of comparison, some of our material has been analysed for strontium by Dr B. A. Loveridge (Chemistry Division, Harwell) by neutron-activation analysis, but with ⁹⁰Sr (β -emitting isotope) and a more elaborate method of radiochemical purification. His results (Table 2) show remarkable agreement with results obtained by us in this laboratory.

RESULTS

Estimations were made of the concentrations of barium and strontium in 35 bone samples. Analyses were made in triplicate whenever sufficient material was available. The results for the various bone samples are presented in four groups according to age (Table 3). The levels of barium and strontium are expressed as $\mu\text{g./g.}$ of ashed bone (p.p.m.).

It can be seen from Table 3 that the results for strontium in the two adult age groups (19–74 years) are higher than those for the two lower age groups (foetus and child 0–13 years). Statistical comparison of groups 1 and 2 with groups 3 and 4 shows that the level of strontium in the group 19–74 years is significantly higher than in the group 0–13 years

Fig. 2. Typical decay curves for barium and strontium after radiochemical separation from bone ash: ◇, standard 1; ○, standard 2; ●, ×, +, ▽, △ and □, fractions 1-6 from bone ash.

Table 1. Reproducibility of results of replicate determinations of barium and strontium in bone samples

Case no.	Barium concn., mean and s.d. (p.p.m.)	No. of determinations	Strontium concn., mean and s.d. (p.p.m.)	No. of determinations
B37	11.4 ± 0.6	8	144 ± 4.5	8
B38	5.5 ± 0.6	9	146 ± 8.0	8
C16	4.7 ± 0.2	5	90.4 ± 5.0	6
C14	7.9 ± 0.6	6	53.4 ± 2.5	6
B16	4.0 ± 0.9	5	85.0 ± 2.0	6

Table 2. Comparison of strontium values obtained for three bone samples, in different laboratories

Case no.	Loveridge (see Methods)		Sowden & Stitch (present work)	
	Mean and s.d. (p.p.m.)	No. of determinations	Mean and s.d. (p.p.m.)	No. of determinations
M2	61 ± 4	4	64 ± 3	12
B37	143 ± 5	4	144 ± 4.5	8
B38	136 ± 9	8	146 ± 8	8

Table 3. *Concentration of stable barium and strontium in bones of human males and females*

Group	...	1	2	3	4
Age	...	0-3 months	1-13 years	19-33 years	33-74 years
No. in group	...	7	9	9	10
Barium					
Range (p.p.m.)		1.9-13.0	2.1-21.0	4.3-7.9	3.7-17.3
Mean (p.p.m.)		7.0	7.6	5.1	8.5
S.D.		±4.0	±7.0	±0.12	±4
Strontium					
Range (p.p.m.)		41.6-108.0	53-129	66.5-130	77-157
Mean (p.p.m.)		79.1	73.8	107	114
S.D.		±20	±26	±23	±28

Table 4. *Distribution of strontium ($\mu\text{g./g.}$ of ash) in different bones of single individuals*

	Age of individual (years)			
	0	6	18	78
Rib	72	100	131	138
Tibia	66	98	114	179
Femur	66	91	127	157
Skull	75	—	126	180
Vertebrae	65	—	115	150
Sternum	—	—	113	135
Humerus	64	—	—	—
Pelvis	67	—	—	—
Clavicle	70	—	—	—
Fibula	67	—	—	—
Ulna	65	—	—	—

at $P < 0.01$. No variability with age was noted in the barium level of these two groups. The greater range of barium results within an age group could not be related to any specific factor.

Analyses of several bones of the same individual were carried out on four specimens in the age range 0-78 years. Significant variability in the strontium (Table 3) or barium levels was not observed in the different bone samples of each individual examined, although higher values for the strontium content of the tibia and skull of one male, aged 78, were noted (Table 4).

The presence of barium and strontium was confirmed in all the bone samples analysed. There was no evidence of relationship between the concentrations of barium and strontium and the sex of the individual. No relationship of barium and strontium with disease was noted in the additional pathological cases investigated, e.g. cases of haemorrhage, ulcerative colitis, peritonitis, heart failure. Bone samples from patients with specific bone diseases have not been examined.

DISCUSSION

Most remarkable in this series of results is the constant presence of strontium and barium in all the bones investigated. Earlier work, which failed to

determine these elements, was based on spectrographic methods. The poor sensitivity of this method probably accounts for the recorded absence of barium in bone.

We have found barium in bone in relatively lower concentration than strontium and the method of neutron-activation analysis described has made it possible to determine the levels with accuracy. The extensive physiological experiments which have been carried out with radioactive strontium tracers (Jowsey, Owen & Vaughan, 1953; Pecher, 1941; Kidman, Tutt & Vaughan, 1950) provide evidence that injected strontium is retained in bone in preference to other organs.

The conclusions of Hodges *et al.* (1950) are in agreement with certain points of our work, but the normal level of strontium recorded by these workers (0.024%) is considerably higher than estimated in this Laboratory. The range of results for strontium in bone given by Tipton *et al.* (1953), from 6 to 255 p.p.m., is greater than that observed by other authors, and the average figure of 64 p.p.m. of strontium is lower. However, consideration must be given to the possibility of dietary variability in different geological locations, when comparing results from different countries. There is no obvious relationship between strontium and barium levels and locality within the British Isles, although specimens from areas where the soils are known to be comparatively rich in strontium have not been examined.

We are generally in agreement with Hodges *et al.* (1950) on the constancy of the strontium level in different bones of the same individual. MacDonald *et al.* (1951) produced data for the strontium content of the femurs and vertebrae of mice fed on diets containing strontium and noted that the strontium level was similar in the two bones. Experiments by Pecher (1941) with injected radioactive strontium suggest that strontium is preferentially deposited in certain bones; this author reported a higher activity in the vertebrae. Lisco, Finkel & Brues (1947), however, found that osteogenic sarcoma caused by the ionizing radiations of the strontium

were more common in the long bones. These observations may be explained by the realization that strontium is laid down in sites of active bone formation, which will vary with each experiment.

Hodges *et al.* (1950) found a difference in the strontium content of foetal and adult material. The foetal material was obtained from foetuses of length 10–18 cm. ($4\frac{1}{2}$ –5 months intrauterine life), and these authors suggest that the lower values of foetal strontium may be due to the slight degree of calcification at this stage of development. Foetal material of this age was not examined by us. Comparison of our analyses of bones obtained from infants with those of bones from adults shows a slight but significant increase in the strontium content; this is not exhibited as a uniform increase throughout the period associated with bone calcification. The most marked increase appears to be in the first few months of life, with isolated results of high strontium content in the upper age group.

SUMMARY

1. A method is described for the determination of strontium and barium in human bone by radio-activation analysis.

2. Results of analyses of 35 bone samples, from normal persons of both sexes and different ages, are given. The concentrations of barium and strontium were found to be of the order of 7 and 100 $\mu\text{g./g.}$ of ashed tissue respectively.

3. No relationship between sex or disease of individuals with strontium and barium concentration was noted. The concentration of strontium in the age group 0–13 years was significantly lower than that in the group 19–74 years.

4. No significant difference was found in the concentrations of strontium and barium in the various bones of those individuals examined.

5. Results obtained in this survey are discussed and compared with those of other workers.

We are indebted to Mrs A. Harrison for her help in the initiation of the programme; to Dr B. A. Loveridge (Chemistry Division, A.E.R.E., Harwell, Didcot, Berks.) for his

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