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The Determination of Strontium in Human Serum Using Neutron Activation Analysis[1]

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INTRODUCTION

The measurement of the content of stable strontium in small volumes of serum, 1.0 ml or less, was found to be necessary in the course of performing strontium kinetic studies in infants and children. For similar physiological studies in adults (1, 2) and in older children (3), flame emission spectroscopy has been used. This technique limited the duration of these studies inasmuch as it was not suitable to quantitate the small serum concentration of strontium encountered five or more days after intravenous administration. X-ray fluorescent spectroscopy has also been used to detect and quantify strontium in normal serum (4, 5), but it was also of limited usefulness, as large sample sizes were required and inter-laboratory differences were observed. Another technique used in the measurement of serum strontium was thermal neutron activation and subsequent radiometric measurement (6, 7, 8). These methods required separation prior to activation (a step which may introduce small additive errors), long periods of activation, large samples, and cumbersome methods of chemical separation following neutron activation. In order to obviate these difficulties, a new method of neutron activation for strontium was developed and has been found to be satisfactory for human serum. This new method and the results of its application are presented in this report.

METHOD

Reagents: Deionized water, 0.04% bromthymol blue, 3 N HCl, 2.5% (w/v) sodium oxalate, 0.8 N perchloric acid, 3 N NaOH, carrier-free ^{85}Sr, and 0.12 M strontium nitrate (Sr "carrier"). Strontium standards were prepared from re-

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crystallized strontium nitrate (in fuming nitric acid) dried at 155° C for 48 hours. All chemicals were reagent grade, and all solutions were stored in polyethylene containers at room temperature.

Materials: Sample holders (Fig. 1a) were made of Nalgene® (grade HH) and had a capacity of one ml; each had its own cover. Thirteen samples were stacked on Nalgene® rods during activation (Fig. 1b). Post-irradiation chemical separation was performed in non-wetable Teflon® funnels fitted with glass fiber filtered paper (Fig. 1c). Other required items are noted in the procedure.

Reactor facility: Ninety to 120 minute thermal neutron irradiation was performed in the Brookhaven Medical Research Reactor. Gold-foil monitored thermal neutron flux varied from 1.05 to 1.15 $\times$ 10^{13} neutrons/cm^2/sec when the reactor was run at three megawatts. The epicadmium neutron flux density was 2.7 $\times$ 10^{11} neutrons/cm^2/sec (cobalt wire). A pneumatic tube was used to deliver and retrieve samples from the core of the reactor.

Counting facility: A multichannel analyzer[1] was used to sort and store pulses from a 3 in $\times$ 2 in NaI (Tl activated) crystal atop a 6393 DuMont photomultiplier tube fitted with a cathode-follower pre-amplifier. Both printed and punched papertape digital output was obtained.[2]

Procedure: Sera obtained from venous or capillary blood were muffled overnight at 600°C and redissolved in deionized water. No anticoagulants of any type were used, because all were found to contain strontium. The dissolved ash and standards were pipetted into the Nalgene® sample holders which already contained a known, constant amount of strontium-85. After drying under a heat-lamp, all unknown samples were stacked with strontium standards on the top and bottom. The stack was sealed in a plastic bag and inserted into a "rabbit." During neutron irradiation the flux varied both across the sample stack, six per-cent, and longitudinally two-to-four per cent. The former variation was minimized by uniform distribution of dried sample in the holder, while the latter was taken into account in calculation.[3]

Chemical separation of strontium was performed after activation to exclude or minimize other radioisotopes, principally sodium-24. Following irradiation the stack of samples was dismantled with long-handled instruments; individual sample holders with their covers were placed in 100 ml beakers. The holders were then rinsed with 5 ml of 0.8 N perchloric acid and deionized water and removed. Five drops of indicator, 3 ml of Sr "carrier," 5 ml of 2.5 per cent sodium oxalate, and sufficient NaOH to alkalinize were successively added to each beaker. All additions were performed with pre-set, spring-loaded syringes. The precipitate was washed twice with water and redissolved with 15 ml 3 N HCl. A repeat oxalate precipitation was performed as noted above. The precipitate was then dried by gently drawing air through the funnels which were then dismantled

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[1]400 channel RIDL analyzer, Model 34-12B.

[2]Friden Model 44-7 add-punch digital printed using a parallel-to-series converter and a Friden Model 44-9 translating matrix.

[3]The fall-off in neutron flux was found to be linear, so that interpolated counting rates were determined from actual counting rates of standards located at the top and bottom of the sample stack.

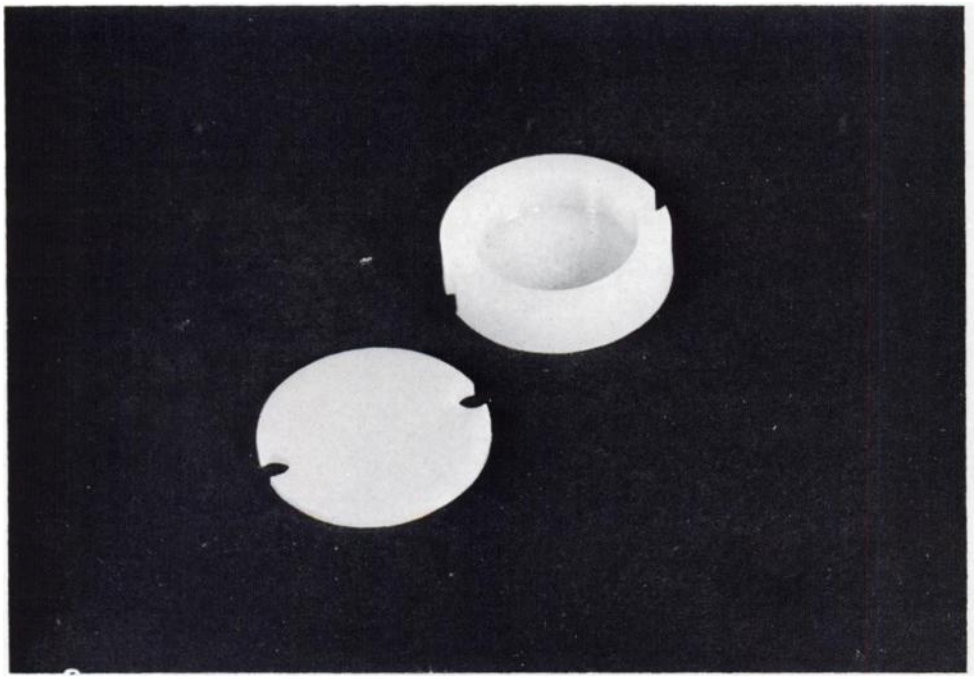

Fig. 1a.

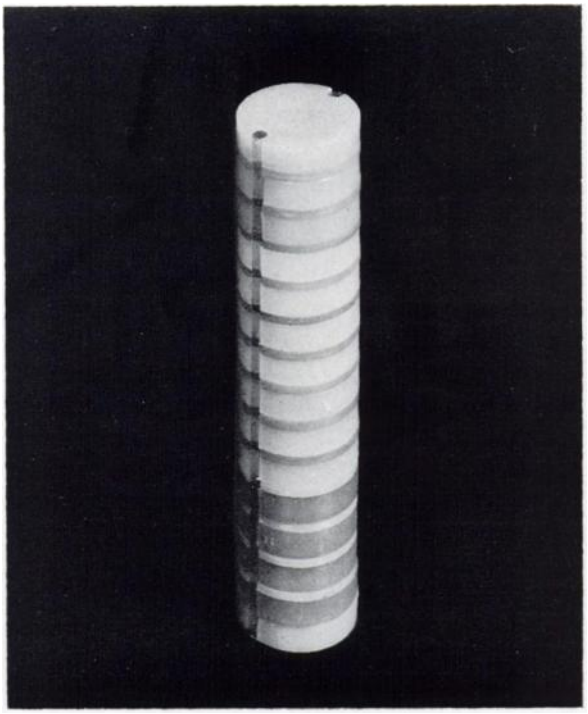

Fig. 1b.

and the samples transferred to Teflon® ribbon (2 in wide, 0.003 in thick), dried, identified, and mounted with Scotch tape® for counting.

All samples were counted two inches above the crystal for five minutes ("live time"). The midpoint of each counting period was carefully noted, to permit correction for physical decay.

Calculations: The principal radioisotope measured was ^{87m}Sr produced from ^{86}Sr by the (n, γ) reaction. The natural abundance of ^{86}Sr is 9.86 per cent. Under the conditions noted above, there were no other nuclear reactions resulting in strontium-87m. The amount of ^{87m}Sr was determined by summing the counts beneath its photoelectric peak at 0.39 MeV. This summation was performed by using one of two methods: 1) a straight line was drawn across the base of the peak and the area of the peak alone was arithmetically determined, and 2) using a gamma-ray spectal stripping porgram (8), an IBM 7094 computer fitted and integrated the peak area. All ^{87m}Sr counts were corrected for physical decay ($\lambda = 0.00417\ min^{-1}$). All samples were counted again two weeks after activation (only ^{85}Sr present at this time). These counts were compared to ^{85}Sr counting standards geometrically similar to the samples prepared from the irradiated material. The calculated recovery factors together with the corrected counts from

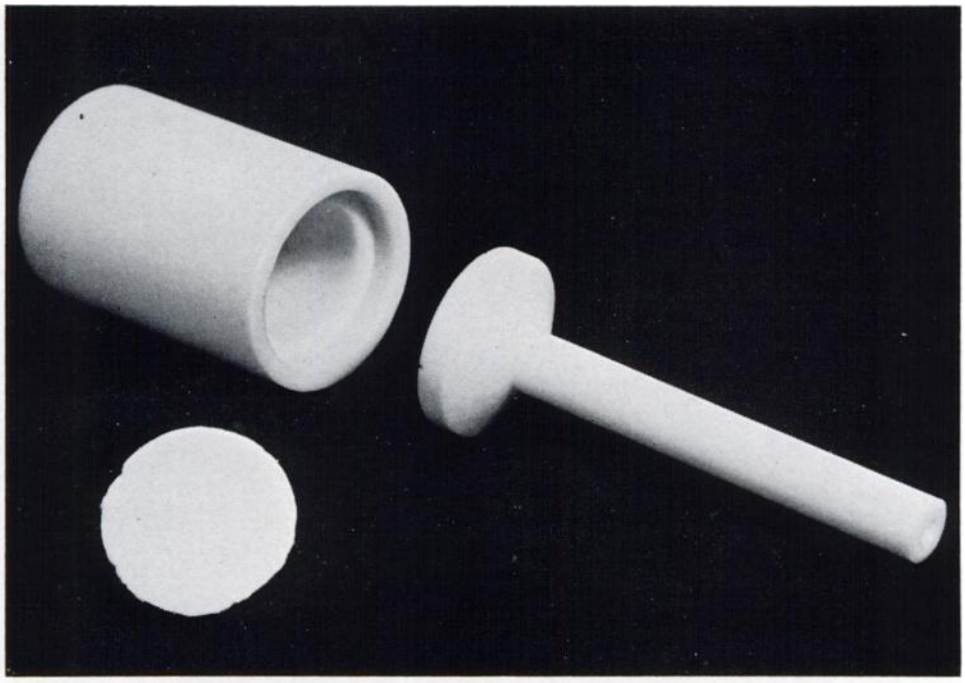

Fig. 1c.

Fig. 1. (a) Nalgene® sample holder, capacity 1 ml with cover (2.6 cm O. D.), (b) stacked samples ready for insertion into plastic bag, the standard samples are at the top and bottom, and (c) Teflon® funnels used to isolate the oxalate precipitate (12 cm length, assembled).

standards were used to determine the amount of stable strontium present in the unknown samples. All concentrations were expressed as micrograms per milliliter of serum.

RESULTS

The gamma-ray spectrum of 0.28 μg of strontium (after activation and chemical separation) is presented in Figure 2 (no added ^{85}Sr). The counting rate attributable to ^{87m}Sr approximated 1000-2000 actual counts/min/μg stable strontium (photoelectric peak at 0.39 MeV only) when the samples were counted three hours after the end of the activation. The double oxalate precipitation did not remove all other radionuclides. Specifically, ^{56}Mn was present in all standard and unknown specimens (0.84, 1.81, and 2.12 MeV), and ^{24}Na in all biological specimens (1.37 and 2.75 MeV). All spectral data were obtained in 100 channels precalibrated by placing the photoelectric peak of ^{137}Cs (0.66 MeV) on channel

TABLE I

REPLICATE DETERMINATION OF STRONTIUM IN STANDARD SOLUTIONS

Concentration of Strontium μg per ml	*Number of Determinations*	*Mean of Determinations μg per ml*	*± 2 Std. Dev. μg per ml*	*± 2 Std. Dev. per cent*
11.17	14	(11.17)[a]	± 0.38	± 3.4
1.12	12	1.13	± 0.045	± 4.0
0.28	10	0.28	± 0.028	± 10.0
0.10	6	0.09	± 0.020	± 22.2
0.02	13	0.03	± 0.010	± 33.3

[a]—used as reference standard

TABLE II

REPLICATE DETERMINATIONS OF STRONTIUM ADDED TO SERUM ASH

Amount of Strontium Added μg per ml	*Number of Determinations*	*Mean of Determinations μg per ml*	*± 2 Std. Dev. μg per ml*	*± 2 Std. Dev. per cent*
11.17	10 (4)[a]	11.15	± 0.40	± 3.6
1.12	10 (5)	1.14	± 0.067	± 6.1
0.28	12 (10)	0.29	± 0.040	± 13.8
0.10	6 (3)	0.11	± 0.032	± 29.1
0.04	8 (5)	0.05	± 0.020	± 40.0

[a]—number in parentheses denotes number of individual serum specimens used

33. Each channel was 20 KeV wide and the total gamma-ray spectrum visualized was two MeV. Although gamma-rays of higher energy were not calculated, such activity was found to be negligible. A gamma-ray peak at 0.51 MeV was always found, largely due to the ^{85}Sr added to each sample before activation.

In Figure 3, the corrected ^{87m}Sr counting rates are plotted against the amounts of standard stable strontium activated, both with and without corrections for recovery. Log coordinates were chosen simply to demonstrate the linear relationship over a wide range of sample size. Because of this relationship, only two standard samples were included in each stack of 13 samples. Recovery of ^{87m}Sr was assumed to be identical to the recovery of ^{85}Sr and varied from 75 to 90 per cent. Such variation was attributed to the varying composition of the samples and to the rapidity of the oxalate precipitations. Each run of 13 specimens exposed the operator to approximately 20 mr (dosimeter at mid-chest).

In Table I the variability of replicate determinations of standard strontium solutions is shown. As judged by these data, the threshold for detection of strontium was about 0.02 μg.

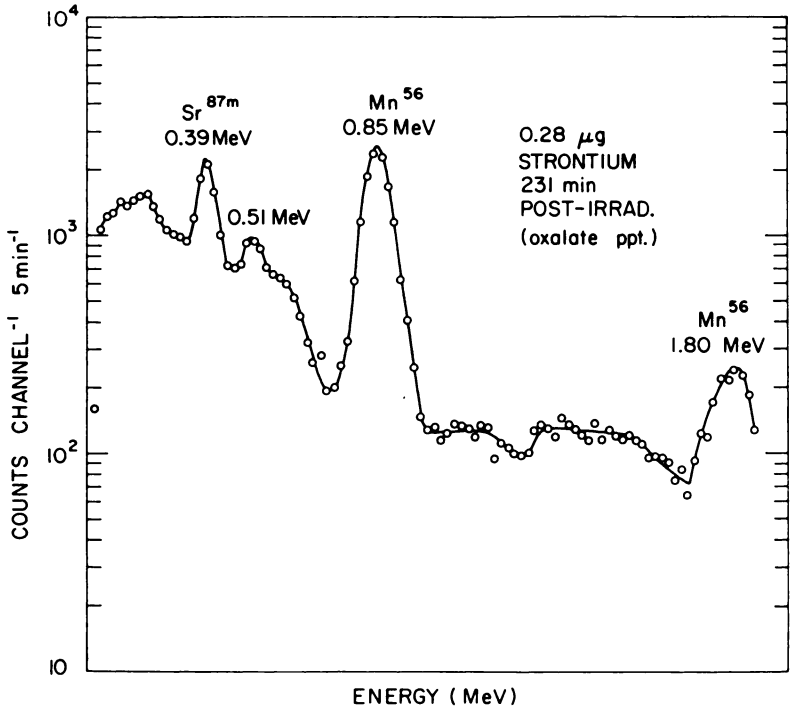

Fig. 2. Gamma-ray spectrum of 0.28 micrograms strontium after activation and separation (see text).

Table II presents the results found when variable amounts of strontium were added to one milliliter aliquots of dissolved serum ash, equivalent to 1 ml serum. Variability in the serum samples was greater than in the standard samples, due to the presence of more ^{56}Mn and ^{24}Na and to trace amounts of stable strontium present in the original serum samples. Serum from 18 infants and children were used in this study; no pools of individual sera were analyzed.

Evident from the averaged data in Table II and from the strontium concentrations in sera from individual subjects noted in Table III, the amount of strontium naturally present in the serum of healthy infants and children is extremely small—considerably less than 0.04 μg per ml.

DISCUSSION

This technique was developed primarily to calculate the concentration of stable strontium in small samples of serum obtained from children to whom strontium gluconate had been administered intravenously. It was necessary that the technique be simple, yield reproducible results and be applicable to very small samples. The data presented validate the method and indicate its potential usefulness in the determination of trace quantities of strontium in biological material.

In the large number of reports dealing with radioactive strontium (principally ^{85}Sr and ^{90}Sr) in animals and man, the results are generally expressed in terms of "per cent administered dose per gram of calcium." Although such studies have added greatly to our knowledge of skeletal dynamics and, indirectly, of calcium metabolism, the specific study of strontium metabolism has been retarded because of an inability to express results of studies in terms of true specific activity (cpm Sr^*/mg Sr).

With this same method, it has also been possible to determine stable strontium in urine and in bone. However, others (9,10,11,12) using larger samples and considerably more complex separation techniques have reported this application of neutron activation analysis. In its present form, it is not possible to use the technique reported here for the determination of strontium in tissue (other than bone) or in feces, because of the large amounts of manganese and other divalent trace metals found in such material. It is possible to minimize, although not exclude, the coprecipitation of manganese with oxalate by carefully adjusting the pH of the final precipitation mixture to 4.5 (13), but other separation techniques (6,14) would appear to be necessary to effect maximum utility. When larger samples are available, flame and absorption spectrophotometry and X-ray fluorescence spectroscopy may be used (4, 15, 16, 17).

Koch has calculated the maximum sensitivity for the neutron activation determination of strontium to be 0.0001 μg, assuming a neutron flux of $10^{14}/cm^2/sec$, a detection of limit of 1000 cpm, eight hours of irradiation, and radiometric assay immediately after activation (18). Theoretically the limit found in the present study may be lowered by increasing the period of neutron bombardment, by further reducing the amounts of contaminating radionuclides, including the added ^{85}Sr, and by longer and more efficient counting.

TABLE III

STABLE STRONTIUM AND CALCIUM CONCENTRATIONS IN HUMAN SERA

Patient	*Age yr.*	*Diagnosis*	*Calcium Concentration*[a] *μg per ml*	*Strontium Concentration μg per ml*
		Healthy		
B. R.	2/12	———	93	0.03[b] <0.03
C. S.	2–11/12	———	98	<0.03
J. E.	5– 2/12	———	96	<0.03
W. E.	7– 4/12	———	98	<0.03
L. C.	14	———	99	<0.03 <0.03
R. C.	1/12	———	94	<0.03
J. D.	5/12	———	96	<0.03
		Diseased		
P. C.	3/12	Prematurity Vitamin D deficiency rickets, untreated	78	<0.03 <0.03
B. McC.	3– 2/12	Metaphyseal dysostosis	108	<0.03 <0.03 <0.03
K. S.	9/12	Multiple congenital anomalies	92	<0.03
J. McG.	3/12	Osteogenesis imperfecta congenita	89	0.31 0.28
B. M.	5– 2/12	DeToni-Fanconi syndrome	101	<0.03 <0.03
P. E.	6/12	Hypophosphatasia	132	0.03
M. E.	7/12	Hypophosphatasia	108	<0.03
R. P.	3–10/12	Idiopathic osteoporosis, untreated	98	<0.03
	4– 9/12	Idiopathic osteoporosis treated	105	<0.03
L. T.	10– 2/12	Vitamin D-resistant rickets, untreated	94	<0.03
S. W.	6– 6/12	Vitamin D-resistant rickets, treated	96	<0.03
F. K.	8– 3/12	Acute leukemia in relapse, osteoporosis	122	0.04 0.03

[a]—Method of Kingsley and Robnett (19).

[b]—Replicate determinations on the same serum sample.

The statistical variation noted in Table II did not permit the presentation of actual concentrations of strontium found in the serum samples noted in Table II. When values were calculated to be 0.00, 0.01, and 0.02 μg per ml, they were reported as <0.03 μg per ml. The data from Table II would suggest a mean concentration of strontium to be of the order of 0.01 μg per ml. When different amounts of stable strontium were added to aliquots of the same serum sample, the determinations performed, and the results plotted, an extrapolation to "0 added Sr" revealed a normal concentration varying from 0.00 to 0.02 μg per ml (five samples). The serum sample obtained from patient J. McG. having an elevated strontium concentration remains unexplained.

A relatively wide variation has been noted in the reporting of normally occuring strontium in human serum (Table IV). In addition to problems in detection; strontium intake, calcium intake, strontium-calcium ratios in foodstuffs, age, or other factors may be responsible for these variations. Wider application of the recent advances in analytic technology, including neutron activation analysis, will extend our knowledge and understanding of strontium in the biosphere.

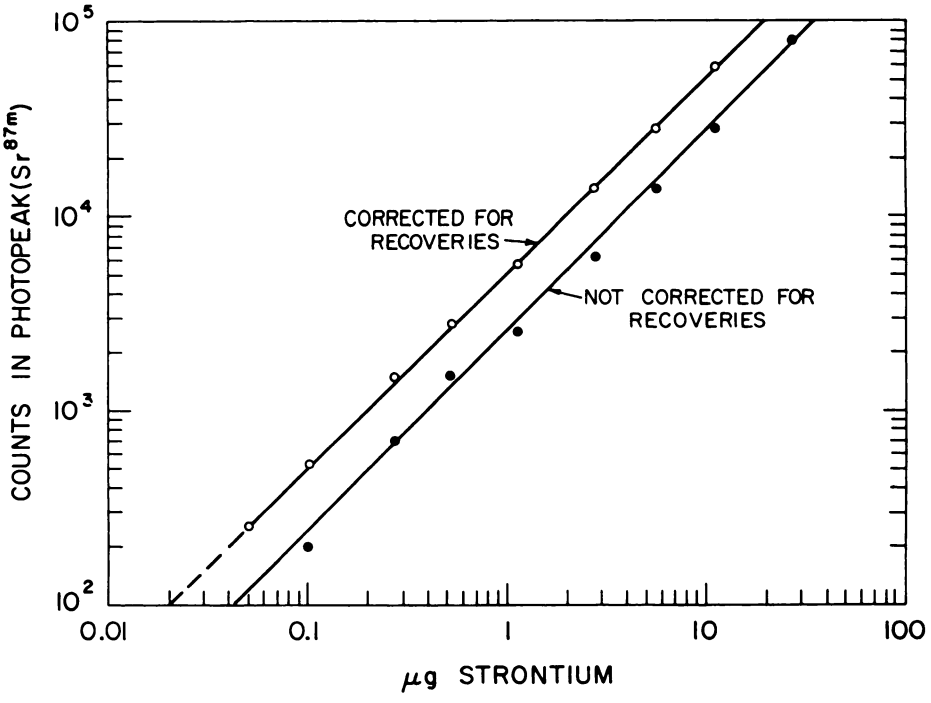

Fig. 3. Counts from ^{87m}Sr photopeak vs. amount of stable strontium activated, with and without corrections for recovery

TABLE IV

REPORTED SERUM STRONTIUM CONCENTRATIONS

Technique	*Number of Samples Assayed*	*Range of Values*		*Mean*	*Reference*
		Low	*High*		
		μg per ml		*μg per ml*	
X-ray fluorescence spectroscopy	15	0.16	0.31	0.25	(4)
Neutron activation analysis	6[a]	0.007	0.03	0.01	(6)
X-ray fluorescence spectroscopy	43	——	——	0.00[b]	(5)
Neutron activation analysis	17[c]	0.055	0.246	0.0948[d]	(7)
Neutron activation analysis	17[e]	0.032	0.145	0.075[f]	(7)
Neutron activation analysis	19	<0.03	0.30	<0.03[g]	Present study

a—whole blood
b—90% confidence limits 0.00–0.03 μg per ml
c—maternal blood from placenta
d—standard deviation 0.045 μg per ml
e—fetal blood from umbilical cord
f—standard deviation 0.036 μg per ml
g—patient J. McG. excluded

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