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STABLE STRONTIUM BALANCES IN MAN

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The development in recent years of sensitive methods of trace metal analysis have made it possible to analyse diet and excreta for stable strontium and so establish balance data under normal conditions. Previously, strontium metabolism was studied using radioactive tracers or as in an early study, by the administration of small but non-physiological amounts of stable strontium (McCance and Widdowson 1939). In the present study, atomic absorption spectroscopy has been used for the determination of stable strontium balances in adult men in control conditions and during oral and intravenous administration of the element.

Experimental

Strontium balances were performed in eleven ambulatory adult male patients with normal kidney and gastrointestinal function, and, (with the exception of Patient 4) who had no skeletal abnormalities. The low calcium-low phosphorus diet contained an average of 200 mg calcium and 820 mg phosphorus per day. In two cases (Patients 4 and 11) balances were determined with the addition of a milk supplement to the diet. This increased the calcium intake to about 800 mg/day. The fluid intake was adjusted according to each patient's requirements and kept constant throughout the studies.

Supplemental strontium given orally was administered as the lactate in a dosage of 1536 mg/day divided into three parts with meals, while the intravenously injected stable strontium was given as the gluconate. The doses in these cases varied from 318-954 mg stable strontium and were infused daily for 6 consecutive days in 500 ml 5% glucose in water over 4 hours. Both the orally and the intravenously administered stable strontium were well tolerated by the patients and no toxic effects were observed.

Balances of strontium and calcium were determined by analysing aliquots of the complete 6-day pool collections of urine and stool as well as aliquots of diet in each 6-day period using atomic absorption spectroscopy as the method of analysis for both strontium (Warren and Spencer 1972) and calcium (Willis 1960, Willis 1961, Osis et al. 1969).

Results

(a) Control Conditions of Strontium Intake

Figure 1 shows intake and excretions in 12 balance studies on low strontium intake ranging from 0.8 to 1.15 mg/day as contained the low calcium diet and 1.5 mg/day when the diet was supplemented with milk. The wide variation in urinary strontium from 0.03 mg/day in Patient 1 to 0.68 mg/day in Patient 4 was similar to the wide variation in urinary calcium excretion from one individual to another. The average urinary strontium excretion of all patients was 0.18 mg/day (omitting the abnormally high excretion of Patient 4). The faecal strontium excretion was also variable and ranged from 0.53-1.56 mg/day and averaged 0.91 mg/day. The average strontium balance of this group of patients was very close to equilibrium.

Although there is wide variation from patient to patient, each individual has been shown to be consistent from one study period to the next on a constant intake. Three consecutive 6-day control periods were determined for Patient 5. The strontium intake varied from 0.79 to 0.81 mg/day. The urinary excretion ranged from 0.22 to 0.24 mg/day or about 30% of the intake while the faecal excretion varied from 0.52 to 0.65 mg/day or about 70% of the intake so that the patient was in equilibrium with regard to strontium. The calcium balances for the same study periods showed a similar degree of uniformity with the patient in slightly positive calcium balance.

(b) Effect of Oral Stable Strontium on the Strontium and Calcium Excretions

Figure 2 shows the effect of orally administered stable strontium on the urinary strontium and faecal excretions and urinary calcium excretion in two studies of Patient 5. The intake in both cases was 1537 mg strontium/day. The patient was in equilibrium during the control period. During administration he excreted 18.5% and 25% of the dose in the urine in the two studies respectively. The calcium balance for the same period of time showed that oral stable strontium administration increased urinary calcium excretion markedly for the duration of the administration only and had no effect on the faecal calcium excretion.

(c) Effect of Intravenously Administered Stable Strontium

Table 1 shows stable strontium excretions of six patients who were given intravenous doses of stable strontium in eight studies. The dose varied from 318 mg to 954 mg per day and was given for six days in each case and the results are expressed as percentages of the dose. During the period of administration the main route of strontium excretion is

via the urine. Figure 3 shows the urinary excretion during one of these studies in more detail and also includes the urinary calcium excretions. Urinary calcium is increased markedly during this time although there is little effect on faecal calcium. Levels of strontium in the urine decrease rapidly following discontinuation of the dose although there is a time lag of one metabolic period before faecal strontium levels decrease. However after 30 days strontium excretion is still considerably higher than baseline.

Discussion

Stable strontium ingested with diet has been shown to range from 0.8 to 1.5 mg/day. Milk increased the calcium intake by a greater proportion than the strontium intake. This would be expected from the low Sr/Ca ratio in milk compared to that of other dietary items. Studies by Harrison et al. (1955) and Tipton et al. (1966) who used neutron activation analysis and emission spectroscopy respectively as methods of estimation stable strontium, showed dietary calcium and strontium levels which are in agreement with the present findings. The proportions of the element in the excreta are in good comparison in the three studies. Fallout strontium 90 is a suitable tracer for stable strontium being ingested in small amounts over a long period of time (Kahn et al. 1969). Indeed it was the presence of this radionuclide in normal diet that provided the motivation for the upsurge of interest in strontium metabolism in the 1950's. Its behaviour would be expected to resemble that of dietary stable strontium more closely than other tracers which are usually given in single doses or stable strontium administered in 'unnatural' amounts. Samachson and Spencer (1967) used strontium 90 in this way and obtained similar results to those in the present study.

Oral and intravenous administration of the element have produced results which are comparable with the many isotope studies. For instance Spencer et al. (1960) giving ^{85}Sr as a tracer showed wide variation in absorption which resulted in a wide variation in urinary levels of the isotope. Although only two patients had stable strontium administered orally in the present study the urinary excretion varied from 7-25% of the dose. Although the urinary excretion increases promptly when oral stable strontium is administered, it continues to increase during the first six days after which equilibrium levels are reached. Similar results were obtained by Samachson 1963 with ^{85}Sr data.

Intravenously administered stable strontium seems to behave in a similar fashion to intravenously administered isotopes of strontium. The isotope studies of Spencer et al. (1960) and Harrison et al. (1966) in man and Bauer et al. (1955) and Lloyd (1964) who worked with

experimental animals, established that the main route of excretion of strontium in the bloodstream is via the kidney. In the present experiment this is confirmed. In two cases where the patients were subjected to varying doses it appeared that renal tubular reabsorption was less effective with the larger dose. However further investigation would be necessary to confirm this.

Both orally and intravenously administered stable strontium continued to be excreted for many months following discontinuation of the dose, and there was no evidence of longterm retention. This is in agreement with the work of Likins et al. (1960) who found that strontium was easily remobilised from bone tissue and appears to contradict the recommendation of Schorr and Carter (1950) who proposed the use of strontium as an adjuvant to calcium in the treatment of osteoporosis. Strontium toxicity has been observed in experimental animals (Bartley and Reber 1961) and although there was no evidence of this in the present experiment, insufficient information is available in man. The urinary excretion of calcium increased during administration of stable strontium by either the oral or intravenous route. The effect of stable strontium infusions has been reported previously Spencer et al. (1967).

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TABLE I

Excretion of Sr following Intravenous Administration

Patient	Dose mg/day	% Dose		
		Urine	Stool	Balance
10.	318	22.3	11.3	+66.4
	Post Sr	10.5	11.6	-21.8
	Post Sr	2.8	2.9	- 5.3
11.	318	25.0	7.2	+67.7
	Post Sr	13.6	10.0	-23.2
	Post Sr	4.3	2.9	- 6.7
5.	636	43.2	9.4	+47.4
	Post Sr	15.0	3.2	-18.1
	Post Sr	5.0	3.2	- 8.0
7.	636	37.6	7.9	+54.8
	Post Sr	15.9	7.6	-23.4
	Post Sr	5.1	2.4	- 7.3
8.	612	38.3	9.8	+51.9
	Post Sr	14.9	9.9	-24.7
	Post Sr	3.5	4.1	- 7.5
9.	636	36.1	4.1	+59.8
	Post Sr	8.8	3.7	-12.4
	Post Sr	2.7	1.3	- 3.8
10.	954	32.3	5.9	+61.8
	Post Sr	8.8	11.9	-19.7
	Post Sr	2.2	2.7	- 4.8
11.	954	35.6	4.5	+59.9
	Post Sr	9.4	8.4	-17.6
	Post Sr	2.4	3.1	- 5.4

(1) All results are based on average excretions during six-day periods.

(2) The diet during the administration and in the post periods contained an average of 1 mg Sr./Day

Figure 1 INTAKE AND EXCRETIONS DURING LOW INTAKE

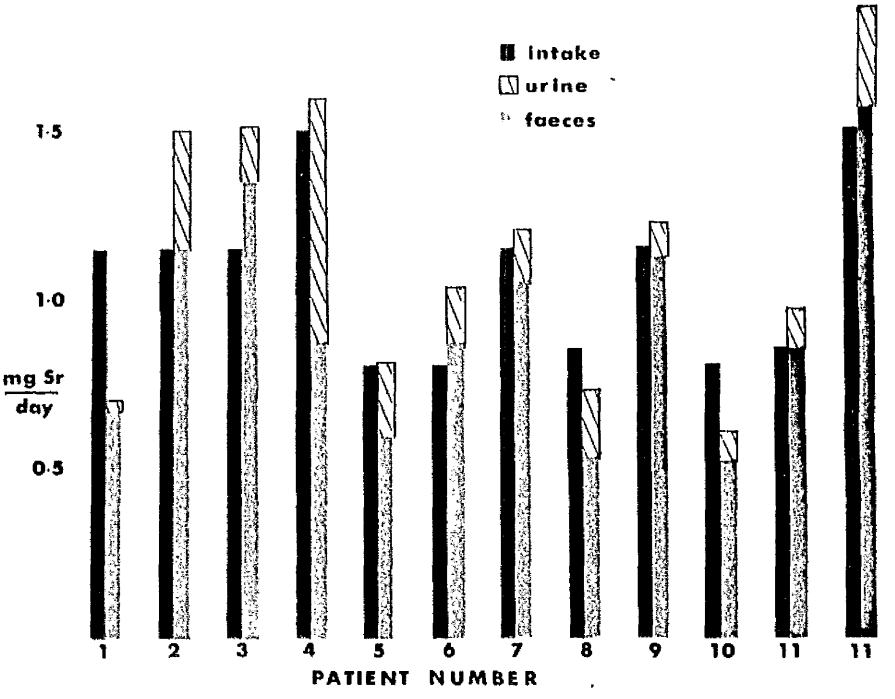


Figure 2 EFFECT OF ORAL STABLE STRONTIUM ON URINARY AND FECAL STRONTIUM EXCRETION

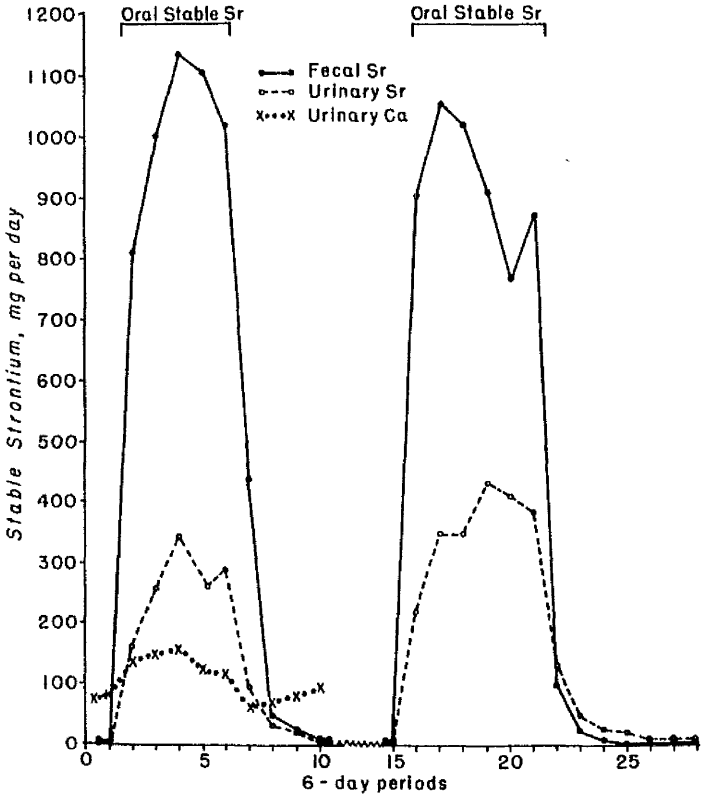
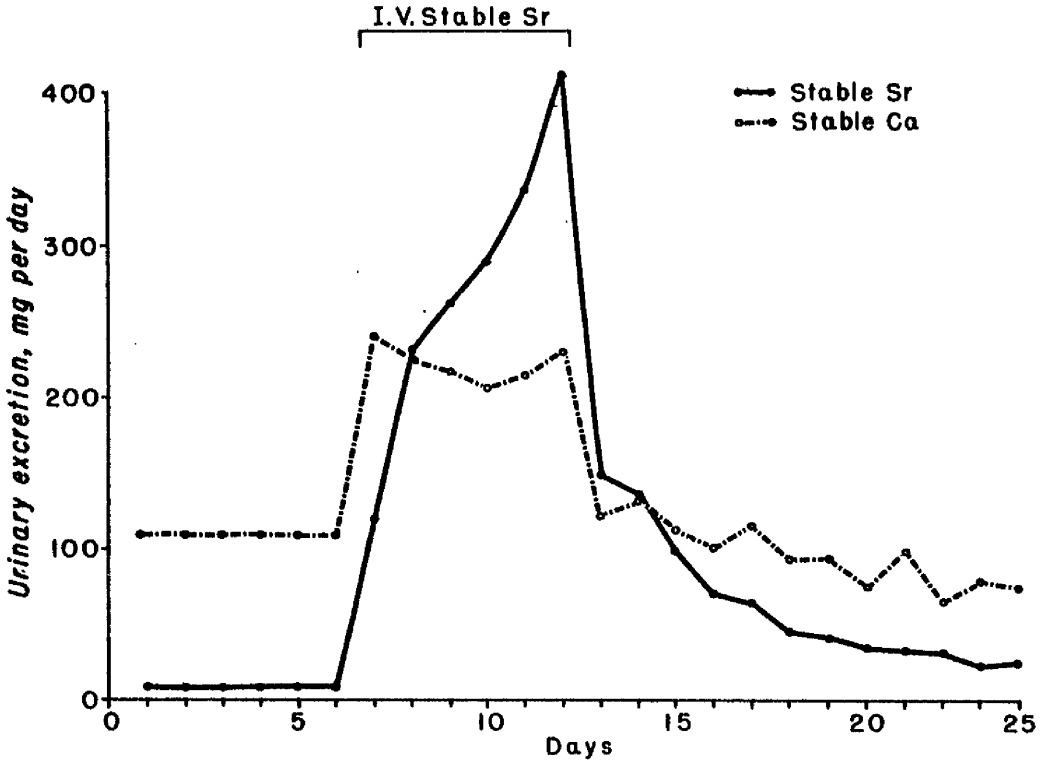


Figure 3 EFFECT OF INTRAVENOUS STABLE STRONTIUM ON URINARY STRONTIUM AND CALCIUM EXCRETION



STUDIES IN STRONTIUM METABOLISM

by

JANET M. WARREN

Summary

1. This thesis reports work done on strontium metabolism at the University Department of Child Health and Department of Clinical Physics and Bio-Engineering, Glasgow and at the Veterans Administration Hospital, Hines, Illinois during the period October 1966 - September 1969.

2. The main object of the work was to study the mechanisms of discrimination in the transfer of strontium and calcium across biological membranes.

3. The experimental work was done in rats and in human subjects using radioactive and stable tracers and intravenous and oral doses of stable strontium. All experiments were carried out under in vivo conditions.

4. The studies using the rat as experimental animal investigated the passage of strontium-85 with respect to calcium-47 across the gastrointestinal membrane from the bloodstream to the intestine. The isotopes were given intravenously and the rats were examined both feeding and fasting. From the first study which was over a 24 hour interval it appeared that strontium was excreted to a slightly greater extent than calcium into the intestine during feeding. When the rats were fasted, less of both isotopes was excreted by this route although the difference between strontium and calcium was increased. The Sr/Ca faecal excretion ratio increased from 1.2 in the feeding rats to 2.2 in the fasting animals

Intravenously administered strontium-85 was excreted equally in urine and faeces - approximately 10% of the dose by each route of excetion. Calcium-47 however was mainly excreted in stool (approximately 8%) while less than 1% was excreted via the kidney. Fasting caused reduced levels of both ^{85}Sr and ^{47}Ca in the urine. These excretory differences resulted in (a) greater bone deposition of ^{47}Ca than ^{85}Sr both during feeding and fasting and (b) greater levels of both isotopes in the skeleton of the fasting rats than of the feeding animals.

A second rat study was performed to investigate the effect of time on the distribution of the isotopes compared to the 24 hour study. In this case animals were sacrificed at $\frac{1}{2}$, 1, 2 and 4 hours after injection of the dose. The results of this experiment indicate that calcium is excreted into the intestine slightly faster than strontium and the differences between ^{85}Sr and ^{47}Ca at 24 hours were due to discrimination in reabsorption by the gut. It appeared that there was little difference between the isotopes between 1 and 4 hours. In fasting the reabsorption of the secreted isotopes was greater than during feeding.

The passage of strontium with respect to calcium across the human placenta was investigated. Neutron activation techniques were used to measure stable strontium and flame emission spectrometry for calcium. It was shown that calcium passes more readily than strontium across the membrane from mother to child by a factor of about 2. The concentration of calcium

in the serum of the newborn was about 20% higher than that of the mother.

The effect of stable strontium on the excretions of strontium and calcium was studied in man during both oral and intravenous administration of the element. It was shown that a large dose of stable strontium administered orally was similarly distributed in the excretions as was the trace level of an average diet. Retention of such a dose was for a short period of time in a subject who had no error in calcium metabolism. Intravenously administered stable strontium was mainly excreted via the kidney.

Ammonium Chloride and Aluminium Phosphate Gel have been used in attempts to modify stable strontium excretion but with little success.

Both intravenously and orally administered stable strontium had a marked effect on the urinary calcium excretion. This was reduced to about half of the normal level when strontium was administered by either route.

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STUDIES IN STRONTIUM METABOLISM

by

JANET M. WARREN

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Introduction

In 1791 Thomas Charles Hope, Professor of Medicine and Lecturer in Chemistry at the University of Glasgow recognised a new mineral when examining some material from a lead mine in Strontian, a village in Argyllshire. Later this mineral was identified as the carbonate of a new alkaline earth which was given the name 'strontites' (Hope, 1798). Although a study of the strontium compounds was first attempted in the Chemistry Department at Glasgow it was not until Humphry Davy began to work on the alkaline earths in 1807 after isolating and naming sodium and potassium that the metal could be separated. Davy, who used the technique of electrolysis, finally isolated Strontium and gave it its name in 1808 (Davy, 1808).

Strontium remained a relatively insignificant element until the 1950's. It had some commercial uses, mainly in sugar refining and in providing the red colouration for fireworks. A few medical uses have been reported in this time, the most important being as a treatment for osteoporosis, but little work was done on the metabolism of strontium in biological systems since there was no incentive for such study and analytical techniques were inadequate. With the advent of atomic energy, however, strontium became an element of considerable importance and both the motivation and the methods of investigation were simultaneously provided. Radionuclides have been used

extensively in the last 15 years in the measurement of strontium metabolism and several more refined chemical techniques have also been developed. In addition to the examination of metabolic pathways and mechanisms much effort has been made to find ways of reducing strontium-90 deposition in the body and of increasing removal of the deposited radionuclide.

The present thesis reports the extension and further development of work presented to the University of Glasgow in March 1965 for the degree of Master of Science. The investigations described here are aimed at examining the passage of strontium with respect to calcium across certain membranes of the body. A study has been made of the passage of the two elements from the bloodstream across the gastrointestinal membrane of the rat using radioactive tracers. The effect on this excretion of food in the intestine has been observed, together with age of the animals and the study has been made as a function of time following injection of the isotopes.

Stable strontium balances have been measured in man and the excretions compared during normal dietary intake and during oral and intravenous administration of large doses of the element. Some attempts at modification of the metabolism have been made. All the experiments have been made in vivo. These studies were performed by the author in the Metabolic Unit of the

Veterans Administration Hospital, Hines, Illinois under the guidance of Dr. Herta Spencer.

In the final experiment, measurements were made of strontium and calcium in blood samples in an attempt to measure placental discrimination which occurs in the passage of the two elements from the maternal bloodstream to that of the foetus. This work was done in Glasgow at the Department of Clinical Physics and Bio-Engineering using material supplied by the Staff of the Paediatric Unit at the Queen Mother's Hospital, Yorkhill. Neutron activation analysis was carried out at the Scottish Research Reactor Centre, East Kilbride.

STRONTIUM METABOLISM - REVIEW

Metabolism of Strontium

Strontium is chemically and biologically similar to calcium. It is therefore usual to consider the metabolism of strontium in conjunction with that of calcium although some investigators have preferred to treat strontium independently. McLean and Hastings (1953) described calcium concentration in body fluids as "one of nature's physiological constants". Calcium is homeostatically controlled by the hormone system. If hypocalcaemia occurs the parathyroid glands release parathyroid hormone or parathormone which acts on bone to increase osteolysis in an attempt to raise the serum calcium level of normal (Albright and Reifenstein, 1948, Howard 1956, 1957, Munson 1955). On the other hand, the stimulus of hypercalcaemia on the ultimobranchial cells of the thyroid causes the release of a calcium lowering hormone, calcitonin, which inhibits bone catabolism (Copp et al. 1961, Copp, 1963, Hirsch, Gauthier, Munson, 1963). The effectiveness of this endocrine system appears to diminish with increasing age (Copp 1969). Although hormones do have some effect, the control is not homeostatic in the case of strontium metabolism which appears to be dependent to a large extent on calcium levels.

The early studies on animals using radionuclides have shown that strontium was similar but not identical to calcium in behaviour in animals, Pecher (1941), Hamilton (1943). Extensive studies were undertaken, starting in 1955, to establish quantitatively the relationship between the two elements and to determine the physiological processes responsible for the differential behaviour. It was

found that where there was transfer of ions across a membrane under metabolic control major differences occurred between the passage of strontium and calcium. These distinct differences were found to be in gastro-intestinal absorption, renal excretion, lactation and placental transfer.

In an early pioneer study on strontium metabolism, stable strontium was administered in comparatively large doses to normal subjects (McCance and Widdowson, 1939). However the chemical techniques available at that time were the limiting factor of such investigations. Later radioisotopes became available and tracers were used extensively in experimental animals and in man. The Annotated Bibliography of Strontium and Calcium in Man and Animals by Wasserman and Comar (1962) gives a comprehensive list of the publications in this field up to 1959. Recent refinements in chemical and physical techniques to include such methods as emission spectrometry, atomic absorption spectrometry, neutron activation analysis and X-ray fluorescence have made it possible to obtain data for stable strontium in diets and excretions of normal individuals under physiological conditions. Fallout strontium-90 can be used as a tracer for stable strontium when ingested under similar conditions (Warren 1965). Widespread survey data of stable strontium and strontium-90 in human bone has given insight into various relationships of strontium and calcium (Bryant and Loutit, 1964; Loutit, 1969; Fletcher et al. 1961; Rivera 1965).

To simplify discussion of the comparative movement of strontium and calcium, Comar et al. (1956) have proposed the following

terminology which is now widely used. The strontium-calcium Observed Ratio (OR) represents the overall discrimination

$$OR\ (\text{sample/precursor}) = \frac{Sr/Ca \text{ in sample}}{Sr/Ca \text{ in precursor}}$$

Discrimination Factor DF is used to quantitate the discrimination between strontium and calcium in a physiological process and several such factors may be involved in the Observed Ratio. These terms are explained in more detail by Comar (1967).

Dairy products are the main source of the average person's dietary strontium in the U.K. - contributing 60% of the strontium and 56% of the calcium intake. (In parts of the world where vegetables rather than milk provide the main source of calcium these proportions are different due to the cow's discrimination against strontium in milk secretion [Comar et al. 1961]). Following ingestion, both elements are absorbed into the bloodstream from the gastro-intestinal tract. Calcium is more readily absorbed than strontium by a factor of 2-4 (there is a variation from species to species). In man there is wide variation from individual to individual. Calcium absorption ranges from 40-80% of the ingested amount while that of strontium is 20-40% in normal subjects. (Spencer et al. 1960, Harrison et al. 1955). The average absorption ratio in man is 2.6 (Spencer et al. 1960). Samachson (1963) used plasma values to determine that ^{45}Ca was absorbed more rapidly than ^{85}Sr. Calcium was favoured by a ratio of 3 to 1 during the first hour and of 2 to 1 for total absorption.

Methods of absorption have been divided into two mechanisms, passive diffusion and active transport. Passive diffusion is passage of water soluble molecules through water filled pores in the membrane. This can generally be increased by conversion of ionised forms to undissociated molecules by altering pH. Active transport is the term used to denote transport of substances against an electro-chemical gradient. It appears that calcium can move from the intestine to the blood both by active transport and by passive diffusion while it was believed that strontium moves by passive diffusion only (Wasserman 1960). However the identification of a calcium binding protein (Wasserman, and Taylor 1966) associated with the brush borders of the duodenal mucosa led to an expansion of the knowledge of absorptive processes. It has been known for some time that Vitamin D has a major function in the intestinal absorption of calcium (Nicolaysen and Eeg Larsen 1953, Harrison and Harrison 1951) and more recently Wasserman and his co-workers have investigated this mechanism (Wasserman 1962, Wasserman and Taylor, 1962, 1963, Taylor and Wasserman 1965) and explain the active transport mechanism by the presence of a calcium binding protein which is Vitamin D_3 stimulated (Ingersoll and Wasserman 1971). Deluca and his co-workers have carried out intensive investigations of Vitamin D elucidating the biologically active forms (Norman et al. 1964, Ponchon and Deluca 1969, Olson et al. 1972).

The absorbed calcium and strontium is deposited in the skeleton although a small percentage together with that mobilised from bone

tissue will be re-secreted into the intestine and excreted in the faeces with the unabsorbed fraction. A considerable proportion of the absorbed calcium and strontium are excreted via the kidney in the urine. The alkaline earths are reabsorbed from the glomerular ultrafiltrate by the renal tubules and the different rates of reabsorption of calcium and strontium result in renal discrimination (Walser and Robinson, 1964, 1965). More than 99% of the calcium is reabsorbed by the tubules so that only a very slightly lower reabsorption of strontium would result in a large difference in terms of discrimination For example, if calcium is 99.5% reabsorbed and strontium 98.5% then three times more strontium than calcium would be excreted.

More than 99% of calcium retained by the body is laid down in bone tissue (Copp 1969) and strontium is similarly localised. Bone deposition of strontium has been the subject of much investigation in the last 20 years since the β emitting isotope strontium-90 became an environmental contaminant and radiation hazard (Engstrom et al. 1958; Vaughan, 1954, 1955, 1961). Many of these studies are long term experiments to measure the toxicity and dosimetry of chronically ingested fallout strontium-90 (Goldman and Della Rosa 1967; Pool et al. 1971). Strontium-90 will induce tumours in the skeletal tissue (Owen et al. 1957; Barnes et al. 1970). The carcinogenic risk is related to both strontium-90 intake and bone turnover rate (Vaughan and Williamson, 1967).

It seems that there is little or no discrimination between calcium and strontium at the bone surfaces (Bryant and Loutit, 1964) although not all investigators are agreed on this point. Samachson and Lederer (1960) in vitro studies showed that when bone tissue was in contact with solutions of ^{85}Sr and ^{45}Ca more calcium was taken up than strontium by factors of between 1.2 and 1.4. Bauer et al. (1955) reported no marked difference between strontium-90 and calcium-45 in skeletal accretion but attributed lower retention of strontium-90 to preferential excretion of strontium both by the kidneys and in the gastro-intestinal tract. Della Rosa and Wolf (1965) also attribute higher bone levels of calcium to preferential excretion of strontium.

Other discriminations have been observed and investigated. There is a marked difference between strontium and calcium in the passage across the placenta resulting in an Observed Ratio foetus/mother of 0.6 in man (Bryant and Loutit, 1961) and between 0.4 and 0.7 for other species (Wasserman et al. 1957). It has also been shown that calcium is preferentially secreted into milk as compared to strontium. In the human, the Observed Ratio milk/diet is an average of 0.10 (Lough et al. 1960). If, for example, the dietary strontium-90 intake is 10 pCi/gCa (which was the approximate level in 1969) then the ratio in mother's milk would be 1.0 pCi/gCa. The Sr.90/Ca ratio in cows' milk is similar to that in total human diet so that an infant fed by the mother has this protective factor. Since the newborn infant appears to discriminate to a much lesser extent than the adult in gastro-intestinal absorption of strontium and calcium (O.R. body/diet 0.9-1 in the first month of life,

Lough et al. 1963) the protection afforded by maternal feeding could be of importance.

When the basic pattern of the body's handling of the element has been established, under normal conditions, then the conditions can be varied and investigations can be carried out in an attempt to modify the metabolism of strontium (Nelson 1963). It might be desirable to know if modifications can be made in case of release of large amounts of the hazardous isotope strontium-90 into the environment or in the case of accidental ingestion of any radioactive isotope of the element. Many investigators have published papers on this subject. Most experiments have been carried out on laboratory animals although some investigations in man are also reported.

The first and most obvious method of reducing uptake of strontium would be to remove the element from the diet. This is difficult to do without destroying the character of the food and the only items which lend themselves to such treatment are water (Bonner et al. 1966) and milk (Migicovsky, 1959; Crosslett and Watts 1959). However since milk is the chief source of strontium in diet of the Western hemisphere, this is a worthwhile consideration. Investigators have shown that by an ion exchange process they can remove strontium without affecting the calcium content which is an essential part of the diet. However the process is lengthy and on a commercial scale expensive but it would be useful in conditions where radioactive fallout is known to be high. (Infants whose diet consists largely of milk would benefit most, while adults and older children would have their strontium-90 intake reduced to about half by this method).

This method would be of no value in the case of accidental ingestion however, and the next step in the process would be to inhibit gastro-intestinal absorption in some way. Calcium is already preferred to strontium by a factor of two and methods of enhancing this preference would be advantageous. The presence or absence of food in the intestine has been shown to affect the absorption of calcium and strontium. Spencer et al. (1969) have shown that the differences in the intestinal absorption ratio can be altered to such an extent that the Ca/Sr absorption ratio changes from 2:1 to about unity when tracer doses of ^{85}Sr and ^{47}Ca are given in the fasting state instead of with food. Marcus and Lengeman (1962) have shown in rats that although the ratio between the two elements remained similar, there was considerably greater absorption of both ^{47}Ca and ^{85}Sr from a liquid dose rather than a solid one, in fact the absorption from the liquid dose was more than double that from the solid dose.

Experiments in rats have shown that the addition of calcium to the diet causes a reduction in the absorption of radiostrontium (MacDonald et al. 1955; Wasserman and Comar 1960; Palmer et al. 1958) and MacDonald et al. and Palmer et al. showed that this reduction effect could be further enhanced by the addition of phosphate to the higher calcium diet. Other studies however have shown less positive results for the effect of calcium on radiostrontium absorption. Hegsted and Bresnahan (1963) showed that growing rats required a five-fold increase in calcium intake to reduce the bone deposition

by one half. Spencer et al. (1961) showed that the addition of calcium to the diet of thirteen patients decreased the ^{85}Sr absorption in only five cases.

Stable strontium might also be expected to be a useful additive to the diet to reduce the radioactive strontium uptake by the method of isotope dilution (Catsch, 1957; Kawin 1959; Nelson et al. 1963). However the application is limited by several factors. All investigators seem to agree that the efficacy is very time dependent and treatment must be within a few hours of the administration of the radiostrontium. Spencer et al. (1967b) showed that intravenously administered strontium increased urinary strontium-85 excretion in man.

Sodium alginate has been similarly reported as an inhibitor of radiostrontium absorption (Skoryna et al. 1964, 1965; Stara and Waldron Edward 1969). Haug (1961) showed that sodium alginate derived from seaweed has a higher affinity for strontium than calcium. Waldron-Edward et al. (1965) showed that sodium alginate markedly reduced the bone uptake of strontium-89 in rats while the uptake of calcium-45 was affected to a lesser degree. Hesp and Ramsbottom (1965) markedly reduced body retention of strontium-85 in a human subject but gave no indication of the effect on the calcium. Sutton (1967) demonstrated reduced absorption of strontium-87m in man when alginate was added to the diet. In this case the effect on calcium was inconclusive. Carr et al. (1968) showed similar results.

Aluminium phosphate gel which is normally used as an antacid has been shown by Spencer and her co-workers (1967d, e, 1969) to decrease the absorption of strontium-85 in man. If given prior to the dose it has been shown that the decrease in absorption is on average 87%. If the delay in giving the inhibitor is half an hour after ingestion of the isotope, the decrease in absorption is 57% and 43% if the delay is one hour. Similar results have been obtained in rat experiments (Friedland et al. 1969).

Many investigators have explored the use of complexing agents and chelating agents but few have had more than borderline positive results and usually very high doses of the agent are required to produce the negligible effect. This subject has been investigated and reviewed by Catsch (1967).

A third method of approach is to enhance the urinary output of strontium. The use of ammonium chloride has been reported by several authors and while Spencer et al. (1958, 1965) showed increased urinary excretion in man, Della Rosa et al. (1961) showed negative results in dogs and Van Putten (1962) had no success in mice. Ammonium chloride causes metabolic acidosis and demineralisation of bone ensues. Magnesium is another calciuric agent and several investigators have shown that salts of magnesium are effective in the removal of radiostrontium from the body if administered early. Clark et al. (1964) showed that magnesium ions administered concurrently with radiostrontium decreased skeletal retention of the isotope although magnesium chloride

administered after the ^{85}Sr was well fixed in bone, had only a small effect. Spencer et al. (1967a) showed that infusions of magnesium resulted in increased urinary ^{85}Sr excretion in man. Nelson et al. (1963) showed that magnesium sulphate decreased retention of radiostrontium. However Volf (1967) has shown that oral sulphates reduced the retention of radiostrontium.

The action of citrate has been described (Smith and Bates, 1965; Smith 1967). Citrate is used as an anticoagulant and it appears that although it has no influence on the rate of disappearance of ^{85}Sr from the blood, it does affect the renal excretion by the formation of a citrate complex. Citrate is only effective in the removal of radiostrontium if administered early after injection since strontium is rapidly removed from the circulating fluids and is incorporated into bone.

Hormones have also been used to affect strontium metabolism with varying degrees of success. This work has been reviewed by Spencer et al. (1967c) who were responsible for many of the investigations.

ANALYTICAL TECHNIQUES - REVIEW

Stable Strontium - Methods of Analysis

Three methods have been used for the determination of stable strontium in biological materials

(a) Emission Spectrophotometry

(b) Atomic Absorption Spectrophotometry

(c) Neutron Activation Analysis

(a) and (b) are similar in sensitivity and applicability and so far as the work described here is concerned the only criterion which determined the choice was that of availability of an instrument. The work carried out in Glasgow used a Carl Zeiss PM QII Spectrophotometer with flame emission attachment while the Hines experiments were made using a Perkin Elmer 303 Atomic Absorption Spectrophotometer. Neutron activation analysis (c) was used where only small samples of material were available for analysis. The main advantage of this method is sensitivity. Activation analysis is the most sensitive technique known for the estimation of most of the elements, Bowen and Cawse (1963).

(a) Flame Emission Spectroscopy is described in detail by Dean (1960) and in broad outline by Weberling and Cosgrove 1965.

The basic principles of flame photometry were established by Bunsen and Kirchhoff in 1860, but the technique was not used extensively until after 1945.

When a solution of a metal salt is sprayed into a flame, the salt decomposes and vaporises to produce atoms or simple molecules. Thermal energy of the flame causes some of these atoms to be raised to an excited state from which return to the ground state is accompanied by the emission of spectra characteristic of the particular elements involved. The temperature of the flame should be such that as many atoms as possible are excited to the higher state so that a strong emission is produced. A suitable optical system is required to measure the intensity of the emission. This should consist of

i) a monochromator or another type of wavelength selector to isolate the emission of the element being analysed from that of the other elements present and from the flame background

ii) a photosensitive detector, preferably the photomultiplier tube which permits the use of narrow slit widths and so increases the signal to background ratio. This lowers the limit of detection - an important consideration in trace element analysis.

(b) Atomic Absorption is a more recent technique for trace analysis. It was first suggested by Walsh in 1955 and has become of major importance since that time.

Atomic absorption spectroscopy is the study of absorption of radiant energy by atoms. Thermal energy is used to decompose compounds to atoms as in the flame emission methods. However the unexcited or ground state atoms absorb radiation of the same wavelength as would be emitted in transitions from the excited to the ground state. The proportion of light absorbed bears a relationship to the number of atoms in the ground state and therefore to the total concentration of the element in solution (provided ionisation is negligible). Hence metals not excited to emission by the flame may be determined by absorption provided that they are capable of existence in the atomic state and a suitable source of radiation is available. The temperature of the flame is not so critical as with emission spectrometry.

The instruments used in atomic absorption and emission spectrometry are similar with the exception of the atomic line source which is the critical component of the absorption flame photometer. Walsh recommended a hollow cathode discharge tube for this purpose. This consists of a sealed tube filled with rare gas at low pressure and containing an anode and a hollow

cylindrical cathode lined with the metal under consideration. When the power supply is connected to the lamp, discharge takes place which ionises the rare gas. Ions bombard the cathode and knock out metal atoms which in turn become excited by collision with atoms of the rare gas and emit its characteristic spectrum. A vaporiser is required to produce atoms to absorb the radiation and, as with emission spectroscopy, this is most commonly the flame. Burners are similar for both methods and may be (a) the total comsumption type where the sample liquid is aspirated directly into the flame or (b) the Lundegårdh burner which has a barrel where the gases are mixed with the vaporised sample before the whole mixture is drawn into the flame. This type of burner has an elongated flame which introduces more atoms into the light path and so improves sensitivity. However since only the smaller droplets of vaporised sample reach the flame there is a possibility of unrepresentative samples especially with mixed solvents; also it may be necessary to use more sample material than with the total consumption type.

The monochromators used are similar to those in emission methods and consist of prisms, gratings and filters. The system used in the Perkin Elmer 303 is a high dispersion Czerny—Turner grating with U.V. dispersion of 6.5 Å per m/m, visible dispersion of 13.0 Å per m.m and wavelength range 1950 Å to 8521 Å.

Photomultipliers are normally used as detectors.

Atomic Absorption spectroscopy has an advantage over the flame emission technique in that it is not so much affected by spectral interferences or interferences due to flame background. Chemical interference is, however, common to both methods, although atomic absorption is less susceptible to inter-element effects. The important advantage of the absorption method is that it involves measuring the ratio between two intensities: the intensity of the monochromatic line source in the presence of and absence of absorbing atoms. Ratios are easier to measure accurately than emission intensities in absolute units.

The methods described later for strontium analysis by both flame emission and atomic absorption spectrometry employ an additive standard technique which compensates for chemical interference and matrix differences (important because in the low level samples large quantities of solid material are present). By using this technique it was found that chemical separation could be eliminated although this would improve the sensitivity of the analysis (but reduce the number of samples that could be handled).

(c) Neutron Activation Analysis

When biological material is subjected to a large flux of thermal neutrons many of the elements present are transformed into radionuclides. If strontium is in the sample the following reaction takes place

$$^{86}Sr + {}^{1}n \longrightarrow {}^{87m}Sr + \gamma$$

Other radionuclides of strontium are also produced by the irradiation of other component isotopes of the stable element but these (^{85m}Sr, ^{85}Sr and ^{89}Sr) are present to a lesser extent due to either longer half life (requiring longer irradiation time for production) or smaller cross section of the nuclear reaction.

Activation analysis requires post-irradiation separation of the desired activity. This can be achieved (a) by chemical means which has the advantage that the added carrier allows a yield estimation - ultimate sensitivity can be reached by using a few relatively simple chemical manipulations: or (b) by instrumental methods (γ-ray spectrometry) which have the advantages - possibility of assaying more than one element in the same sample - the procedure may be automated - there may be repeated study of the same sample - but the sensitivity is limited and often not good enough for biological materials.

In the case of strontium in biological material, chemical separation is necessary and the method is based on that of Harrison and Raymond (1955). Details are included in Appendix 3(3).

EXPERIMENTAL STUDIES

(1) GASTROINTESTINAL EXCRETION

(i) Intestinal Excretion of ^{85}Sr and ^{47}Ca during fasting and feeding in rats

An experiment has been made to study the passage of strontium and calcium from the bloodstream to the gastro-intestinal tract following the intravenous administration of strontium-85 and calcium-47.

It is known from experiments in man that absorption of radiostrontium is affected by the presence or absence of food in the intestine. Spencer et al. (1969) found the Ca/Sr absorption ratio to be unity in subjects who had fasted overnight and approximately 2 when the tracers were given with food. Other investigations have shown that the form of the food, whether solid or liquid, can affect the absorption of calcium and strontium in rats (Marcus and Lengemann, 1962). Absorption from a liquid dose of both strontium-85 and calcium-47 was about twice that when the isotopes were given in a solid form. Taylor et al. (1962) investigated the effect of starvation and absorption of strontium and found no effect in young rats while older rats had increased absorption during starvation compared with the absorption during feeding. However the absorption of calcium was slightly reduced in both age groups during feeding. The evidence in this experiment seems to be inconsistent.

The absorption of calcium and strontium has been shown to be age dependent (Gran, 1960) and decreasing with age. The Ca/Sr absorption ratio varies with species in the range 2-4 and a ratio

Outline of Study

Sprague-Dawley Rats

Weight	Feeding	Fasting
100 g	^{85}Sr i.v. ^{47}Ca i.v.	^{85}Sr i.v. ^{47}Ca i.v.
250 g	^{85}Sr i.v. ^{47}Ca i.v.	^{85}Sr i.v. ^{47}Ca i.v.
400 g	^{85}Sr i.v. ^{47}Ca i.v.	^{85}Sr i.v. ^{47}Ca i.v.

Eight rats were used in each study.

Radioactivity was determined in all intestinal segments.

of 2.6 is reported in man by Spencer et al. (1960). However when the passage of strontium and calcium is considered, in the opposite direction across the gastrointestinal membrane, that is from the bloodstream into the intestine, the ratio for man is found to be only slightly higher than 1 (Spencer et al. 1960).

In the following experiment the secretion of radioisotopes of strontium and calcium was measured under normal feeding conditions in rats of three weight groups. The experiment was then repeated under the same conditions with the exception that the animals were deprived of food for 8 hours before the injection and for the duration of the experiment. Details of the procedure are given in Appendix I.

Results and Discussion

When ^{85}Sr and ^{47}Ca are injected intravenously into rats of varying weights and ages the amounts of the isotopes secreted into the intestine are as summarised in table 1.

At 24 hours post injection the intestinal excretion (that is, the intestinal content together with faecal excretion) is found to increase slightly with age. 10.1 - 12.7% of the administered ^{85}Sr dose was secreted into the intestine where the corresponding range for ^{47}Ca was 8.1% - 9.2% of the dose. Gusmano et al. (1968) found similar results - for example, in rats of 255 g body weight 11.7% of intraperitoneally administered ^{85}Sr was excreted in the faeces in 24 hours. Likins and co-workers (1959) found, however, that considerably more strontium than calcium was

TABLE 1

% Dose of Injected Isotope secreted into the GI tract in 24 hours

Weight Group	Feeding			Fasting		
	^{85}Sr	^{47}Ca	$^{85}Sr/^{47}Ca$	^{85}Sr	^{47}Ca	$^{85}Sr/^{47}Ca$
100 g	10.1 ± 0.41	8.1 ± 0.44	1.24	4.2 ± 0.45	1.95 ± 0.23	2.17
250 g	11.0 ± 0.45	8.6 ± 0.49	1.28	5.0 ± 0.57	2.4 ± 0.24	2.11
400 g	12.7 ± 0.60	9.2 ± 0.63	1.38	7.7 ± 0.43	3.3 ± 0.29	2.34

Values include amounts of ^{85}Sr and ^{47}Ca passed with the feces in 24 hours.

excreted in the faeces after intraperitoneal injection, 12.9% and 5.95% of the dose respectively in the 24 hour time interval. This gives a Sr/Ca ratio of 2.17 compared with a ratio of between 1.24 and 1.38 in the present studies.

In an experiment by Bauer et al. (1955) a 2:1 Sr/Ca ratio was observed when ^{90}Sr and ^{45}Ca were injected simultaneously i.p. - 10% of the injected ^{90}Sr and 5% of the ^{45}Ca were in the GI tract and the feces 24 hours after administration of the isotopes. However Bauer's animals were deprived of food until 8 hours after injection and did not in fact eat for a further 4 hours so that the 12 hour fast may have affected the results.

Fasting has been shown to reduce markedly levels of both strontium-85 and calcium-47 as the results on table 1 demonstrate. Strontium-85 excreted into the GI tract of fasting rats is approximately half of that excreted by feeding animals in all the age groups, the range being 4.2 - 7.7% of the dose administered. Between 1.95% and 3.3% of the ^{47}Ca dose was found in the intestine and feces of fasting rats. This corresponds to a reduction by a factor of 3-4 of the feeding values. The $^{85}Sr/^{47}Ca$ ratio in the fasting state is therefore an average of 2.2 compared with 1.3 in the feeding condition.

It would appear therefore that fasting caused a reduction in the intestinal excretion of both isotopes but more markedly of calcium. However the levels at 24 hours in both feeding and

fasting studies may be the result of considerable recycling of the isotopes in the intestine, Since there is much evidence to show that calcium is more readily absorbed than strontium, the higher levels of strontium in the intestine and faeces may be due to greater re-absorption of the secreted calcium rather than to reduced secretion. If this were so, it would be reasonable to suppose that the re-absorption of calcium would be enhanced by fasting since then more sites would be available for the transport of both isotopes across the gut wall.

Table 2 shows the urinary excretions of strontium-85 and calcium-47 in the 24 hour period following intravenous injection into feeding and fasting rats in the three weight groups (100, 250 and 400 grams). It can be seen that, during feeding, 8-10% of the dose is excreted in the urine while only 0.5 - 0.9% of the ^{47}Ca dose is excreted via the kidney. These levels are similar to those found by Bauer et al. (1955) who showed that, following an i.p. injection 13% of the administered ^{90}Sr and 0.9% of the ^{45}Ca was excreted in the urine. Likins et al (1959) reported 6.6% and 1.7% respectively of i.p. administered ^{89}Sr and ^{47}Ca in the urine of 40-50 g rats. Hansard and Crowder (1957) showed that insignificant amounts of ^{45}Ca were excreted in the urine.

TABLE 2

% Dose of injected isotopes in urine at 24 hours

Weight Group	Feeding			Fasting		
	^{85}Sr	^{47}Ca	$^{85}Sr/^{47}Ca$	^{85}Sr	^{47}Ca	$^{85}Sr/^{47}Ca$
100 g	8.4	0.93	9.0	2.2	0.14	15.7
250 g	8.3	0.46	18.0	3.4	0.21	16.0
400 g	10.5	0.68	15.4	4.9	0.25	19.6

From this evidence there is no doubt that the kidney discriminates between strontium and calcium with a preferential excretion of strontium. Lloyd (1964) showed that the main route of excretion of radioactive strontium administered intravenously to rabbits was via the kidney while calcium was mainly excreted in the faeces. Kidman et al. (1950) showed that the calcium content of the diet affected the route of radiostrontium excretion in rabbits - on a low calcium diet the radiostrontium in the faeces exceeded that in the urine. This was reversed when the dietary calcium was high.

During fasting there was a marked reduction in the urinary excretion of strontium-85 in the rats, to 2-5% of the dose and the calcium-47 excretion was similarly reduced to almost negligible amounts, 0.1 - 0.3%.

This evidence does not seem to agree with the theory of more recycling of the isotopes (secretion into GI tract with subsequent reabsorption into the bloodstream) during fasting when it would be expected that higher proportions of the dose would be excreted via the kidney rather than reduced levels.

Table 3 shows the percentage of the injected dose found in bone at 24 hours, post injection. Although the differences are small at the % dose/g.bone level, it can be seen that calcium-47 levels are consistently higher than the strontium-85 levels and those of the fasting animals higher than for the feeding rats. When the activities are extrapolated to the whole skeleton (table 4)

TABLE 3

^{85}Sr and ^{47}Ca uptake in bone during feeding and fasting at 24 hours

Weight Group	% Dose/g Bone					
	^{85}Sr		^{47}Ca		$^{85}Sr/^{47}Ca$	
	Feeding	Fasting	Feeding	Fasting	Feeding	Fasti
100 g	6.5 ± 0.11	7.2 ± 0.13	7.3 ± 0.23	7.8 ± 0.13	0.89	0.9
250 g	4.1 ± 0.06	4.4 ± 0.22	4.6 ± 0.07	5.0 ± 0.11	0.84	0.8
400 g	2.7 ± 0.13	3.1 ± 0.09	3.0 ± 0.16	3.2 ± 0.09	0.90	0.9

TABLE 4

^{85}Sr and ^{47}Ca in skeleton during feeding and fasting at 24 hours after injection

Weight Group	^{85}Sr % Dose/skeleton		^{47}Ca % Dose/skeleton	
	Feeding	Fasting	Feeding	Fasting
100 g	76.9	92.8	89.1	97.4
250 g	79.6	90.4	91.9	96.9
400 g	74.1	86.2	89.1	95.9

the effect is more marked and it can be seen by comparing this table with tables 1 and 2 that the 'differences' are in good agreement as summarised in table 5. Strontium is preferentially excreted by 9.5 - 13% during feeding while calcium is taken up by bone tissue by 12 - 15% more than strontium. During fasting the difference is reduced since both isotopes are incorporated into bone to a greater extent than during feeding. Again the difference between the two isotopes contained in the excretions - 4-10% more strontium-85 being excreted than calcium-47 - is almost exactly balanced by the preferential retention of calcium in the bone.

The increase in skeletal levels of both ^{85}Sr and ^{47}Ca due to the fasting state is statistically highly significant ($P<0.001$ for ^{85}Sr in all age groups and for ^{47}Ca in the 100 g and 400 g groups and $P<0.01$ for ^{47}Ca in the 250 g rats).

From these results it would appear that strontium passes from the bloodstream into the intestine slightly more easily than calcium. However, since calcium is absorbed from the intestine to a much greater extent than strontium it is possible that the higher intestinal strontium levels in this experiment are due to preferential re-absorption of the secreted calcium. It is postulated that calcium and strontium are secreted from the bloodstream into the intestine equally and there is no discrimination.

TABLE 5

Relationship between increase in ^{85}Sr and ^{47}Ca in carcass and decrease in excretions of the isotopes during fasting

Weight Group	^{85}Sr, % Dose			
	% increase in carcass during fasting	Excretion % decrease		
		Faeces	Urine	Total
100 g	15.9	6.1	6.2	12.3
250 g	10.8	6.0	4.9	10.9
400 g	11.9	5.0	5.6	10.6
	^{47}Ca, % Dose			
100 g	8.7	6.0	0.79	6.8
250 g	5.0	6.2	0.25	6.5
400 g	6.8	5.9	0.43	6.3

This would be supported by the evidence of Bauer et al. (1955) who showed that although at 24 hours there was twice as much ^{85}Sr of an i.p. dose in the intestine as ^{45}Ca, the difference was much less at earlier time intervals. Unidirectional discrimination has been observed in the passage of strontium and calcium across other membranes. For example Wasserman et al. (1957) showed in the rat and the rabbit that discrimination occurs in placental transfer from dam to foetus but not in the opposite direction. Similarly Twardock and Comar (1961) observed a discrimination in the passage of the elements from blood to milk although there is little or none in the opposite direction.

In fasting the effect is more pronounced, presumably because more sites are available in the intestine for the transfer of the radioisotopes than during feeding.

(ii) Intestinal Excretion of ^{85}Sr and ^{47}Ca as a function of time

The previous experiment illustrates that when strontium and calcium are administered intravenously to rats, strontium is preferentially excreted to calcium both via the intestine and in the urine while more calcium than strontium is retained in the skeleton. Although fasting causes a decrease in the amount of strontium-85 excreted there is a more marked decrease in the calcium-47 excreted.

An attempt was made to find whether this difference is caused by (a) preferential transport of strontium from the bloodstream across the intestinal membrane or (b) the preferential absorption (which has been well established by investigators) of calcium giving an indirect net result by the 24 hours interval after injection of the dose.

Since all age groups behaved similarly in the previous experiment only one group (250 g) was used in the next where the protocol was the same with the exception that animals were sacrificed at $\frac{1}{2}$, 1, 2 and 4 hours after injection of the isotope dose. Table 6 summarises the results (24 hour data were obtained from the first experiment). The figures indicate that during feeding both strontium and calcium are rapidly secreted into the intestine. In the first half-hour there is slightly more calcium that strontium which seems to be in agreement with the theory that calcium passes more easily across the membrane than strontium. One hour after injection equal amounts of both isotopes are in the intestine

TABLE 6

% Dose of injected isotope in the GI tract at various time intervals

Time Interval	Feeding			Fasting		
	^{85}Sr	^{47}Ca	$^{85}Sr/^{47}Ca$	^{85}Sr	^{47}Ca	$^{85}Sr/^{47}Ca$
½ hour	4.6	5.1	0.89	4.0	3.8	1.07
1 hour	5.0	5.1	0.99	4.3	3.7	1.17
2 hours	6.4	5.4	1.18	4.8	3.8	1.26
4 hours	7.0	5.6	1.25	5.1	4.3	1.19
24 hours	11.0	8.6	1.28	5.0	2.4	2.11

and the strontium level gradually increases with time up to 4 hours. Between 4 and 24 hours after administration of the dose, most of the secreted isotope, that is, 90%, passes out with the faeces while only 10% remains. It appears, therefore, that with calcium an equilibrium is established between ½ and 4 hours post injection while in the same time interval strontium continues to be secreted to a greater extent than re-absorption takes place.

During fasting the pattern of secretion is similar to that associated with the feeding condition although there is always less of both isotopes in the intestine during fasting. As with the feeding study the increase in activity in the GI tract is more marked in the case of strontium than calcium between ½ and 4 hours. By 24 hours, 50% of the secreted isotopes has been passed out with the faeces while 50% remains in the intestine, although re-absorption is considerable between 4 and 24 hours and the level at 24 hours is about 50% of the 4 hour level in the case of ^{47}Ca. Again this seems to point to the greater re-absorption of calcium than strontium.

When the segments are considered it seems that the greatest secretion is in the jejunum. Jones and Coid (1956) reported that when rats are injected intravenously with ^{89}Sr, three quarters of the radioactivity secreted into the alimentary tract was found in the small intestine and Wallace et al. (1951) showed similar results for ^{45}Ca administered intramuscularly to feeding rats.

These investigators showed a two-fold increase in the total intestinal ^{45}Ca secretion between 1 and 6 hours following injection whereas in the experiment described here there is little change between $\frac{1}{2}$ and 4 hours with a decrease between 4 and 24 hours.

The bone levels of both isotopes are shown in table 7 . During feeding at half an hour following injection there is 20% more calcium than strontium in the femur. The increase in bone deposition however is slightly greater for strontium than calcium between $\frac{1}{2}$ and 24 hours. During fasting the levels of both isotopes are similar to those in the feeding study at half an hou following administration but by 24 hours the percentage dose per gram of bone is higher in fasting than feeding with little difference between the isotopes. Other investigators have produced conflicting reports concerning discrimination between strontium and calcium by bone tissue. Bauer et al. (1955) concluded that there was little or no skeletal discrimination and Comar et al. (1956) showed that the ratio $\frac{\text{Sr/Ca blood}}{\text{Sr/Ca bone}}$ was only slightly greater than 1. Likins et al. (1959, 1960) found that the $^{85}Sr/^{47}Ca$ ratio was less than 1 in bone and was dependent on crystal size in studies with hydroxyapatite. The slower the growth rate and therefore the larger the crystal size, the greater was the selection against strontium. Bone shaken with solutions of ^{45}Ca and ^{85}Sr was found to take up more ^{45}Ca than ^{85}Sr by factors of from 1.2 to 1.4 (Samachson and Lederer 1960).

TABLE 7

^{85}Sr and ^{47}Ca uptake in bone during feeding and fasting

Time Interval	% dose/gm bone					
	Feeding			Fasting		
	^{85}Sr	^{47}Ca	$^{85}Sr/^{47}Ca$	^{85}Sr	^{47}Ca	$^{85}Sr/^{47}Ca$
½ hour	2.7	3.3	0.82	2.7	3.0	0.88
1 hour	3.3	3.8	0.86	3.0	3.7	0.83
2 hours	3.5	4.3	0.80	3.6	4.3	0.83
4 hours	3.7	4.4	0.83	3.5	4.6	0.77
24 hours	3.9	4.3	0.91	4.7	4.9	0.96

This evidence would agree therefore with the results in the present study where slightly more calcium than strontium is taken up by bone initially. At later time intervals the more marked renal discrimination favouring the excretion of strontium becomes effective and less strontium is available for deposition in bone and Della Rosa and Wolf(1965) attribute higher bone levels of ^{47}Ca than of ^{85}Sr to preferential urinary excretion of strontium. Similarly Domanski et al. (1969) report preferential urinary clearance of strontium rather than calcium by a factor of 5 but similar clearances of both isotopes from plasma to bone. It has been shown that when the skeletons of nephrectomised rats were labelled with ^{85}Sr and ^{45}Ca the release of strontium was greater than that of calcium by a factor of 1.3 when the rats were subjected to peritoneal lavage. (Talmage et al. 1957). However it is generally agreed that the discrimination between strontium and calcium by the skeleton is small compared to that of the kidney and in intestinal absorption but operates to favour the retention of calcium in the skeleton.

Table 8

Secretion of ^{85}Sr and ^{47}Ca into Intestinal Segments during feeding and fasting

FEEDING EXPERIMENTS

Time* Hours	Isotope	% Dose per Segment						
		Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	Total
½	^{85}Sr	0.28	0.16	3.14	0.17	0.40	0.45	4.60
	^{47}Ca	0.33	0.22	3.31	0.24	0.49	0.54	5.13
1	^{85}Sr	0.28	0.11	3.41	0.25	0.50	0.50	5.05
	^{47}Ca	0.32	0.14	3.26	0.27	0.59	0.62	5.10
2	^{85}Sr	0.22	0.06	3.62	0.65	1.14	0.72	6.41
	^{47}Ca	0.15	0.10	3.24	0.32	1.08	0.56	5.45
4	^{85}Sr	0.10	0.04	1.65	0.46	3.09	1.70	7.04
	^{47}Ca	0.18	0.05	1.42	0.38	2.42	1.22	5.67
24	^{85}Sr	0.04	0.01	0.22	0.05	0.41	0.30	1.03
	^{47}Ca	0.05	0.01	0.27	0.05	0.42	0.21	1.01

FASTING EXPERIMENTS

½	^{85}Sr	0.37	0.18	2.25	0.26	0.42	0.56	4.04
	^{47}Ca	0.32	0.19	2.32	0.23	0.38	0.38	3.82
1	^{85}Sr	0.21	0.12	2.67	0.34	0.51	0.43	4.28
	^{47}Ca	0.22	0.12	2.06	0.29	0.49	0.51	3.69
2	^{85}Sr	0.17	0.08	1.98	1.11	0.96	0.48	4.78
	^{47}Ca	0.18	0.10	1.44	0.58	0.92	0.57	3.79
4	^{85}Sr	0.10	0.04	0.59	0.40	3.02	0.95	5.10
	^{47}Ca	0.10	0.05	0.72	0.34	2.03	1.07	4.31
24	^{85}Sr	0.04	0.01	0.26	0.22	1.66	0.32	2.51
	^{47}Ca	0.03	0.02	0.19	0.06	0.68	0.18	1.16

* Time after the intravenous injection of ^{85}Sr or ^{47}Ca.

(iii) Rate of Passage through the intestine

Passage of ^{85}Sr and ^{47}Ca through the intestine is measured by the data of this experiment when considered in more detail. Table 8 shows the percentage of the injected isotope found in the various segments of the intestinal tract at the five time intervals of the experiment, and table 9 shows these levels expressed as a percentage of the total intestinal contents at each of the time intervals.

Initially the greatest proportion of the secretion is into the jejunum - two-thirds of the total secretion of each isotope is found in this segment during feeding. When the other portions of the small intestine are included the levels agree with the experiment of Jones and Coid (1956) who found three-quarters of the intestinal secretion of strontium and calcium in the small intestin

The level of activity is maintained in the jejunum during feeding for two hours following injection (of either ^{85}Sr or ^{47}Ca) demonstrating that there is equilibrium between absorption and excretion at this time. Methfessel et al. (1964) found similar results for calcium-47. After two hours following injection, the level of isotope in the jejunum decreases while the activity in the caecum (and to a lesser extent in the colon) increases. This increase is mainly due to movement along the length of the intestine although the half-hour levels show that there is some secretion into all segments of the GI tract.

Table 9

^{85}Sr into Segments as percentage of total

		Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
½ hour	Feeding	6.02	3.42	68.37	3.62	8.67	9.90
	Fasting	9.08	4.56	55.83	6.45	10.29	13.79
1 hour	Feeding	5.47	2.20	67.57	4.99	9.84	9.92
	Fasting	4.94	2.86	62.24	7.99	11.88	10.09
2 hours	Feeding	3.46	1.01	56.38	10.18	17.80	11.17
	Fasting	3.48	1.72	41.36	23.16	20.12	10.16
4 hours	Feeding	1.38	0.34	23.47	6.51	43.90	24.20
	Fasting	1.92	0.84	11.58	7.86	59.20	18.60
24 hours	Feeding	3.84	0.96	21.15	4.81	39.42	28.85
	Fasting	1.59	0.40	10.36	8.76	66.14	12.75

^{47}Ca in Intestinal Segments percentage of Total

		Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
½ hour	Feeding	6.47	4.28	64.53	4.76	9.51	10.45
	Fasting	5.82	4.93	60.73	5.95	9.89	9.97
1 hour	Feeding	4.35	2.81	63.91	5.36	11.51	12.24
	Fasting	5.86	3.39	55.88	7.79	13.22	13.85
2 hours	Feeding	2.69	1.79	59.63	5.82	19.81	10.26
	Fasting	4.63	2.64	37.98	15.31	24.42	15.01
4 hours	Feeding	3.22	0.95	25.01	6.73	42.62	21.44
	Fasting	2.23	1.23	16.63	7.90	47.13	24.87
24 hours	Feeding	4.95	1.00	26.7	4.95	41.6	20.8
	Fasting	2.59	1.72	16.4	5.17	58.6	15.5

Moore and Tyler (1955) have suggested that secretion takes place only across the walls of the small intestine and not across the large intestine but other investigators do not agree with this (Harrison and Harrison, 1951; Jones and Coid, 1956; Cramer and Copp, 1959).

During fasting the amounts of both isotopes initially secreted into the segments are less than during feeding (with the exception of the distal portion of the intestine for ^{85}Sr although this difference is marginal and within the limits of accuracy of the experiment). At 1 hour, the levels are similar to those at $\frac{1}{2}$ hour following injection but by 2 hours there is evidence of movement along the tract - the activity in the ileum has increased at the expense of that in the jejunum. At 4 hours the levels are at a maximum in the caecum and are similar during fasting to the levels during feeding while the levels in the other segments are lower during fasting than during feeding. This seems to indicate that greater re-absorption takes place during fasting - particularly in the jejunum.

In summary it was found that fasting had a marked effect on the secretion of strontium and calcium from the bloodstream to the gastro-intestinal tract.

During feeding more strontium than calcium is found in the intestine and faeces at 24 hours following intravenous administration of radioisotopes of the two elements although at the earliest time interval studied after the dose, the calcium excretion slightly exceeded that of strontium and it appeared that there was greater re-absorption of calcium than strontium from the intestine. Up to 4 hours after injection the excretion into the gastro-intestinal tract of strontium was greater than the re-absorption resulting in a progressive increase in the radioactivity in the tract. The re-absorption of calcium appeared to approximately equal the secretion between $\frac{1}{2}$ and 4 hours so that there was very little increase in the intestinal activity during that time. Fasting reduced the initial secretion of calcium into the intestine to a greater extent than that of strontium and again the excretion of calcium was in equilibrium with the re-absorption for the first two hours and re-absorption exceeded excretion in the 4-24 hour time interval while strontium excretion increased slightly between $\frac{1}{2}$ and 4 hours and was in equilibrium with the re-absorption between 4 and 24 hours. Fasting caused an overall decrease by a factor of two in the intestinal excretion of intravenously administered radiostrontium and a decrease by a factor of 3-4 in the case of radiocalcium excreted in 24 hours post injection. It appeared that this was due

to enhanced re-absorption of the calcium which is normally absorbed from the intestine more readily than strontium.

During fasting the urinary excretion of both ^{85}Sr and ^{47}Ca was reduced although very little calcium was excreted by this route in either the feeding or fasting state (Strontium excretion is almost equally divided between urine and stool under normal conditions).

The effect of these differences in excretion due to fasting is to make more of both ^{85}Sr and ^{47}Ca available for bone deposition although ^{47}Ca deposition always exceeds that of ^{85}Sr.

EXPERIMENTAL STUDIES

(2) STABLE STRONTIUM BALANCES IN MAN

2. Stable Strontium balances in Man

Stable strontium has been recommended and used as a treatment for osteoporosis (Shorr and Carter 1950) but until recently metabolic studies of the element have been hampered by inaccurate analytical techniques (McCance and Widdowson, 1947). Recent refinements in methods of analysis have made it possible to determine stable strontium with accuracy not only during periods of administration of doses of the element but also at the trace levels normally found in biological materials. It is now possible to compare the results of metabolic studies using radioactive tracer techniques with information on the metabolism of stable strontium as found in normal conditions and so evaluate the chronic ingestion situation with regard to strontium-90.

Animal studies have shown toxic effects of stable strontium (Bartley and Reber 1961) but these effects have not been observed in human studies by Spencer et al (1967) or in the experiments now to be described.

Material and Methods

Strontium balances were determined in eleven adult male patients who were maintained under strictly controlled dietary conditions in the Metabolic Research Ward. All patients were fully ambulatory and had normal kidney and gastro-intestinal function. With the exception of Patient 4 who had Paget's disease of the bone, there were no skeletal abnormalities. All patients received a constant low calcium-low phosphorus diet which contained an average of 200 mg calcium and 820 mg phosphorus per day. In two cases a milk supplement was given which increased the calcium intake to about 800 mg/day. The fluid intake was kept constant throughout the studies. Complete collections of urine and stool were obtained in all studies.

When stable strontium was administered orally, the dosage was about 1500 mg strontium per day, given as lactate. This supplement was given in three divided doses with meals. Patient 5 was subjected to two such studies of 28 and 36 days respectively with a time interval of 60 days between. Following discontinuation of the orally administered stable strontium the balance studies were continued for 24 days in each of the two studies.

Patient Protocol

Patient	Age	Diagnosis	Study	Study days	Intake, mg/day	
					Strontium	Calcium
1	56	History of Alcoholism	Control	12	1.1	197
2	55	Pulmonary Emphysema	Control	12	1.1	183
3	48	History of Alcoholism	Control	12	1.1	203
4	54	Paget's Disease of Bone	Control	6	1.5	824
5	49	History of Alcoholism	Control	18	0.8	228
			Oral Sr	28	1536	239
			Post-Sr	24	1.1	245
			Control	6	1.2	234
			Oral Sr	18	1536	224
			Oral Sr	18	1536	720 *
			Post-Sr	24	1.1	727
			Control	12	1.1	237
			i.v. Sr	6	636+	247
			Post-Sr	18	1.2	241
			Control	12	1.6	830
6	49	Psychoneurosis	Control	12	0.8	234
			Oral Sr	24	1536	250
7	41	Hypertension	Control	12	1.1	475
			Control	6	1.5	778
8	45	History of Alcoholism	Control	12	0.80	257
			i.v. Sr	6	612+	267
			Post-Sr	30	1.0	272
9	59	History of Hyper-tension	Control	12	1.1	209
			i.v. Sr	6	636+	219
			Post-Sr	12	1.2	213
10	58	History of Alcoholism	Control	6	0.8	221
			i.v. Sr	6	318+	240
			Post-Sr	24	1.0	244
			Control	10	1.1	237
			i.v. Sr	6	954+	253
			Post-Sr	12	1.2	248
11	43	History of Alcoholism	Control	12	0.8	200
			i.v. Sr	6	318+	208
			Post-Sr	36	1.1	213
			Control	6	0.8	237
			i.v. Sr	6	954+	231
			Post-Sr	6	1.2	218

+ amount of stable strontium infused per day. *Higher calcium intake in this period is due to higher calcium content of enteric coated vitamin B tablets

Six patients received intravenously administered strontium as the gluconate for six consecutive days. The amount of strontium infused was approximately 600 mg/day in patients 5, 7, 8 and 9 while patients 10 and 11 each underwent two studies where the dose was 300 mg and 900 mg/day. The stable strontium was infused in 500 ml of 5% glucose in water over a period of 4 hours daily. A control period of 12 days preceded the experimental infusion period. The excretions were measured for a further 12 days following discontinuation of the dose.

Balances of strontium and calcium (Osis et al. 1969) were determined using Atomic Absorption spectroscopic methods to analyse aliquots of the six-day pool collections of urine and stool as well as representative aliquots of the diet. Nitrogen and phosphorus balances were also measured in each six-day period in order to determine the metabolic status of the patient. The analytical method for strontium is described in detail below (Appendix 3(2).

Results and Discussion

Table 10 summarises stable strontium balance data in 11 patients during control conditions, that is, on low calcium metabolic diet with the stable strontium intake solely derived from the diet. The calcium intake ranged from 200 mg to 800 mg (in two cases where a milk supplement was given) and the corresponding strontium intake was 0.8 mg and 1.50 mg. The average strontium intake was 1.03 mg per day. Urinary strontium was shown to be highly variable on a constant intake. Samachson and Spencer-Laszlo have correlated ^{85}Sr excretion with urinary calcium but report a wide range of ^{85}Sr excretions for any given calcium excretion. The urinary strontium excretion ranged from 0.03 mg per day in Patient 1 who had a low calcium excretion of 9 mg per day to 0.68 in Patient 4 who excreted 200 mg Ca per day. The urinary strontium excretion averaged 0.18 mg per day or 17.5% of the intake, omitting the high excretion of Patient 4 who had Paget's disease of the bone. The faecal excretion ranged from 0.53 to 1.56 mg per day and averaged 0.91 mg per day or 88.3% of the intake. The average strontium balance of this group of patients was in equilibrium.

Table 10

Stable Strontium Balances on Low Intake

	Patient	Study days	Strontium, mg/day			
			Intake	Urine	Stool	Balance
Jones	1	12	1.15	0.03	0.67	+ 0.45
Conroy	2	12	1.15	0.35	1.15	- 0.35
Jeter	3	12	1.15	0.16	1.35	- 0.36
Golembiew-ski	4	12	1.50	0.68	0.87	- 0.09
Swist	5	18	0.80	0.24	0.57	- 0.01
Hunter	6	12	0.80	0.35	0.68	- 0.13
Smith	7	12	1.15	0.15	1.05	- 0.05
Adams	8	12	0.85	0.20	0.53	+ 0.12
Hula	9	12	1.15	0.10	1.12	- 0.07
Norwood	10	6	0.80	0.08	0.52	+ 0.20
Lawrence	11a	12	0.85	0.11	0.85	- 0.11
Lawrence	11b	12	1.50	0.28	1.56	- 0.34
		Average *	1.03	0.18	0.91	- 0.06

* Patient 4 is omitted from averages.

In a study using neutron activation as the method of analysis, Harrison et al. (1955) showed that of an intake of 1.99 mg Sr per day, 1.58 mg was excreted in the faeces daily and 0.39 mg in the urine or 79% and 19.5% of the intake respectively. The higher dietary strontium content of this study corresponds to a higher calcium content of the diet - 1.18 g per day compared with 0.22 g in the present study. Lipton et al. (1966) used spectrographic techniques to determine trace elements in diets and excreta. Two subjects received a diet containing about 1 g calcium per day and 1.3 mg strontium per day. An average of 25% of the strontium intake was excreted in urine and 68% in the faeces. Data obtained from ^{90}Sr determinations in metabolic balance studies show results similar to those in the present study. On a low calcium diet 19% of the intake was excreted in urine and 88% in the stool (Samachson and Spencer 1967). Wasserman and Comar (1964) have shown in studies using ^{85}Sr orally as a tracer that 18.5% was excreted in urine and 78% in stool.

The variability from patient to patient of urinary strontium reflects a wide range of absorption of the element which has been shown to range from 8-35% in sixteen patients in an ^{85}Sr study by Spencer et al. (1960). In these patients the urinary ^{85}Sr excretion ranged from 2-17% of the dose in the 12 days following oral administration. However this variability is not so marked as that of ^{45}Ca which is absorbed to a greater extent than ^{85}Sr.

Table 11

Stable Strontium balances in individual study periods

Study Days	Stable Strontium, mg/day			
	Intake	Urine	Stool	Balance
6	0.79	0.26	0.65	- 0.12
6	0.81	0.22	0.52	+ 0.05
6	0.80	0.24	0.54	+ 0.02
Average	0.80	0.24	0.57	- 0.01

Calcium balances during the same study periods

Calcium, mg/day			
Intake	Urine	Stool	Balance
242	80	130	+ 32
224	72	105	+ 47
219	78	99	+ 42
228	77	111	+ 40

The excretions of one individual from period to period are consistent however, as is demonstrated in the next table. Table 11 shows three consecutive control periods for a patient who was later given both oral and intravenous doses of stable strontium in four separate studies. It can be seen that on an intake of 0.8 mg Sr per day as contained in a low calcium metabolic diet, 30% is excreted in the urine and 72.5% in the faeces so that the patient is in very slightly negative balance.

From these figures it can be calculated that the net absorption is 29.2%.

$$\text{(Net absorption} = \frac{\text{Intake - faecal excretion}}{\text{intake}} \times 100\%)$$

Administration of an oral tracer dose of ^{85}Sr at this time gave a net absorption of 36%.

The calcium balances are also shown for the corresponding periods and have the same degree of uniformity as the strontium balances although the patient is in slightly positive balance on the low calcium diet.

The following table shows summaries of two separate oral studies performed with a time interval of 10 weeks when the normal dietary strontium was the only intake. The oral dose of stable strontium was 1536 mg per day given as the lactate. The absorption in the first study was 30.5% compared with 39.7% in the second study. This higher absorption is reflected in the higher urinary excretion of 385 mg per day in the second period of administration compared with 285 mg per day in the first.

TABLE 12

Period	Study days	Stable Strontium mg/d			
		Intake	Urine	Stool	Balance
Control	18	0.80	0.24	0.57	-0.01
Stable Sr I	22	1536	285	1067	+184
Post	24	1.1	40	131	-170
Control	6	1.15	4	4	-6.85
Stable Sr II	30	1536	385	926	+225
Post	24	1.2	58	37	-94

Overall, the balances are similar and both are slightly positive. Table 12a shows the first balance study in more detail. The first six days of administration of the stable strontium are regarded as a transition of equilibration period and are omitted in the averages although must be included when the retention is calculated. From the figures it is calculated that 4.5 g are retained over the period of 30 days administration. In the 24 days following discontinuation 4.12 g are excreted in urine and faeces so that only 380 mg of the dose remains in the body. However the excretion is 7-8 mg per day higher than the intake 10 weeks following discontinuation of the dose so that by this time almost all of the previously retained strontium has been remobilised and excreted. It appears therefore that this patient is in good equilibrium with regard to strontium and the evidence agrees with the theory that strontium is only loosely bound to bone tissue and becomes remobilised fairly rapidly (Samachson and Lederer, 1960, 1963).

The calcium balance during the stable strontium study is also included. The most obvious effect of the strontium administration is the twofold increase in the urinary calcium excretion. This decreases to baseline levels immediately following discontinuation. There is a slight increase in calcium absorption (from 52 to 56%) due to the stable strontium intake although the balance becomes less positive but in the post periods this is reversed and retention is increased.

Table 12a

Study days	Stable Sr mg/day				Calcium mg/day			
	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balanc
18	0.80	0.24	0.57	- 0.01	228	77	111	+ 40
6	1536	162	812	+ 562	230	138	102	- 10
6	1536	253	1003	+ 280	242	148	111	- 17
6	1536	342	1137	+ 57	234	155	104	- 25
6	1536	257	1108	+ 171	249	127	105	+ 17
4	1536	290	1020	+ 226	243	118	105	+ 20
Average (22)	1536	285	1067	+ 184	242	137	106	- 1
8	0.9	94	439	- 532	245	61	93	+ 91
6	1.1	35	49	- 83	255	69	93	+ 90
6	1.2	24	28	- 51	241	72	93	+ 76
8	1.1	8	9	- 16	242	90	83	+ 69
Average (28)	1.1	40	134	- 171	246	73	91	+ 82

Figure 1 shows the effect of the oral dose of strontium on the urinary excretion of both strontium and calcium. In both study periods there is a sharp rise in the urinary strontium when the element is administered orally and the elevated levels are maintained with minor fluctuations while the dosage is given. Immediately following discontinuation the levels drop rapidly and although many months are required for the urinary strontium to reach base level it decreased by a factor of about 100 within four periods. The urinary calcium excretion is also noticeably affected by the oral stable strontium. In the first study the effect is to increase the urinary calcium level about twofold. In the second study there is a similar increase initially followed by a further increase when the calcium intake was inadvertently increased, when vitamin tablets were given from a batch with a high calcium content. This may also account for the higher urinary strontium at this time. The urinary calcium behaves in a similar manner to strontium following discontinuation of the dose in the first study and immediately decreases to baseline levels within one study period. However in the second study the decrease is delayed until the calcium intake decreased.

EFFECT OF ORAL STABLE STRONTIUM ON URINARY STRONTIUM AND CALCIUM EXCRETION

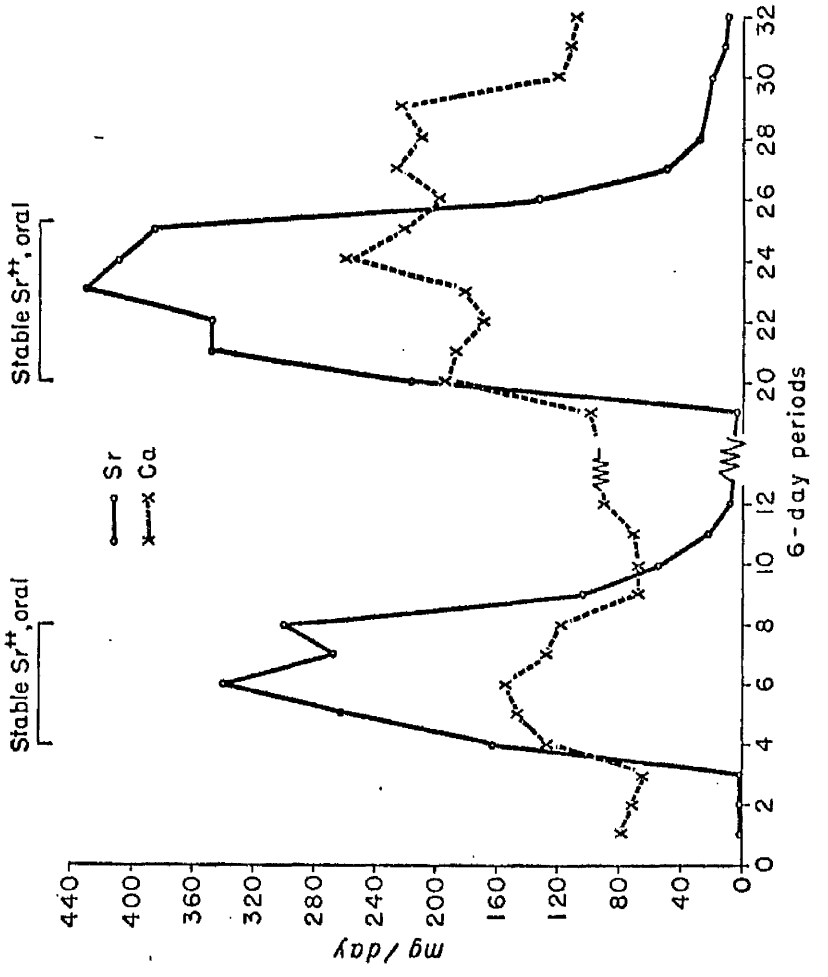


Table 13

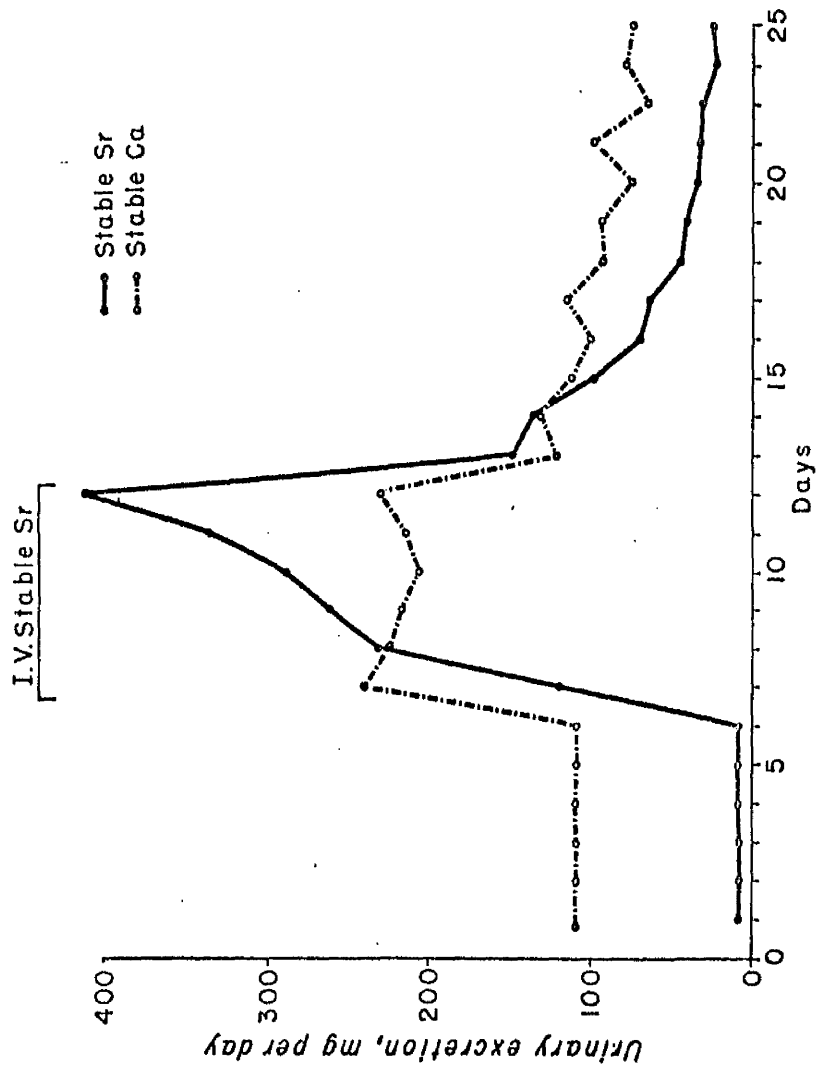
Effect of Intravenous Stable Strontium on Strontium Balances

Patient	Study	Days	Stable Strontium, mg/day			
			Intake	Urine	Stool	Balance
	Control	12	1.1	9.6	5.4	- 13.9
5	Stable Sr	6	636.0	275.0	60.0	+ 301.0
	Post-Sr	18	1.2	46.0	15.2	- 60.0
	Control	12	1.14	0.14	1.05	- 0.5
7	Stable Sr	6	636.0	238.0	50.0	+ 348.0
	Post-Sr	18	1.2	48.0	23.0	- 70.0
	Control	12	0.85	0.21	0.53	+ 0.11
8	Stable Sr	6	612.0	235.0	60.0	+ 317.0
	Post-Sr	30	1.1	26.5	22.2	- 47.6
	Control	12	1.14	0.11	1.12	- 0.09
9	Stable Sr	6	636.0	230.0	26.0	+ 380.0
	Post-Sr	12	1.1	36.8	16.1	- 51.8
	Control	6	0.8	0.08	0.52	+ 0.20
10	Stable Sr	6	318.0	71.0	36.0	+ 211.0
	Post-Sr	24	1.1	11.6	13.6	- 24.1
	Control	6	0.8	0.2	0.7	- 0.10
	Stable Sr	6	954.0	308.0	56.0	+ 590.0
	Post-Sr	12	1.2	52.6	69.9	- 121.3
	Control	12	0.85	0.11	0.85	- 0.11
11	Stable Sr	6	318.0	80.0	23.0	+ 215.0
	Post-Sr	36	1.1	11.2	9.4	- 19.5
	Control	6	0.80	0.2	1.05	- 0.45
	Stable Sr	6	954.0	340.0	43.0	+ 571.0
	Post-Sr	42	1.2	19.8	20.8	- 39.4

In the next table the results of 8 intravenous administrations of strontium are summarised. It has been shown that during intravenous administration about 40% is excreted in the urine and 5-10% in the stool. By 30 days post infusion of strontium a total of 60% of the dose has been excreted in urine and about 30% in stool. By 100 days post infusion, levels of the element in urine and stool were still 6-10 times higher than baseline excretions.

Figure 2 shows the effect of intravenous administration of stable strontium on the urinary excretion of strontium and calcium. Although there is a sharp increase in the urinary strontium as soon as the dose is given this continues throughout the six days and no maximum is established. Samachson who gave small multiple doses of ^{85}Sr for 12 days showed similar results with an increase in the urinary ^{85}Sr throughout the duration of the dosage, with a sharp decrease as soon as the doses were discontinued, and a more gradual decrease thereafter. Administration of strontium had an immediate effect on the urinary calcium levels which increased about twofold but this dropped again as soon as the infusions were discontinued. The urinary calcium was less regular and slightly lower following infusions than it was in the control periods.

EFFECT OF INTRAVENOUS STABLE STRONTIUM ON URINARY STRONTIUM AND CALCIUM EXCRETION



Several studies were made where ammonium chloride was given at the same time as the intravenous infusion of strontium was made. It has been reported that ammonium chloride is a calciuric agent and causes metabolic acidosis and demineralisation of bone. Spencer et al. (1958, 1965) reported increased urinary excretion in man although studies in mice (Van Putten 1962) and dogs (Della Rosa et al. 1961) have had negative results. The results shown here are also negative. (Fig. 3)

In another experiment the effects of phosphalgel were studied. In these cases the calcium content of the diet was higher than previously. Milk was included to bring the calcium up to 800 mg per day. This increased the strontium intake slightly between 1.4 and 1.6 mg per day. The results of these studies are tabulated for the four patients involved. The levels are so low, however, that it is difficult to be sure that during the periods of phosphalgel administration there is an increased output faecally. This effect was expected from previous studies on animals (Friedland et al. 1969) when absorption of strontium-85 was decreased by up to 82% as reflected by bone levels. The decrease depended on the timing of the phosphalgel administration compared with the oral dose of ^{85}Sr and sacrifice of the animal. Studies in man (Spencer et al. 1967 (i) and (ii) and 1969 (iii)) showed similar decreases in absorption of ^{85}Sr of up to 87% when the phosphalgel was given immediately prior to the oral administration of the isotope. However it was also shown that

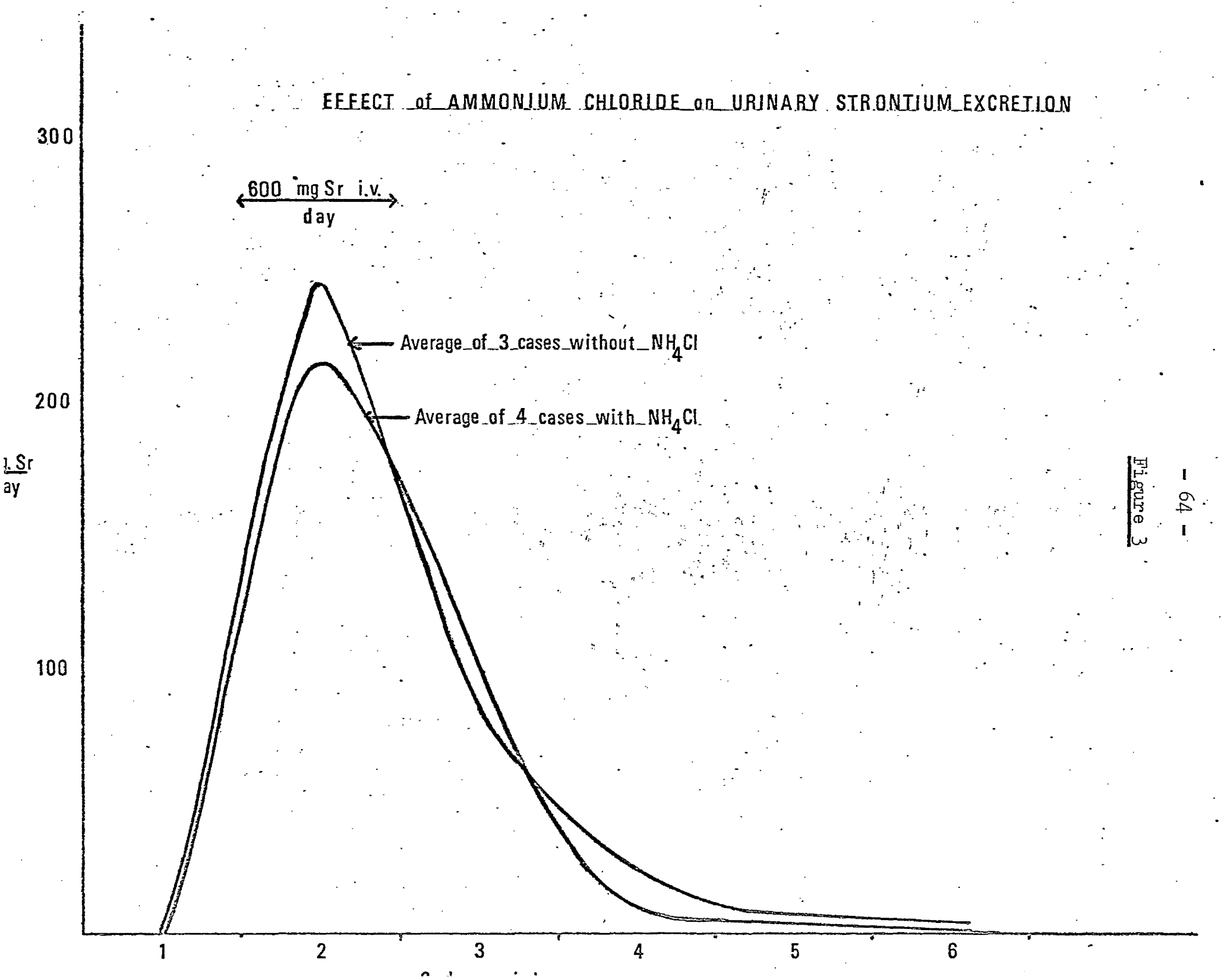

EFFECT of AMMONIUM CHLORIDE on URINARY STRONTIUM EXCRETION
300
200
100
600 mg Sr i.v.
day
Average of 3 cases without NH_4Cl
Average of 4 cases with NH_4Cl
1 2 3 4 5 6

Figure 3

Table 14

Aluminium Phosphate Gel - Effect on Stable Strontium Balances

Patient	Period	Days	Intake	Urine	Stool	Balance
4	Control	6	1.5	0.72	0.87	- 0.09
	Experimental	12	1.6	0.70	0.69	+ 0.21
	Post	6	1.5	0.97	2.11	- 1.58
	Post	12	1.5	0.88	0.85	- 0.23
11	Control	12	1.6	0.28	1.56	- 0.24
	Experimental	6	1.6	0.24	1.35	+ 0.01
	Experimental	6	1.6	0.29	1.78	- 0.47
	Post	12	1.5	0.22	1.49	- 0.21
7	Control	6	1.5	0.73	0.96	- 0.19
	Experimental	6	1.6	0.72	0.79	+ 0.09
	Experimental	6	1.6	0.79	1.03	- 0.22
	Post	6	1.5	0.62	0.72	+ 0.16
5*	Control	12	1.6	3.40	2.40	
	Experimental	6	1.6	1.51	4.50	
	Experimental	6	1.6	2.72	2.15	
	Post	12	1.5	2.07	2.24	

* Patient had previously been administered stable strontium, hence high excretions.

the effectiveness of the aluminium phosphate gel was reduced with increasing time between its administration after the isotope. Therefore in this study where phosphalgel was given with breakfast meal then it could not be expected to affect the absorption of the strontium in the other meals to any considerable extent. This is borne out by the results (Table 14). It is shown in all of the four studies that there is more stable strontium in the faecal samples in the periods of administration of the phosphalgel or in the period immediately following but the change is marginal in most cases.

In summary, on a stable strontium intake of 1 mg per day, approximately 18% was excreted in urine and 88% in the faeces in an average of 12 patients and the balance was in equilibrium. When the oral intake was increased by a factor of 1500 by the addition of strontium lactate to the diet the distribution between urine and faeces was similar to that in the control study of one patient and the strontium retained during the administration period was excreted within the first few weeks following discontinuation. Intravenous administration of the element resulted in 30-40% being excreted in the urine and 5-10% in the stool. By 30 days post infusion the excretions were 60% and 30% respectively. Strontium excretion was 6-10 times higher than baseline levels at 100 days after discontinuation of the intravenous dose.

Stable strontium has been suggested as a treatment for osteoporosis but long term retention would be desirable and this would only be expected in patients who were in positive balance with regard to strontium under control conditions.

The reported increase in urinary strontium excretion due to ammonium chloride administration has not been demonstrated and it has been shown that aluminium phosphate gel has only borderline efficacy if administered in one daily dose.

It has been shown that administration of stable strontium, whether intravenously or orally, has a marked effect on the excretion of calcium via the kidney. The dose of 1500 mg orally or 600 mg intravenously gave a twofold increase in the urinary calcium content.

EXPERIMENTAL STUDIES

(3) PLACENTAL DISCRIMINATION

3. Placental transfer of Strontium and Calcium

It has been shown by direct and indirect methods that the placenta discriminates against strontium and in favour of calcium. Using double tracer studies in the rat and the rabbit, Wasserman et al. (1957) showed that in an overall discrimination of 0.17 between foetus and diet, the contribution due to placental transfer was 0.65. They demonstrated that the major discrimination was from dam to foetus with little or no differential movement in the opposite direction. Other investigators report a range of 0.4-0.7 for the O.R. foetus/mother in various species of experimental animal (Comar et al. 1955; Haggroth and Hoglund, 1961). Bryant and Loutit (1961) used survey values for stable strontium and calcium in foetal and adult human bone to measure placental discrimination which they found to be 0.62 (O.R. foetus/mother). Rivera has analysed serum of the mother and newborn for strontium and used these values to calculate discrimination by the human placenta however his results are not absolute since he assumes the calcium concentrations to be the same on both sides of the placenta and there is evidence that this is not so. Hallman and Salni (1953) have shown that the placental blood entering the infant contains an average of 1 mg/100 ml plasma more calcium than that returning to the mother. Widdowson and Dickerson (1964) compiled values for various elements in human serum before and soon after birth and they report the calcium concentration of a full term baby at birth to be 11.0 mg/100 ml., while that of an adult is 10 mg/100 ml.

An experiment has been carried out in an attempt to measure the discrimination between strontium and calcium in their passage across the human placenta. Samples of venous blood have been taken from the mother as soon after delivery as possible. Venous cord blood has been taken to be representative of the newborn. Aliquots of the blood were also taken for calcium determination (in sequestrenated tubes) and for haemoglobin and packed cell volume measurements (so that the results could be expressed in terms of plasma rather than whole blood).

The samples (about 5 ml) for strontium analysis were dried in silica thimbles, ashed at 400° and stoppered with polythene before determining by neutron activation analysis. The details of the procedure are given in Appendix 3(3). The results are summarised in Table 15. The strontium levels in maternal plasma ranged from 28.3-67.2 ng/g.plasma with an average of 46.7 ng Sr/g.plasma for 7 samples while that of cord plasma was in the range 14.3-48.0 ng/g.plasma and averaged 32.6 ng Sr/g.plasma. This gave a ratio for foetal/maternal plasma of 0.70.

Calcium on the other hand was in higher concentration in cord blood than maternal blood. The calcium content in the mother's plasma was very close to 100 μg/g.plasma in six out of seven cases with an average of 99 μg Ca/g.plasma while that in the newborn was more variable but averaged 122 μg/g plasma.

TABLE 15

		Sr mg/g	Ca ug/g	Sr/Ca ug/g	$\frac{Sr/Ca\ Newborn}{Sr/Ca\ Mother}$
1.	Mother	28.3	102	280	0.51
	Newborn	14.3	102	143	
2.	Mother	40.2	100	402	0.87
	Newborn	43.3	125	346	
3.	Mother	52.6	104	504	0.41
	Newborn	28.8	139	207	
4.	Mother	38.4	101	380	0.46
	Newborn	31.2	179	174	
5.	Mother	56.3	100	563	0.47
	Newborn	32.5	124	262	
6.	Mother	44.1	90	470	0.75
	Newborn	30.0	80	370	
7.	Mother	67.2	98	686	0.67
	Newborn	48.0	104	461	
Average	Mother	46.7	99	472	0.59
	Newborn	32.6	122	282	
Ratio	$\frac{Newborn}{Mother}$	0.70	1.23	0.60	

The O.R. $\frac{\text{Newborn}}{\text{Mother}}$ varied within the range 0.41-0.87 in this group of seven pairs and averaged 0.60. This direct measurement of placental discrimination is in good agreement with the figure of 0.62 deduced by Bryant and Loutit from bone surveys.

DISCUSSION AND CONCLUSIONS

Discussion and Conclusions

This thesis reports three studies with the common element that they investigate the passage of strontium with respect to calcium across membranes of the body.

The first study concerns the effect of fasting on the passage of strontium and calcium from the bloodstream to the gastrointestinal tract. It has been observed that fasting caused the disappearance of discrimination against strontium in the absorption of strontium and calcium (Spencer et al. 1969). To investigate this subject further, the rat was chosen as experimental animal as relevant studies had already been published in this species (Jones and Coid 1956; Cramer and Copp 1959; Cramer 1959a and b).

The low levels of radiostrontium and radiocalcium found in most intestinal segments alone at 24 hours following the intravenous injection indicates little difference between the two isotopes in the passage from the plasma into the intestine during both feeding and fasting. When the amounts of ^{85}Sr and ^{47}Ca remaining in the gastrointestinal tract at 24 hours are considered, it appears that fasting results in a distinct increase of intestinal excretion of ^{85}Sr and in a slight increase of ^{47}Ca. The increase of ^{85}Sr is mainly reflected by the higher ^{85}Sr levels in the caecum. However, the marked decrease in the faecal excretion of both ^{85}Sr and ^{47}Ca during fasting outweighs greatly the increase of the radioisotopes recovered in the intestine, resulting in a markedly lowered total intestinal excretion of the radioisotopes

during fasting, the factor of decrease being about twice as great for ^{47}Ca as for ^{85}Sr. Also, proportions of ^{85}Sr and ^{47}Ca passed with the faeces compared with those retained in the intestinal tract differed during fasting and feeding as only about 50% of the total intestinal excretion of ^{85}Sr and of ^{47}Ca were passed with the faeces in 24 hours during fasting vs. 90% during feeding.

Since the radioisotopes were given intravenously, the amounts of ^{85}Sr and ^{47}Ca recovered in the intestine and faeces are of endogenous origin. The excretions of both ^{85}Sr and ^{47}Ca into the intestine were greater than the urinary excretions of either radioisotope. The ratio of the endogenous faecal/urinary excretion of ^{85}Sr ranged from 1.2 to 1.4 during feeding and the ratio was somewhat higher during fasting, mainly due to a greater decrease of the urinary than faecal ^{85}Sr excretion. The decrease in urinary ^{85}Sr excretion during fasting may be a result of decreased renal function during starvation (Murphy and Shipman, 1963). Since very small amounts of ^{47}Ca were excreted in urine both during feeding and fasting, the ratio of the endogenous faecal/urinary excretions for ^{47}Ca is much greater than that for ^{85}Sr, the values ranging from 8-16 during feeding and from 4-7 during fasting.

Intravenously injected radiostrontium has been reported to be excreted to a greater extent in the faeces than in urine in rabbits on a low calcium diet, while this excretion pattern was reversed on a high calcium diet (Kidman et al. 1950). In another study in

rabbits, intravenously injected radioactive strontium was predominantly excreted via the kidney while the calcium was mainly excreted in faeces(Lloyd 1964). In rats, both radioactive strontium and calcium were mainly excreted with the faeces (Likins et al. 1959). The first study has shown an equal distribution of the excretion of ^{85}Sr in urine and stool, in agreement with results obtained in a recently reported study in rats (Gusmano et al. 1965) and preferential excretion of ^{47}Ca in the faeces. In man intravenously administered radioactive strontium and calcium have been demonstrated to be predominantly excreted in urine (Spencer et al. 1960). Another study in man reported that radioactive strontium is predominantly excreted in urine and radioactive calcium is excreted equally in urine and stool (Bronner et al. 1963).

The preferential intestinal absorption of radioactive calcium compared to strontium and the Ca/Sr discrimination of the intestinal absorption have been well established in animals and man (Marcus and Lengeman, 1962; Comar et al. 1956; Spencer et al. 1957). Also, the absorption of ^{85}Sr and ^{47}Ca has been shown to differ during different study conditions (Marcus and Lengemann 1962; Taylor et al. 1962). The absorption of both ^{85}Sr and ^{47}Ca was about twice as high when the tracers were given in liquid form than when given with solids (Marcus and Lengemann, 1962). Deprivation of food has been reported to have no effect on the absorption of strontium in young rats while the absorption was increased in older rats during fasting

and the absorption of calcium was slightly reduced during starvation in both age groups (Taylor et al. 1962). The presence of food in the intestine has been shown to play a role in the absorption of radiostrontium in man (Spencer et al. 1969).

In constrast to the many studies reported on the comparative passage of radioactive strontium and calcium from the intestine into the vascular space, only few studies deal with the passage of the two radioisotopes in the opposite direction. When both ^{85}Sr and ^{47}Ca were injected intraperitoneally to feeding rats, about twice as much strontium as calcium was found in the gastrointestinal tract (Bauer et al. 1955; Likins et al. 1959). The observations made in the present study during feeding are in partial agreement with these findings as the values for the intestinal ^{85}Sr excretions were similar to those reported while the excretion of ^{47}Ca was higher resulting in an $^{85}Sr/^{47}Ca$ ratio which was only slightly higher than 1 with a maximum of 1.4. Considering the faecal ^{85}Sr and ^{47}Ca excretions alone during feeding, the faecal $^{85}Sr/^{47}Ca$ ratios ranged from 1.3 in the youngest group to 1.6 in the oldest group. During fasting, however, the faecal ^{47}Ca levels decreased by a factor of 7 and the ^{85}Sr levels by a factor of 3 to 4. This resulted in an overall increase in the $^{85}Sr/^{47}Ca$ ratio of 2 to 4 in the three age groups. In a study in dogs 20% more radioactive strontium than calcium was excreted into the intestine 25 minutes following the intravenous injection of the radioisotopes (Singer et al. 1957), similar to the present results obtained at 24 hours. Two possible mechanisms may

explain the presence of approximately equal amounts of ^{85}Sr and ^{47}Ca in the intestine following the intravenous injection of the radioisotopes during feeding:

1) the preferential passage of calcium from the intestinal tract into the bloodstream is reversed when the radioisotopes pass across the intestine in the opposite direction and slightly more strontium than calcium passes into the intestine, or

2) strontium and calcium are equally secreted from the bloodstream into the intestinal tract and the differential passage from the intestine into blood is unidirectional and calcium is preferentially reabsorbed so that a somewhat higher residual amount of strontium remains in the intestine. It is also conceivable that both processes are operative. The high $^{85}Sr/^{47}Ca$ ratio of the intestinal excretions during fasting may be a result of the greater intestinal reabsorption of ^{47}Ca. This may be a result of the competition of calcium and strontium for the calcium binding sites, calcium being preferentially bound and transported across the intestine so that a larger amount of strontium remains in the intestine.

The marked preferential urinary excretion of radioactive strontium as compared to calcium observed in the present study during both feeding and fasting has been previously reported by others. This has been shown following intraperitoneal injection of the radioisotopes in rats (Bauer et al. 1955, Likins et al. 1959) and following intravenous and oral administration of the two radioisotopes in man (Spencer et al, 1957; Samachson and Spencer-Laszlo, 1962; Harrison et al. 1966). However, fasting decreased the urinary

excretion of both isotopes considerably, the decrease in excretion being more marked for ^{47}Ca than for ^{85}Sr.

The bone uptake of ^{47}Ca in the first study was slightly greater than than of ^{85}Sr during feeding and fasting and the $^{85}Sr/^{47}Ca$ uptake ratio in bone was always less than 1 during both study conditions. This observation is in agreement with studies reported by other investigators (Likins et al. 1959; Kshirsagar et al. 1966). However, some investigators report that bone does not significantly discriminate between strontium and calcium (Bauer et al. 1955; Della Rosa and Wolf, 1965; Bauer 1957). During fasting the bone uptake of ^{85}Sr and ^{47}Ca was greater than during feeding. This difference became more apparent in each weight group when the uptake of the two radioisotopes was calculated for the total skeleton. This increase in bone uptake is related to the decrease in urinary and faecal excretion of both radioisotopes during fasting and may be due to greater intestinal and/or renal reabsorption of ^{85}Sr and of ^{47}Ca.

In regard to the effect of age on strontium and calcium metabolism the present studies have shown that the intestinal excretions increased with age during both feeding and fasting and that the increase was more marked for ^{85}Sr than for ^{47}Ca. This observation is in agreement with that reported by others (Gusmano et al. 1968). The urinary excretion of ^{85}Sr increased slightly with age in the present study while the excretion of ^{47}Ca decreased slightly. Insignificant amounts of ^{45}Ca were reported to be excreted in the urine of rats of all ages (Hansard and Crowder, 1957). Lowering

of the urinary excretion of calcium in persons of the older age group has been observed previously (Spencer et al. 1964). In studies of the passage of radioactive calcium from the intestine into the bloodstream, it has been shown that the intestinal absorption decreased with age (Hansard and Crowder, 1957; Hertoghe, 1967; Friedland et al. 1969) while an increase in intestinal absorption of calcium in old rats has also been reported (Hironaka et al. 1960). The calculated uptake of ^{85}Sr and ^{47}Ca in the total skeleton was similar in the young and older rats in the present study as were $^{85}Sr/^{47}Ca$ ratios in the three age groups. This finding is not in contradiction to the lowered uptake of the two radioisotopes per gram bone with age as the skeletal size increased with age. The finding of the similarity of the ^{85}Sr and the ^{47}Ca retention in the skeleton with age is in disagreement with studies reported by other investigators which indicate a decrease in skeletal retention of radiostrontium with age determined by total body counting (Gusmano et al. 1968).

The second part of the animal study was carried out in order to investigate the intestinal discrimination further. An attempt was made to elucidate the mechanisms discussed earlier (page 76) by introducing a time factor.

Strontium and calcium are shown to be rapidly secreted into all segments of the gastrointestinal tract following intravenous injection of tracers. However secretion is not equal in the various segments. Two-thirds of the total intestinal secretion of both

^{85}Sr and ^{47}Ca are found in the jejunum indicating that most of the endogenous faecal calcium and strontium is secreted into this segment of the small intestine. It would be expected that most of the secreted calcium and strontium is also absorbed from this site. A report that three-quarters of the total intestinal ^{85}Sr was found in the small intestine of rats one hour after intravenous injection of the dose (Jones and Coid 1956) is in agreement with this observation. In another study in rats, radioactivity was found in all segments of the intestine as early as 15 minutes following intramuscular injection of ^{45}Ca, the largest proportion being in the small intestine (Wallace et al. 1951). Some investigators have suggested that calcium is secreted only across the walls of the small intestine and not across the large intestine (Moore and Tyler 1955) although others (Harrison and Harrison 1951) have shown that calcium was absorbed mainly from the proximal part of the small intestine but also from the distal part and from the large intestine.

From the present experiment it appears that during feeding, ^{47}Ca was excreted into the intestine to slightly greater extent than ^{85}Sr in the initial stage although the ^{47}Ca in the intestine maintained the same level up to four hours while the ^{85}Sr in the intestine increased considerably in this time interval. This is in contrast to the results of Wallace et al. (1951) who showed a two-fold increase in intestinal ^{45}Ca between 1 and 6 hours after intramuscular administration.

The distribution of the secreted isotopes in the various intestinal segments are similar in the first hour of the experiment. The jejunum contained about two-thirds of both the intestinal ^{85}Sr and ^{47}Ca and the remainder was distributed between the other segments. The small percentage in the duodenum and ileum may be a reflection of the small size of these segments to some extent but the levels of isotope are not in direct proportion to the length of the segment. With the passage of time the large percentage of both isotopes in the jejunum decreased as the more distal segments increased. This change in the distribution of the ^{85}Sr and ^{47}Ca is due to reabsorption of the secreted isotopes as well as passage along the tract of the gastrointestinal contents. In studies of the movement of radio-strontium along the intestine of feeding rats receiving ^{85}Sr orally Cramer (1959b) has shown that the caecium reached a peak at four hours which was maintained for a further two hours. The absorption of ^{85}Sr from ligated caecal loops was slow and reached a peak at 3-4 hours after instillation of the radioactive strontium (Cramer and Copp 1959). It has also been shown that there is a time lag of about 20 minutes before any significant amounts of strontium were absorbed from the intestine (Cramer 1959a) while the absorption of calcium has been demonstrated within a few minutes after the oral dose of a tracer.

When the results of the fasting rats are compared with those obtained in the feeding condition, it appears that fasting causes a reduction in the secretion of both isotopes across the tract and that they are more evenly distributed between the segments.

Volf and Polig (1971) have shown the net intestinal excretion of injected ^{85}Sr to be distinctly lower in fasting than in feeding rats and the high degree of reabsorption might lead to underestimation of endogenous faecal excretion (if the method of calculation using faecal excretion following intravenous injection were used). From the present experiment the error in endogenous faecal calcium excretion estimations would be more marked than that of strontium. The proportion in the jejunum is slightly less at the earliest time interval and decreases more rapidly thereafter than during feeding. This appears to be due to a greater degree of reabsorption during fasting than feeding particularly from the jejunum. The amount of both isotopes excreted with faeces at 24 hours is very markedly decreased and this results in a slightly greater amount remaining behind in the caecum, where there is a very low degree of reabsorption. This leads to the anomaly at 24 hours that fasting causes an increase in the levels of ^{85}Sr and ^{47}Ca secreted into the intestine as compared with during feeding when the intestine is considered alone.

The present study has shown that the secretion of ^{47}Ca into the intestine reaches equilibrium within one hour following intravenous administration of the isotope which is in agreement with a study in rats by Methfessel et al. (1964). Such an equilibrium has not been established with ^{85}Sr. The data indicate that there is also preferential reabsorption of calcium from the intestine. It appears

that the reabsorption of both ^{85}Sr and ^{47}Ca is greater during fasting than during feeding and again that of ^{47}Ca exceeds that of ^{85}Sr.

From this it might be concluded that gastrointestinal discrimination against strontium absorption in preference to calcium is unidirectional. There is little or no discrimination in the direction from the bloodstream to the intestine. Such a unidirectional discrimination across a membrane is not unprecedented. Similar results have been shown in discrimination across the mammary gland (Twardock and Comar, 1961) and across the placenta (Wasserman et al. 1957) Placental discrimination in human subjects is the topic of the final section of this report.

In the second study certain aspects of human metabolism of strontium and calcium were considered. Firstly, an assessment was made of the metabolism of the traces of strontium available in a normal diet. A method of analysis using atomic absorption spectroscopy was devised for this purpose (Warren and Spencer, 1972). This technique was equally useful in analysing the trace levels of strontium in biological materials and in performing balance experiments where large doses of strontium were administered to patients.

Stable strontium ingested with diet has been shown to range from 0.8 to 1.5 mg/day. Milk increased the calcium intake by a greater proportion than the strontium intake. This would be expected from the low Sr/Ca ratio in milk compared to that of other dietary items.

Studies by Harrison et al. (1955) and Tipton et al. (1966) who used neutron activation analysis and emission spectroscopy respectively as methods of estimating stable strontium, showed dietary calcium and strontium levels which are in agreement with the present findings. The proportions of the element in the excreta are in good comparison in the three studies. Fallout strontium-90 is a suitable tracer for stable strontium being ingested in small amounts over a long period of time (Kahn et al. 1969). Indeed it was the presence of this radionuclide in normal diet that provided the motivation for the upsurge of interest in strontium metabolism in the 1950's. Its behaviour would be expected to resemble that of dietary stable strontium more closely than other tracers which are usually given in single doses, or stable strontium administered in "unnatural" amounts such as the large doses recommended for the treatment of patients with osteoporosis (Schorr and Carter, 1950). Samachson and Spencer (1967) used fallout strontium-90 in this way and obtained similar results to those in the present study. Of an average intake of 1.03 mg Sr/day 88% was excreted in the faeces while 17.5% was excreted in the urine (Samachson and Spencer reported 88% and 19% respectively under similar conditions).

Oral and intravenous administration of the element have produced results which are comparable with the many isotope studies. For instance Spencer et al. (1960) giving ^{85}Sr as a tracer showed wide variation in absorption which resulted in a wide variation in urinary levels of the isotope. Although only two patients had stable strontium administered orally in the present study the urinary

excretion varied from 7-25% of the dose. Although the urinary excretion increases promptly when oral stable strontium is administered, it continues to increase during the first six days after which equilibrium levels are reached. Similar results were obtained by Samachson (1963) with ^{85}Sr data.

Intravenously administered stable strontium seems to behave in a similar fashion to intravenously administered isotopes of strontium. The isotope studies of Spencer et al. (1960) and Harrison et al. (1966) in man and Bauer et al. (1955) and Lloyd (1964) who worked with experimental animals, established that the main route of excretion of strontium in the bloodstream is via the kidney. In the present experiment this is confirmed. In two cases where the patients were subjected to varying doses it appeared that renal tubular reabsorption was less effective with the larger dose. However further investigation would be necessary to confirm this.

Both orally and intravenously administered stable strontium continued to be excreted for many months following discontinuation of the dose, and there was no evidence of longterm retention. This is in agreement with the work of Likins et al. (1960) who found that strontium was easily remobilised from bone tissue and appears to contradict the recommendation of Schorr and Carter (1950) who proposed the use of strontium as an adjuvant to calcium in the treatment of osteoporosis. Presumably the strontium balance would remain positive for as long as the element is administered. However strontium toxicity has been observed in experimental animals (Bartley and Reber, 1961) and although there was no evidence of this in the present

experiment, insufficient information is available in man. The urinary excretion of calcium increased during administration of stable strontium by either the oral or intravenous route. This effect of stable strontium infusions has been reported previously (Spencer et al. 1967; Mazzuoli et al. 1961).

Aluminium phosphate gel although effective in decreasing retention of ^{85}Sr if administered with or soon after the dose (Spencer et al. 1969; Friedland et al. 1969) was shown to have little effect on dietary stable strontium metabolism. This may be due to the fact that the aluminium phosphate gel was given in one daily dose with breakfast while the stable strontium was ingested at regular mealtime intervals. Any decrease in absorption would therefore refer only to the breakfast meal as the efficacy of the gel was shown to decrease markedly with time (Friedland et al. 1969).

In the third and final section an estimate of placenta discrimination in human beings has been made. Concentrations of both strontium and calcium were measured by emission spectroscopy and neutron activation analysis in samples of maternal blood and infants blood at the time of delivery. Previously Rivera (1963) measured strontium in a similar experiment. However as he made no measurement of calcium, his results were inconclusive. An estimate of placental discrimination has been made by Bryant and Loutit (1961) who used stable strontium and calcium levels in adult and foetal bone. This method of determination gave a value of 0.62 $\frac{Sr : Ca\ (foetus)}{Sr : Ca\ (mother)}$ which compared very well

with the ratio 0.60 obtained by the present experiment. This ratio is derived from the differential passage of both strontium and calcium across the placenta. Calcium in foetal and newborn blood is slightly higher than that of the maternal circulation (Hallman and Salni, 1953; Widdowson and Dickerson, 1964; Radde, 1971). This has been attributed to an active transport mechanism (Delivoria-Papadopoulos et al. 1967; Kronfeld et al. 1971). Strontium is at a lower level in the foetus than in the mother, (Bryant and Loutit, 1961) and as shown in this experiment. It has been suggested that the discrimination against strontium in placental transfer occurs only in early pregnancy (Borisov, 1972). Although such a theory might be consistent with the bone samples of newborn having a lower strontium/calcium ratio than adult bone it cannot be reconciled with the present work. However the Borisow's paper is not supported by conclusive evidence particularly regarding calcium so that more detailed information must be obtained before placental transfer of strontium and calcium can be expressed as a function of gestation time.

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APPENDICES

APPENDIX I

Intestinal Excretion of ^{85}Sr and ^{47}Ca by the rat

Material and Methods

Male Sprague-Dawley rats in weight categories 100, 250 and 400 grams having respective ages of 4-5 weeks, 10-11 weeks and 16 weeks were studied during feeding and fasting. The animals were purchased at weights of 20-30 grams below that required and maintained on standard laboratory pelleted food containing 1.4% calcium and 1.2% phosphorus until the experimental weight was attained (an average of 6-7 days). They were then weighed individually and ten animals matched for weight in each category. The feeding rats were given access to the food and water throughout the experiment while the diet was removed from the fasting groups 8 hours prior to injection. The fasting animals had de-ionised water and libitium until sacrifice.

At time of injection each animal was reweighed. The injection was made via the tail vein. The dose was approximately 1 μCi of either ^{85}Sr or ^{47}Ca as chloride in saline solution containing 10 μCi/ml - that is, 0.1 ml was injected. The calcium isotope as obtained from Oak Ridge was in strongly acid solution which caused the animals to react violently making it very difficult to complete the injection.

This was counteracted by neutralising the solution partially with NaOH before diluting the isotope to the required activity with physiological saline solution for injection. The strontium isotope was effectively carrier free therefore this complication did not arise.

Eight feeding and eight fasting animals were injected in each weight group and with each isotope.

After injection the rats were put into separate metabolic cages for the 24 hour duration of the experiment so that collections of faeces and urine could be made. Sacrifice was by decapitation and exsanguination - samples of blood being taken for assay. The peritoneal cavity was opened and the gastrointestinal tract removed and divided into six segments - stomach, duodenum, jejenum, ileum, cecum and colon. Samples of muscle, skin, hair, kidney, liver and both femora were also taken. All samples were put in pre-weighed counting vials and the weights noted.

Recovery of the isotope was measured by the following method. The carcass of each animal was weighed after removal of the tissues. A known volume of water was added and the carcass homogenised. Four aliquots of each homogenate were taken for counting. The activity of each of the aliquots was measured and the sum used to calculate the activity of the entire carcass.

The radioactivity in the separately analysed tissued was added to the carcass activity to determine the recovery of isotope in each animal. These were averaged for each group.

The counting technique is described on page 94 .

Rate of Intestinal Excretion of ^{85}Sr and ^{47}Ca by the rat

Material and Methods

Male Sprague Dawley rats having weight 250 g and between 10 and 11 weeks old were studied during both feeding and fasting conditions.

As with the previous experiment the animals were purchased at weight 220-230 grams and maintained on the standard rat diet for about 6 days. Immediately prior to injection the rats were matched for weight and the exact weight of each animal noted. The experiment was carried out in the same way as previously with the following exceptions:

(1) Animals were sacrificed at time intervals of $\frac{1}{2}$, 1, 2 and 4 hours post-injection.

(2) Groups of six rats were sacrificed at each time interval.

Appendix 2

Counting techniques

Where radioactive isotopes of strontium and calcium were used as tracers, the assay was by gamma-ray spectrometry. ^{85}Sr and ^{47}Ca with half-lives of 65 days and 4.7 days respectively were the isotopes chosen; both of these isotopes emit gamma radiation. The ^{85}Sr produces a daughter ^{85m}Rb which has only one gamma-ray with energy 0.513 MeV while for the calcium isotope the gamma-ray with energy 1.299 MeV was chosen. The instrument used was a Nuclear Chicago well type NaI scintillation spectrometer equipped with a single channel pulse height analyser.

Counting time was selected to be a minimum of one minute or to give at least 4000 counts with the exception of very inactive samples such as muscle tissues where only much lower counts could be accumulated in longer times of up to 20 or 30 minutes.

Background radiation was allowed for by counting two empty sample vials at intervals of 2-3 hours. One hundred counts were accumulated on each vial and an average count rate was subtracted from the subsequent standard and sample count rates.

Standards were made up by introducing the injected dose into a sample vial using the syringe that was used to administer the isotope to the experimental animals. This was made up to

10 ml with water and if necessary further dilution made to give count rates of approximately 100,000 counts per minute. The final counting standard was in a volume of 10 ml. At least four such standards were made up with each group of animals studied, two doses being measured out for standards before the animals were injected and two after. If a large group of animals were used then an extra two standards were taken in the middle of the administration. These standards were counted at intervals of 2-3 hours for ^{47}Ca so that decay rates did not have to be considered in the calculations. The timing of the standard counting for ^{85}Sr was not quite so critical for the longer lived isotope.

Sample geometry was taken into consideration. The height of each sample in the vial was measured and compared with vials containing known volumes of water to determine the sample 'volume'. A factor was then applied to the sample count rate to correct this to the standard volume. The factors were derived by comparing the count rate of equal doses of isotope in varying volumes of solution.

Analysis of Stable Strontium by the Flame Emission Method (Harrison 1958)

Aliquots of diet or faecal ash were weighed into centrifuge tubes. About 2-3 gms of diet ash and 0.5 of faecal ash gave a satisfactory analysis. Strontium-85 was added to the ash as a means of determining the yield of the separation. The ash was dissolved in 5 ml concentrated HCl with the addition of 5-10 ml de-ionised water. When solution was complete (any unsoluble material was removed by centrifuging and then pouring the supernate into a clean tube) a few ml of saturated ammonium oxalate were added and the solution made alkaline to pH 8-9 with 0.88 NH_3 solution. The supernate was discarded after centrifugation and the precipitate dissolved in a few ml of fuming nitric acid (95% HNO_3). When solution was complete more nitric acid was added until precipitation of the nitrates of calcium and strontium was seen. The tube was cooled in an ice waterbath before centrifugation and the supernate again discarded. The nitrate precipitate was dissolved in a minimum of de-ionised water and 95% nitric acid added to reprecipitate the nitrates. The acid supernate was removed as before and the precipitate was dissolved in water and transferred to a 10 ml graduated tube which was made up to the mark. 3 ml of this solution was pipetted into a counting vial and the activity measured using a well

crystal scintillation counter. The count rate was compared with a standard made up by taking an identical aliquot of ^{85}Sr to that added to the ash sample at the beginning of the separation procedure, diluting this to 10 ml and counting 3 ml of this solution. Hence the percentage recovery can be calculated.

After counting the 3 ml was returned to the 10 ml tube and the whole transferred to a 25 ml graduated flask and made up to the mark with de-ionised water (Solution D_1)

0.1 ml of a solution containing 200 ppm Sr and made up from "Specpure" Sr CO_3 (Johnson Matthey and Co.) was pipetted into a 10 ml graduated flask and the volume made up with solution D_1. This gave a solution D_2 of concentration (C + 2) ppm where C was the concentration of the unknown solution D_1.

The urine sample was measured into a beaker and 1 ml syrupy phosphoric acid added and the sample made alkaline with 0.88 ammonia solution. The beaker was warmed gently and after stirring allowed to stand overnight. The supernatant liquid was siphoned off and the residual liquid removed by centrifuging. The precipitate was transferred to a silica crucible and ashed at 500°C. The ash was then treated as before.

After a suitable 'warming up' period the instrument (a Zeiss Spectrophotometer PM QII with double monochromator and flame attachment) was set at wavelength 460.7 mμ or 4607 Å (checked for maximum deflection with a standard solution of 2 ppm strontium) slit width 0.02 mm and acetylene pressure 100 mm.

The emission due to the additive standard solution D_2 and that due to the sample solution D_1 were measured in turn and the deflection of these solutions at about 5 mμ ± the strontium wavelength was also noted. The mean of these off peak readings was a measure of the background interference effects.

The concentration of strontium in the unknown was then calculated using the formula

$$X = \frac{(D_1 - D_M) \times 2}{D_2 - D_1} \text{ ppm}$$

where X is the unknown concentration of strontium

D_1 is the deflection of the unknown solution

D_2 is the deflection of the unknown solution + 2 ppm

D_M is the mean deflection of the solutions measured off peak

Appendix 3 (2)

Analysis of stable strontium in biological materials by Atomic Absorption Spectroscopy

A method has been developed for the analysis of stable strontium in biological materials using atomic absorption spectroscopy. Chemical separation is eliminated to increase the speed with which samples can be handled. The technique is equally applicable to the measurement of the traces of the element found in a normal diet as it is to the determination of metabolic balances during the administration of relatively large doses of strontium to patients. The additive standard technique is used as has been described by Harrison (1958) for a method using flame emission spectrophotometry and by David (1960, 1962) who used atomic absorption spectrophotometry. This technique ensured that there was no matrix difference between standard and sample. This is of particular importance at trace levels where large quantities of solid material are present. Solid material (which affects viscosity and drop size of the solution sprayed into the flame) can be removed by chemical means (Harrison, 1958 Robbins, 1967, Tompsett, 1968, and Elfers et al. 1964) but such treatment greatly increases the time required for the analysis. It has been reported by Trent and Slavin (1964) that the addition of 1% lanthanium to the solutions controlled to a large extent

interference effects, such as depression of the absorbance by phosphate, silicate and aluminate and alkali metal enhancement of the signal. All solutions examined were therefore made up to contain 1% lanthanum.

Sample preparation

Complete 24-hour urine collections were obtained daily and all stool specimens were collected in acid washed polythene containers throughout the studies. Analyses were performed on aliquots of the 6-day pools of urine (which was acidified with conc. HCl to ensure that the alkaline earths stayed in solution) and of 6-day pools of homogenised stool. When doses of the element had been administered intravenously or orally the urinary strontium analyses were determined on samples of daily 24-hour collections. Aliquots of homogenised diet were analysed in each 6-day study period. A more detailed description of the sample preparation for analysis using the atomic absorption spectrometer has been previously reported (Osis et al. 1969) Analytical grade reagents are used throughout.

Urine

Duplicate 500 ml aliquots of the 6-day urine pool were evaporated to dryness in pyrex beakers before ashing at 550°C. The ash was then dissolved in a minimum amount of 3N HCl, (approximately 5 ml) and the solution was transferred to a 25 ml

flask, 5 ml lanthanum chloride solution (5% lanthanum as the oxide in 25% HCl) was added and the solution made up to the 25 ml mark with triple distilled water (Solution D_1).

Diet and faeces

These samples were treated in a similar manner. 100 g samples of the homogenised 6-day faecal pool and the same weight of the homogenised diet were dried before ashing and the ash was dissolved, again using a minimum of 3N HCl. The solution was transferred to a 25 ml flask, 5 ml of lanthanum chloride solution added and the volume was made up to mark. Whenever necessary the solution was filtered to remove undissolved material. When the strontium content of the samples was high, smaller aliquots were used, and an intermediate dilution was usually required for the faecal ash in order to bring the final solution (solution D_1) within the required range for analysis. At the settings used the range 2-5 ppm Sr was found to give linear readings. This linearity was important since the additive standard technique involves extrapolation.

An additive standard was prepared by pipetting 0.1 ml of a standard solution containing 200 ppm into a 10 ml flask (i.e. adding 2 ppm strontium) and the volume was made up to mark using part of the solution (D_1) of urine, diet, or faecal ash to be analysed, the new solution being Solution D_2.

Spectrophotometric Assay

The Atomic Absorption Spectrophotometer used was a Perkin Elmer Model 303 using a Digital Concentration Readout. The settings of the instrument were those recommended for strontium by Trent and Slavin (1964). The wavelength was 4607 Å in the visible range. The slit width used was 13 Å or slit setting 4. The hollow cathode discharge tube for strontium was allowed to warm up for 20-30 minutes before the air acetylene flame was ignited. A further 5 minutes were allowed to elapse before any readings were taken.

Solutions containing 5, 2, 1, 0.5, 0.25, 0.1 and 0.0 ppm strontium and 1% lanthanum as chloride were used as calibration standards for the instrument. Solutions D_1 and D_2 were sprayed into the flame consecutively for each sample of the batch being analysed, before the duplicate samples were measured.

Strontium in the sample was calculated by using the formula: $X = \frac{d_1 \times C}{d_2 - d_1}$ where C equals the added concentration of strontium, d_1 equals the deflection of sample D_1 and d_2 equals the deflection of sample D_2, i.e. (sample + added strontium) (Harrison, 1958, David, 1960, 1962)

When the samples contained high concentrations of strontium, i.e. where high dilution factors were involved, the solution D_2 gave a reading corresponding to (D_1 + 2)ppm Sr so that in these

cases direct comparison with the aqueous standards gave the same results as additive standard technique. But when the concentration of strontium was low, the readings were depressed due to the presence of high concentrations of interfering ions and only the additive standard formula could be used for calculation of the results.

The reproducibility of the analyses is indicated by data listed in Table 16. The patient numbers on this table are not comparable with those on the previous tables since different patients were generally used for this test. Duplicate analysis were made on two successive days on the urine collections of nine patients who received a normal dietary strontium intake of about 1 mg/day. The higher urinary strontium excretion of Patient 9 is due to the previous intake of orally administered stable strontium. The analyses listed give an average urinary stable strontium excretion of 0.19 mg per day (excluding the values for Patient 9). From the results listed in Table 16 it was calculated that at these concentrations of strontium in urine the difference between duplicates should not exceed 0.06 mg.

The recovery of strontium added to samples of diet before ashing is summarised in table. 17. Six aliquots of the homogenised diet were analysed without any addition to give the average strontium content of the diet, 0.96 mg/day. The recoveries of

TABLE 16

mg Strontium Excreted Per Day

Patient	DAY 1			DAY 2		
	Aliquot 1	Aliquot 2	Average	Aliquot 1	Aliquot 2	Average
1	0.22	0.20	0.21	0.21	0.16	0.10
2	0.83	0.74	0.79	0.80	0.93	0.87
3	0.28	0.28	0.28	0.32	0.24	0.28
4	0.20	0.17	0.19	0.25	0.19	0.22
5	0.31	0.20	0.27	0.23	0.29	0.27
6	0.24	0.22	0.23	0.18	0.17	0.17
7	0.14	0.11	0.13	0.13	0.17	0.15
8	0.16	0.15	0.15	0.12	0.15	0.14
9	0.15	0.18	0.17	0.11	0.08	0.10

Average excretion 0.19 $\pm$ 0.04* mg/day (Patient 2 omitted)

*Standard deviation from the mean.

TABLE 17

Recovery of added Strontium to Diet Samples

	mg* Present	mg Added	mg Recovered	% Recovery
Aliquot 1	0.96	0.40	1.32	90.0
2	0.96	0.40	1.40	110.0
3	0.96	0.40	1.37	102.5
4	0.96	0.40	1.34	95.0
5	0.96	0.80	1.84	110.0
6	0.96	0.80	1.86	112.5
7	0.96	0.80	1.71	93.8
8	0.96	0.80	1.72	95.0
9	0.96	1.60	2.62	103.8
10	0.96	1.60	2.47	94.4

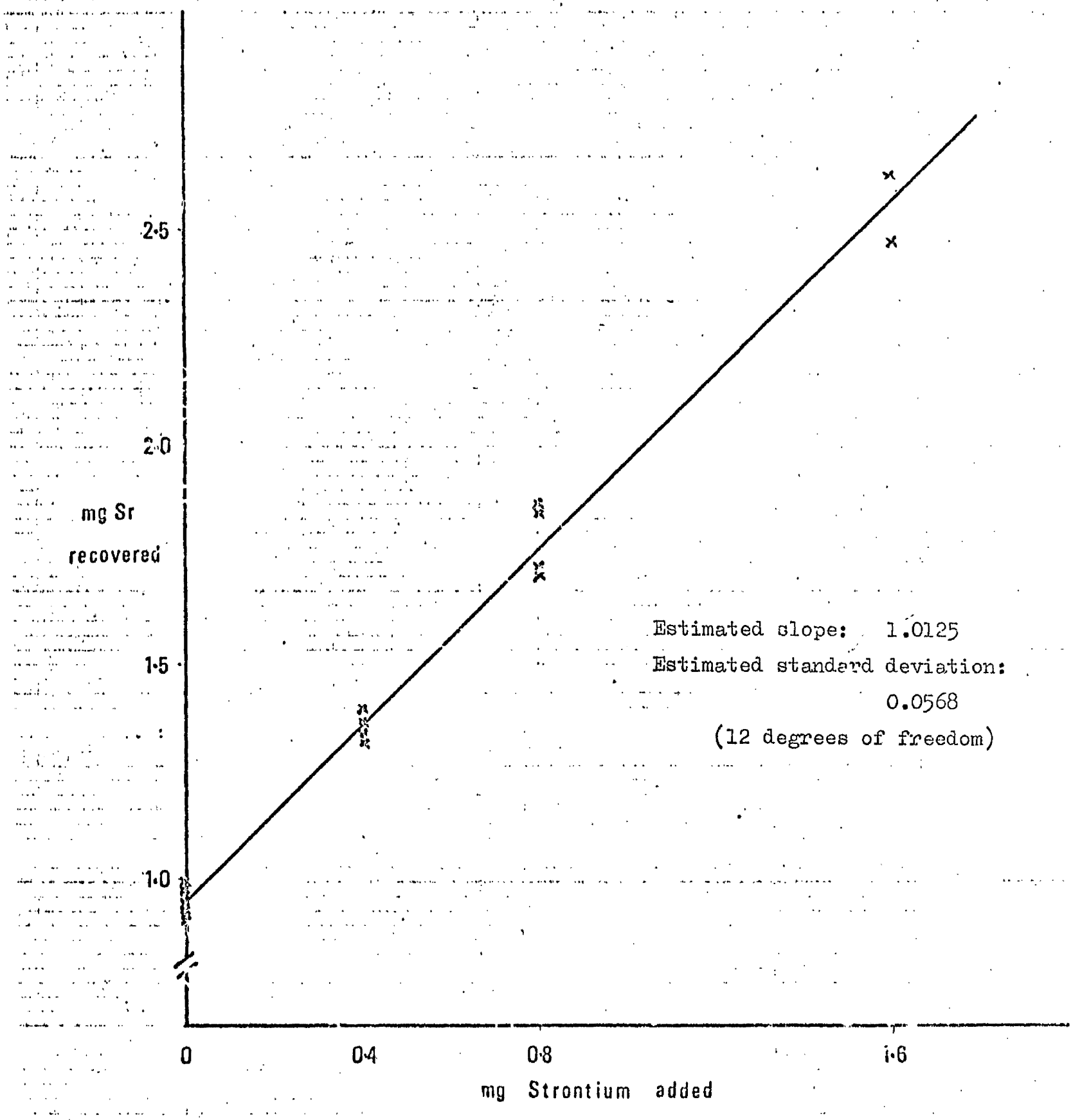
* The average of six determinations on replicate samples.

varying amounts of strontium added to further aliquots of this diet ranged from 90-112% with an average of 100.7%. When the results are expressed graphically (Fig. 4) good correlation is obtained between the observed and the expected results and a straight line with slope 1.0125 can be drawn from the experimental points. Assuming equal variability throughout the range of 1 - 2.5 mg, the difference between duplicate analyses should not exceed 0.175 mg (0.95 probability).

The recovery of strontium added to samples of urine ranged from 94 - 100% with an average of 98% and the corresponding figures for stool analyses were 92 - 101% and 96%.

Figure 4

Correlation of observed and expected recoveries of strontium.



Stable Strontium Analysis by Neutron Activation Analysis

This method of analysis has been employed mainly for the assay of blood samples where the sample size is too small to enable any other techniques to be used. The method is based on that of Harrison, Raymond (1955).

The blood was collected into small silica thimbles in duplicate and at the same time a sample was taken for the analysis of calcium and for the measurement of P.C.V. and Hb.

The silica containers were put into a drying oven at 110°C until the sample was dried (3-4 days) and then transferred to a muffle furnace. The furnace temperature was raised very slowly to 400°C. Blood tends to froth at 200-250°C probably due to the breakdown of the haemoglobin with the release of O_2 and CO_2. When ashing was complete, the silica containers are allowed to cool and then stoppered tightly with polythene. The samples were irradiated with a neutron flux of 10^{12} neutrons/cm^2/second for 3 hours

$$^{86}Sr\ (n\ \gamma)\ ^{87m}Sr \qquad ^{86}Sr + n \quad ^{87m}Sr + \gamma$$

^{87m}Sr has a half life of 2.7 hours and emits a gamma-ray of 0.39 MeV.

After irradiation about 30 minutes was allowed to elapse so that the undesirable short-lived isotopes decayed considerably before the containers were opened and the contents dissolved in concentrated nitric acid with the addition of a few drops of strontium carrier, barium copper and manganese carriers and transferred to a centrifuge tube. The nitric acid concentration was increased to 80% and the mixture cooled by standing the tube in ice water to precipitate the strontium as nitrate. The supernate was removed by centrifugation and the precipitate redissolved in a minimum of water before reprecipitation with nitric acid (as nitrate) again in the presence of the carriers. The strontium nitrate was dissolved in water, the solution made alkaline by the addition of ammonium hydroxide and sodium carbonate (saturated solution) added cautiously. After removal of the supernate, the carbonate precipitation was repeated twice (three times in all)followed by two more nitrate precipitations. In cases where the red colouration was visible in the blood ash, an iron scavenge was also carried out after the first nitrate precipitation. A few drops of iron carrier solution were added to the solution derived from the second nitrate precipitation, and this was then made alkaline by the addition of ammonia solution and the tube was placed in a boiling water bath for a few minutes. The hydroxide precipitate

was removed by centrifugation before additon of Na_2Co_3 (satd. solution) to the supernate.

The final nitrate precipitate was dissolved in 5 ml water and the solution transferred to a vial for counting before estimation of the radioactivity by gamma spectrometry using a Laben analyser with 512 channels. The photopeak was compared with that of a standard obtained by irradiating a known amount of Specpure strontium carbonate (Johnson Matthey and Co.) in a polythene container placed in the same rabbit as the sample being analysed. The standard (approximately 20-30 mg weighed accurately) was dissolved in a minimum of hydrochloric acid, made up to 100 ml, 2 ml of that solution diluted to 250 and 3-5 ml taken.

The chemical yield of the sample was determined by adding ^{85}Sr to the irradiated sample before the separation technique was carried out. The photopeak due to the ^{85}Sr was also measured and compared with a standard.