STRONTIUM STUDIES IN CHILDREN

A Thesis for the degree of M.Sc.

by

Wilma M. M. Brown, B.Sc.

University of Glasgow.

June, 1966.

STRONTIUM STUDIES IN CHILDREN

by

Wilma M. M. Brown, B.Sc.

S U M M A R Y

The localisation of radio-active strontium within the bone matrix of the young animal has given rise to a modern hasard unknown before the advent of thermonuclear explosions and fallout. The metabolism of this element has been investigated by many workers but results for work on children are somewhat limited.

This thesis describes a series of measurements in hospitalised children during which the stable isotope of strontium was determined in various samples using a spectrochemical method. The relative calcium content of these samples was also estimated by this means. Optimum working conditions for the instrument were first obtained and are detailed in the text.

The variation of urinary calcium content in ageing samples has been studied and found to be minimised by acidification.

A group of nine strontium-calcium balances under additional calcium are described and the effect of the salicylate ion considered. This appears to be dependent on the calcium salt employed.

The strontium content of some 45 samples of children's urine has been determined using a direct method which omits the separation of the strontium. Strontium/calcium ratios for these samples have also been calculated and found to occupy a considerable range in value. One sample of urinary calculus has been investigated and is shown to differ markedly from the urine of the same patient.

Values for natural strontium/calcium in bone and teeth have been determined and found to be reasonably comparable, giving rise to O.R. $\frac{\text{bone or teeth}}{\text{diet}}$ of 0.25 (approx.). One case of osteogenic sarcoma is described showing the uptake of strontium in both the tumour and surrounding tissue.

Balances in rachitic children suggest that vitamin D. produces an increased absorption of calcium but not of strontium.

Studies in infants on milk diets indicate that the O.R. bone/diet is higher than in older children or adults, which suggests a lower discrimination in favour of calcium in infants. A large variation in O.R. $\frac{\text{urine}}{\text{diet}}$ is also exhibited in this age group.

Throughout this thesis, various interlaboratory comparisons of results are given, as are comparison of results obtained on the same sample by different methods.

The above results have awakened many questions which it is hoped to answer in further research.

The Village of Strontian

Argyllshire, Scotland.

ACKNOWLEDGMENTS

The advice and encouragement received from Dr. J. M. A. Lenihan, Regional Physicist, Western Regional Hospital Board, has been much appreciated.

Bone samples from post mortem examinations were provided by the hospital pathologist, Dr. A. M. Macdonald.

Thanks are due to Miss Whitaker, the hospital dietician, and her staff, for the work involved in the production of duplicate diets in balance studies.

The photographs contained in this thesis were the work of Mr. Maxwell Steven of Hamilton.

The author is indebted to the Department of Child Health, University of Glasgow, for the use of hospital laboratories and apparatus and to the Medical Research Council for the financing of this research.

CONTENTS

Illustrations

The element strontium has been known for nearly two hundred years. Its carbonate ore was recognised as 'a probable new species of earth' by Adair M. Crawford in 1789, when he and the chemist Cruikshank were working at Woolwich on a mineral purchased from a mine at Strontian, Argyllshire (Partington 1962). Crawford's chief interest was in the new ore's possible medicinal properties. These observations were recorded in Medical Communications for 1790 (Crawford 1790) and are the first published information on the subject.

Previous to 1790, Thomas Charles Hope had described many of the chemical properties of the ore in lectures to his students, but publication did not take place until 1798 (Hope 1798). Many chemists including Sulzer (1791), Klaproth (1793-94) and Kirwan (1794) investigated the Scottish ore describing its mineralogical characteristics, carmine red flame and isolating such compounds as the oxide and chloride (Partington 1941-47).

The element itself was not obtained until 1808 when Humphry Davy separated calcium, barium and the related strontium by heating amalgams (Davy 1808). From that time until fairly recently little of note had been added to the

initial knowledge of strontium. It had been estimated in sea water (Desgnez and Meunier 1926) obtained in animal bone and teeth (Gerlach and Muller 1934, Geis 1918), sold as a red-flame producing component of fireworks (Kleinberg et al. 1960), and generally considered as a relatively harmless chemical. With the production of the radioactive isotope of mass 89 (Strontium - 89) in the cyclotron (Lawrence 1936) a means of investigating the metabolism of this element was obtained. The path of strontium in the human and animal body using the 'tracer' technique was studied by such workers as Treadwell et al. (1942) and Pecher et al. in the early 1940s. Their work showed that strontium was metabolised in the same way as calcium, concentrating in the skeleton.

The first large thermonuclear explosion in 1954 subjected the human race to the new phenomenon of 'fallout' which is the descent to earth of the products of nuclear fission (Noel - Martin 1962). Among the radionucleides produced by such an explosion are the strontium isotopes 89 and 90; the uptake of these isotopes has become a matter of some concern (M.R.C. Report 2, 1960).

This is especially felt with regard to strontium - 90 since it has a radiological half life of 28 years and a biological half life of 7.5 years. (Hawthorne 1959). Strontium

and calcium are generally found associated in nature and thus with entry of strontium into the body's stores of calcium (i.e. bones and teeth) would come this radioisotope with the possibility of danger from the formation of localised radio-active areas or 'hot spots'. These hot spots are capable of irradiating the surrounding tissue both of bone and blood-forming bone marrow. Furthermore, with the decay of Sr-90 to its daughter Yttrium (Y-90), a radio-isotope of greater penetration is produced (Engstrom et al. 1957).

Experiments on strontium uptake and metabolism have therefore assumed a greater importance since the recognition of Sr-90 as a constituent of fallout. Since 1959, much work on the analysis of post mortem samples of children's bones for Sr-90 has been carried out here in Glasgow; a method for the estimation of urinary Sr-90 has also been developed (Warren 1965).

From the conception of the word isotope, it is realised that the various isotopes of an element are chemically analogous (Soddy 1913) and thus both natural and radio-active strontium are treated metabolically in the same manner. The use of the stable strontium content of biological tissues to trace the path of the element through the body has been adopted by many workers.

In 1963 we obtained a Zeiss Flame Spectrophotometer of extremely good sensitivity capable of determining very low concentrations of strontium. Using this instrument we were able to extend

the scope of the work to include stable strontium estimations. Several studies of strontium metabolism under various influences and dietary additives were begun.

This thesis describes the investigations carried out and results obtained. The work was on children of less than 18 years of age in whom there is a higher rate of metabolic activity and a lower mineralization of bone tissue than in the adult. (Mitchell et al. 1945). As is normal in strontium investigations, the relative amounts of calcium were also determined spectrometrically. The work was carried out in the Strontium Research Laboratory, The Royal Hospital for Sick Children, Yorkhill, Glasgow, and the subjects studied were all patients in the hospital.

S E C T I O N I

STRONTIUM IN THE BIOSPHERE

a) Strontium in Soil

The soil or upper layer of the surface of the earth forms a nutritive medium for the growth of plants which in turn are ingested by animals and humans. The lithosphere of which the soil forms part contains 0.035% natural strontium. Since the alkaline earth group of elements, to which both strontium and calcium belong, is most reactive, neither element occurs in the neutral state. These elements are divalent, forming electropositive chemical bonds with negative ions, thus it is as the carbonate (stronianite) and the sulphate (celestine) that strontium occurs. (Haissinsky and Adloff 1965). Generally the ores of these two elements are found together in nature, so that in ingesting calcium, all organisms to a much lesser extent ingest strontium. Little information on the natural strontium to calcium relationship in the soil is obtainable, probably due to the fact that the complexity of equilibria between calcium and strontium in soil is such that no simple relationship is possible. (Comar et al. 1957).

An additional source of strontium in soil is the radioactive Sr-90 and Sr-89 released in fallout, but it has been shown that these radionucleides penetrate to a depth of only 5 cm. below the surface of the soil. (Benison et al. 1964). Several workers have attempted to reduce the radio-strontium to calcium content of plants grown on contaminated soil by the addition of

calcium salts (Fuller and Flocker 1955, Romney et al. 1956). Generally it was found that, where soil contained sufficient lime for good growth of crops, further additions provided little protection against radio-strontium.

b) Strontium in Water

Strontium has been shown to be present in both salt water and fresh water.

The values for salt water show considerable discrepancy, varying from 8.7-50 mgms. Sr./litre for a salinity of 35 parts per million (ppm.) (Desgnez and Meunier 1926; Thomas and Thompson 1932; Ramage 1933). However, 235 determinations of strontium to calcium ratio made on 160 samples from diverse parts of the Atlantic gave a value of 9.23 atoms Sr./1000 atoms Ca or 8.10 mgms. Sr./litre for a salinity of 35 ppm (Odum 1951). This result is slightly lower than that of Smales (1951). It has been suggested from the results on the 160 samples of Atlantic water that strontium is a conservative element varying with salinity.

The Agricultural Research Council's figures for impounded water in Glasgow in 1963/64 give a mean value of 0.013 mgm./l., resulting in a strontium to calcium ratio of 6.0 mgm. Sr/gm. Ca (Burton and Russell 1964).

The importance of the strontium content of fresh water is in its ingestion both as drinking water and as a constituent in the preparation of other food stuffs - for example, in the reconstitution of dried milk for infants. Burton and Russell show that the ratios of strontium to calcium in different water sources lie within a

fairly narrow range (1.8 mgm. Sr/gm. Ca - 6.0 mgm. Sr/gm. Ca) but actual concentrations of both elements vary by factors exceeding 60. (1964). The possible effects of this are shown by contrasting the composition of synthetic milk reconstituted using water from widely differing sources. Variations of a factor of 2 in the ratio of stable strontium to calcium are thus obtained.

c) Strontium in Plants

Figures for natural Sr/Ca ratios in leaf and other vegetables and fruit are of the order of 2.3 mg. Sr/gm. Ca (Burton and Russell 1964). This strontium is obtained by the plant via two distinct routes:

1) Absorption by the roots from the soil with the uptake of both stable and radioactive strontium.

2) Absorption by the foliage of airborne radio-strontium.

The major part of dietary strontium obtained by man or animal from plants comes from the leaves. The passage of strontium and calcium through the plant appears to be unidirectional with a slight discrimination against strontium in favour of calcium on passage from root to leaves. This bias is best shown by use of the 'OBSERVED RATIO' which is the ratio of Sr/Ca in the sample divided by the ratio of Sr/Ca in the precursor (Comar et al. 1956). In the case of plants, this is the $\frac{\text{Sr/Ca ratio in the plant}}{\text{Sr/Ca ratio in the soil}}$

Various authors report O.R.s $\frac{\text{plant}}{\text{soil}}$ of 0.7-1.3 (Menzel and Heath

1955, Collander 1941) but the majority of values are below 1.0. This discrimination is not fully understood but some retention of strontium appears to take place at the stem nodes.

G. H. Sidrak et al. give a figure of 1.0 for the O.R. between plant stem and soil with the O.R. for the root portion greater, and for the inflorescence less. These workers also state that wide variations in the ratio of Sr/Ca and in absolute concentrations of both elements in culture solution have little effect on discrimination between the two ions (Sidrak et al. 1964). This result is further supported by the fact that in the water plant Elodea, the ratio of Sr/Ca in the plant tissue approximates to that of its growth medium (Squire and Russell 1955).

Thus it is seen that in the first of the biological systems there is little discrimination against strontium in favour of calcium and therefore in animals obtaining most of their calcium from plants (herbivores) the uptake of strontium relative to calcium is greater than in omnivores such as man. In eastern rice-eating areas the effect of increased radio-strontium in soil is much enhanced (Hawthorne 1959). In western countries, such an increase is offset by the effective discrimination against strontium taking place in the animal, resulting in a reduced uptake of strontium relative to calcium by the human.

d) Strontium in Animals

The presence of strontium in the skeleton of animals was postulated by Gerlach and Muller in 1934, Ascari in 1950, and fully reported by Sowden and Stitch in 1950. Recently, using emission spectrography and a radiation buffer technique, strontium was found in the bones of all animals investigated (Macdonald et al. 1956). The values obtained in animals on controlled diets ranged from 240 ug. Sr/gm. Ca in mice (omnivores) to 2,200 ugm. Sr/gm. Ca in guinea pigs (herbivores). In most cases the calcium content was somewhat lower than the 38% accepted for human bone.

Pioneer work on strontium metabolism in animals was carried out by Pecher et al. in the early nineteen forties. These workers showed that strontium, like calcium, concentrated in bone and that there was little localisation in other tissues. Also the administration of compounds of calcium and strontium to cows resulted in the rapid appearance of these elements in the milk within a few hours. These results show the close relationship of the animal diet to the mineral content of the milk produced (Erf and Pecher 1940). In 1953 it was shown that an injection of Ca-45 in dairy cows, 20 days prepartum, resulted in a deposit of radio-calcium in the mammary gland.

This calcium was not further metabolised but eventually appeared in the milk. Thus it would seem that there are temporary non-exchangeable pools of calcium formed in pregnancy which help to reduce the relative strontium uptake of the animal offspring (Visek et al. 1953).

A mean value of 320 ug. Sr/gm. Ca was obtained for cows' milk in the United Kingdom during the period 1963/64 (Burton and Russell 1964). The availability to man of this strontium has been found to be extremely high, the uptake from milk being almost as high as from an aqueous solution of the stable inorganic salt (the chloride). The relative availability of the strontium in such a solution has been reported as almost complete ($0.9 \pm .05$) (Carr et al. 1962).

Once the young animal has been weaned from its mother's milk the amount of strontium absorbed becomes dependent upon the strontium content of the dietary constituents. Originally it was suggested that as in plants, the ratio of Sr/Ca in the animal body would approximate to the ratio of Sr/Ca in the soil. This was soon disproved. Working with rats, it was found that there was a physiological differentiation by the body in favour of calcium.

Employing a double tracer technique using Ca-45 and Sr-89, Comar et al. discovered two major discrimination processes. (Comar et al. 1956).

a) Preferential absorption of calcium from the gut (shown to be a factor of 2 (Vaughan 1959).

b) Preferential excretion of strontium in the urine (shown to be a factor of 3-5 (Della Rosa et al. 1961).

As a measure of the net result of such processes, the use of the O.R. bone-diet was suggested. This ratio was found to vary with such factors as composition of diet or age of animal. Where the diet was of milk, a higher O.R. bone-diet was obtained than with animals on a non-milk diet. The influence of milk was thought to be due to the presence of such substances as lactose, lysine and arginine which, while increasing calcium absorption from the gastro-intestinal tract also increased strontium absorption to an even greater extent (Wasserman et al. 1956). Much work has been done on the effect of the phosphorus content of the diet. When dietary phosphorus is increased the primary effect on rats appears to be a diminished absorption of both strontium and calcium from the gut, with a diminished excretion of both elements in the urine. As would be expected, faecal excretion of both elements is increased. (Kostial et al. 1963).

Smith and Bates (1965) investigated the effect of various chemicals on the process of tubular reabsorption in the rat kidney. They found that the salicylate ion induced tubular block as a delay phenomenon, resulting in an increased excretion of strontium in the urine. If administered with strontium, the effect was contrary and a reduced excretion of urinary strontium relative to control animals was obtained. No calcium results were given and thus the effect of salicylate on the urinary Sr/Ca ratio may not be determined.

In the absence of the kidney, as in nephrectomized rats, there is a greater output of strontium relative to calcium from the peritoneal cavity by lavage - O.R. lavage / bone of 1.3 (Talmage et al. 1957). This experiment removed the effect of renal discrimination which has been shown by other workers to be somewhat vulnerable to chemical and metabolic influences. Walser et al. (1961) found that at high rates of sulphate infusion, tubular reabsorption of strontium almost ceased. Infusion of either sodium bicarbonate or citrate increased excretion of both strontium and calcium, but countered renal discrimination (Della Rosa et al. 1961). When bone tissue was actively calcifying discrimination between strontium and calcium in the kidney ceased (Macdonald et al. 1957). This last result gives a further indication

of the connection between the phosphate ion and strontium metabolism.

The uptake of strontium in the rabbit bone has been studied by means of radioactive tracers and autoradiographs. The results obtained show a retention of 12% of the dose in mature animals and of 46% of the dose in the young (Vaughan 1959). A recent publication gives a discrimination factor of 1.6 against strontium in favour of calcium in its passage from blood to bone in rabbits (Kshirsagar et al. 1966).

Strontium was first shown to be taken up by animal teeth in 1918 when work on dogs showed it to be present both in the calcified portion and also the soft tissue of the pulp (Geis 1918). The apposition of organic dentine was much affected both quantitatively and qualitatively by the presence of strontium. This effect was found to be accentuated by low Ca/P ratios in the diet (Comar and Bronner 1960).

From the foregoing, it is obvious that the animal body is capable of differentiating between calcium and strontium in its diet, in favour of calcium. Calcium is preferentially absorbed from the gastro-intestinal tract, secreted into milk and transported to the foetus, and reabsorbed in the kidney tubules. The overall discrimination against strontium in favour of calcium in the non-pregnant, non-lactating animal in normal health is of the order of a factor of 4. This is shown by the reduction in

the ratio of Sr/Ca in the animal products as compared with the high Sr/Ca ratio occurring in plants from which they derive their nutrient (Table I - below).

i.e.

Food-stuff	Average Ratio Sr mg./Ca gm.
Vegetables and Fruit	2.3
Milk and cream	0.32
Cheese	0.58
Meat	0.6

(Burton and Russell 1964)

In the United Kingdom where some 30% of the total intake of stable strontium comes from animal products, this discrimination on passage through the animal considerably reduces the relative uptake of strontium from the diet.

e) Strontium in Humans

Experimentation with radioactivity in the human is naturally more limited than with animals. In the last decade or so, however, many workers (Harrison et al. 1955; Hesp and Ramsbottom, 1965; Dow and Stanbury 1960) have used various isotopes of strontium both natural - generally as Sr/Ca ratios, and radioactive - Sr-85, Sr-90, and combinations of Ca-45 and radiostrontium, in double tracer methods, to study the metabolism of strontium in the human body after its introduction by various routes, i.e. orally (Comar et al. 1957; Spencer et al. 1956, 1957; Spencer-Laszlo et al. 1963) or intravenously (Bishop et al. 1960; Bauer and Ray 1958).

These projects represent an artificial introduction to the body which nevertheless, give much information on the normal uptake of strontium in humans. Since strontium is present in the air we breathe, the water we drink and the food we eat, we cannot hope to prevent easily its introduction to the body. We may, however, develop methods to control the extent to which strontium is retained by the bone.

There are three main routes of uptake:-

1) Via the lungs - by ingestion of air-suspended particles. This route is reserved for radio-strontium produced in fallout from thermonuclear explosions.

2) From drinking water - both radioactive and stable isotopes are absorbed by this route.

3) From food - in food there is the possibility of either
   a) a high Sr/Ca ratio where the component has been obtained directly from plants in which little or no discrimination against strontium is evident. Such foods are cereals, cereal products (bread, flour, rice and leafy vegetables.)

or b) a lower Sr/Ca ratio where the component has been obtained indirectly from plants after passage through a biological filter, such as a cow or sheep, where a definite discrimination in favour of calcium is known to be active (Comar and Wasserman 1956; Comar et al. 1956).

Such foods are meat and meat products. Milk, with its almost complete availability of strontium, represents a special case (Carr et al. 1962).

Since section (1) above may be disregarded as far as stable strontium is concerned and as it represents only a small proportion

of uptake of radio-strontium, it is proposed to ignore this route of absorption and to consider only the oral route of administration with regard to dietary constituents.

The Human Diet

Because of the wide divergence of eating habits and the many nutritive prejudices of the orient, it is proposed to study only the diet of the western world, with especial regard to that of the United Kingdom.

Some 80% of the total intake of strontium in the normal adult diet in the United Kingdom in 1962 was stated to be derived from cows' milk, wheat flour, creta-praeparata (or added chalk) and drinking water, in all of which the strontium may be considered fully available. The remaining 20% was obtained from meat, vegetables and fruit (Carr et al. 1962). Burton and Russell in 1963 considered the relative proportions be nearer 30% for meat, vegetables and fruit, giving a value of 1.2 mgms. Sr/gm. Ca as the mean ratio of Sr/Ca in the average diet. In British adult diet, although subject to large variations due to differing quantities and constituents, the overall variation in Sr/Ca ratio rarely exceeds 10% (Burton and Russell 1964).

Strontium-calcium balance for one subject on a general diet gave results of average daily intake of Sr = 1.99 mgms., Ca = 1.18 gms. and a resulting ratio of 1.7 mgm. Sr/gm Ca

(Harrison et al. 1955). In preparation of a strontium-calcium model, Dolphin and Eve considered figures of 1.6 mgms. Sr/day and 1.2 gms. Ca/day as representative of strontium and calcium intake in the standard man on a diet similar to that of the United Kingdom (Dolphin and Eve 1963). These values give a ratio of $\frac{\text{Sr mgm.}}{\text{Ca gm.}} = 1.33$, which is closer to that recorded by Burton and Russell above.

When considering the diet of children larger variations both in Sr/Ca ratio and also in the absolute amounts of these elements ingested are noted. Such variations are due to the change from the fairly low Sr/Ca ratios of milk to the higher ratios of the mixed diet. This increase is greatest where the infant was initially breast-fed. Also, as already mentioned (p. 7), considerable variation in amounts of strontium and calcium ingested results from variation of the source of water used to reconstitute dried milk for infant feeds. The published results for the strontium-calcium content of diets for various age groups are summarized overleaf:-(TABLE II)

Age Group	Ratio: $\frac{\text{Sr mgms.}}{\text{Ca gms.}}$	Source of References
Adults	1.2	Burton and Russell 1964
Infants		
a) Breast Fed	0.246	
b) Bottle Fed	0.515	Widdowson et al. 1960

The Absorption Process

The absorption of dietary strontium and calcium takes place from the upper part of the intestine where the reaction of the bowel contents is slightly acid. In acid solution, soluble salts of these elements are formed which may be absorbed through the wall of the gastro-intestinal tract. In alkaline solution, the salts formed are insoluble-phosphate or phytate and these are excreted in the faeces (Bell et al. 1953). Absorption of both strontium and calcium is accelerated by factors which promote the formation of soluble salts. The presence of large amounts of unabsorbed fatty acids (i.e. coeliac disease) or the condition of gastro-enteritis diminishes

absorption (Alstead and MacArthur 1965). Other factors governing the absorption of calcium (and therefore strontium) are stated to be

a) Total quantities of calcium and phosphorus present.

b) Relative proportions of these elements.

c) Thyroid and parathyroid function.

d) Gestational age.

e) Intestinal mobility.

f) Vitamin D availability.

g) Carbohydrate, protein and fat content of gut (Calcagno 1966).

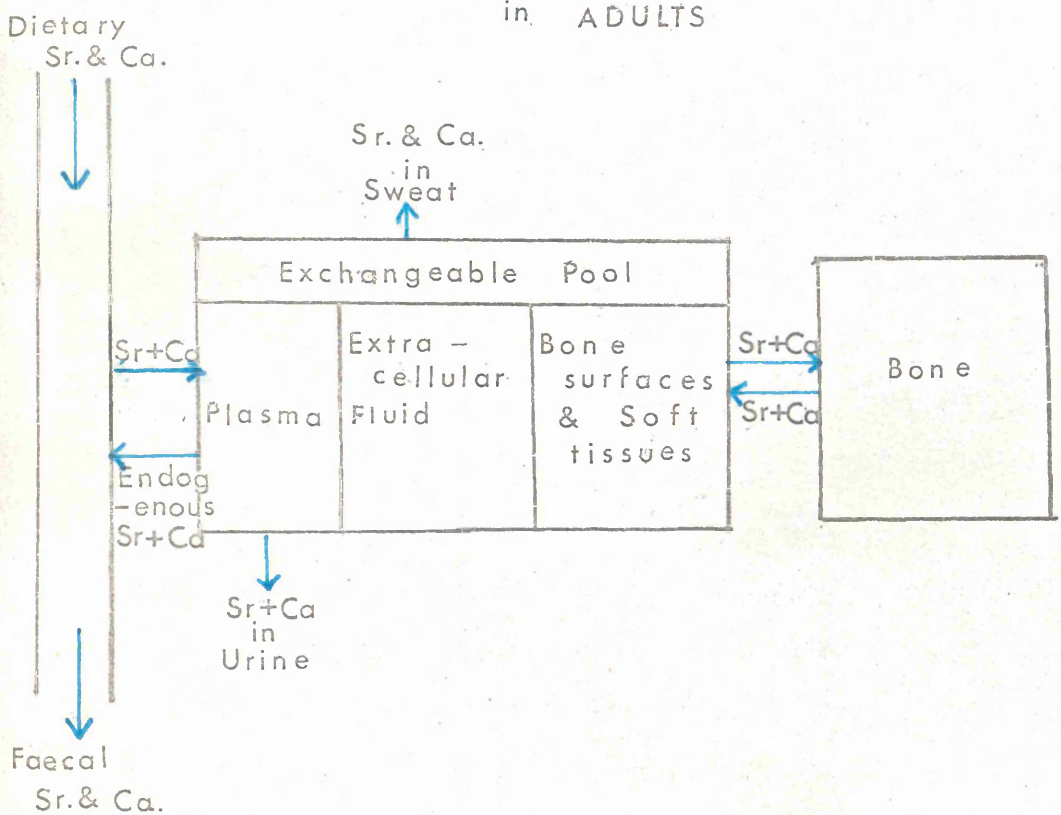
If in balance the absorption of each equals the endogenous excretion of the element in the urine, faeces and sweat. Values for the absorption of calcium by the adult man are of the order of 40% of the ingested calcium (Dolphin and Eve 1963) while the absorption of strontium is stated to be only 20% of that ingested (Laszlo and Spencer 1959). Thus there is a two - fold discrimination against absorption of strontium from the gut (Bailey et al. 1960). In infants a lower discrimination against strontium is to be expected due to the larger volumes of milk ingested.

Since strontium and calcium are considered

Fig I

STRONTIUM – CALCIUM METABOLISM

in ADULTS



After Dolphin and Eve.

to be similarly metabolised, although a quantitative differentiation occurs in some processes, their passage through the body may be diagrammatically represented by the same model. Many such models have been proposed (Comar and Wasserman 1956; Loutit 1961; Bauer and Ray, 1958) but the most recent which accounts for all known compartments in strontium-calcium metabolism is shown opposite (figure 1).

The excretion of strontium in sweat is usually neglected. In any such model the endogenous excretion of both calcium and strontium into the gut via the various digestive juices and by reabsorption must be recognised (Nordin et al. 1962). Thus absorptions calculated from dietary intake minus faecal output are not 'true' absorptions, but 'net' absorptions, resulting in false low absorption values. A difference of only 2% between such values has been calculated for calcium absorption (Dolphin and Eve 1963).

Contrary to results obtained in rats (Kostial et al. 1963) on increased dietary phosphate, Widdowson et al. found that in very young babies absorption of calcium from the gut was enhanced (Widdowson et al. 1963).

After absorption through the intestinal wall, the strontium and calcium became associated with extra-cellular fluid from

which they are absorbed into the blood. Unabsorbed dietary strontium and calcium, together with endogenous faecal strontium and calcium, are excreted from the gut in the faeces. This will be dealt with under excretion.

The Blood

The blood consists of two distinct entities, the 'formed' elements (or corpuscles and platelets) and the plasma in which they are suspended. This fluid acts as a transport mechanism for the food passing to the tissues and the waste products excreted by them (Bell et al. 1953). Strontium and calcium are carried mainly in the plasma fraction of the blood, both attached to the plasma protein (protein bound) and also ionized and diffusible. The normal calcium content of blood plasma in the adult man is 100 mgm./litre (Dolphin and Eve 1963) but only approximately 65% of this is capable of diffusion through the vessel walls. Relatively less strontium than calcium is bound to the proteins of the plasma and a plasma concentration of 30 µg. Sr/litre on an average diet containing 1.3 mg. Sr/gm. Ca has been recorded (Harrison et al. 1955). The blood calcium content is maintained at the normal level through the action of

the parathyroid glands. These glands affect the state of the bone calcification and may give rise to demineralization in hyperparathyroidism. This result is thought to be due to their action on renal tubular reabsorption of phosphate, together with a direct action on bone tissue leading to the release of calcium (Bergstrom 1956).

Work on the clearance from the blood of an intravenous injection of Sr-85 in five adult males shows a rapid initial decrease in circulating radio-strontium amounting to approximately 80% of the dose. This rapid decrease has been attributed to diffusion into extracellular fluids surrounding the bone tissue (Bauer and Ray 1958). Other workers have shown that the clearance of strontium from the plasma was approximately independant of the plasma concentration of injected strontium (Bishop et al. 1960).

The Bone Tissue

The bone tissue may be divided into two distinct types

a) Skeleton b) Teeth

a) The Human Skeleton

The functions of the skeleton as a supportive structure and shelter for the blood forming bone marrow have long been

known. It is only in the last sixty years that it has become recognised as an electrolyte reservoir. Bone of the skeleton is now recognised as having an active part in physiological processes, particularly mineral metabolism (Bergstrom 1956).

Skeletal Bone is thought to consist of

a) Inorganic crystals - physically very thin tablets of extremely small size giving rise to a tremendously high specific surface area, the smallest recurring unit structure being the calcium hydroxyapatite crystal of chemical composition - $Ca_{10}(PO_4)_6.(OH)_2$. These crystals are highly hydrated, capable of binding 2.5 times their own volume of water.

b) Water - forming a hydration shell surrounding the calcium hydroxyapatite crystal.

c) Polysaccharide gel - this is the continuous phase or ground substance.

d) Protein - present as collagen fibres associated with the apatite (Comar and Wasserman 1956).

The growth of this tissue is believed to be a combination of two processes, simultaneous erosion (resorbtion) and deposition (accretion). These processes are extremely active in the young but limited in the mature human, although still

evident in the Haversian systems of the fully grown adult. Compared to the dense, poorly hydrated areas of the well established bone, the bone tissue of the young animal is found to be of a cancellous character denoting a lower mineralization, much more chemically reactive and with a metabolic turnover rate of 10%/annum (Smith and Bates 1965).

The high concentration of calcium in bone and the chemically similarity of strontium and calcium lead to the proved uptake and concentration of strontium in bone (Treadwell et al. 1942). This uptake is most marked where there is actively calcifying tissue as in the young, and since 99% of the body strontium is found in bone, localisation is almost complete (Dolphin and Eve 1963).

In 1959, Boyd et al. suggested that the skeletal fixation of strontium arose from the following mechanism.

a) Diffusion of strontium ions from the extracellular fluid into the bone areas.

b) Entry of these ions with hydration jackets of the apatite crystals.

c) Exchange with the calcium ions on the surface of these crystals.

d) Eventual penetration of these ions into the interior of the crystal lattice after recrystallisation has

taken place (Boyd et al 1959).

The levels of both radio-active and natural strontium relative to calcium in children's bones have been reported in the Medical Research Council's six-monthly reports. The natural strontium to calcium in various age groups has been reported (Bryant et al. 1964). These show that levels are of the order of 250 µg. Sr/gm. Ca with a range of 197-335 µg. Sr/gm. Ca for the age range of still birth - twenty years. The level of strontium relative to calcium in bone is dependent on the dietary uptake of the individual and the effect of his various discrimination processes against strontium. The net result may be gauged by use of the O.R. bone-diet. In adults this generally is of the order of 0.25 (Loutit et al. 1964). In children and infants this ratio is not found to be reasonably constant as in adults but to show a considerable variation. In children less than two years old, the O.R. exceeds 0.25 and may go as high as 1.0 (Rivera 1965). This lowered discrimination in infants is considered to be due to their greater uptake of milk which has been shown to reduce the gastro-intestinal absorptive discrimination against strontium. The variation in observed ratios reflects the fact that the infant can gain or lose strontium from the body, depending on its rate of growth

and comparative Sr/Ca ratios of the body at birth and the diet (Comar et al 1965).

The question of a discrimination in the uptake of either strontium or calcium in their passage from blood to bone and vice versa is the subject of some controversy at the time of writing. In vitro results for the comparative fixation of calcium and strontium by synthetic calcium hydroxyapatite give a discrimination against strontium of 1.6. (Likins et al. 1960).

The Teeth

The chemical composition of teeth very closely resembles that of bones, the predominant inorganic salt being calcium hydroxyapatite. However, the water content of tooth tissue is lower than that of skeletal bone (Bell et al. 1953).

The various layers of teeth which are well defined are described below:-

a) Enamel - covers external, orally exposed crowns and is composed of densely packed rods or prisms. This layer is completely acellular and avascular.

b) Dentine - again acellular and avascular, but here we have a close association with the cells lining the pulp cavity.

The metabolism of teeth is essentially a unidirectional

process of apposition with no resorbtion as shown in bone tissue. Only in the root of the deciduous tooth is resorbtion exhibited (Bryant et al. 1960). These workers also found Sr/Ca ratios in teeth to range from 250-340 µg. Sr/g. Ca in third molars and from 240-290 µg. Sr/g. Ca in premolars. These results show that the overall discrimination against strontium uptake is similar for both teeth and bone.

The Excretory Process

In all higher animals there are two main routes for excretion of unwanted materials, either in the urine after filtration of the blood plasma by the kidney or via the gastro-intestinal tract in the faeces. Minor processes of excretion not considered in this work are excretion through the skin in sweat and in the hair.

The excretion of strontium and calcium from the human body has been studied and found to vary with the route of administration of these elements (Spencer et al. 1957). In the normal course of events, the excretion of an orally ingested dose of strontium and calcium (as from diet) results in some 80% of the ingested strontium and 60% of the ingested calcium appearing

in the faeces (Dolphin and Eve 1963). When the administration is by intravenous means, relatively small amounts of either element appears in the faeces and the predominant route is via the kidney in the urine (Harrison et al. 1958).

Excretion from the Gastro-intestinal Tract

The material excreted in the faeces is derived from two sources:

a) unabsorbed dietary strontium and calcium.

b) endogenous strontium and calcium obtained from re-absorption through the intestinal wall and from various secretions of the digestive glands into the lumen of the gut.

The amount of faecal endogenous calcium has been shown to be independent of dietary calcium changes over short periods of up to three months during which faecal endogenous calcium did not vary to any great extent (Comar and Wasserman 1956).

Strontium and calcium appear to be equally transferred back to the gut from the extracellular fluids so that there is no apparent discrimination in this process (Dow and Stanbury 1960).

Work in children gives a mean faecal excretion of 78% of ingested dose of strontium which is similar to that quoted for

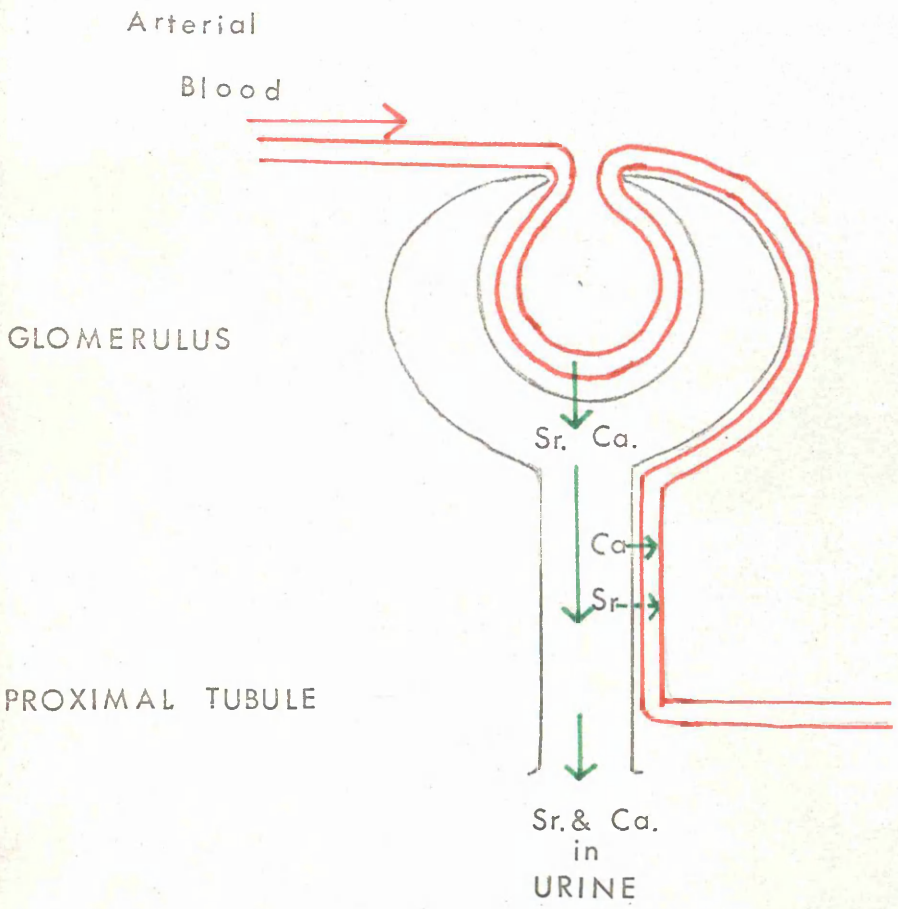
adults (Bedford et al. 1960). In newly born infants, an extremely high Sr/Ca ratio in the meconium exists. Corresponding figures for infants are much lower. The various figures for different age groups are contrasted below. (Table III).

Age Group	Ratio: $\frac{\text{Sr in mgm.}}{\text{Ca in g.}}$	Source of Reference
Adults	2.3	
Children (4-14 yrs.)	1.9	
Infants		
Breast Fed	0.9	
Bottle Fed	0.5	Widdowson et al. 1960
Perinates (<24 hrs.)	5.9	Widdowson et al. 1962

Excretion by the Kidneys

The production of urine by the kidneys is a two-fold process involving ultrafiltration of the blood plasma through the glomerular membrane and modification of the filtrate by reabsorption taking place in the tubules. This action is

Fig 2 DIAGRAM of KIDNEY ACTION



After BELL et al.

diagrammatically represented opposite (fig. II).

Ionic strontium and calcium together with unionised complexes with citrate etc. are filtered at the glomerulus and reabsorption takes place in the proximal tubule. This is not a quantitative process but relatively more calcium than strontium is reabsorbed. The ratio of Sr/Ca in the urine compared to the Sr/Ca ratio in the blood plasma gives a measure of this discrimination against strontium reabsorption.

Human renal discrimination was found to vary from 2.8-4.3 with a mean value of 3.5 in favour of calcium reabsorption (Barnes et al. 1961). The excretion in urine is of entirely endogenous calcium and strontium and reflects the previous day's intake, rather than the body burden (Comar et al. 1957). The phosphate ion appears to exert an influence on the relative reabsorption of strontium and calcium, although almost quantitatively reabsorbed itself. This effect is thought to be due to increased need for minerals in the young skeleton during calcification (Macdonald et al. 1957).

In breast-fed babies (low phosphate intake), the urinary excretion of strontium and calcium exceeds that of bottle-fed babies, in spite of a lowered intake of these cations from breast milk. The ratio of Sr/Ca in the urine of breast-fed

babies is also greater (Widdowson et al. 1960).

In older children there appears to be a lowered renal clearance of both strontium and calcium, compared to adults, while the Sr/Ca ratio in children's urine tended to be higher (Bedford et al. 1960).

Urinary Sr/Ca ratios obtained from literature are given below (Table IV).

Age Group	Ratio: $\frac{\text{Sr in mgms.}}{\text{Ca in gms.}}$	Source of Reference
Adults	0.96	Bedford et al. 1960
Children (4-14 yrs.)	1.2	
Infants		
Breast Fed	2.6	Widdowson et al. 1960
Bottle Fed	0.8	
Perinates (<24 hrs. old)	3.2	Widdowson et al. 1962

Net Result of Excretory Process

There is a two-fold discrimination against the passage of strontium through the gut wall. The relative retention of strontium compared to calcium is further lowered by a factor of

approximately 4. in the passage of blood through the kidney. Thus the net discrimination against strontium in the human body is a factor of 4.

Pregnancy and Lactation

The effect of these two physiological processes is to reduce the intake of strontium relative to calcium, first of all by the foetus and secondly by the breast fed infant.

The strontium content of the foetal calcium is governed by the strontium content of the calcium ingested by the mother shortly before birth. It has been shown that the relative transfer of strontium across the placenta is only half of the calcium transfer. The effect of breast feeding infants has been studied. The results thus obtained show that, although the average strontium intake of bottle fed babies was 4-8 times higher than breast fed infants, the latter excreted approximately 7 times more strontium in urine. Also, in most cases, their excretion was greater than intake and thus they were in negative balance for strontium, although in positive balance for both calcium and phosphorus (Widdowson et al. 1960).

Values of Sr/Ca in human milk are stated to be approximately 0.25 mg. Sr/gm. Ca (Burton and Russell 1964) although a lower

figure of 0.12 mgm. Sr/gm. Ca was postulated by Lough et al. in 1960. This lower figure would give an O.R. milk-diet of 1:10.

- - - - - - - - - - - - - -

In humans, as in animals, strontium and calcium are metabolised similarly and affected by same influences, such as age, health, dietary constituents, hormones, etc. The net result of all the physiological processes in the human body is a preferential laying down of calcium in bone which is achieved by:

a) Preferential absorption of calcium from the gut.

b) Preferential reabsorption of calcium in the kidney tubule.

c) Preferential transfer of calcium across the placenta and into the milk.

Due to the passage through animals, the human diet already has a reduced Sr/Ca ratio on ingestion.

In Vienna at a Scientific meeting in 1962, J. F. Loutit is quoted as saying -

"Even when strontium is injected intravenously into the normal adult, only about 10-15% is retained after a year, so that all the small factors (i.e. renal and intestinal excretion) of normal physiological function provide 70, 80-90% removal of

strontium. If each of these factors could be increased slightly, the gain by summation would be considerable." (Loutit 1962).

S E C T I O N II

EMISSION FLAME SPECTROPHOTOMETRY

The History of Spectral Analysis

In A.D. 40, Seneca noted the similarity between the rainbow and the colours produced when light passed through a piece of glass with a sharp edge (Weise 1960). This must surely be the first recorded dispersion of light to produce a spectrum.

The emission of salts when introduced into a flame was studied by Herschel in 1823-28 and he published emission spectra as plots of intensity varying with spectral colour (Herschel 1828). He also suggested the use of flame emission to detect extremely small amounts of an element. This was further investigated in 1826 by Talbot, who studied the spectrum of strontium and lithium and developed the idea of a characteristic spectrum for each element (Talbot 1826). In 1860, cesium and rubidium were discovered by means of their emission spectra in a bunsen flame (Kirchoff and Bunsen 1860).

Up until this time, flame emission had been used as a qualitative chemical method, but in 1869, Janssen suggested that the emission intensity varied with the concentration of the element in the flame and was therefore of use as a quantitative method - 'Thus the estimation of a certain quantity of

matter can be reduced to a measurement of light.' (Janssen 1870).

The 'spectronatrometre' developed in 1873 by Champion et al. was the first quantitative chemical instrument employing the principles of flame emission measurement. This instrument was, as the name suggests, specific for sodium estimation (Champion et al. 1873).

This new chemical method was greatly advanced by the work of the Swedish scientist, Lundegardh, who devised the first satisfactory method for the introduction of solutions into flames at a constant rate. His method was to use a concentric atomizer to disperse the solution into droplets, which passed through a spray chamber for removal of larger particles before entering the oxyacetylene flame used as an excitation source. He also developed the means of measuring the emitted radiation using a galvanometer connected to the amplified output from a photo-cell which was placed so as to receive an appropriate portion of the flame spectrum dispersed by a prism (Lundegardh 1929; 1934).

Using this apparatus, Lundegardh was able to measure almost 50% of the elements of the periodic table.

Thus is Lundegardh deservedly regarded as the 'Father of

modern flame photometry'.

Further advances in instrumentation were the introduction of filters, the development of photo-multipliers in place of photo-tubes with a resulting gain in sensitivity of 10^6, the use of integral atomizer-burners and better methods of lens production. All these factors have contributed to the much increased accuracy of modern spectral analysis.

1) Pressure Regulators

2) Burner Housing

3) Double Monochromator

4) Indicating Instrument

Fig 3 ZEISS FLAME SPECTROPHOTOMETER

Instrumentation

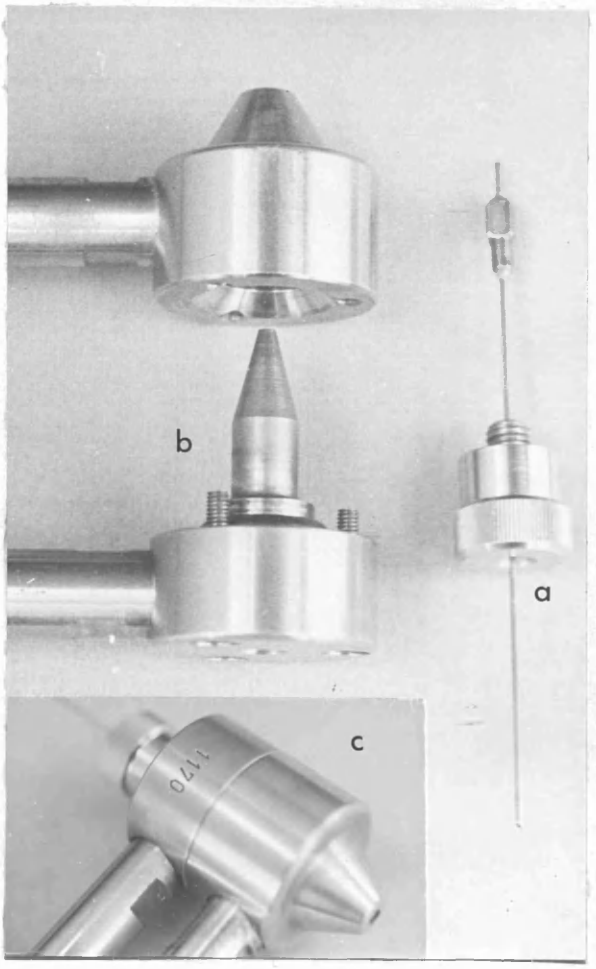
It is barely a century since the first quantitative use of light emission for chemical analysis. Yet in this time the development in instruments has been prolific. In place of bunsen burners and sample tubes, we have fuel gases producing temperatures in excess of 3000°C and atomizer-burners. In place of the human eye we have photo-detectors, photomultipliers and a recording system. Thus the many advances of the physicist are being used to increase the chemist's potentialities with resulting additions to scientific knowledge.

All modern emission photometers have certain fundamental components. These are six in number:-

1) pressure reducers
2) flow meters
3) atomizer
4) burner
5) an optical system
6) photosensitive detector with some instrument for indicating or recording the output of the detector.

The instrument used in the work herein described was a Zeiss spectrophotometer P.M.Q. II with double quartz mono-

Fig 4 INTEGRAL ATOMIZER-BURNER UNIT



a) Cannula

b) Gas Nozzles

c) Assembled Unit

chromator M.M. 12 and flame attachment employing an enlarged fuel inlet (fig. 3 overleaf). The important functions of this instrument are described below.

a) Control of Flame Temperature: This is achieved by variation of the proportion of fuel gas to oxygen in the flame. In the 'Zeiss' instrument, since the oxygen flow is also responsible for introduction of the sample to the flame, this is maintained at a constant pressure of 0.3 Kg./sq. cm. The respective gas pressures are obtained by action, firstly, of regulation of the valves in the flow meters. These valves are extremely finely adjusted and once obtained, the pressures remain constant.

b) Atomization and Combustion : In this instrument the atomizer and burner form an integral unit spraying the sample solution directly into the flame at a stable and reproducible rate. The oxygen and combustible gas are introduced into the flame by concentric annuli through the centre of which passes the sample cannula (see fig. 4 opposite).

c) The Optical System: The purpose of such a system is to

collect the emitted light, render it monochromatic and focus it upon the surface of a photosensitive detector. The 'Zeiss' employs a concave mirror placed behind the flame with its centre of curvature in the flame by which means the emission recorded is almost doubled. A double monochromator is used to disperse this light.

d) Detection and Indication of Emission: In this instrument, photomultiplier tubes are utilised. These are fairly recent developments (circa 1940) with exceptional sensitivity. Amplification takes place within the tube and the intensity is indicated on a galvanometric scale.

Emission Spectra

These arise from the transfer of an outermost electron of an element from the ground state to an orbit of higher energy further from the nucleus. The energy for such a transfer is absorbed from the energy source - i.e. the flame. Emission of light which accompanies this release of energy is specific for each element and found at distinct wavelengths. This radiation is characterised by wavelength, frequency and wave number.

Units of wavelength are:-

1) Angstroms (Å) $1 \text{ Å} = 10^{-8}$ cms.

2) milli microns (mμ) $1 \text{ mμ} = 10^{-7}$ cms.

$\therefore$ $1 \text{ mμ} = 10 \text{ Å}$. (Delahay 1957)

In this work, all wavelengths are given in millimicrons.

The complexity of the spectrum emitted is dependent on the electronic configuration of the element. When a solution of a salt is introduced into a flame, a complex series of rapidly occurring reactions takes place (Dean 1960; Robinson 1960).

a) The water envelope of the droplet evaporates.

b) The remaining salt particle is heated and vaporizes to gaseous salt molecules.

c) These molecules are then dissociated by the heat of the flame into atoms.

d) Some of these atoms combine with the components of the flame.

e) Others become ionized.

It is this mixed population (ions, atoms and molecules) in the flame which give rise to the various components of the spectrum:- see below.

Atomic Spectra

These are produced when the electron of a neutral atom is excited to a higher energy level and on return to the resting state, the energy absorbed is released.

Resonance line - the most easily excited line of an element, the upper level of energy of which is lower than all others in the spectrum of that element.

Ionic Spectra

Such spectra arise where there is more than one electron in the outermost shell of an element. If during excitation the energy absorbed causes an electron to be released from the atom, then an ion is produced and an ionic line results. The energy required is equal to the ionization potential of the atom (measured in electron volts - e.v.). This spectrum is

unlike the neutral atom spectrum but resembles that of the element of preceding atomic number.

Band Spectra

Band spectra are emitted when a molecule is excited. Here there is possibility of excitation of energy levels other than electronic due to the movement of the constituent atoms of the molecule. Such spectra represent the sum of energy transitions and are therefore exhibited as a broad band extending through a considerable spectral range.

Continuous Radiation

This is a weaker, continuous background radiation derived from metals present in large quantities in the flame.

(Dean 1960)

- - - - - - - - - - - - - - - -

Emission of Spectrum of Calcium

There are five main 'peaks' in the emission spectrum of calcium. Measured in millimicron, these are:

Resonance line	Atomic Ca	422.7 mμ
	Ionic Doublet	393.3 mμ)
		)
		396.8 mμ)

$Ca(OH)_2$ + CaO band peaks	554 mμ 602 mμ 622 mμ	(Robinson 1960)

The excitation potential of calcium = 2.92 e.v. The blue line at 422.7 mu is clear of band systems and thus at this wavelength background is low. The region of maximum intensity for this line is closer to the inner cone of the flame. Calcium is generally estimated using an oxyacetylene flame with fairly high fuel gas pressure. (Dean 1960).

Emission Spectrum of Strontium

The peaks in the emission spectrum of strontium occur at the following wavelengths:

Resonance line	Atomic Sr	460.73 mμ	
	Ionic Doublet	407.8 mμ 421.6 mμ	
	Band peaks due to $Sr(OH)_2$	605 mμ 666 mμ 680 mμ	(Robinson 1960)

The excitation potential for strontium = 2.68 e.v. The

blue-green atomic line at 460.73 mμ is extremely strong. Maximum emission for this line occurs immediately above interconal gases and decreased rapidly up the flame mantle. Strontium is also estimated using an oxyacetylene flame but the proportion of fuel gas is lower than with calcium. (Dean 1960).

Various factors cause distortion in the relationship between the emission of an element and the concentration of the element in the solution. Such factors give rise to interference effects which may inhibit or enhance the emission intensity of the element.

Interferences

Several classifications of interference effects exist; the following list is gathered from many sources (Ahrens and Taylor 1950; Ikeda 1956: MacIntyre 1961; Dean 1960).

A) Spectroscopic Interferences

(1) Direct Spectral Interferences - these are caused by either background due to continuous spectra, which all metals emit when present in large amounts, or overlapping and

adjacent emissions where elements are present in the solution which emit at wavelengths similar to the wavelength of the required element.

Background emissions and variations must always be taken into account.

(2) Cationic Interferences - these generally lead to enhancement of the emission intensity and occur mainly in hot flames with alkali and alkaline earth metals. A high temperature flame gives rise to ionization in these groups with a resulting decrease in atomic spectra, due to fewer neutral atoms present, and an increase in ionic spectra. When small quantities of another easily ionized element are introduced in the test solution, the ions produced depress the ionization of the test element and thus more neutral atoms exist giving a stronger atomic line.

This enhancement may be compensated for by the addition of an excess of an easily ionized element such as cesium or by working with lower flame temperature.

(3) Anionic Interference - interference from this source is due

to the formation of difficultly vaporizable compounds of certain metals with certain anions. It is generally considered that the compounds formed are of the formula M.I.O., where M = metal investigated

I = interfering ion

O = oxygen from flame

The compounds are of high melting point and low volatility, more troublesome in low temperature flames. The results of such interferents is a reduction in emission intensity which can be corrected for by the addition of substances which complex with the metal and prevent formation of the refractory compound.

(4) Self Absorption This effect is due to absorption of emission from the excited particles in the centre of the flame by the ground state particles in the periphery. The result is a reduction in emission intensity which causes a non-reduction in emission intensity proportionally with the concentration of the element above a certain limiting concentration. Ground state transitions only are involved. This interference may be eliminated by dilution of the test solution.

B) Effect of Solution Properties

Where there is a difference in the physical properties of the test solution and the standard, correct results will not be obtained. Such factors as differing viscosity, density, volatility or surface tension may produce completely erroneous results.

Under such circumstances, the use of a lithium internal standard is effective.

C) Instrumental Factors

Other variables such as design of burner, region of flame in optical path, rate and mechanism of sample introduction, may have effect on intensity (West 1964).

Practical Aspects of Flame Spectrophotometry

A flame spectrophotometer is capable of estimation of any element which produces an emission spectrum, providing a suitable method of analysis may be obtained. It is important to determine the most useful spectral line for the element to be estimated. This is not necessarily the line of greatest emission intensity since such a line may be subject to interference from overlapping or adjacent emissions produced by other elements in the sample. The accuracy of any estimation is also governed by the care and correctness of dilution techniques. Operational variables such as

a) mechanical slit width,

b) relative fuel pressures,

c) sensitivity, must be carefully chosen to give the maximum ratio of signal to background emissions.

- - - - - - - - -

General Methods

All quantitative methods should employ the principle "the general chemical composition and physical properties of standards and unknowns should be the same or closely similar" (Ahrens and Taylor 1960).

In the work here described, two distinct methods of analysis have been employed, these being a radiation buffer technique as described by MacIntyre (MacIntyre 1961) and an additive standard method either following enrichment procedures (Harrison 1958) or directly on the sample.

a) Radiation Buffer Technique

Since the mutual interference between two elements tends to reach a maximum for large amounts of interferent, inclusion of a large quantity of interferent in both standards and sample solutions swamps the effects of the cations and anions in the sample. Such methods have been employed for a large range of samples by many workers (Mitchell and Robertson 1936; Hegemann et al. 1954).

b) Additive Standard Technique

In this method, two solutions of the sample are estimated, one of which contains a known quantity of a standard solution of the required element. This is the additive standard solution (D_2). The concentration of the unknown solution is determined by the proportion of the emission intensity of one solution above the background emission, to that of the other. Backgrounds are read at $\pm$ 5 mu on either side of the analysis line using the both solutions of the unknowns.

If D_1 = emission of unknown solution,

and D_2 = emission of unknown solution + x ppm. of standard solution,

and D_m = average of emissions of both D_1 and D_2 when read at $\pm$ 5 mu on either side of the analysis line,

and X = concentration of unknown solution,

then $$\frac{D_1 - D_m}{D_2 - D_m} = \frac{X}{x + X}$$

$$\therefore \quad \frac{(D_1 - D_m)x}{D_2 - D_1} = X$$

Since only micro-volumes of the standard solution are added, this does not appreciably alter the solution characteristics. The incorporation of a minute quantity of a non-ionized detergent allows for the reduction of surface tension in the micropipette and the complete addition of the standard.

SECTION III

EXPERIMENTAL

With the exception of one small group of urine samples for strontium, all samples were estimated for strontium and calcium by flame spectrochemical procedures. Since the instrument was newly acquired at the start of this series of investigations, considerable time was spent in acquiring a working knowledge of the Zeiss P.M.Q. II flame spectrophotometer. Several of the spectrochemical experiments are described before the biological studies are considered.

Flame Spectrochemical Analysis

All standard solutions contained the standard cations in spectroscopically pure form and all water was deionised of not less than 4 megohms resistivity as measured on the Elgastat purity meter. The spectral lines employed were those recommended by other workers i.e. for calcium, Atomic line at 422.7 mu (MacIntyre 1961), for strontium, Atomic line at 460.7 mu (Harrison 1958).

Determination of Optimum Fuel Gas Pressure

The oxygen pressure was set at 0.3 Kg./sq. cm. and the acetylene pressure varied to give a maximum value for the ratio: $\frac{\text{emission due to element}}{\text{emission due to flame}}$ for solutions of both strontium and calcium (Alcock et al. 1960). Both experiments were carried out using a slit width of 0.02 mm. and the atomic line for each element.

a) A strontium standard solution of concentration 2 ppm. Sr was aprayed at acetylene pressures varying from 100-130 mm. water pressure. The instrument was operating at a sensitivity of 7/10/2 and the solution emission was read at

peak wavelength while the background emission was that of the solution measured at ± 5 mu on either side of the maximum emission. The ratio of signal to background for each pressure was calculated and is given in Section IV.

b) A similar experiment was carried out for calcium, using a standard solution of concentration 10 ppm. Ca. Acetylene pressures were varied from 100-250 mm. water pressure. In this case, since the solutions were not aqueous, but radiation buffer solutions, the flame background was read at peak wavelength using the standard solution of the blank.

The sensitivity was maintained at 4/1/2 throughout.

Instrumental Drift

Soon after the spectrophotometer had become operational, it was noted that galvanometer readings for any specific solution showed a definite reduction with progressing time. This was true of all solutions of any concentration, other than deionised water.

a) Solutions of 20, 14, 12 and 8 ppm. Ca were sprayed at 10 minute intervals for a period of 2.5 hours, and the resulting readings noted. A sample of deionised water was sprayed at the same time.

b) When other calcium solutions of concentrations = 10, 6 and 2 ppm. were sprayed, a similar effect was noted. If, however, the flame was extinguished at the end of 60 minutes on relighting 100 minutes later, the galvanometer readings obtained were most interesting.

Determination of Optimum Slit Width

In the above experiments, it was noted that constant galvanometric readings could be maintained if the slit width was increased with time. This was further investigated with the following experiments:

a) Strontium solutions of 20, 10, 5, 2, 1 and 0.2 ppm. were sprayed at slit widths of 0.02 mm. and 0.01 mm. Sensitivity was 10/1/2 and 10/10/2, respectively.

b) Aqueous solutions of concentration = 6, 2 and 1 ppm. Sr together with a sample of deionised water, were sprayed at 15 minute intervals, using constant full sensitivity (10/10/2) and increasing the slit width from 0.01-0.02 mm.

Determination of Calcium

In biological fluids, both sodium and phosphate are present

in large amounts, giving rise to cationic and anionic interference effects respectively. The effect of sodium is minimised by estimating calcium from its blue atomic line using narrow slit widths. Phosphate interference is overcome by addition of excess phosphate to both standards and samples and the use of high temperature oxyacetylene flame.

The method used for the estimation of calcium in this survey was that of MacIntyre (1961). The standards employed were, however, measured in parts per million (ppm.), ranging from zero to 20 ppm. Ca.

Operational Procedure: The flame spectrophometer meter assembly is allowed to warm up to working temperature before setting the electric zero point. The oxygen pressure is adjusted to 0.3 Kg./sq. cm. and deionised water sprayed to check atomization. Then the acetylene pressure is adjusted to 150 mm. W.S. and after 30 seconds, the mixture of gases ignited. The slit width is now opened to 0.02 mm. A standard solution of 20 ppm. Ca is introduced and the wavelength setting adjusted to 422.7 mu. By variation of sensitivity settings, this emission is adjusted to give a scale deflection of 100 divisions. The remaining standard solutions are now sprayed in order using the deionised water reading to check the flame emission.

The samples may now be estimated. These have already been diluted either 10 or 20 fold with diluting fluid containing a deproteining agent. The order of spraying is deionised water, sample (to check approximate concentration), standard below sample, sample, standard above sample, deionised water, standard above sample, sample, standard below sample, and finally, deionised water once more. By spraying in this order and bracketing the sample between two adjacent standards, any fluctuations of the flame emission are compensated for.

A stock solution of bone ash of 38% calcium is estimated at the beginning and end of each series of determinations. All samples are estimated from duplicate prepared solutions, diluted in duplicate giving four estimations for each unknown.

Preparation of standards and reagents involved are detailed in the appropriate appendix to this work (No. 1).

Calibration of Standards

When newly prepared, the standards are sprayed under the above conditions and a graph of intensities minus blank against concentration drawn. This is found to be linear as shown in the next section. Furthermore, successive dilutions from the stock bone ash solution are estimated for calcium before new

standards are used to estimate an unknown solution. The accuracy of estimation of the bone solution must be within $\pm$ 2.5%.

Preparation of biological samples for estimation of calcium is described in the appendix. Intercomparisons of calcium results are given in the next section (No. IV).

Determination of Strontium

At a wavelength of 460.7 mμ strontium emits a strong resonance line with fairly low excitation potential, allowing for the use of lower temperature flame and reducing the cationic interference effect.

The method used for the estimation of strontium was that of Harrison (1958) which entails a lengthy separation procedure, described in appendix II. Because of the design of the flame spectrophotometer used, the author found after some experimentation that most samples could be estimated directly on acid solutions of the samples.

Procedure: The spectrochemical technique is the same whether the sample has been chemically separated or not. For strontium, however, the fuel gas pressure is only 115 mm. W.S. and the spectral line employed, 460.7 mu. The peak wavelength is found by spraying an aqueous strontium solution of 2 ppm. concentration, at a

sensitivity of 6/10/2.

Two solutions of the unknown are employed, in one of which a microvolume of an aqueous strontium standard is included to give a standard concentration of 2 ppm. Sr (additive standard solution D_2). Both solutions are sprayed at peak wavelength and also at $\pm$ 5 mu on either side of peak wavelength. The concentration of the unknown solution is obtained by proportion as explained in the previous section (page 55). All samples are estimated in triplicate and the sensitivity so chosen as to give a scale deflection of 30-40 for D_1 compared to 90-100 for D_2.

Calibration of Strontium Standards

a) Dilutions from an accurately prepared strontium standard of 0.0-5 ppm. Sr are sprayed at 460.7 mu and at $\pm$ 5 mu on either side of this wavelength. Sensitivity is unvaried throughout. The results are graphed and shown in the next section.

b) A bone solution of known strontium concentration is also estimated, as in calcium calibration, using each new set of standards.

Preparation of samples for analysis is described in appendix IV and various intercomparisons given in the next section.

Biological Investigations

The strontium content of any biological tissue is always considered with regard to its calcium content, thus it is most important that this should not vary in any one sample. Urine samples, if estimated when received, always give a higher value for calcium than if left for some time. This is because calcium remains in solution when the pH. is low, whereas in alkaline urine, on standing, it is gradually precipitated.

The effect of Acidifying Urine

A 24 hour urine specimen of volume = 860 mls. and pH. = 7 was divided into two equal volumes of 430 mls. Urine A. had 5 mls. conc. HCl added, while 5 mls. of deionised water was added to the second specimen. Duplicate analysis for calcium by flame spectrophotometry were carried out on each specimen for one month, by which time the unacidified urine calcium had become reduced considerably while the acidified urine remain reasonably constant. The acidified urine had a pH. of 2 throughout this time, while the unacidified urine became slightly less alkaline. After several months, the unacidified urine calcium had fallen to less than 1/3 of the original value.

At this point, four 10 ml. samples of each urine were removed and evaporated to dryness in silica crucibles over a bunsen flame, taking care to prevent spluttering or boiling over. These were then ashed at 650°C to destroy phosphate and the ash dissolved in the minimum volume HCl/HNO_3, then transferred to 10 ml. volumetric flasks and made up to the mark with deionised water.

These solutions were now diluted tenfold with diluting fluid and estimated according to MacIntyre's calcium method (MacIntyre 1961). Table VIII.

Strontium - Calcium Balances

Various studies on the effect of increased dietary calcium on strontium metabolism have been conducted in both animals and adults. Macdonald et al. (1954) investigated the effect of dietary calcium on the deposition of Sr-90 in the skeleton. The secretion of strontium into milk under high dietary calcium has been studied by Comar and Wasserman (1956) and Spencer-Laszlo et al. carried out a series of balance studies on young adults (Spencer-Laszlo et al. 1963). Little is known, however, on the effect of such an increase in the strontium-calcium balance in children. An investigation of this nature was begun in late 1963 using young patients in the hospital as subjects. Each

child was on balance study in a special metabolic ward attached to Ward 1. for approximately one month. During this time, the composition of the basic diet was kept reasonably constant and any drugs etc. recorded. The balance study was divided into four distinct periods of not less than six days, each period ending at midnight on a day in which a stool had been passed. These periods are explained below.

Period I:- during this time the child received a diet suitable for his/her age. Any obvious dislikes could be eliminated at this point. All faecal and urinary specimens were collected, although not analysed, in order to estimate approximate amounts of these specimens to be expected.

Period II:- this period was similar to I above but now duplicate diets were obtained from the diet kitchen and together with any ingested drugs, analysed for strontium and calcium. All urine and faecal specimens were collected and analysed.

Period III:- an additional 1 gm. Ca per day was given with the diet. This was a period to allow for a new mineral metabolic equilibrium to be attained. Excretory specimens were collected but not analysed.

TABLE V STRONTIUM-CALCIUM BALANCES CASES INVESTIGATED

(1) E.C. (M). Age = 11½ yrs; Wt. = 34Kg; Ht. = 141 cm.
Diagnosis:- Primary Tuberculosis; Therapy:- P.A.S. I.N.A.H. Streptomycin
wks. 3 and 4 - Additional Calcium (as carbonate) = 1 gm/day

(2) J.A. (F). Age = 10 yrs; Wt. = 23.5Kg; Ht. = 120 cm.
Diagnosis:- Acute Rheumatism; Therapy:- ASPIRIN, Penicillin G.
wks. 3 and 4 - Additional Calcium (as carbonate) = 1 gm/day

(3) B.M.(M). Age = 9 yrs; Wt. = 27.5Kg; Ht. = 136 cm.
Diagnosis:- Acute Rheumatism; Therapy:- ASPIRIN, Penicillin G.
wks. 3 and 4 - Additional Calcium (as carbonate) = 1 gm/day

(4) E.McI. (F). Age = 12½ yrs; Wt. = 38Kg; Ht. = 139 cm.
Diagnosis:- Rheumatic Fever; Therapy:- ASPIRIN, Penicillin G.
wks. 3 and 4 - Additional Calcium (as carbonate) = 1 gm/day

(5) J.McD. (M). Age = 13½ yrs; Wt. = 29Kg; Ht. = 139 cm.
Diagnosis:- Acute Rheumatism; Therapy:- ASPIRIN, Thyroxine, Penicillin G.
wks. 3 and 4 - Additional Calcium (as phosphate) = 1 gm/day

(6) K.McG. (F). Age = 6½ yrs; Wt. = 22Kg; Ht. = 120 cm.
Diagnosis:- Encephalomyelatis; Therapy:- NO DRUGS
wks. 3 and 4 - Additional Calcium (as phosphate) = 1 gm/day

(7) L.C. (F). Age = 9½ yrs; Wt. = 25.8Kg; Ht. = 126 cm.
Diagnosis:- Tubercular Meningitis; Therapy:- Streptomycin; I.N.A.H.
wks. 3 and 4 - Additional Calcium (as phosphate) = 1 gm/day

(8) A.D. (F). Age = 10y. 5m; Wt. = 26.7Kg; Ht. = 135 cm.
Diagnosis:- Primary Tuberculosis; Therapy:- P.A.S. Isoniazid, I.N.A.H.
wks. 3 and 4 - Additional Calcium (as phosphate) = 1 gm/day

(9) S.C. (M). Age = 4y. 9m; Wt. = 18.6Kg; Ht. = 108.6 cm.
Diagnosis:- Primary Tuberculosis; Therapy:- P.A.S. Streptomycin, I.N.A.H.
wks. 3 and 4 - Additional Calcium (as phosphate) = 1 gm/day

P.A.S. = Para - amino - salicylic acid

ASPIRIN = Acetyl - salicylic acid

Period IV:- supplementary calcium was continued as in Period III. Duplicate calcium samples were added to the duplicate diets after allowance had been made for any uningested calcium in the medicine glass. All faecal and urinary specimens were collected, measured and analysed.

Choice of Subjects

The children studied were nine hospitalized cases of both sexes aged from 4 years 9 months to 13 years 6 months. These were either convalescent or chronically ill but without kidney abnormality, continent, capable of co-operation and likely to be in hospital for a period of not less than six weeks. Their parents were told of the proposed investigation and were at liberty to withhold permission. As can be imagined, it was most difficult to find children satisfying all these criteria and indeed the youngest child required practically constant supervision. For these reasons, only nine such studies were completed. Details of the completed cases are to be found in Table V, which includes diagnosis, drugs and calcium compounds given.

Collection and Treatment of Samples -

a) Diet: During periods 2 and 4 of these balances, the total dietary constituents ingested each day were obtained from the diet kitchen the following day. These were collected and transported to the laboratory in a covered polythene beaker where they were then homogenized. The resulting mixture was put into an evaporating basin and heated to dryness in an oven at 110°C. This was then ashed in an electric muffle furnace at 550°C-600°C. All ash for a particular period was combined and the total ash weight obtained. Any supplementary constituents were added before homogenization.

b) Faeces: Faecal samples were collected in a polythene covered bed pan and dried in an evaporating basin on an electric hot plate. When completely dry, the evaporating basin with complete collection for that particular period was ashed at 550°C-600°C.

c) Urine: Successive, twenty-four hour specimens for each period were collected in winchester bottles which had been washed with acid and then rinsed in deionised water before drying. The volumes were measured and 5 mls. of concentrated HCl added. The total acidified urine for each period was bulked and stored in covered polythene buckets.

Chemical Treatment of Samples

a) Diet and Faeces:- Accurately weighed samples of diet and faecal ash were processed in triplicate for the separation of strontium according to Harrison's method, using Sr-85 as a "spiking" agent for the estimation of chemical recovery (Appendix II). The separated strontium was then estimated by flame spectrophotometry.

Separate duplicate samples of ash were removed and accurately weighed for estimation of calcium. These were dissolved in a minimum volume of HCl/HNO_3, diluted to volume and further diluted tenfold with diluting fluid for estimation on the flame spectrophotometer.

b) Urine:- Urine samples were precipitated as phosphate by the addition of orthophosphoric acid and the supernatant decanted off. The phosphate precipitate was ashed 550°C, dissolved in 2 N HCl and thereafter the separation for strontium carried out as for solid samples.

Urine was measured directly for calcium using MacIntyre's method.

Interlaboratory comparisons of results for strontium and calcium in these samples are given in section IV.

Direct Spectral Analysis for Strontium

Because of the time-consuming separations involved in the previous estimations for strontium and since the instrument in use had an extremely high sensitivity and employed an integral-atomizer burner, capable of consuming fairly acid solutions, it was decided to attempt estimations for strontium in biological samples without preliminary separation.

Procedure: Diet and faeces - Accurately weighed, triplicate samples of ash which had previously been estimated by the longer method were dissolved in the minimum volume of HCl/HNO_3 in volumetric flasks which were made to volume with deionised water. These solutions were the D_1 components of the additive standard method.

Urine - Two samples of urine which had previously been estimated using Harrison's separation method were estimated directly by the additive standard method. In this case, D_1 = the acidified, but otherwise untreated, urine.

The results obtained are contrasted in section IV (Table X1).

This method was particularly useful since it eliminated the spiking procedure and thus was unlikely to cause contamination of samples being estimated in the same laboratory for very low levels of radio-strontium.

Investigation of Non-Ionic Detergent

The volumes of additive standard used are extremely small, being in the micro range and therefore the inclusion of a surface tension reducant in the 200 ppm. Sr standard is beneficial. The proposed detergent was in non-ionic form but was nevertheless investigated for strontium and calcium content, as detailed in section IV.

Excretion of Natural Strontium via the Kidney

Much work has been done on strontium metabolism in both adults and animals but much remains to be elucidated with regard to the metabolism of strontium in children. Very few figures for the excretion of strontium and calcium in children's urine are available (Bedford et al. 1960). Such figures give some indication of the differential treatment of these cations by the kidney. With the abbreviated method described above, these estimations were much simplified. Some forty-five samples have

been estimated by this method for strontium and for calcium by MacIntyre's method.

All such samples were collected in specially cleaned winchester bottles, the volume measured and acidified with conc. HCl.

Investigation of one case of Urinary Calculus

The calculus from a case of Urinary Calculus in a three-year old girl was investigated. The particle of stone was extremely small and somewhat hygroscopic and had been received from the biochemistry department of the hospital. Since neither the total weight of the calculus or the amount of water absorbed could be ascertained, absolute values for strontium and calcium content could not be obtained. The portion was dissolved in acid in a volumetric flask and the solution made to the mark with de-ionised water. This solution was used for the estimation of both strontium and calcium by the abbreviated, additive standard method and MacIntyre's method respectively.

Comparison of the Natural Strontium Content of Urine estimated by two Distinct Methods

Eight samples of children's urine, which had previously been

estimated using the abbreviated additive standard method were investigated for natural strontium content by neutron activation analysis procedure as described by Harrison and Raymond (1955). The activation of the samples was carried out in the Scottish Universities Research Reactor at the National Engineering Laboratories, East Kilbride, Scotland. The urinary strontium was activated to Sr-87 m. ($t_{\frac{1}{2}}$ = 2.8 hrs.) by neutron bombardment at a flux of 10^{12} Neutrons/cm²/sec. for approximately 2.8 hrs. Duplicate 5 ml. samples were employed and before activation, these were dried at 110°C and then ashed at 550°C in small silica tubes. During activation, a sample and standard of accurately weighed 'spec-pure' strontium carbonate were in the same container and therefore the same position in the reactor. After activation, the standards were dissolved in 2N HCl and diluted to volume. The strontium in the urine samples was obtained after a lengthy separation procedure employing precipitation with carriers (Appendix III).

The radioactivity of both sample and standard was determined using a scintillation counter and pulse height analyser. All activity was corrected for decay from a given zero time.

The results obtained are presented, together with those obtained by flame spectrophotometry, in the next section (Table XIII).

Effect of Vitamin D on Strontium-Calcium Metabolism

Vitamin D is thought to regulate calcium and phosphate balance by direct action on phosphate metabolism (Documenta Geigy 1962) with a resulting increase in the mobilization of bone salts (Bauer et al. 1956). From the similarity in metabolism of strontium and calcium, an effect on strontium metabolism is also to be expected.

Calcium, strontium and phosphorus balances were carried out on two suspected rachitic children. In one of these cases, a second balance was studied after treatment with 100,000 I.U. Vitamin D. Samples were collected as described in the previous balance studies. The periods of collection were of varying duration but results given are corrected for 24 hours.

a) Diet was collected, homogenized, dried at 110°C and ashed at 500-600°C. Calcium and strontium were estimated spectrochemically on the Zeiss P.M.Q. II and absolute values for total ash weight calculated.

b) Faeces were collected, dried on a hot plate and ashed at 550°-600°C. Total ash weight was obtained. Calcium and strontium content was again determined spectrochemically.

c) Urine was measured and acidified. Calcium and strontium were determined directly by flame spectrophotometric procedures.

The phosphorus content of all samples was obtained from the biochemistry department of the hospital. The method used was a colorimetric one and samples were measured on an Eel colorimeter with red filter (Appendix V).

The Natural Strontium Content of Children's Bones

In contrast to the lack of information on renal excretion of natural strontium in children, much information is available for the natural strontium content of children's bones (Bryant et al. 1964). This is possibly because the estimation of strontium and calcium in bone is reasonably simple compared to urine. In conventional photometry using larger slit widths and having lower sensitivity, the sodium interference in urine analysis is considerable, whereas the relative sodium content of bone being so much lower allows for the easier estimation of the alkaline earth constituents of bone on simplier instruments.

Procedure:- Accurately weighed duplicate samples of bone ash were dissolved in the minimum volume of HCl/HNO_3 and made

up to the required volume with de-ionised water. As before, these solutions represent D_1, while D_2 was prepared from them by addition of solutions D_1 to accurately pipetted micro-volumes of standard strontium solution to give the required volume. These solutions were used to estimate the strontium content of bone by additive standard method.

Accurately weighed duplicate bone ash samples were dissolved in acid as above made to volume and diluted with diluting fluid for estimation of calcium by MacIntyre's method.

Duplicate analysis of bone by these methods and at Capenhurst laboratories gave most encouraging results (Table XVl). These results established this method of investigation and gave credence to the results obtained in the one sample of osteo-genic sarcomatic bone investigated.

Natural Strontium Content of Children's Teeth

In this survey, aggregates of whole, unashed teeth were investigated. These teeth were dissolved in dilute nitric acid and the acid solution estimated for strontium and calcium as in methods described for bone.

Studies in Infants (overleaf)

Most of the foregoing biological studies have been on older children. In infants, metabolic factors are much different. Milk, with its lower Sr/Ca ratio, but greater availability than some other foodstuffs, forms the complete diet of the young baby.

Natural Strontium and Calcium Content of Infants' Diet

Four samples of synthetic milk ingested in 24 hours by babies in the hospital were analysed. The analysis were conducted in triplicate. The milk was first evaporated to dryness in a silica crucible and then ashed at 600-700°C in an electric muffle furnace to destroy phosphate and organic constituents. The ash was dissolved in acid and reconstituted with de-ionised water. The resulting solutions were then used for analysis for strontium and calcium by flame spectrophotometry.

Comparison of Dietary Intake and Urinary Output of Natural Strontium and Calcium in Infants

Studies over 48 hours were carried out on five male infants up to six months old. These babies were given milk diets, duplicates of which were analysed for strontium and calcium following the above procedure. The total urinary

output over this period was collected by catheter into polythene bottles. This volume was measured and acid added to bring the pH to 2.

Triplicate analysis were carried out on both milk and urine samples. Strontium and calcium were estimated by flame spectrophotometric procedures.

Natural Strontium and Calcium Content of Glasgow Tap Water

A supply of drinking water in the hospital was investigated for strontium and calcium content. The importance of water in the reconstitution of infant feeds has already been mentioned (page 7). The water was estimated directly for calcium after dilution with diluting fluid, but a ten-fold concentration was used for strontium estimation.

- - - - - - - - - - - - - -

In this section, the basic principles of the analytical procedures are given but more detailed chemical methods may be found in the appropriate appendix.

SECTION IV

RESULTS AND CONCLUSIONS

DETERMINATION OF OPTIMUM FUEL GAS PRESSURES

TABLE VIa STRONTIUM: Using aqueous strontium standard, conc. = 2ppm. Sensitivity = 7/10/2. Oxygen Pressure = 0.3 Kg/sq.cm.

Acetylene Pressure	Background	Signal	Ratio : $\frac{\text{Signal}}{\text{Background}}$
100	31.0	61.0	1.97
105	32.0	64.0	2.0
110	31.0	68.0	2.2
115	32.0	71.0	2.22
120	33.0	73.0	2.2
125	36.0	77.0	2.14
130	39.0	83.0	2.13

TABLE VIb CALCIUM: Using calcium standard solution, conc. = 10ppm. Sensitivity = 3/1/2. Oxygen Pressure = 0.3 Kg/sq.cm.

Acetylene Pressure	Background	Signal	Ratio : $\frac{\text{Signal}}{\text{Background}}$
100	2.0	15.5	7.75
125	2.25	33.5	15.0
150	2.5	44.0	17.6
175	2.75	46.5	17.0
200	3.0	48.0	16.0
225	7.0	56.0	8.0
250	11.0	66.5	6.0

PHOTOMETRIC STUDIES

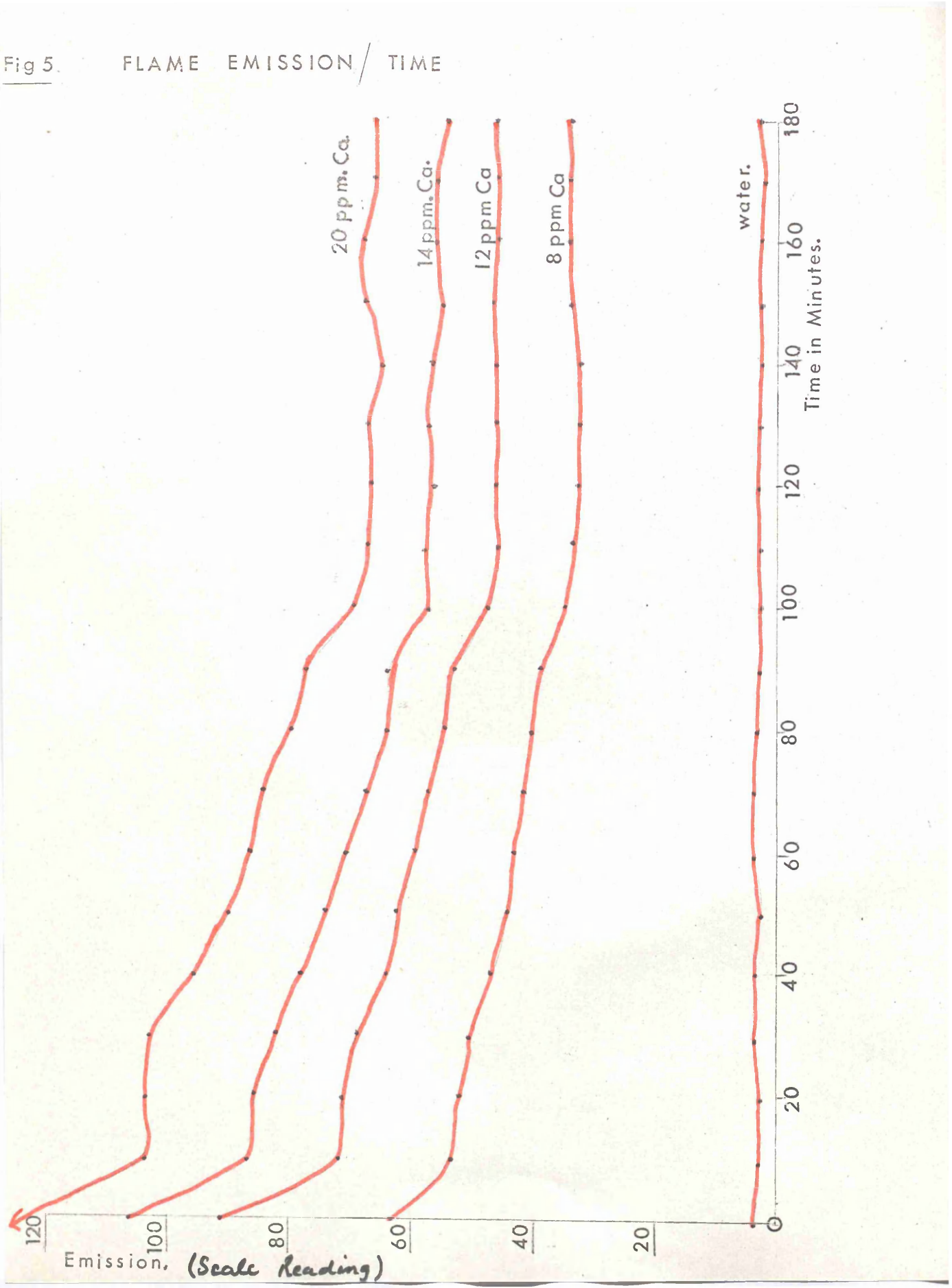
Determination of Optimum Fuel Gas Pressures

The temperature of the flame and consequently the energy of the flame may be altered by varying the fuel gas pressure. Since the energy of excitation (the excitation potential) of any element is specific for that element, it is essential that the optimum fuel gas pressure be determined.

The results of two such experiments as described in section III of this work are shown opposite (Tables VIa and VIb).

Conclusion: The optimum fuel gas pressure for strontium was equal to 115 mm. water pressure while that for calcium was found to be 150 mm. water pressure. Such a result is to be expected since the excitation potential for calcium exceeds that of strontium.

Fig 5. FLAME EMISSION / TIME



Instrumental 'Drift'

a) A graph of the galvanometric readings at 10 minute intervals for a series of calcium standards is shown opposite. (Fig. 5).

Result:- It can be seen that after a period of 110 minutes the emission readings for all calcium solutions as read on the galvanometric scale were reduced by more than 40% of the initial emission reading.

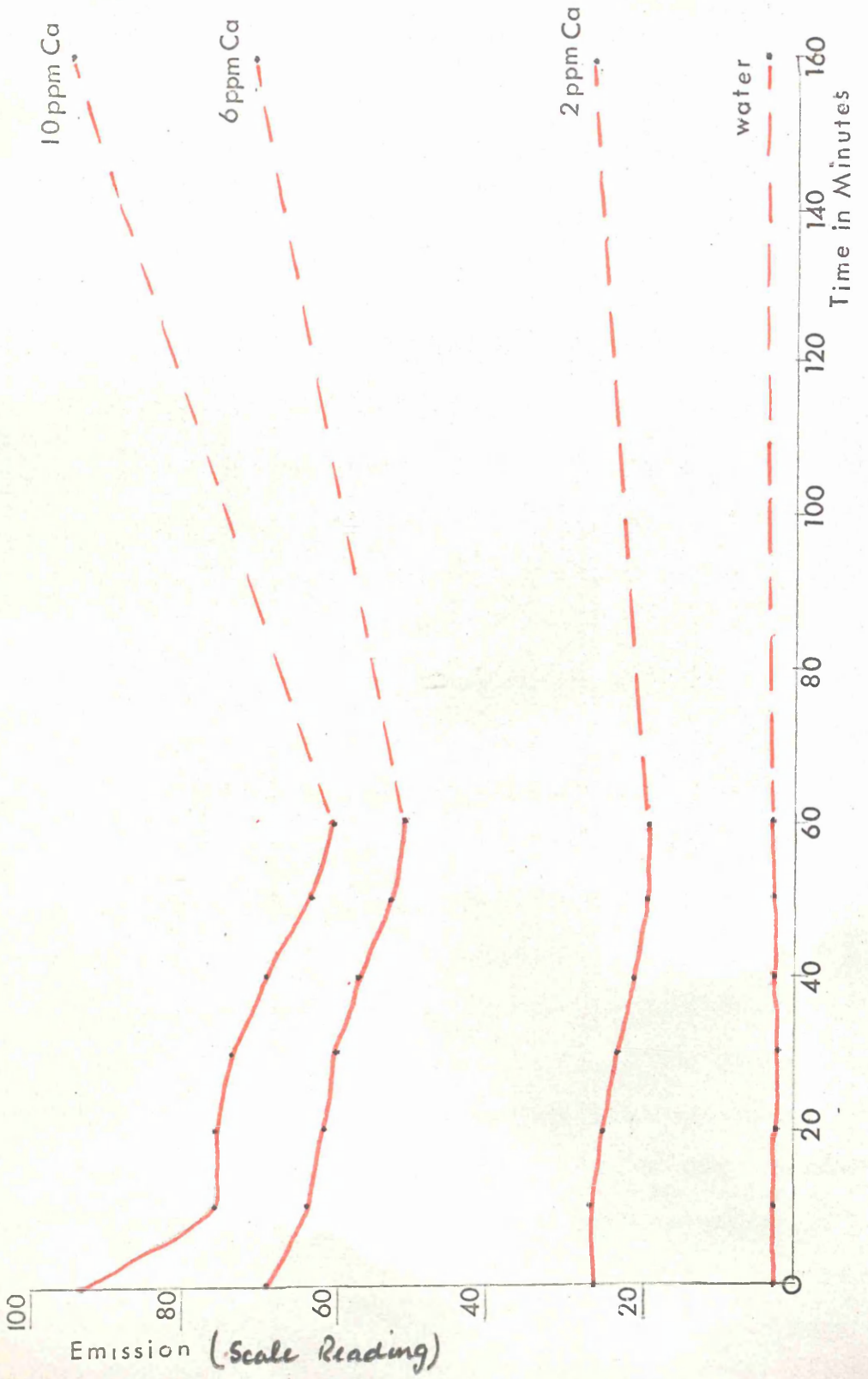
No such effect on de-ionised water was noted.

b) The effect of extinguishing the flame after one hour is graphically shown overleaf. (Fig. 6).

Result:- When the heating effect of the flame is removed, no further reduction in emission reading takes place although all electronic devices are still operational. As in the previous experiment, therewas little or no effect on the de-ionised water readings. On relighting the flame the original reading is obtained.

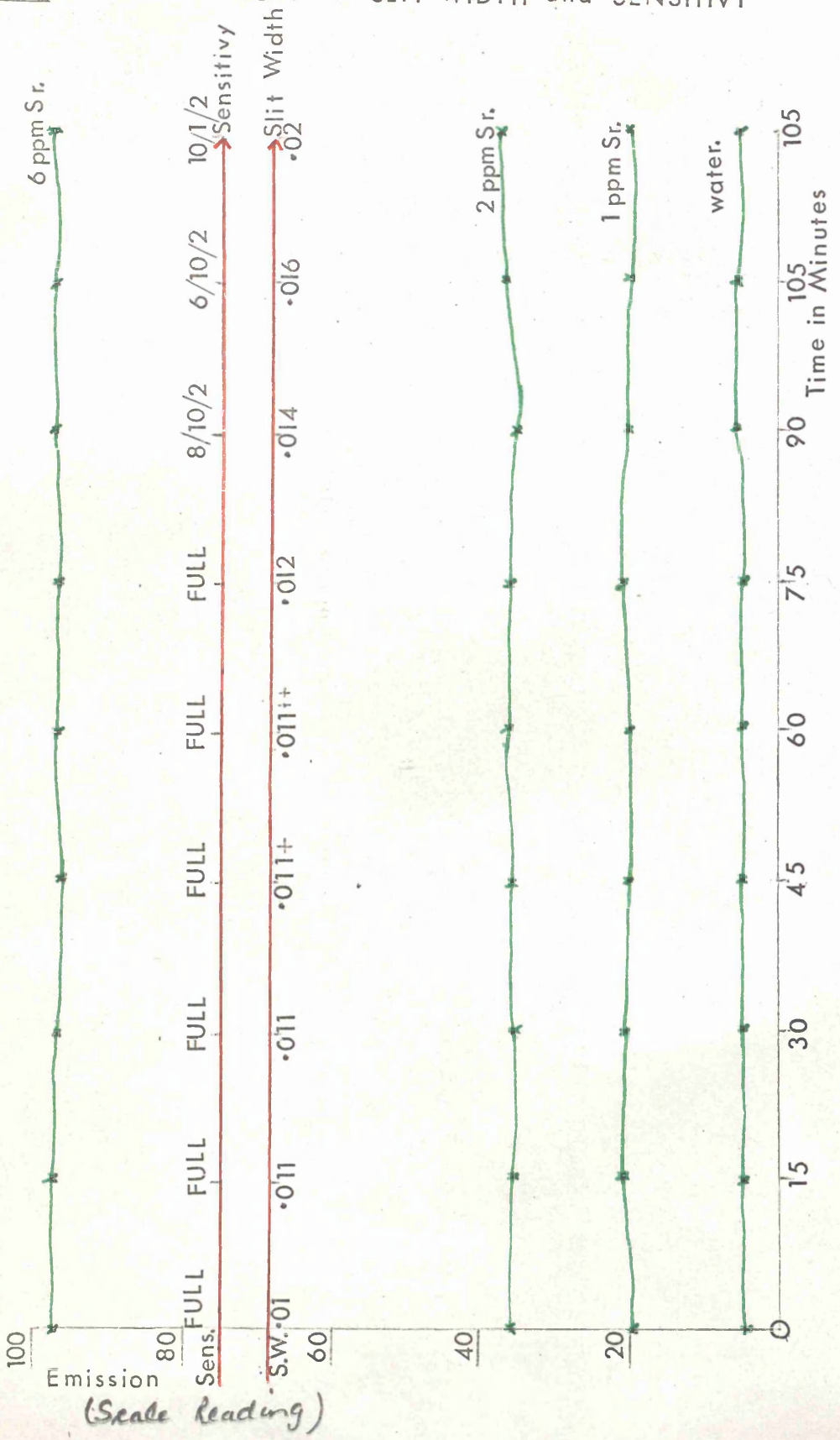
Conclusion:- The effect would appear to be due to the heating effect of the flame and to give rise to a diminishing of a specific spectral line intensity. Possibly due to contraction of the effective slit width.

Fig 6. EFFECT of EXTINQUISHING the FLAME



An intermediate optical device between the burner housing and the slit width was installed (see Fig. 3) and this all but eliminated the deleterious heating effect of the flame.

Fig 7 VARIATION of SLIT WIDTH and SENSITIVY



Determination of Optimum Slit Width

a) Results of a series of aqueous strontium standards emissions at two different slit widths and sensitivities are shown below (Table VII).

Conc. of Sr in ppm.	20	10	5	2	1	
Emission Intensity	100	45	22	15	10	Slit width = 0.02 mm. Sens. = 10/1/2
Emission Intensity	100	45	21	15	10	Slit width = 0.01 mm. Sens. = 10/10/2

Wavelength = 460.7 mu.

Acetylene press. = 115 mm. W.S.

Oxygen press. = 0.3 Kg/sq. cm.

Thus it can be seen that for a slit width of 0.01 mm. full sensitivity is required to equalize readings at a sensitivity = 10/1/2 and a slit width = 0.02 mm.

b) The effect of increasing slit width as emission intensity diminished with time is shown opposite (Fig. 7). From this it can be seen that the fall in emission intensity readings may be compensated for by increasing the slit width. Such

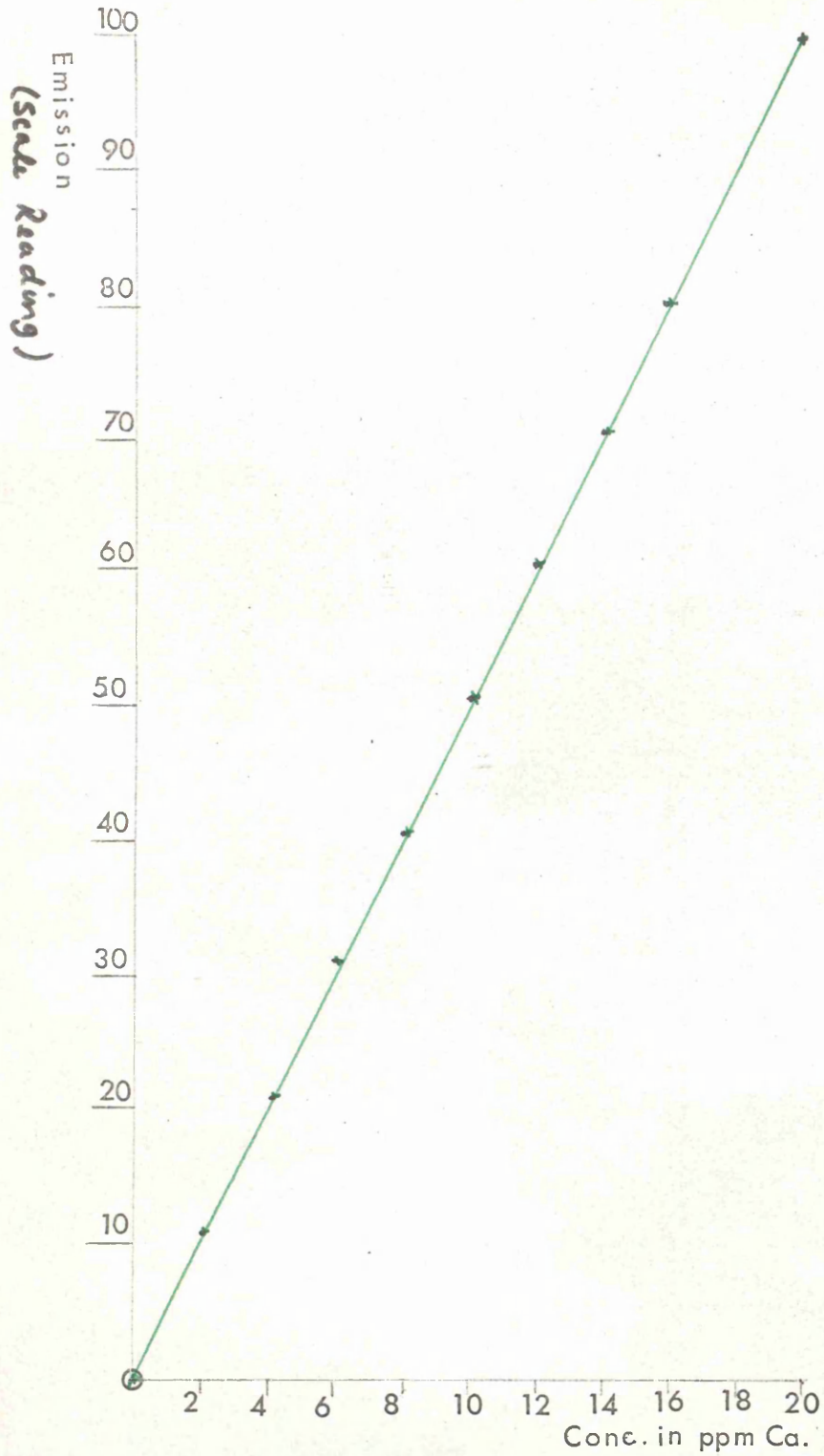
a procedure is to be avoided as constant conditions for investigations are not then possible. From these results it can be seen that at a slit width of 0.02 a considerable reduction in the required sensitivity takes place.

- - - - - - - - - - - - - -

Conclusions: From the foregoing set of experiments, optimum operational conditions for the Zeiss P.M.Q. II flame spectrophotometer when estimating calcium and strontium are as follows:-

	Wave-length in mu.	Slit width in mm.	Oxygen Pressure	Acetylene Pressure	Sensitivity
For estimation of calcium	422.7	0.02	0.3 Kg/ sq. cm.	150 mm. W.S.	Dependent on concentration of unknown solution
For estimation of strontium	460.7	0.02	0.3 Kg/ sq. cm.	115 mm. W.S.	Dependent on concentration of unknown solution

Fig 8 CALIBRATION of CALCIUM STANDARDS.



Calibration of Standards for the Estimation of Calcium

A typical graph of emission intensity/concentration for calcium standards is shown opposite (Fig. 8). Such standards are prepared as described in Appendix I. The graph is seen to be linear, obeying the law

$I \propto C$ or $I = KC$

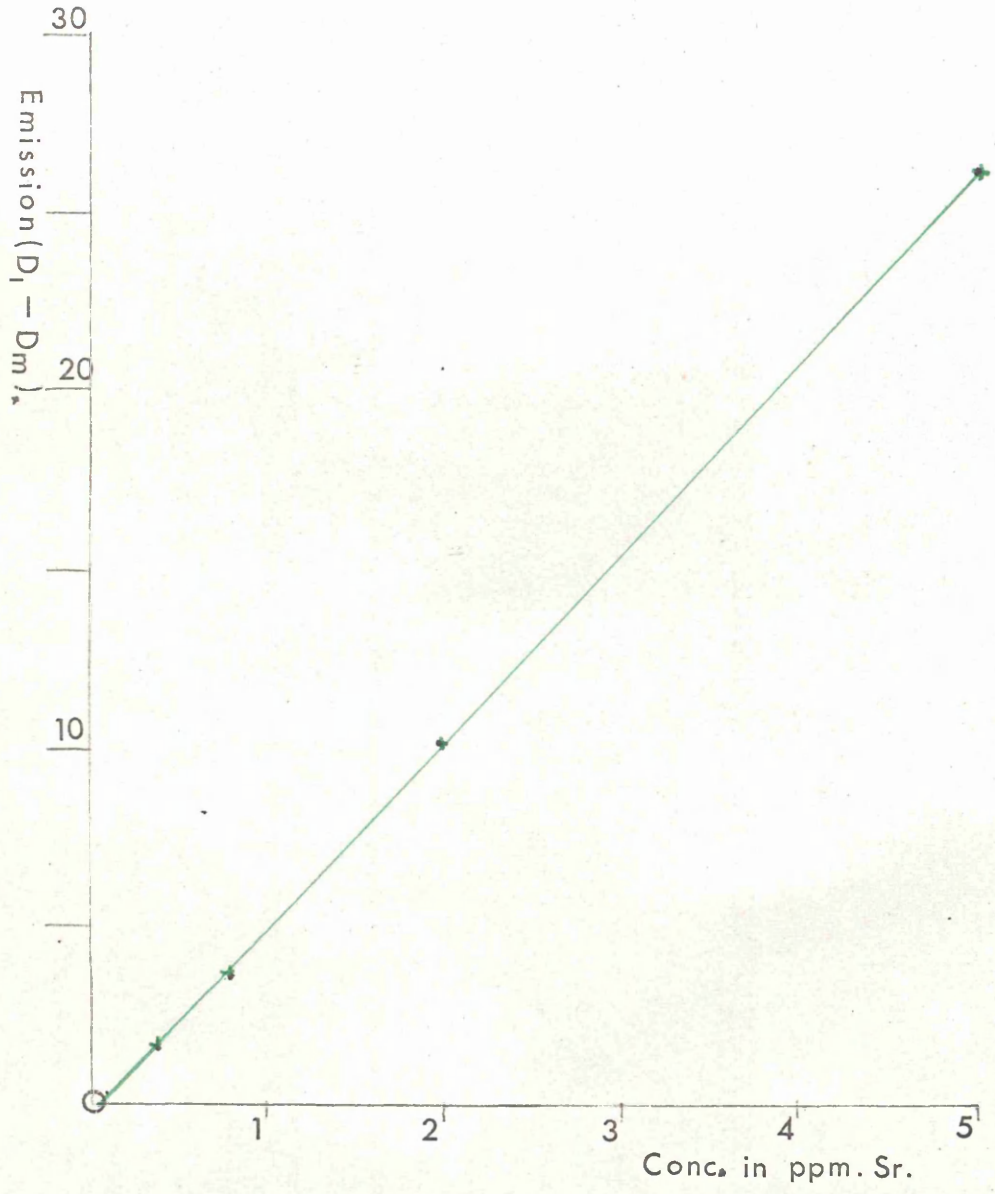
I = emission intensity.

K = constant.

C = conc. of calcium in the standard.

Various dilutions of a bone ash sample previously estimated by the oxalate precipitation methods (Bryant et al. 1959) and found to have a calcium concentration of 38% were estimated using each new set of standards.

Fig 9 CALIBRATION of STRONTIUM STANDARDS.



Calibration of Standards for the Estimation of Strontium

Flame emissions of dilutions from an accurately prepared 200 ppm. strontium standard as described in Appendix II were graphed as shown opposite (Fig. 9). The graph was found to be linear to above a concentration of 5 ppm. strontium, provided correction was applied for emission of the solutions at $\pm$ 5 mu on either side of the emission peak at 460.7 mu.

Estimations were controlled so that the unknown solution plus additive standard did not exceed this concentration since a slight deviation from linearity was noted at concentrations exceeding 5 ppm. strontium.

As in calibration calcium standards a bone solution of known strontium concentration was estimated using each new set of standards.

- - - - - - - - - - -

Conclusions: Provided conditions explained above are met, the methods for the estimation of both strontium and calcium give a linear relationship for the concentration of Solution/Intensity of Emission both in pure and biological solutions.

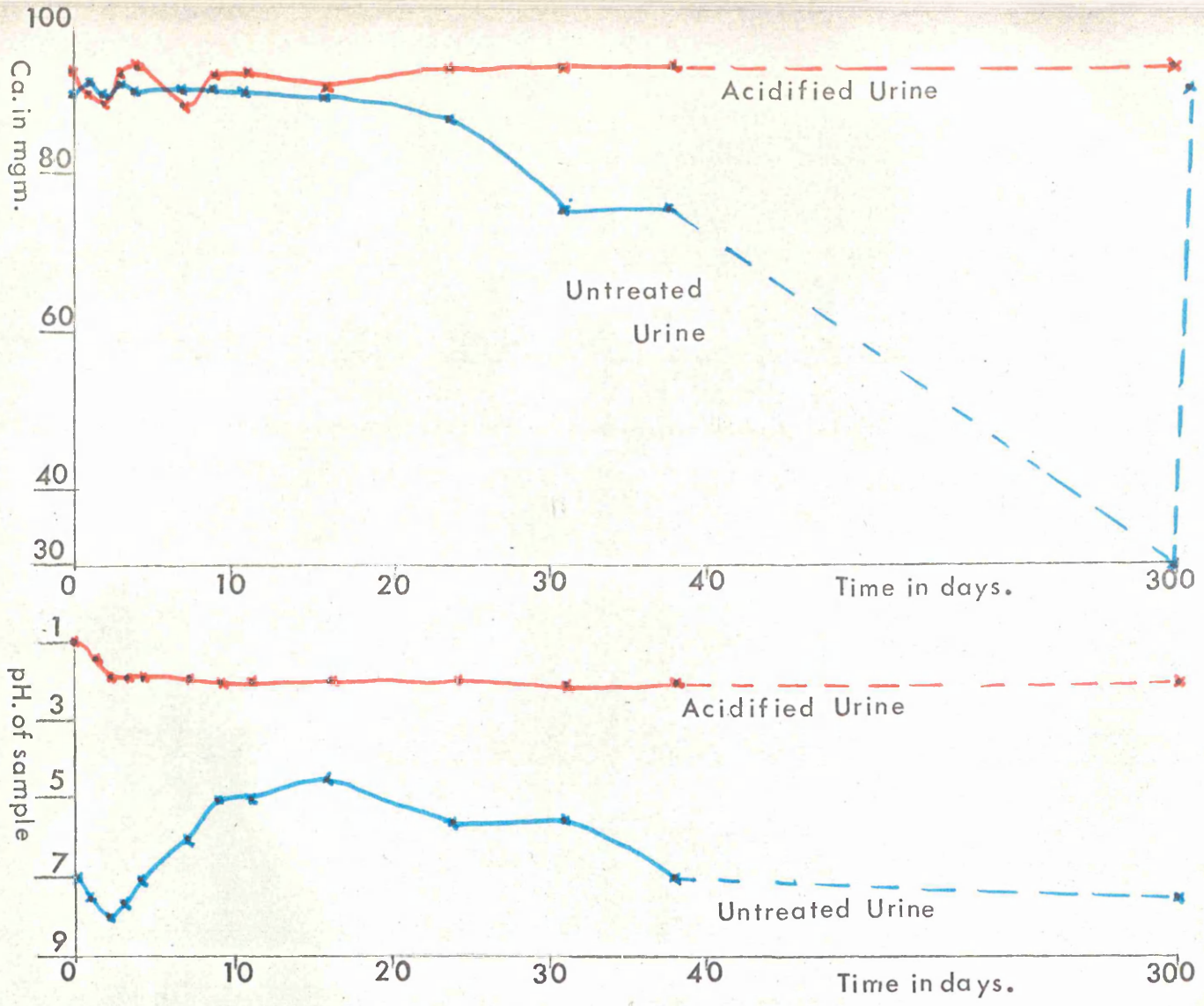


Fig 10 EFFECT of ACIDIFYING URINE.

BIOLOGICAL INVESTIGATIONS

Effect of Acidifying Urine

The results obtained on repeated estimation of the calcium content of a urine sample are shown overleaf (Table VIII). These were found to be totally different where the urine had been acidified when freshly collected. In the untreated urine after 31 days the calcium content was found to be reduced to 80% of the original value and at the end of 300 days the value was less than 33% of the original.

The original value for this sample was re-obtained by evaporating to dryness, ashing at 700°C to remove phosphate and reconstituting with de-ionised water after dissolving in a minimum volume of HCl/HNO_3.

In the acidified sample the calcium content varied although the pH was maintained at 2 or less. This variation was always less than 5%.

The results obtained are expressed graphically opposite (Fig. 10).

Conclusion: Since the calcium content of the urine could be regained by ashing, it appears that in alkaline urine, on ageing,

TABLE VIII EFFECT OF ACIDIFYING URINE

Day	Sample	pH	Mean Ca content (in mgms)
0	Acidified (A)	1.0	93.03
	Untreated (U)	7.0	90.18
1	A	1.5	91.25
	U	7.5	91.77
2	A	2.0	89.8
	U	8.0	90.35
3	A	2.0	92.8
	U	7.5	92.75
4	A	2.0	93.9
	U	7.0	90.7
7	A	2.0	88.6
	U	6.0	91.1
9	A	2.0	92.6
	U	5.0	91.1
11	A	2.0	93.0
	U	5.0	90.8
16	A	2.0	91.0
	U	4.5	90.1
24	A	2.0	93.6
	U	5.5	87.2
31	A	2.0	93.7
	U	5.5	75.5
38	A	2.0	93.78
	U	7.0	76.0
300	A	2.0	93.53
	U	7.5	29.22
302	A	2.0	93.53
	U (reconstituted)	-	93.3

some compound of calcium must be formed. The formation of this compound is prevented by maintaining the urine at a sufficiently low pH.

TABLE IXa. STRONTIUM-CALCIUM BALANCES

Balance and Period	Mean Sr. Content (mgms) Intake Diet	Output Urine	Output Faeces	Output u + f	IN - OUT.	Mean Ca. Content (gms) Intake Diet	Output Urine	Output Faeces	Output u + f	IN - OUT.
(1) E.C.										
period 2	7.9	1.01	8.8	9.81	-1.91	5.1	1.4	5.5	6.9	-1.8
period 4	12.4	1.67	10.2	11.87	+0.53	11.28	1.59	7.4	8.99	+2.29
(2) J.A.										
period 2	6.82	0.5	6.7	7.2	-0.38	6.2	0.22	6.38	6.6	-0.4
period 4	9.98	0.77	6.9	7.67	+2.31	9.78	0.49	6.3	6.79	+2.99
(3) B.M.										
period 2	5.59	0.71	4.38	5.09	+0.5	5.56	1.47	4.8	6.27	-0.71
period 4	8.65	0.62	5.5	6.12	+2.35	11.82	1.13	9.08	10.21	+1.61
(4) E.McI										
period 2	5.82	0.9	5.47	6.37	-0.55	5.78	1.08	4.88	5.96	-0.18
period 4	8.7	1.08	6.57	7.65	+1.05	11.3	1.64	7.21	8.85	+2.45
(5) J.McD										
period 2	4.98	0.72	6.03	6.75	-1.77	5.01	1.68	4.95	6.63	-1.62
period 4	7.43	0.75	8.49	9.24	-1.81	10.45	1.54	10.86	12.4	-1.94
(6) K.McG										
period 2	5.02	0.51	2.7	3.25	+1.77	7.03	0.38	4.09	4.47	+2.56
period 4	7.77	0.38	4.62	5.0	+2.77	12.84	0.45	7.75	8.2	+4.64
(7) L.C.										
period 2	4.39	0.83	3.7	4.53	-0.14	5.62	0.4	3.89	4.29	+1.33
period 4	6.23	0.89	4.97	5.86	+0.37	9.65	0.87	8.26	9.13	+0.52
(8) A.D.										
period 2	4.6	0.51	6.53	7.04	-2.44	4.38	0.84	5.68	6.52	-2.14
period 4	7.11	0.73	11.75	12.48	-5.37	10.65	0.66	15.9	16.56	-5.91
(9) S.C.										
period 2	4.86	0.24	4.72	4.96	-0.1	4.63	0.29	5.02	5.31	-0.68
period 4	5.33	0.30	8.25	8.55	-3.22	9.97	0.25	11.56	11.81	-1.84

Strontium - Calcium Balances

The results of strontium-calcium balances in 9 hospitalized children of 4 years 9 months - 13 years 6 months are detailed opposite (Table IXa). These children were on fairly normal diets for their age group and the calcium content approximates to the recommended value of 1-1.2 g./day (Documenta Geigy).

These results are summarized below where mean values and standard deviations from the mean are given.

PERIOD	DIET - mean content		FAECES - mean content	
	Ca - gms.	Sr - mgms.	Ca - gms.	Sr - mgms.
No. 2				
Mean Value	5.48	5.55	5.02	5.45
Std. Dev.	±0.77	±1.13	±0.73	±1.72
Range	4.38-7.03	4.39-7.9	3.89-6.38	2.7-8.8
No. 4				
Mean Value	10.86	8.18	9.37	7.47
Std. Dev.	±0.99	±2.03	±2.77	±2.28
Range	9.65-12.84	5.33-12.4	6.3-15.9	5.5-11.75

All results overleaf are for 6 day periods. Mean results for urinary excretion are not given since these appear to be under special influences. The results are given below for 24 hour periods.

	DIET		FAECES	
PERIOD	Ca in gm.	Sr in mgms.	Ca in gm.	Sr in mgms.
2	0.91	0.93	0.84	0.91
4	1.81	1.36	1.56	1.25

TABLE IX (b) STRONTIUM - CALCIUM BALANCES

Balance No.	Period	Salicylate	Compound of Calcium added	State of balance Sr	State of balance Ca
1	period 2	P.A.S.	Carbonate	-	-
	period 4			+	+
2	period 2	Aspirin	Carbonate	-	-
	period 4			+	+
3	period 2	Aspirin	Carbonate	+	-
	period 4			+	+
4	period 2	Aspirin	Carbonate	-	-
	period 4			+	+
5	period 2	Aspirin	Phosphate	-	-
	period 4			-	-
6	period 2	-	Phosphate	+	+
	period 4			+	+
7	period 2	-	Phosphate	-	+
	period 4			+	+
8	period 2	P.A.S.	Phosphate	-	-
	period 4			-	-
9	period 2	P.A.S.	Phosphate	-	-
	period 4			-	-

\- IN NEGATIVE BALANCE

\+ IN POSITIVE BALANCE

When these results are considered in light of the drugs and chemicals given (Table IXb opposite) some interesting results are noted. All children on salicylate therapy are in negative balance for both strontium and calcium in period 2. (exception B.M. period 2 Sr). When results for period 4 are considered, it is noted that, whereas the children on supplementary calcium carbonate and salicylate (balance nos. 1-4) now show a positive balance, those on supplementary calcium phosphate and salicylate show an increasingly negative balance (balance nos. 5, 8 and 9). The two children on supplementary calcium phosphate without salicylate show positive balances for periods 2 and 4 (exception L.C. period 2 Sr).

TABLE IXc Strontium-Calcium Balances

Balance No.	Sr. (mgms) period 4 - period 2 Intake Diet	Output Urine	Output Faeces	Ca. (gms) period 4 - period 2 Intake Diet	Output Urine	Output Faeces	Balance period 4 - period 2 Sr. (mgms)	Ca. (gms)
1	4.5	0.66	1.4	6.18	0.19	1.9	+2.44	+4.09
2	3.16	0.27	0.2	3.58	0.27	-0.08	+2.69	+3.39
3	3.06	-0.09	1.12	6.26	-0.34	4.28	+2.3	+2.32
4	2.88	0.18	1.1	5.52	0.56	2.33	+1.6	+2.63
5	2.45	0.03	2.46	5.44	-0.14	5.91	-0.04	-0.32
6	2.75	-0.13	1.92	5.81	0.07	3.66	+1.0	+2.08
7	1.84	0.06	1.27	4.03	0.47	4.37	+0.51	-0.81
8	2.51	0.22	5.22	6.27	-0.18	10.22	-2.93	-3.77
9	0.47	0.06	3.53	5.34	-0.04	6.54	-3.12	-1.16

When the increase between periods 2 and 4 in dietary strontium and calcium and excretory strontium and calcium is compared (Table IXc opposite), it can be seen that all balances (exception - J.A. Ca) show an increased faecal excretion which is especially marked in balances 5, 8 and 9. Indeed in these balances faecal increase exceeds dietary increase for both strontium and calcium. Urinary excretion shows no such consistency of result.

The change in the state of the balances between periods 2 and 4 is also given in this table. Balances nos. 1-4 and 6 have all become considerably more positive whilst balances nos. 5, 8 and 9 have become more negative.

TABLE IXd Strontium-Calcium Balances

Balance No. and Period		Ratio $\frac{\text{Sr. in mgms}}{\text{Ca in gms}}$			Observed Ratios	
		Intake	Output		$\frac{\text{Urine}}{\text{Diet}}$	$\frac{\text{Faeces}}{\text{Diet}}$
		Diet	Urine	Faeces		
(1)	period 2	1.55	0.72	1.6	0.47	1.03
	period 4	1.1	1.05	1.38	0.96	1.25
(2)	period 2	1.1	2.27	1.05	2.03	0.96
	period 4	1.02	1.57	1.1	1.54	1.07
(3)	period 2	1.0	0.48	0.92	0.48	0.92
	period 4	0.73	0.55	0.66	0.75	0.91
(4)	period 2	1.02	0.83	1.12	0.81	1.1
	period 4	0.67	0.66	0.68	0.99	1.02
(5)	period 2	0.99	0.43	1.22	0.46	1.23
	period 4	0.71	0.49	0.78	0.69	1.12
(6)	period 2	0.72	1.32	0.66	1.8	0.92
	period 4	0.61	0.85	0.6	1.39	0.99
(7)	period 2	0.8	2.04	0.95	2.55	1.19
	period 4	0.65	1.02	0.6	1.57	0.93
(8)	period 2	1.05	0.6	1.15	0.57	1.09
	period 4	0.67	1.1	0.74	1.64	1.1
(9)	period 2	1.05	0.83	0.94	0.79	0.9
	period 4	0.54	1.2	0.71	2.22	1.32

In Table IXd results for the ratio of strontium in mgms. to calcium in gms. is given. In all cases for diet the ratio in period 4 is lower than in period 2. Faecal ratios were also reduced or remained approximately the same. Urinary ratios varied considerably. When the observed ratio urine/diet is considered, it is seen that 6 out of 7 children on salicylate therapy exhibit an increased observed ratio in period 4 as compared with period 2. Observed ratios of faeces/diet do not appear to be similarly affected.

- - - - - - - - - - - - -

Conclusions:-

Salicylate therapy appears to produce negative calcium and strontium balances in growing children in spite of the fact that the dietary calcium is in excess of the recommended minimum requirements. This result is somewhat surprising since para-amino-salicylic acid and acetyl-salicylic acid are both acidic and should therefore produce conditions conducive to absorption from the gut. Such a result suggests that calcification of these children's skeletons may be adversely affected by the salicylate ion. This negative balance may be corrected by the addition of supplementary calcium compounds to the diet. The

phosphate is not recommended since this merely increases the negativity of the balance.

The effect of increased phosphate appears to be to increase the excretion of both strontium and calcium via the gut in the faeces. This result is in agreement with Kostial et al. whose work on rats shows that increased dietary phosphate decreases strontium and calcium absorption from the gut (Kostial et al. 1963). In the three children on salicylate with increased phosphate the urinary excretion of strontium is increased - if only slightly - while the urinary excretion of calcium has fallen. This suggests that the combination of salicylate and phosphate affects gut excretion of strontium and calcium similarly but kidney excretion differently.

Smith and Bates, working with rats, show that the effect of salicylate is on the renal tubular reabsorption of strontium. No calcium content is given in this work (Smith and Bates 1965). In this experiment, all children (except balance 2) on salicylate had a lower Sr/Ca ratio in urine than those children not on salicylate. The effect of increased calcium was to increase this urinary Sr/Ca ratio in five of these children, as opposed to the decrease in the ratio shown by children not on salicylate. The effect on the strontium/calcium ratios of faeces and diet

is interesting since for each period of each balance the ratios Sr/Ca in faeces and diet corresponds reasonably well with each other, as do the new ratios obtained on increased calcium in period 4. Thus, there appears to be no definite discrimination exhibited in the passage through the gastro-intestinal tract of children.

Thus the greatest effect of supplementary calcium in period 4 appears to be on the kidney action to cause a rise in the Sr/Ca ratio where salicylate has been administered and a fall where no salicylate has been given. If salicylate causes tubular block, then this rise could be attributed to a lowered reabsorption of both strontium and calcium with the greater effect on strontium.

Where the observed ratios are considered, no significant difference is to be found for O.R. faeces-diet; however, in the O.R. urine-diet, balances 1, 3, 4, 5, 8 and 9 all on salicylate give an increased O.R. urine-diet, which is especially noticeable in two of the balances with phosphate supplements (nos. 8 and 9).

TABLE Xa. Interlaboratory comparison of Calcium content in Biological Samples

Laboratory	A.E.R.E. Harwell	Vet.College Glasgow	Victoria Inf. Glasgow		P.G.M.S. London	M.R.C. Strontium RES. Glasgow	
Instrument	Unicam SP.900		Eel Titrator	Unicam SR.900	Zeiss P.M. QII.	Zeiss P.M. QII.	
						Direct.	After separ.
Faeces	30.7	34.0	34.0	34.0	30.06	31.2	31.0
Diet	25.7	27.0	25.0	26.0	24.05	27.0	27.0
Diet	24.8	29.0	24.0	26.0	24.05	26.0	27.0
Faeces	30.6	35.0	33.0	35.0	30.06	33.0	30.4

Notes:- All concentrations in p.p.m.
Samples 1 and 4 duplicate samples
2 and 3 duplicate samples

TABLE Xb. Interlaboratory comparison of Strontium content in Biological Samples

Laboratory		A.E.R.E. Harwell	M.R.C. Glasgow
Faeces	1	6.8	6.7
	2	7.3	6.9
	3	8.4	8.8
	4	10.6	10.2

Notes:- All results in mgms.

Intercomparisons of Estimations in Balance Studies

Results:- (Table Xa)

The calcium content of faecal and diet balance samples were investigated independently by five laboratories. The results obtained were reasonably consistent, as shown.

(Table Xb)

Faecal samples for strontium were investigated independently by the author and the Radiobiological laboratory A.E.R.E. Harwell. Results are shown opposite.

TABLE XI. Comparison of Results for Strontium.

Obtained by two different methods.

Method Used	Diet: Sr. content (mgms)	Faeces: Sr. content (mgms)	Urine: content (mgm./l.)
Harrison's Separation	4.7	5.57	Sample I = 0.13
(Solid samples processed in triplicate)	4.8 4.96	5.44 5.50	Sample II = 0.085
(H)	Mean = 4.82	Mean = 5.5	
Direct Spectral Analysis	4.8	5.5	Sample I = 0.12
(Estimations in triplicate)	4.95 4.85	5.42 5.48	Sample II = 0.09
(D.S.)	Mean = 4.9	Mean = 5.46	
Ratio: $\frac{H}{D.S.}$	0.98	1.07	Sample I = 1.08 Sample II = 0.94

Direct Spectral Analysis for Strontium

Results for samples of urine, diet and faeces estimated both by Harrison's separation of strontium and directly, using the additive standard method are compared opposite (Table XI).

All results are seen to be within 10% of each other. Conclusion - the results obtained by direct estimation of biological samples compare favourably with those obtained by much more difficult means.

- - - - - - - - - - - - -

Investigation of Non-Ionic Detergent

Results obtained for strontium and calcium content of this solution are detailed below:-

Strontium - no strontium could be detected at full sensitivity of the instrument (10/10/2).

Calcium - a value of 2 ppm. was obtained.

Since the dilution of this non-ionic detergent was to be 1:10,000, the calcium present may be neglected in strontium estimations.

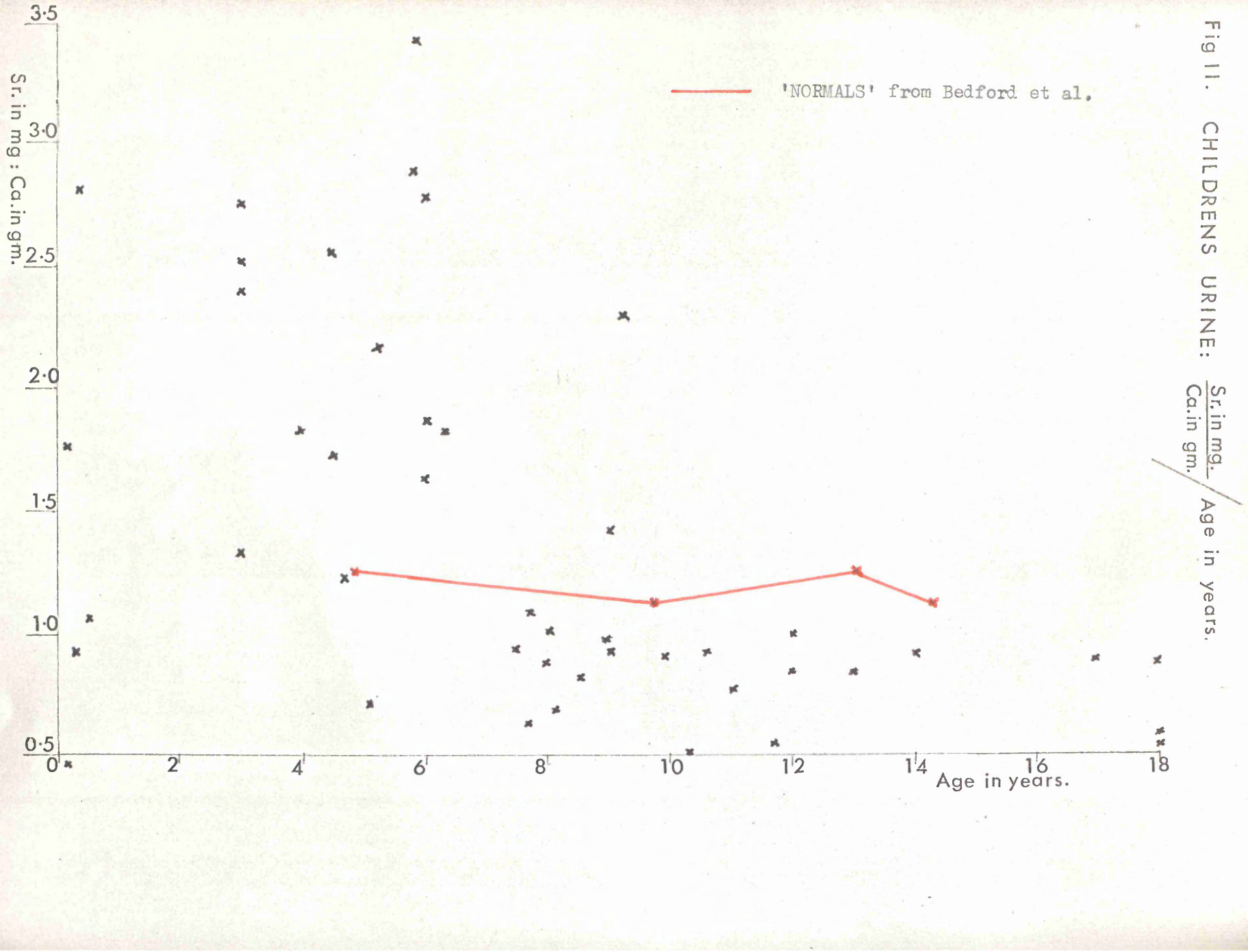


Fig 11. CHILDRENS URINE: $\frac{\text{Sr. in mg.}}{\text{Ca. in gm.}}$ / Age in years.

Excretion of Natural Strontium via the Kidney

Results for the natural strontium (in mgms.) and calcium (in gms.) content of some forty-five samples of children's urine are given overleaf (Table XIIa). The age range is from 6 weeks-18 years and since the children were all hospital patients, diagnosis and therapy, where known, are also given. The ratio of Sr. in mgm./Ca in gms. has been calculated and is graphed against age (figure 11).

A second graph (figure 12) shows the effect of various abnormal conditions and drugs on this ratio.

Conclusions: Very few figures for natural strontium content in children's urine are to be found in literature. Those of Bedford et al. for four children of 4-14 years give an average result of 1.3 mgm. Sr/gm. Ca. The results in this survey being from hospitalized children are necessarily under many extraneous influences. The spread of results is large, especially in infants, but similar conditions or drugs appear to produce similar ratios for Sr in mgm./Ca in gms. from different groups. The salicylate ions give rise to ratios of less than 1.0 mgm. Sr/ gm. Ca which is the result found in samples from balance studies. See overleaf—

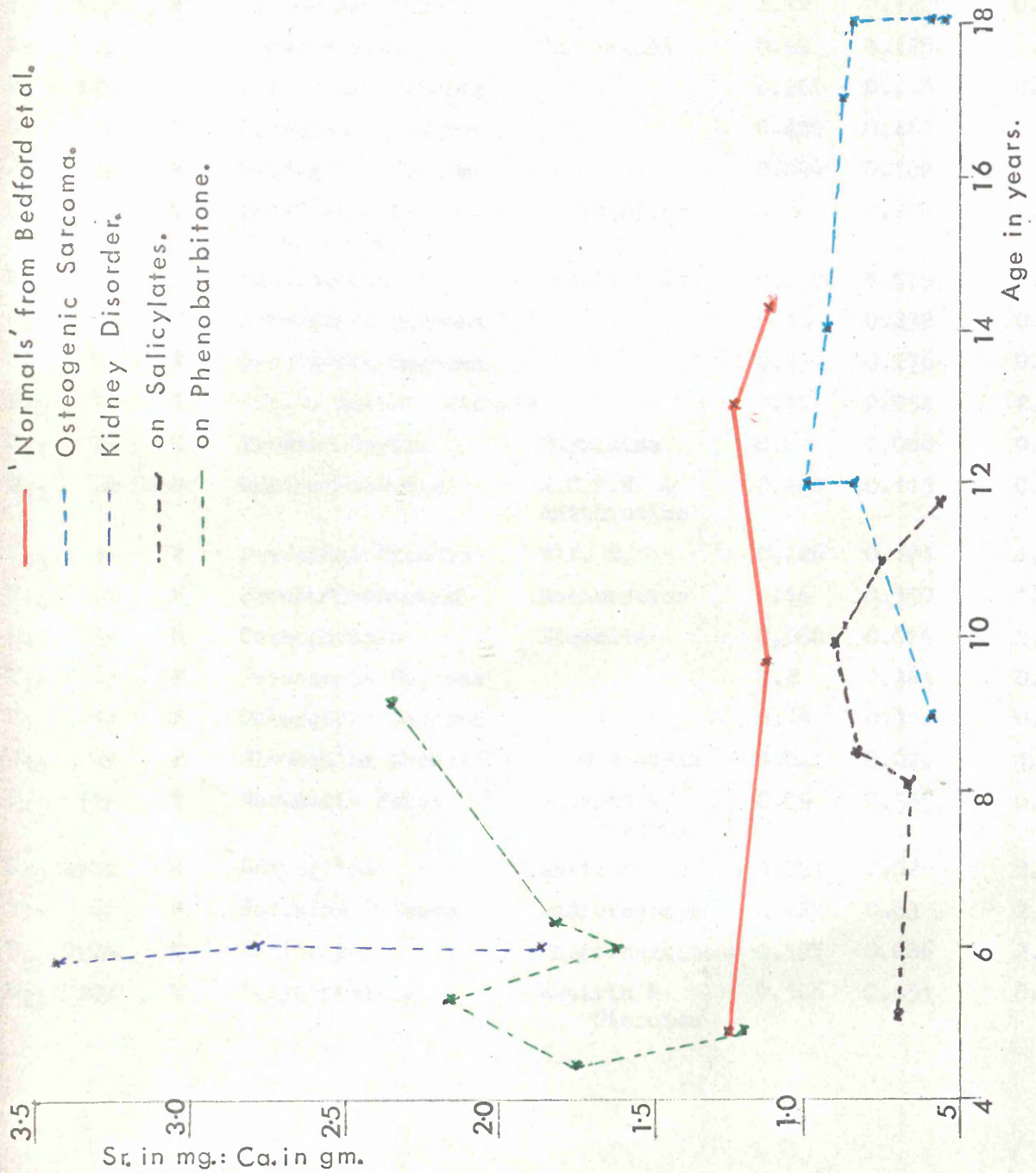
TABLE XIIa Natural Strontium in Urine (2)

No.	Age	Sex	Diagnosis	Therapy (where known)	Sr mgm/l	Ca g/l	$\frac{\text{Sr. (mgm)}}{\text{Ca. (gm)}}$
U_{24}	13y	M	Muscular Atrophy	Antibiotics	0.194	0.235	0.826
U_{25}	8y 6m	M	Thrombocy.topenia	Steroids & Salicylates	0.11	0.136	0.809
U_{26}	11y 8m	M	Arthritis	"	0.167	0.313	0.534
U_{27}	4y	F	Coeliac Disease		0.079	0.043	1.837
U_{28}	8y	F	Haematemesis & Splenomegaly		0.080	0.08	1.0
U_{29}	7y 9m	F	Vomiting		0.136	0.216	0.63
U_{30}	10y 3m	F	Exothalmic Goitre	Carbimazole	0.057	0.114	0.5
U_{31}	7y 6m	F	Pyelonephritis	Sulphur drugs	0.058	0.062	0.932
U_{32}	9y 11m	F	Cervical Adenitis	Aspirin	0.088	0.098	0.9
U_{33}	5y 10m	M	Acute Glomerular Nephritis	Antibiotics	0.03	0.009	3.45
U_{34}	4y 6m	F	Congenital Stenosis	Phenolbarbitone	0.094	0.054	1.75
U_{35}	4y 9m	M	Congenital heart disease	"	0.175	0.144	1.22
U_{36}	5y 3m	M	"	"	0.128	0.059	2.17
U_{37}	6y 9m	M	Asthmatic Bronchitis	Ephedrine	0.172	0.16	1.075
U_{38}	10y 7m	M	Duodenal Ulcer		0.126	0.14	0.9
U_{39}	6y	M	Congenital heart disease	Phenolbarbitone	0.154	0.095	1.63
U_{40}	9y	M	Hereditary Ataxia		0.179	0.193	0.93
U_{41}	8y	F	Pneumonia & Portal hypertension	Antibiotics	0.140	0.158	0.88
U_{42}	6y 3m	F	Heart Disease	Phenolbarbitone	0.123	0.067	1.83
U_{43}	8y 2m	M	Rheumatic Fever	Aspirin	0.191	0.284	0.67
U_{44}	3y	F	Coeliac Disease		0.019	0.008	2.4
U_{45}	6y	M	Nephrotic Syndrome		0.023	0.008	2.8

TABLE XIIa Natural Strontium in Urine (1)

No.	Age	Sex	Diagnosis	Therapy (where known)	Sr mgm/l	Ca g/l	$\frac{\text{Sr. (mgm)}}{\text{Ca. (gm)}}$
U_1	12y	M	Osteogenic Sarcoma		0.12	0.122	0.98
U_2	9y	M	Renal Rickets	Calciferol	0.18	0.125	1.44
U_3	17y	M	Osteogenic Sarcoma		0.265	0.298	0.888
U_4	14y	M	Osteogenic Sarcoma		0.420	0.463	0.908
U_5	12y	M	Osteogenic Sarcoma		0.090	0.108	0.832
U_6	3m	M	Non-Tubercular Atelectasis	Antibiotics	0.36	0.128	2.812
U_7	6w	M	Septicaemia	Antibiotics	0.139	0.079	1.760
U_8	9y	F	Osteogenic Sarcoma		0.138	0.232	0.595
U_9	18y	F	Osteogenic Sarcoma		0.237	0.276	0.859
U_{10}	3y	F	Vit.D. Resist. Rickets		0.11	0.054	2.052
U_{11}	7w	M	Treated Cretin	Thyroxine	0.03	0.066	0.452
U_{12}	3m	M	Gastroenteritis	A.C.T.H. & Antibiotics	0.108	0.115	0.943
U_{13}	3y	F	Resistant Rickets	Vit. D.	0.226	0.171	1.322
U_{14}	6m	M	Bronchopneumonia	Antibiotics	0.16	0.150	1.067
U_{15}	3y	M	Osteoporosis	Adexolin	0.068	0.026	2.576
U_{16}	18y	F	Osteogenic Sarcoma		0.2	0.364	0.55
U_{17}	18y	F	Osteogenic Sarcoma		0.19	0.32	0.594
U_{18}	6y	F	Glomerular Nephritis	low protein	0.044	0.024	1.864
U_{19}	11y	F	Rheumatic Fever	Aspirin & Steroids	0.29	0.385	0.753
U_{20}	4y8m	M	Laryngitis	Antibiotics	0.051	0.02	2.55
U_{21}	6y	M	Hodgkins Disease	Radiotherapy	0.087	0.03	2.9
U_{22}	9y2m	M	Epilepsy	Phenolbarbitone	0.197	0.086	2.3
U_{23}	5y2m	M	Polyarthritis	Aspirin & Steroids	0.106	0.151	0.7

Fig 12 EFFECT of VARIOUS CONDITIONS on the Sr:Ca RATIO in CHILDRENS URINE.



Strontium - Calcium Balances - Urine Results

Subject	Sr in mgm./Ca in gm.	
E.C.	0.72	
J.A.	2.27	Exception
B.M.	0.48	
E.McI.	0.83	
J.McD.	0.43	
A.D.	0.60	
S.C.	0.83	

TABLE XII (b) URINARY EXCRETION OF STRONTIUM IN 24 HOURS

Sample	Age	Diagnosis	Sr/24 hrs (μg)	Therapy (where known)
* U18	6y	Glomerular Nephritis	48	Penicillin G
U19	11y	Rheumatic Fever	116	Salicylates and steroids
U21	5y 10m	Hodgkins Disease	69	Radiotherapy
U22	9y 2m	Epilepsy	114	Phenolbarbitone
U23	5y 2m	Polyarthritis	117	Aspirin
U25	8y 6m	Thrombocytopenaemia	196	Salicylates and steroids
U26	11y 8m	Arthritis	245	Salicylates and steroids
* U27	4y	Coeliac Disease	41.5	-
U28	8y	Haematemesis	113	-
* U29	7y 9m	Vomiting	60.5	-
U30	10y 3m	Exophthalmic Goitre	67	Carbinazole
* U31	7y 6m	Pyelonephritis	58	Sulphadinidine
U32	9y 11m	Cervical Adenitis	145	Aspirin
* U33	5y 10m	Glomerular Nephritis	24.5	Penicillin G
U34	4y 6m	Congenital Heart Disease	72	Phenolbarbitone
U35	4y 9m	Congenital Heart Disease	80	Phenolbarbitone
U36	5y 3m	Congenital Heart Disease	83	Phenolbarbitone
U37	6y 9m	Asthmatic Bronchitis	117	Ephedrine
U38	10y 7m	Duodenal Ulcer	123.5	-
U39	6y	Congenital Heart Disease	72	Phenolbarbitone
U40	9y	Hereditary Ataxia	104	-
U41	8y	Pneumonia	82	Penicillin G
U42	6y 3m	Heart Disease	106	Phenolbarbitone
U43	8y 2m	Rheumatic Fever	153	Aspirin
* U45	6y	Coeliac Disease	27.2	-

Urinary Excretion of Strontium in 24 hours

Results for excretion of strontium in 24 hour samples of children's urine are given opposite (Table XIIb).

These are somewhat variable but it is noted that cases of kidney disease or abnormal absorption from the gastro-intestinal tract (*) are consistently low, being less than 61 µg natural strontium in 24 hours.

Conclusion: Only twenty five results are as yet available and thus not a great deal of information is forthcoming. It is perhaps significant that some of the lowest values are obtained in cases of abnormal kidney function suggestive of an impairment of the renal tubular discrimination against strontium.

Investigation of a Single Case of Urinary Calculus

Results for the strontium and calcium content of both the urine and calculus from a case of renal calculus are shown below (Table XIIc). The samples are from a three year old girl.

Sample	Sr mg./l.	Ca g./l.	$\frac{\text{Sr mgm.}}{\text{Ca gm.}}$	Sr mgm. in 24 hrs.
E.W. Urine	0.11	0.024	4.580	0.036
Soln. of Calculus in HCl/HNO_3	0.204	0.23	0.887	

Conclusion: The Sr/Ca ratio in urine sample is extremely high compared with that of the calculus formed. This is thought to be due to the fact that the predominant cation in a calculus is calcium, generally in combination with oxalate or phosphate (Laskowski 1965). Therefore, after its formation, the calcium content of the urine may be expected to be lower.

Comparison of the Natural Strontium Content of Children's Urine estimated by two Distinct Methods

Results for the natural strontium content of eight samples of urine are given below (Table XIII). These were estimated both by the abbreviated Harrison method and by neutron activation analysis.

Sample	Zeiss P.M.Q. II nat. Sr ug/l. (Z)	Activation Analysis Nat.Sr ug/l. (A/A)	Ratio $\frac{Z}{A/A}$
D.T.	197	219	0.90
S.H.	45	42	1.07
S.C.	167	190	0.88*
S.B.	80	86	0.93
J.H.	30	30.2	1.00
C.McE.	58	54.5	1.06
J.M.	136	143	0.95
L.M.	175	180	0.97

* In only one case was there a variation of greater than 10% between the two sets of results.

TABLE XIVa Strontium-Calcium Balances in Rachitic Children

Subject and Period	Ca.gms/24hrs. Intake Diet	Ca.gms/24hrs. Output Faeces	Ca.gms/24hrs. Output Urine	Sr.mgm/24hrs. Intake Diet	Sr.mgm/24hrs. Output Faeces	Sr.mgm/24hrs. Output Urine	P.gms/24hrs. Intake Diet	P.gms/24hrs. Output Faeces	P.gms/24hrs. Output Urine	Diagnosis and Therapy
B.M. (M) 9 years 7 day balance	1.11	0.542	0.14	1.2	0.7	0.2	1.07	0.25	0.59	Renal Rickets
M.G. (F) 3 years 9 day balance	0.82	0.61	0.02	0.78	0.508	0.041	0.83	0.41	0.3	Vitamin D Resistant Rickets
M.G. (F) 3 years 10 day balance	0.75	0.28	0.05	0.62	0.358	0.066	0.77	0.175	0.26	Following dose of Vitamin D (100,000 I.U.)

TABLE XIVb

Subject	State of Balance	Ratio: $\frac{\text{Sr.mgm}}{\text{Ca.gms}}$ Intake Diet	Output Faeces	Output Urine	Observed Ratio $\frac{\text{Faeces}}{\text{Diet}}$	Observed Ratio $\frac{\text{Urine}}{\text{Diet}}$	Net Absorption (Diet-Faeces) Ca(gm)	Sr(mgm)	P(gm)	Net Retention [Diet-(urine+faeces)] Ca(gm)	Sr(mgm)	P(gm)
B.M.	+	1.08	1.29	1.44	1.2	1.3	0.57	0.5	0.82	0.43	0.3	0.23
M.G. (before Vit. D)	+	0.91	0.84	2.05	0.92	2.25	0.21	0.27	0.42	0.19	0.23	0.12
M.G. (after Vit. D)	+	0.82	1.25	1.32	1.53	1.6	0.47	0.26	0.6	0.42	0.2	0.33

Effect of Vitamin D on Strontium - Calcium Metabolism

Results:- Two cases of rickets in children have been investigated, In the case of M.G. a second balance was studied after administration of a massive dose of Vitamin D. The results obtained are tabulated opposite (Tables XIVa and XIVb). Calcium, strontium and phosphorus values are given for 24 hour periods for diet, faeces and urine. Sr/Ca ratios, observed ratios, net absorptions and retentions are also given.

Conclusions:- All three balances are positive for all elements investigated. The phosphorus intake closely follows the calcium intake but the output of calcium is predominately in the faeces while a larger proportion of phosphorus output is via the kidney in the urine. Excretion of strontium is, like calcium, predominately in the faeces. Following Vitamin D ingestion there is a decided drop in faecal content of all three elements.

When the ratios of Sr/Ca are studied (Table XIVb) it can be seen that both in Vitamin D Resistant Rickets and in Renal Rickets studies, the highest ratio is obtained in urine. This is especially true of untreated Vitamin D Resistant Rickets. Similarly in this case the O.R. urine/diet is extremely high.

The net absorption and retention of calcium is more than doubled

after treatment with Vitamin D with no corresponding effect on strontium. The percentage absorption of calcium rose from 25% - 62% after treatment with Vitamin D.

TABLE XV NATURAL STRONTIUM AND CALCIUM IN BONE ASH

Sample No.	Age	Calcium % of Ash	Ratio: $\frac{\text{Sr in } \mu g}{\text{Ca in gm}}$
B1	4y 6m	38.08	273
B2	7y	37.51	241
B3	5y	38.24	207
B4	1y 10m	38.01	283.5
B5	9y 8m	32.61	205
B6	5y 5m	38.03	267
B7	9y 8m	38.38	208
B8	4y 2m	38.31	176.3
B9	1y 8m	38.63	151.2
B10	4y 10m	27.04	349.3
B11	3y	38.09	170.6
B12	3y	38.0	261.0
B14	1y 6m	38.5	342
B15	10y 9m	38.4	200
B16	11y 6m	38.9	312
B17	8y	38.9	270
B18	4y 2m	39.0	316
B19	6y 2m	38.5	306
B20	6y 8m	38.0	182
B21	11y	39.0	271
Mean			249.6
Standard Deviation			± 57.8
B13	25y	39.25	710

Natural Strontium Content of Children's Bones

Results from twenty-one samples of human bone ash for natural Sr/Ca content are shown opposite (Table XV). The bones are post mortem samples from children of less than twelve years old. One sample from a twenty-five year old mother is also given but not included in the calculation of the mean. Values range from 151.2 ugm Sr/gm. Ca in a 1 year 8 month old to 349.3 ug Sr/gm. Ca in a 4 year 10 month old child. There are two low calcium values but all others range from 37.51-39% of bone ash.

Intercomparisons of results on bone ash samples obtained by the author and the laboratory at Capenhurst are given below (Table XVI).

Sample No.	Calcium. % of Ash		Nat.Sr. ug/gm.Ca		Ratio Ca % Glasgow / Capenhurst	Ratio: Sr ug/Ca gm. Glasgow / Capenhurst
	Glasgow	Capenhurst	Glasgow	Capenhurst		
G/HB.1001	38.9	38.7	312	290	1.005	1.07
G/HB.1003	38.9	38.9	270	260	1.0	1.04
G/HB.1012	39.0	38.6	316	300	1.01	1.05
G/HB.1027	38.5	38.5	306	290	1.0	1.055
G/HB.1036	38.0	38.3	182	160	0.99	1.13
G/HB.1057	39.0	39.2	270	270	0.995	1.0

Natural Strontium/Calcium Levels in a Case of Osteogenic Sarcoma

The sample was obtained from an amputation operation on a boy of 14 years. The femur including the sarcoma was investigated after division as detailed below (Table XVII). All determinations were on bone ash.

Section of Femur	% Ca in Ash	µg. Sr/gm. Ca
a) Head (non-tumorous)	38.4	302
b) Mid Shaft	38.6	302
c) Lower End (surrounding tumour)	38.6	324
d) Tumour	27.6	306

Conclusions:- This case of osteogenic sarcoma has been reported clinically by Davidson et al. 1965. It is interesting to note the agreement between this case and those of Treadwell et al. (1942). Strontium is not only taken up by the tumour but also by the tissue surrounding it where the highest Sr/Ca ratio is found. The tumour tissue exhibits an extremely low % calcium. From Bryant's figures, the normal value of natural Sr/Ca in bone for an 11-16 year old = 289 ±12.5 µgm./gm. Thus this osteogenic sarcomatic bone does not appear to differ markedly from the normal Sr/Ca ratio.

TABLE XVIII NATURAL STRONTIUM AND CALCIUM IN TEETH

Sample No.	Age	% Calcium (wet wt.)	Ratio: $\frac{\text{Sr in } \mu g}{\text{Ca in } g}$
T3	8y	27	222
T4	8y	26	322
T5	7y	26	256
T6	9y	28	186
T7	10y	23.4	268
T8	11y	26.3	202
T9	6y	31.0	215
T10	5y	25.3	295
T11	-	26.8	225
T12	-	28.2	190
T13	4y	28.0	252
T14	12y	29.2	354
T15	5y	27.1	156
T16	6y	24.7	196
T17	7y	26.0	245
T18	8y	27.3	274
T19	9y	28.3	227
Mean		26.9	240.3
Standard Deviation		± 1.73	± 49.24

Natural Strontium to Calcium Levels in Children's Teeth

Seventeen samples of teeth from children aged 12 years and under have been investigated and the results are shown opposite (Table XVIII). The percentage calcium in the unashed teeth and the ratio of Sr/Ca in these samples is also given. The percentage calcium content was found to range from 23.4-31.0% with a mean value of 26.9% ± 1.73. The ratio of natural strontium/calcium in µgm/gm. varied from 156-354 with a mean value of 240.3 ± 49.24.

Conclusion:- These results are in fair agreement with others previously reported (Bryant et al. 1960) and show that a similar relationship exists between the natural strontium - calcium content of both teeth and bone.

STUDIES IN INFANTS

TABLE XIXa. Milk Feeds

Sample	Age of Infant	Vol. consumed (in mls.)	Mean Content Ca(g)	Sr(mgm)	Ratio Sr.mg./Ca.g.
Cow's milk (test)		1 litre	1.224	0.3	0.25
Synthetic milk feeds	7 wks.	865	0.754	0.421	0.558
	3 wks.	950	1.095	0.238	0.217
	2 mths.	1350	1.29	0.304	0.236
	10 wks.	1000	1.161	0.3	0.258
Moribund Leukaemia case	4 yrs.	665	0.562	0.266	0.473

TABLE XIXb.

Dietary Intake and Urinary Output in 24 hours

Patient and Age	Sample	Vol. (mls.)	Mean Content Ca(g)	Sr(mgm)	Ratio Sr.mgm/Ca.g.	O.R.:- Urine/Milk	Diagnosis and Therapy
J.G.(3m) 1	Milk	1350	1.62	0.378	0.233	12/1	Non-Tubercular Atelectasis-Anti-biotics
	Urine	250	0.032	0.09	2.813		
D.McL(5w) 2	Milk	1300	1.5	0.336	0.224	7.9/1	Septicaemia -Colomycin
	Urine	480	0.038	0.067	1.763		
S.T.(7w) 3	Milk	1740	2.28	0.791	0.347	1.3/1	Treated Cretin -Thyroxine
	Urine	850	.0564	0.0255	0.452		
W.A.(3m) 4	Milk	1500	1.63	0.555	0.340	2.8/1	Gastroenteritis - No drugs
	Urine	530	.061	0.0575	0.943		
B.S.(6m) 5	Milk	1720	2.44	1.08	0.443	2.4/1	Bronchopneumonia - Antibiotics
	Urine	645	.0966	0.103	1.068		

Studies in Infants

Natural Strontium and Calcium content of Infants Diet

Results:- With the exception of the cows' milk, all feeds are duplicates of these ingested by infants in the hospital. The mean content of calcium in grams and strontium in mgms. per feed is given and also the Sr/Ca ratio. (Table XIXa).

Comparisons of Dietary Intake and Urinary Output of Natural Strontium and Calcium in Infants

Results of investigations on five male infants of up to 6 mths. are detailed opposite (Table XIXb). These show content of milk and urine in gms. of calcium and mgms. of strontium per 48 hours. Ratio of Sr/Ca in µg./g. is also given for each sample.

Observed ratios urine/milk have been calculated.

Conclusion:- In all milk feeds the ratios of strontium to calcium was found to be less than one half that of the normal adult (1.2 mg. Sr/gm. Ca). One of the synthetic milk feeds had a particularly high ratio - greater than fresh cows' milk, while the others were in fairly close agreement and possibly belonged to the same batch of dried milk.

Milk/urine studies gave the same type of Sr/Ca ratio in milk but widely varying results for urine leading to O.R.s urine/milk of from 1.3-12.0. Since the strontium content of bone in the young has been shown to be extremely labile (Kostial et al. 1964) and also since no knowledge of the state of development of kidneys etc. in these infants is known, such results defy explanation.

Other urinary ratios of Sr/Ca in the infant have been found to vary from 11.8 in the breast fed to 3.2 in the bottle fed. No evidence of breast feeding is available but if this was the case in subjects (1) and (2) then these results would be more understandable.

- - - - - - - - - -

Natural Strontium and Calcium Content of Glasgow Tap Water

Results obtained in this investigation are given below.

Sr = 23 µg./litre

Ca = 2.55 mgm./litre

$$\text{Ratio} \quad \frac{\text{Sr in mgm.}}{\text{Ca in gm.}} = 9:1$$

This is comparable with previously published figure of a ratio 6:1 for impounded Glasgow water (Burton and Russell 1964).

SECTION V

APPENDICES

APPENDIX I

The Estimation of Calcium in Biological Samples

This method employs a radiation buffer technique and is basically that of MacIntyre 1961. The unknown solution is diluted either 10 or 20 fold with a combined diluting and deproteinizing fluid and its flame emission is compared with that of a set of standards containing calcium in 'spec-pure' form.

Reagents: All solutions are prepared from Analar grade chemicals and stored in polythene bottles. The water used in preparation of solutions is deionised of not less than 4 megohms resistivity as measured on the Elgastat purity meter. All glassware is first washed in 50% HCl, then soaked in deionised water for 24 hours before final rinsing in fresh deionised water.

The following stock solutions are required.

I Calcium Stock Solution (conc. = 1000 ppm. Ca)

Prepared by dissolution of 2.497 gms. 'spec-pure' calcium carbonate (Johnson, Matthey & Co. Ltd., London) in a minimum volume HCl and making up to 1 litre.

II Mixed Salt Solution

Containing the following in 1 litre:

Potassium Chloride 30 m.M. = 2.2367 gm.

Potassium Sulphate 5 m.M. = 0.8713 gm.

Sodium Chloride 1.4 M. = 81.8272 gm.

Potassium Dihydrogen Phosphate 50 m.M. = 6.8046 gm.

III Magnesium Solution (conc. = 8 m.M.)

Containing 1.627 gms. magnesium chloride/litre.

IV Perchloric Acid Solution 60% w/v

V Phosphate Solution (conc. = 44.4 m.M.)

Containing 6.0424 gm. potassium dihydrogen phosphate/litre.

VI Sodium Solution (conc. = 200 m.M.)

Containing 11.6896 gms. sodium chloride/litre

- - - - - - - - - - -

Preparation of Calcium Standards

10 ml. mixed salt solution (No. II), 10 mls. magnesium solution (No. III) and an appropriate volume of calcium stock solution (No. I) are added to 700 mls. water in a litre flask. 50 mls. perchloric acid (No. IV) is then added and the total volume made up to 1 litre with de-ionised water. The range of standards is from 0-20 ppm. Ca at 2 ppm. intervals, omitting 18 ppm. Ca.

- - - - - - - - - -

Preparation of Diluting and Deproteinizing Fluid

10 mls. phosphate solution (No. V) and 25 mls. sodium solution (No. VI) are diluted to 700 mls. in a volumetric flask. 55.5 mls. perchloric acid (No. IV) is added and the volume made up to 1 litre.

- - - - - - - - - -

Preparation of Samples for Analysis

Samples are all estimated in liquid form so as to be capable of dilution with diluting fluid. Preparation of such solutions is described in Appendix IV.

APPENDIX II

Estimation of Strontium in Biological Samples

Initially all strontium estimations were carried out using Harrison's method which involves the separation of strontium as the nitrate before estimation on the flame spectrophotometer using the additive standard technique. A solution of Sr-85 (1500 c.p.m.) was used as a 'spiking' agent for estimation of recovery rate.

Reagents: These are of analar grade with the strontium for standard solutions being in the form of 'spec-pure' strontium carbonate (Johnson Matthey and Co. Ltd., London). Water is de-ionised of not less than 4 megohms resistivity.

The following stock solutions are required:-

I Ammonium Oxalate 4% (w/v)

II Ammonium Hydroxide Solution (0.88)

III 2 N Hydrochloric Acid

IV Universal Indicator Solution (B.D.H.)

V Orthophosphoric Acid

VI Nitric Acid (Specific gravity = 1.42)

VII Nitric Acid (Specific gravity = 1.5)

VIII Standard Strontium Solution (conc. = 1000 ppm. Sr)

This solution is prepared by dissolution of 1.6848 gm. 'spec-pure' strontium carbonate in a minimum volume of HCl and the addition of 0.1 mls. of non-ionic detergent (obtainable from Thompson, Skinner and Hamilton, Glasgow) before making up to 1 litre with de-ionised water.

Procedure: Aliquots of ashed solid samples (0.5 gm. wt.) were treated in triplicate. 1 ml. of Sr-85 solution (1,500 c.p.m.) was added to each and the ash dissolved in the minimum volume of conc. HCl. The solution was made alkaline (pH = 8-9) using 0.88 ammonia and B.D.H. universal indicator solutions. The strontium and calcium oxalates were then precipitated by the addition of 4% ammonium oxalate solution and left overnight. The oxalates were spun off and dissolved in HNO_3 (Sp. grav. = 1.42). The concentration of acid was now increased by addition of fuming nitric acid (Sp. grav. = 1.5) to precipitate the strontium nitrate.

This was cooled on ice, spun off, redissolved and reprecipitated as before. Finally the nitrate was spun off, dissolved in de-ionised water and made up to 10. mls. for estimation on the flame spectrophoto-

meter. 3 mls. of this final solution was removed for counting on the I.D.L. scintillation counter and chemical recovery calculated by proportion from the original Sr-85 activity.

Urine samples were first precipitated as phosphates by addition of orthophosphoric acid. The phosphate precipitated was ashed at 550°C overnight and then dissolved in 2 N HCl. Thereafter, separation of strontium proceeded as for solid samples.

APPENDIX III

Estimation of Strontium in Urine by Neutron Activation Analysis

Method:- The strontium contained in the urine sample is activated to Sr-87 m. ($t_{\frac{1}{2}}$ = 2.8 hours) by neutron bombardment in the Scottish Universities Reactor at East Kilbride, Scotland. Activation is for one half-life of Sr-87 m. An accurately weighed standard of 'spec-pure' strontium carbonate is activated at the same time and in the same container. Comparison of activity of this standard and the unknown using a scintillation counter with pulse height analyser and estimating recoveries by means of Sr-85 added after removal from the reactor gives the strontium content of the unknown (Harrison and Raymond 1955).

Reagents:- All reagents are of the Analar grade with the exception of the standard which is of 'spec-pure' strontium carbonate (Johnson Matthey & Co. Ltd., London). Water was de-ionised of not less than 4 megohms resistivity.

Required:- 2N Hydrochloric Acid.

2N Nitric Acid.

Fuming Nitric Acid (specific gravity = 1.5).

5% solution (w/v) of the following as carriers:

Strontium Nitrate or Chloride.

Barium Nitrate or Chloride.

Manganese Sulphate.

Copper Nitrate or Sulphate.

Sodium Carbonate.

Ferric Chloride.

0.88 Ammonia solution.

Chemical Procedure:- After removal from the reactor 0.5 hours was allowed for decay of undesired short-lived isotopes.

The standard was removed from the 'rabbit', dissolved in 2N HCl/HNO_3 and diluted to volume for counting under conditions similar to those of the samples

0.1 mls. of a 40 uc. solution of Sr-85 was added to each sample as a 'spiking' agent then these were dissolved in 2N HCl/HNO_3 and transferred quantitatively to 50 ml. centrifuge tubes. Two drops of each of the following carrier solutions were added; strontium chloride, barium chloride, manganese sulphate and copper nitrate. The nitric acid concentration was increased by addition of fuming nitric acid to precipitate the nitrates. The samples were cooled in iced-water for 10 minutes before retesting with strontium carrier solution. The nitrates were spun off, dissolved in water, strontium and manganese carriers added and the nitrates reprecipitated. After spinning, these were dissolved in water, 2 drops of ferric carrier solution added (to

remove iron and trace amounts of activity due to rare earths) and the solution heated in a water bath. Ammonia solution was used to raise the pH of the solution for precipitation of the hydroxides which were spun off and discarded. Sodium carbonate was added to the supernatant to precipitate the cations as carbonates and after heating in a water bath these were obtained by centrifuging. The carbonate precipitated was dissolved in dil. HNO_3 and reprecipitated.

The precipitate was dissolved in nitric acid and the concentration increased by means of fuming nitric acid to precipitate the nitrate. This was dissolved and reprecipitated. This final nitrate precipitate was dissolved in de-ionised water and the γ ray activity of the Sr-87 m. obtained. The activity due to Sr-85 was also estimated at this point.

APPENDIX IV

Preparation of Samples for Direct Spectral Analysis

Estimation of Calcium

a) Diet:- Duplicate samples of diet ash = 0.2 gms. wt. were dissolved in minimum volume HCl/HNO_3 and made up to 500 mls. in a volumetric flask. These were then diluted, in duplicate, either ten or twenty-fold with diluting fluid.

b) Milk:- Four 10 ml. samples of milk were pipetted into silica crucibles and evaporated to dryness with a bunsen flame, taking care to prevent boiling or spluttering. These were then ashed at 700 °C to remove phosphate. The resulting ash was dissolved in the minimum volume HCl/HNO_3 and transferred to a 10 ml. volumetric flask and made to volume with de-ionised water. These solutions were diluted ten-fold with water and then ten-fold with diluting fluid.

c) Water:- The calcium content of water was determined by evaporating 1000 mls. tap water to less than 100 mls. and transferring by means of de-ionised water to a 100 ml. flask and making to volume. This experiment was performed in triplicate. These solutions were diluted 1/10 with diluting fluid.

d) Faeces:- Duplicate samples of faecal ash of 0.15 gms. wt. were dissolved in the minimum volume HCl/HNO_3 and made up to 500 mls. with de-ionised water. These solutions were diluted 1/10 with diluting fluid, in duplicate.

e) Urine:- Urine samples when freshly collected were measured, acidified to a pH = 2 and diluted 1/20 in triplicate with diluting fluid.

Where the calcium content was found to be less than 100 mgm./litre, an additional oxalate precipitation was carried out as follows.

Duplicate 10 ml. samples of urine were pipetted into 50 ml. centrifuge tubes, made alkaline (pH = 8-9) with 0.88 M. ammonium hydroxide and the oxalate precipitated by addition of saturated solution of ammonium oxalate. This precipitate was isolated by centrifuging after the supernatant had been tested with further ammonium oxalate. It was then dissolved in HCl/HNO_3 and made up to 10 mls. in a volumetric flask.

Urinary Calculus

The result for the urinary calculus was obtained by the dissolution of the calculus in the minimum volume HCl/HNO_3 and making

up to 25 mls. in a volumetric flask. This solution was diluted in duplicate with diluting fluid.

f) Bone:- Duplicate 0.2 gm. wt. samples of bone ash were dissolved in the minimum volume of HCl/HNO_3 and made up to 1000 mls. with de-ionised water and then diluted in duplicate with diluting fluid.

g) Teeth:- The teeth samples available were also to be estimated for radio-strontium content. Thus the solutions obtained were aliquots of solutions prepared for radio-strontium analysis. These were solutions of 5-10 gms. unashed tooth tissue dissolved in dilute nitric acid, filtered to remove organic matter and made up to 500 mls. Such aliquots were diluted 1/100 in duplicate with de-ionised water and then 1/10 with diluting fluid.

h) Non-Ionic Detergent:- estimated directly after tenfold dilution with diluting fluid.

- - - - - - - - - - -

All solutions thus obtained were estimated by MacIntyre's method on the Zeiss P.M.Q. II using normal calcium operational conditions.

Estimation of Strontium

a) Diet - Duplicate diet ash samples of 0.5 gms. weight were dissolved in a minimum volume HCl/HNO_3 and made up to 25 ml. with de-ionised water. Each solution was used to prepare duplicate 5 ml. solutions containing additive standard.

b) Milk - Four 10 ml. samples of liquid milk were evaporated to dryness in crucibles using a bunsen flame, ashed at 700°C and dissolved in a minimum volume HCl/HNO_3. These were then washed into four 10 ml. volumetric flasks with de-ionised water and made to volume.

Each was used to prepare 5 ml. additive standard solution.

c) Water - Ordinary tap water was concentrated to 1/10 volume by evaporation. This was done in triplicate. These solutions were used to prepare the additive standard solution.

d) Faeces - Duplicate faecal ash samples 0.25 gms. weight were dissolved in a minimum volume HCl/HNO_3 in a 25 ml. volumetric flask and made to volume with de-ionised water. These were used in the preparation of the additive standard solutions.

e) Urine - Urine samples were measured, acidified to pH = 2 and the strontium content of the acidified urine estimated in duplicate, both directly and after oxalate precipitation as described for estimation of calcium.

Urinary Calculus - The solution prepared in the estimation of calcium was also used for direct estimation of strontium, in duplicate.

f) Bone - Duplicate bone ash samples of 0.3 gms. weight were dissolved in HCl/HNO_3 and made up to 25 mls. in a volumetric flask. Each was used to prepare duplicate additive standards.

g) Teeth - Strontium content of teeth was estimated in duplicate from the aliquot of the solution obtained for radio-strontium analysis.

h) Non-Ionic Detergent - This was diluted in duplicate 1/10 with de-ionised water and each solution used to prepare additive standards.

- - - - - - - - - - -

All samples were finally estimated on the Zeiss flame spectrophotometer using normal strontium operational conditions. The additive standard was prepared from the above solutions by adding 0.05 mls. of 200 ppm. Sr standard solution to a 5 ml. volumetric flask and making to volume with the prepared solution.

APPENDIX V

Estimation of Phosphorus in Biological Samples

(As carried out in the Biochemistry
Laboratory, R.H.S.C., Glasgow)

Diet and Faeces:- Approximately 1 gm. ash was dissolved in hydrochloric acid or trichloracetic acid, treated with molybdic acid, hydroquinone and sodium sulphite to produce a colour reaction and estimated on an Eel colorimeter using a red filter.

Urine:- Protein was precipitated with 10% trichloracetic acid and removed by centrifuging. The solution was diluted 1/50 with de-ionised water and an aliquot taken for estimation on the colorimeter, as above.

APPENDIX VI

STATISTICAL NOTES

a) Standard Deviation

The standard deviation of results from the mean was calculated from the formula:-

$$\text{Standard Deviation} = \sqrt{\frac{\sum_{i=1}^{i=n} (x_i - \bar{x})^2}{n}}$$

(Sherlock, 1964)

where x_i = observed value,

$\bar{x}$ = mean, n = no. of observations.

b) Replicate Photometric Analysis

Results for replicate analysis of bone ash sample known to be 38% Ca content are as follows:-

37.5%. 38%, 38%, 38%, 38.5%, 38%, 38% and 38.5%

These results give a mean = 38.06% with a standard deviation from the mean of $\pm$ 0.3 and a standard error of the mean = 0.106.

SECTION VI

REFERENCES

Alexander, G. V., R. E. Nausbaum and N. S. MacDonald (1956).
J. Biol. Chem. 218. 911.

Ahrens, L. H. and S. R. Taylor. "Spectrochemical Analysis"
Addison-Wesley Press, Cambridge, Mass. 1950.

Alcock, N., I. MacIntyre and I. Radde (1960). J. Clin. Path. 13 506.

Alstead, S. and J. Gordon MacArthur. "Clinical Pharmacology",
Bailliere, Tindall and Cassell, London. 1965.

Ascari, T. (1950). J. Chem. Soc. Japan (Pure Chem. Sect.) 71 287.

Bailey, N. T. J., F. J. Bryant and J. F. Loutit.
A.E.R.E. R 3299, H.M.S.O., London. 1960.

Barnes, D. W. H., M. Bishop, G. E. Harrison and A. Sutton (1961).
Internat. J. Rad. Biol. 3 637.

Bauer, G. C., A. Carlsson and B. Linquist (1956). Metabolism 5 573.

Bauer, G. C. and R. Ray (1958). J. of Bone and Joint Surgery
49A No. I.

Bedford, J., S. E. Harrison, W. H. A. Raymond and A. Sutton (1960).
Brit. Med. J. 1 589-592.

Bell, G. H., J. N. Davidson and H. Scarborough. "Textbook of
Physiology and Biochemistry", E. & S. Livingstone Ltd.,
Edinburgh and London. 1953.

Benison, D., E. V. Elst and E. Ramos. 3rd United Nations Inter-
national Confer. on Peaceful Uses of Atomic Energy. 1964.
Argentina. A/Conf. 28/P/769.

Bergstrom, W. H., (1956). Metabolism 5 no. 4. 433.

Bishop, M., G. E. Harrison, W. H. A. Raymond, A. Sutton and R. Rundo (1960). Internat. J. of Radiat. Biol. 2 No. 2. 126.

Boyd, J., W. F. Neuman and H. C. Hodge (1959). Arch. of Biochem. and Biophys. 80 part II 105.

Bryant, F. J., E. C. Blacklock, E. H. Henderson, R. V. Jury, A. Parker and E. R. Webber. M.R.C. Report no. 9. H.M.S.O., London. 1964.

Bryant, F. J., E. H. Henderson and D. W. Holgate (1960). Brit. Dent. J. 108 No. 8. 291.

Bryant, F. J. and J. F. Loutit. A.E.R.E. R. 3718. H.M.S.O. London. 1961.

Bryant, F. J., A. Morgan and G. S. Spicer. A.E.R.E. R. 3030. H.M.S.O. London. 1959.

Burton, J. D. and R. Scott Russell. A.R.C.R.L. No. 12. H.M.S.O. London. 1964.

Calcagno, P. L. (1966). Lancet i 320.

Carr, T. E. F., G. E. Harrison and A. Sutton (1962). Proc. Soc. Exper. Biol. and Med. 110 151.

Champion, P., H. Pellet and M. Grenier (1873). Spectrometry: The Spectronatrometre. Comp. Rend. 76 707.

Collander, R. (1941). Plant Physiology 16 691.

Comar, C. L., and F. Bronner. "Mineral Metabolism" No. I part B. - Academic Press. New York and London. 1961.

Comar, C. L., R. Scott Russell and R. H. Wasserman (1957). Science 126 485.

Comar, C. L. and R. H. Wasserman. Progress in Nuclear Energy Series VI. Vol. I. Biological Sciences - Pergamon Press Ltd. 1956. Editors: J. C. Burgher, J. Coursaget and J. F. Loutit.

Comar, C. L., R. H. Wasserman and M. M. Nold (1956). Proc. Soc. Exper. Biol. 92 859.

Comar, C. L., R. H. Wasserman, S. Ulberg and A. S. Andrews (1957). Proc. Soc. Exper. Biol. and Med. 95 386.

Comar, C. L., K. Kostial, N. Gruden and S. E. Harrison. Health Physics. Vol. II. 609 - Pergamon Press. 1965.

Crawford, A. M. (1790). Medical Communications 2 301.

Davidson, J. W., P. B. Cha-Cha and W. James (1965). J. of Bone and Joint Surgery (Brit.) 47B 537.

Davy, H. (1808). Phil. Frans. 98 333f.

Dean, J. A. "Flame Photometry". McGraw-Hill Book Company Inc. New York. 1960.

Delahay, P. "Instrumental Analysis" MacMillan and Company. London. 1957.

Della Rosa, R. J., F. A. Smith and J. N. Stannard (1961).
Internat. J. Rad. Biol. 3 557.

Desgrez and Meunier (1926). J. Compt. Rend. 183 689.

Documenta Geigy. "Scientific Tables" 6th Edit. Geigy Pharmaceuticals Company Limited. Manchester. 1962. Editor: K. Diem.

Engström, A., R. Bjørnestedt, C. F. Clemedsen and A. Nelson
"Bone and Radiostrontium" John Wiley and Sons Inc.
New York. 1957.

Erf, L. A. and C. Pecher (1940). Proc. Soc. Exp. Biol. and Med.
45 762.

Fuller, W. H. and W. J. Flocker (1955).
Univ. of Arizona Tech. Bull. 130.

Geis, W. J. (1918). J. Amer. Dent. Assoc. 5 527.

Gerlach, W. and R. Muller (1934).
Arch. Path. Anat. and Physiol. Verchow 294 210.

Haissinsky, M. and J. P. Adloff. "Radiochemical Survey of Elements"
Elesvier Publishing Co. London. 1965.

Harrison, G. E. (1955). Brit. J. Pharmoc. and Chemotherapy 12 336.

Harrison, G. E. (1958). Nature 182 792.

Harrison, G. E. and W. H. A. Raymond (1955). J. Nucl. Energy. 1 290.

Harrison, G. E. W. H. A. Raymond and H. C. Trethaway (1955)
Clin. Science. 14 681.

Hawthorne, J. (1959). Chem. and Indust. 1294.

Hegemann, F., H. Kostyra and B. Pfab (1957). Glastech. Ber. 30 14.

Herschel, J. F. Encyclopedia Metropolitina. 2nd edit. rev. 438. J. Griffen. London. 1848.

Hesp. R. and B. Ramsbottom (1965). Nature 208 1341.

Hope, T. C. (1798). Trans. Roy. Soc. Edinburgh. 4 3.

Ikeda, S. (1956). Tohoku Univ. Res. Instit. Science Reports. Series A. Physics, etc. 8 457.

Janssen, J. (1870). Compt. Rendu. 71 626.

Kleinberg, J., Argensinger, W. J. and E. Griswold. "Inorganic Chemistry" - Boston Heath. 1960.

Kostial, K., N. Grunden and G. E. Harrison (1964). Nature 201 1240.

Kostial, K., A. Lutkic, N. Grunden, S. Vojvodic and G. E. Harrison (1963). Internat. J. Radiat. Biol. 6 431.

Kshirsagar, S. G., E. Lloyd and J. Vaughan (1966). Brit. J. Radiol. 39 131.

Laskowski, D. E. (1965). Anal. Chem. 37 1399.

Laszlo, D., M. Blau, H. Spencer and J. Swernov (1955). Geneva Conf. Paper. No P./212.

Laszlo, D. and H. Spencer (1959). Progress in Nuol Energy. Series VII Medical Sciences. Editors: Burgher, Coursaget and Loutit.

Lawrence, E. O. and D. Cooksey (1936). Physiol. Rev. 50 1131.

Likins, R. C., H. G. McCann, A. S. Posner and D. B. Scott (1960)
J. Biol. Chem. 235 2152.

Lough, S. A., G. H. Hamada and C. L. Comar.
Proc. Soc. Exper. Biol. and Med. 104 194.

Loutit, J. F. (1961). Irish J. Med. Science. 427 292.

Loutit, J. F. 'Diagnosis and Treatment of Radioactive Poisoning'.
I.A.E.A. Vienna. 1963.

Loutit, J. F., R. Scott Russell, R. S. Bruce and B. O. Bartlett (1964)
Nature 201 770.

Lundegardh, H. 'Quantitative Spectral Analysis of Elements' I
G. Fischer. Jena. 1929.

Lundegardh, H., 'Quantitative Spectral Analysis of Elements' II
G. Fischer. Jena. 1934.

Macdonald, N. S., P. Noyes and P. C. Lorick (1957)
Amer. J. Physiol. 185 131.

Macdonald, N. S., P. C. Spain, F. Ezmirlian and D. E. Rounds (1954)
Univ. of California Project report no. U.C.L.A. 317.

MacIntyre, I. (1961). Advances in Clin. Chem. 4 1.

Med. Res. Council. Report no. 2. "Hazards to Man of Nuclear
and Allied Radiations". H.M.S.O. London. 1960.

Menzel, R. G. and W. R. Heald (1955). Soil Science 80 287.

Mitchell, H, H., H. S. Hamilton and F. R. Steggerda and H. W. Bean.

J. Biol. Chem. 158 629 (1945).

Mitchell, R. L. and I. M. Robertson (1936).

J. Soc. Chem. Ind. (London) 55 269.

Noel-Martin, C., "Atom Friend or Foe".

G. C. Harrap and Co. Ltd. London. 1962.

Nordin, B. E. C. and W. G. Robertson (1966). Brit. Med. J. i 450.

Odum, H. T. (1951). Science 114 211.

Partington, J. R. (1941-47). Annals of Science 5 157.

Partington, J. R. "History of Chemistry" Vol. III.

MacMillan and Co. Ltd. London. 1962.

Pecher, C., (1941). Proc. Soc. Exper. Biol. 46 86.

Ramage, H. (1933). S. E. Nat. and Antig. Norwich Confer.

Rivera, J. (1965). Nature 207 1330.

Robinson, J. W., "Encyclopedia of Spectroscopy".

Rheinhold Publishing Corp. New York. 1960.

Edit. G. L. Clark.

Rothe, C. F. and L. A. Saperstein. (1955).

Amer. J. Clin. Path. 25 2. 1076.

Sherlock, A. J., "An Introduction to Probability and Statistics".

Edward Arnold. London. 1964.

Sidrak, G. H., L. J. Middleton, P. Newbould and H. M. Squire.
A.R.C.R.L. No. 12. H.M.S.O. London. 1964.

Smales, A. A. (1951). Analyst. 76 348.

Smith, H. and T. H. Bates. (1965) Nature 207 799.

Soddy, F. (1913). Nature 92 399.

Sowden, E. M. and S. R. Stitch. (1957). Biochem. J. 67 104.

Spencer, H., M. Brothers, E. Berger, H. E. Hart and D. Laszlo. (1956)
Proc. Soc. Exper. Biol. Med. 91 155.

Spencer, H., D. Laszlo and M. Brothers. (1957).
J. Clin. Invest. 36 680.

Spencer-Laszlo, H., J. Samachson, E. Hardy and J. Rivera. (1963).
Clin. Science. 24 405.

Squire, H. M. and R. S. Russell. (1955).
J. Exper. Biol. 9 262.

Talbot, W. H. F. (1826). Edin. J. Science 5 77.

Talmage, R. V., J. C. Schooley and C. L. Comar. (1957).
Proc. Soc. Exper. Biol. and Med. 95 413.

Thomas, R. and T. G. Thompson.
Bull. Nat. Res. Council. U.S. 95 No. 85.

Vaughan, J. (1959). New Scientist. 5 130.

Visek, W. J., R. A. Monroe, E. W. Swanson and C. L. Comar. (1953).
J. Dairy Science 36 373.

Walser, W., J. W. Payne and A. A. Browder. (1961).

J. Clin. Invest. 40 234.

Warren, J. M. M.Sc. Thesis. University of Glasgow. 1965.

Wasserman, R. H., C. L. Comar and M. M. Nold. (1956).

J. Nutrition. 59 371.

Weise, E. K. "Encyclopedia of Spectroscopy".

Rheinhold Publishing Corp. New York. 1960.

Editor: C. L. Clark.

West. (1964). Anal. Chem. 36 310.

Widdowson, E. M., R. A. McCance, G. E. Harrison and A. Sutton.

(1962). Lancet. ii 373.

Widdowson, E. M., R. A. McCance, G. E. Harrison and A. Sutton.

(1963). Lancet. ii 1250.

Widdowson, E. M., J. Slater, G. E. Harrison and A. Sutton. (1960).

Lancet. ii 941.