NUCLEAR AND ATOMIC METHODS OF ANALYSIS IN THE DETERMINATION OF ELEMENTAL COMPOSITION AND DISTRIBUTION OF BIOLOGICAL MATERIALS

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DEDICATED TO MY PARENTS
Abstract

Several 'in-vitro' and 'in-vivo' physical techniques of analysis have been used to examine the elemental composition and distribution of human bone for both contemporary and archaeological samples. Instrumental neutron activation analysis (INAA), a powerful tool, which had to be adapted for the particular requirements in the analysis of a bone matrix, determined a wide range of elements whereas electron microprobe analysis, using a scanning electron microscope, although not sensitive for trace element detection gave information about the distribution of major elements in bone, i.e. Ca and P, in much smaller areas with a spatial resolution of microns.

The contemporary samples were human tibiae which were analysed for a range of elements whose distribution in transverse sections and along the length of the bone was investigated. A number of elements showed increased metabolism towards the knee and ankle ends of the bone. The bones were also scanned in X-ray transmission mode (CT); by using the dual energy CT method values were obtained for the Ca/P ratio along the bone. These were found to be in agreement with the results obtained using INAA indicating that the dual energy technique is able to determine differences in the Ca/P ratio.

A Romano-British cemetery at Dorchester provided a population of skeletons and 'uniquely' some hair samples, for a study of trace element composition. The hair samples were found in a certain type of burial condition and the bone samples examined were taken from this same type of grave, and both were studied for the effects due to burial. These must be allowed for before any comparison can be made.
of trace element levels with contemporary populations. For bone Au, Sb and Zn were all increased in concentration in the poorly preserved samples and for hair Ca, P, Pb and Fe were found to be present as contamination due to burial. The bone and hair elemental concentrations were intercompared since it is thought that hair may act as a biological monitor of changes of body intakes and internal body burdens of environmental origin. However no correlation between elements for bone and hair was found for this very small population.
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INTRODUCTION

Bone is the principal calcified tissue of vertebrates constituting most of the hard mineralised component of the skeleton in most species. The structure and function of bone is extremely complex and the factors governing skeletal and mineral homeostasis and the mechanisms by which they act are not completely understood. The bone mineral consists of calcium, phosphate and carbonate which are present as a mixture of hydroxyapatite crystals and amorphous calcium phosphate. These hydroxyapatite crystals are small in size thus exposing an enormous surface area and they also have a high capacity for ion exchange and chemisorption. It is due to these factors and to the dynamic nature of bone metabolism that bone can be studied using bone seeking compounds labelled with radionuclides.

Many workers have studied the uptake and washout of such radionuclides (Hughes et al., 1978) in order to try to understand the mechanism of distribution in normal bone and to determine the differences when abnormal bone (lesions, fractures etc.) is present. Research work on animals indicates that cortical bone consists of four components through which minerals pass from the capillaries to the bone crystal (Hughes et al., 1978, Khan 1977). Marshall (1969) has divided bone elements into (a) 'volume' seekers (e.g. Ca, Sr, Ba) which are taken up during active mineralisation in high concentration and diffusely in low concentration throughout the whole bone mineral and (b) surface seekers (Am, Ce, Th, Cf and Y) which concentrate onto the surface of the bone. It is speculated that the increased avidity of bone for certain elements/compounds may depend upon the structure of the hydroxyapatite crystal.
The principal components of bone mineral are hydroxyapatite and amorphous calcium phosphate but smaller quantities of such elements as Mg, F, Na as well as several trace elements are also present. It is now suggested (Miller et al., 1980) that bone mineral is intermediate in structure between an amorphous solid and a perfect crystal. Also various elements may substitute into the apatite crystal e.g. Sr, Ba may exchange with Ca, whereas F exchanges with the hydroxyl ion. It is this lack of perfection in bone crystal structure which makes the bone mineral metabolically active (Posner, 1978) and therefore it is of great interest to measure the composition of bone. 'In-vivo' techniques generally give an indication of Ca concentrations in bone and although 'in-vivo' neutron activation analysis does allow the measurement of Na, Cl, P and N as well, these values reflect total body content rather than just the concentrations of these elements in bone. Therefore 'in-vitro' methods are required to determine the presence of other elements in bone mineral.

In this work the main technique used to analyse bone in order to study its elemental composition and distribution was 'in-vitro' neutron activation analysis. A variety of irradiation conditions were used and this enabled a total of 12 elements to be detected. Scanning electron microprobe analysis was also used to study the variation of Ca and P in transverse sections cut from along the length of the bone. The samples examined were human tibiae and this enabled X-ray transmission tomography to be performed before any 'in-vitro' analysis was carried out. X-ray transmission tomography is widely used for examining the bone mineral status of patients and its main advantage is in being able to outline
trabecular and cortical bone areas. The scanner provides reconstructed images of the sections of bone representing a normalised distribution of linear attenuation coefficients. This information may be used to give a measure of bone density and also the Ca/P ratio in cross-sections of the bone. These data can then be compared with the subsequent 'in-vitro' results to determine whether any variations in elemental composition measured by the in-vitro methods were reflected and could be detected, by an 'in-vivo' technique.

An excavation site at Dorchester revealed a Romano-British cemetery of the 3rd to 4th centuries A.D. providing a 'population' of human skeletons. A preliminary study was undertaken to examine the elemental composition and distribution of bone and to compare the results with a contemporary population. Due to diagenic effects it was important to examine the bones to determine the uptake or loss of elements before the results could be compared. In particular samples were taken from tibiae in order that the results may be compared with those obtained from the 'fresh' bone. In-vitro neutron activation analysis was used to obtain the concentrations of a wide range of elements in the bone.

A unique feature of this cemetery was that due to a particular type of burial, hair, in some cases, was also found to be well preserved. This gave the opportunity to study the elemental concentrations of Romano-British hair compared to contemporary values. Contemporary hair has been analysed for trace elements for a wide range of reasons in particular to determine whether it acts as either an epidemiological or an environmental monitor. Again diagenic
effects may have affected the elemental concentrations and in this case the scanning electron microprobe analyser was used to examine contamination on the hair whilst instrumental neutron activation analysis was employed to determine the actual trace elements present and their concentrations. The results obtained were compared with those from bone since it is thought that mineral levels in hair may reflect body intakes and internal body burdens of environmental mineral pollutants.
CHAPTER 1  STRUCTURE AND FUNCTION OF BONE

1.1  Introduction

Bone is a highly specialised form of connective tissue composed of interconnected cells in an intercellular substance, forming the skeleton or framework of the bodies of most vertebrates. It differs from other connective tissues, in particular because of its hardness, this being due to the deposition of a mineral substance, composed mainly of calcium, phosphate, carbonate and citrate within a soft organic matrix.

The main functions of bone are:-

i)  it constitutes a rigid skeleton whilst allowing movement due to muscle attachments to different bones.

ii) it provides protection for vital organs e.g. the brain,

iii) it plays an important role in the maintenance of mineral homeostasis through its high content of mineral ions.

The structure and function of bone is complex at all levels and the factors governing both skeletal and mineral homeostasis and the mechanisms by which they act are not completely understood. Skeletal homeostasis is affected both by hormones, for example, and by external factors such as mechanical stress and pressure. Mineral homeostasis is maintained by a complicated system of controlling factors acting on the skeleton, on the processes of absorption from the gastrointestinal tract and excretion from the bloodstream by the kidney. Therefore the skeleton is never metabolically at rest and is constantly
being remodelled. The patterns of skeletal renewal and loss have been well characterised in gross morphological terms but little is known about changes in skeletal ultrastructure or chemical composition with age.

1.2 The Structure of Bone

Macroscopically there are two types of bone structure distinguishable in the mature skeleton (see fig. 1.1):

i) Compact or cortical bone which is found largely in the shafts of long bones and surrounds the marrow cavities,

ii) cancellous or trabecular bone which is found in the vertebrae, the ends of long bones and most flat bones; it consists of a network of fine interlacing partitions, the trabeculae, enclosing cavities that contain the marrow.

There is also another type known as woven bone, which appears first in embryonic development or in the repair of fractures. It is usually more cellular and is replaced by mature bone but some may remain especially near tendon insertions and ligament attachments.

1.2.1 Bone Cells

In all kinds of bone there are three different types of cells present:

1) The Osteoblasts

They are derived from stromal marrow cells and are important in the process of the formation of bone. Their size varies but they
Fig. 1.1: Sagittal section through the head and shaft of the human tibia.
are usually ~10 μm in diameter and their shape is dependent upon their function e.g. 'active osteoblasts' are cuboidal or columnar in shape whereas resting osteoblasts are attenuated in shape. Osteoblasts lay down the osteoid, which is the ground substance of bone, by the synthesis of collagen and some of the carbohydrate protein complexes. Mineralisation then occurs, involving the initial deposition of a mainly amorphous calcium phosphate which is slowly converted into hydroxyapatite, in two stages (Frost, 1966):

a) Primary Mineralisation (under the control of osteoblasts) - about 70-75% of the mineral is deposited, within a few days, in the matrix.

b) Secondary Mineralisation (not under the control of osteoblasts) - the density gradually increases to about 90% of the maximum over a period of several months.

Osteoblasts lie closely together and appear to form a barrier between the surface of the forming bone on one side and the connective tissues and blood vessels of the marrow on the other. Next to the osteoblasts is a layer of as yet unmineralised matrix (the osteoid seam) which is separated from the fully mineralised bone by an intermediate-zone (the mineralisation front) where bone seeking agents are deposited. The osteoblast has also been implicated in the deposition and exchange of calcium and other ions, and may play at least three roles in the mechanism of calcification:

1) as the origin of matrix vesicles (Anderson, 1976),
2) by exerting some control on the movement of calcium ions in and out of bone fluid (Talmage, R.V., 1970).
3) by its capacity to store calcium in its mitochondria and possibly pass it onto the matrix (Matthews and Martin, 1971).

2) The Osteocyte

As bone grows some of the osteoblasts become surrounded by matrix and develop into osteocytes. These osteocytes continue to lay down a small amount of matrix which ultimately becomes calcified (Baylink and Wergedal, 1971), reducing the size of the lacunae in which they rest. A series of cytoplasmic processes that extend radially from each cell in cannaliculae maintain the connection between osteocytes and osteoblasts. These processes are probably connected with one another by intercellular junctions that could form a communication network in bone (Holtrop and Weinger, 1972). It has also been suggested that nutrients circulate in the extracellular fluid between the cells and the bone, since labelled extracellular proteins can penetrate into this space.

The shape of these cells varies from round to flat and elongated depending upon the plane of view and the size may also differ with age and site. When the osteocyte dies then the surrounding matrix although persisting for a time becomes structurally inadequate, eventually crumbling away to be removed and replaced by living bone. 'Osteocytic osteolysis' is a process in which it is thought that the osteocytes are involved in the removal of not only the mineral but also the perilacunar matrix. It is important, however, to dissociate osteocytic osteolysis from the relatively large lacunae characteristic of immature and woven bone which are formed
this way so interpretation may therefore be difficult. The mechanism of this process is obscure, it could be a one-way cycle for the cells culminating in death, autolysis and empty lacunae or else in the light of the evidence that osteocytes are able to form bone, it could be part of a recovery and restoration phase.

3) The Osteoclast

This is a large, multinucleated cell found lying in Howship's lacuna on the resorbing surface of bone and has a 'ruffled' or 'brush' border. Recent electron microscope studies indicate that there is a difference between a 'ruffled border' and a 'brush border'. Ham and Cormack (1979) describe the ruffled border as the cause of resorption, for it is composed of fine finger-like cytoplasmic processes fanning out from the cell to terminate on the bone surface, while the brush border is an effect of resorption. It is composed of the straight collagenic fibrils of calcified bone or cartilage at sites where they are disposed more or less at right angles to a surface undergoing resorption, in which process the mineral between the fibrils is removed so that they are freed from it. The osteoclast is very mobile, leaving the bone to 'melt away' after it has moved from its original site of action. Originally it was thought that the osteoclast shared a common stem cell with the osteoblast, but there is now evidence that the osteoclast is derived from the mononuclear cell of the blood and not from a bone forming precursor (Vaughan, 1981).

There is considerable debate as to how exactly the osteoclast exerts its resorptive effect on bone but no agreement has been
reached between different workers. One suggestion is that the osteoclast secretes an acid which dissolves the mineral, releasing the collagen fibres to be digested by other mononuclear cells (Heersche, 1978). Other workers consider that the calcium is first removed and the matrix constituents are ingested and digested by the many lysosomes present in the cytoplasm (Hancox, 1972). The removal of the apatite crystals has been attributed to the production of both citric and lactic acid, so freeing the collagen (Vaes, 1968), but the evidence on the part played by both acid and enzymes in relation to organ and tissue culture experiments, is confused. However there is no doubt that the osteoclast is involved in the resorption, since the factors such as calcitonin and parathyroid hormone which affect resorption produce marked changes in both the appearance, the number and behaviour of the osteoclast (Vaughan, 1981).

1.2.2 Bone Modelling

In the embryo bone develops by two methods, one being membranous in character, the other occurring inside a cartilaginous model of the future bone. The fundamental process of osteoblasts laying down the osteoid which later becomes calcified, occurs in both cases.

1) Intramembranous Ossification

This process begins when a cluster of mesenchymal cells differentiate into osteoblasts thus forming an ossification centre. These osteoblasts secrete osteoid and some become buried and form osteocytes, whereas the remainder continue to form osteoid and surround ingrowing capillaries which will bring in the haemopoietic cells of the future marrow. Spicules of bone are found radiating out from the
ossification centre thus creating a spongy type of bone which then becomes calcified. In early foetal life resorption and apposition begin to take place so that trabecular bone occupies the centre of the mass whilst a layer of compact bone is formed on each surface by the continuous addition of new sheets of bone by the active osteoblasts. This type of formation of bone is found, for example, in the bones of the calvarium of the skull.

2) Endochondral Ossification

A cartilage model is formed by chondroblasts (derived from mesenchyme cells) which lay down an intercellular substance, and is enclosed in perichondrium, a dense fibrous connective tissue layer that is membranous on the outside and becomes cartilage-like on the inside. The cartilage model grows in width by apposition of new layers of cartilage on the surface whereas growth in length occurs by an interstitial mechanism involving chondrocyte/chondroblast division and further synthesis of intercellular material, at the ends of the model.

The inner layer of cells of the perichondrium differentiate to form osteoblasts which lay down a layer of osteoid which calcifies forming a collar of periosteal bone directly in contact with the cartilaginous model. Meanwhile cartilage cells in the centre of the model have undergone degenerative changes associated with some calcification. Capillaries from outside the perichondrium push through the perichondrium and the periosteal bone and invade the degenerating cartilage cells carrying osteogenic cells, forming the periosteal bud (Ham, 1969), and this is the site of the internal ossification centre of the diaphysis. Here osteoblasts deposit bone on the remnants of the calcified cartilage, and the primary spongiosa
so formed consists of trabeculae with cores of calcified cartilage. As the cortex expands some trabeculae disappear and the marrow cavity becomes larger. Ossification stops short of replacing all the cartilage; articular cartilage remains, together with a disc of cartilage between the diaphyseal and epiphyseal ossification centres which forms the epiphyseal plate, see figs. 1.2 and 1.3. Elongation of the long bones is thus dependent upon the proliferation and subsequent calcification of this epiphyseal cartilage. The shaft of the bone increases in width by subperiosteal apposition of cortical bone, see fig. 1.4. Simultaneous resorption inside the shaft prevents the shaft from becoming too thick.

1.2.3 Bone Remodelling

Upon skeletal maturity growth stops but internal remodelling of the bones continues at localised sites where old, damaged tissue may be directly replaced by new bone or trabeculae may be differentially reinforced or resorbed to cope with changes of stress. There are two types of remodelling to be considered since there are two main types of bone.

1) Haversian Remodelling

Cortical bone consists of cylindrical columns, 150-300 μm in diameter, known as Haversian systems or osteons, whose long axis runs approximately parallel to that of the bone, with interstitial lamellae filling the gaps between them, see fig. 1.5. A typical osteon consists of a central canal 30-70 μm in diameter, containing a nutrient vessel, lymphatics, nerves and connective tissue, surrounded by concentric lamellae. The boundary of the osteon is
Fig. 1.2: The epiphyseal plate. Arrows (top) indicate the direction of growth.
Fig. 1.3: The development of the long bone

Fig. 1.4: The development of haversian systems at the periosteal surface. (I) Channels containing blood vessels occur at the surface of the bone. (II) Osteoblasts differentiate from the periosteum on either side of the channel. (III) Osteoblasts deposit ridges of new bone (a). (IV) More layers of new bone (b) and (c) are formed on the ridges and the channel becomes a tunnel. (V) Osteogenic cells from the periosteum trapped in the tunnel begin to produce bone (d). (VI) Further concentric layers complete the new haversian system.
Fig. 1.5: A model of bone.
indicated by a cement line, a 1-2 µm thick layer of mineralised ground substance with few or no collagen fibres, which separates pieces of bone made at separate times.

Formation of a new osteon begins with the creation of a resorption space and osteoclastic activity begins near the centre of the future osteon and advances centrifugally for about 100 µm. Refilling of the hole is achieved by centripetal deposition of successive lamellae of new bone which continues until it has narrowed to the diameter of a mature Haversian canal. Thus in this sequence a newly formed batch of osteoclasts arrange themselves as at the point of a drill, forming the so-called cutting zone which advances longitudinally through the cortex either along or parallel to an existing Haversian system. Behind these is a zone of undifferentiated spindle shaped cells and behind these are the blood vessels which will eventually traverse the mature Haversian canal. A variable distance behind the advancing resorptive front and separated from it by a zone of inactivity, osteoblastic refilling of the hole begins, forming the closing zone. This anatomically discrete longitudinally oriented structure persists for a variable but unknown period of time, travelling through the cortex to carry out the functions of bone turnover in an ordered manner and is referred to as a cortical remodelling unit by Parfitt (Parfitt, 1976).

Remodelling may occur at multiple sites in the same osteon, travelling either in the same direction or in opposite directions to form a double-ended cone. Variations occur in the duration of the switchover from resorption to formation and the formation process may not be continuous. Mesenchymal cell proliferation, osteoclastic
resorption and osteoblastic formation follow one another in succession but the nature of the coupling between these processes is still unknown.

2) Trabecular Remodelling

There is strong evidence for the same sequence of resorption followed by formation as in Haversian remodelling from the examination of cement lines, the shape of which indicates the state of the surface just before the bone within the cement line began to form (Parfitt, 1976). A smooth cement line indicates that new bone was deposited on a quiescent surface whereas a scalloped line indicates it was deposited on a surface covered by Howship’s lacunae due to previous osteoclastic activity. However this does not establish whether resorption and formation are coupled together temporally or functionally. It was found that in three dimensions (Jaworski, 1971) the trabecular remodelling unit resembled a Haversian system which had been cut open longitudinally and unfolded over the trabecular surface. The osteoclasts spread out over a front of up to 800 µm wide and advance across the surface excavating a shallow furrow between 10 and 100 µm deep and about 1000 µm long. The time relationship between resorption and subsequent formation is less well defined for trabecular than for cortical bone but the interval between them may be estimated roughly from the relative prevalence of the different types of remodelling activity on the trabecular surface.

It has been suggested (Frost, 1964) that bone remodelling is similar to energy which can only be transferred in quanta i.e. that bone turnover occurs in anatomically discrete foci, within which
remodelling activity lasts about 4-6 months. This occurs in both types of remodelling with one quantum being the bone remodelling unit. Therefore this indicates that the imbalance between resorption and formation in a person with metabolic bone disease e.g. osteoporosis (Parfitt, 1979) should be examined not at the level of the whole skeleton but at the level of the individual bone modelling unit. Measurement of whole body turnover by biochemical or isotopic methods at present gives no indication as to whether remodelling imbalance is due to an increase in the amount of bone removed or to a decrease in the amount of bone formed.

1.2.4 Mineralisation of bone

Calcification is concerned with the mechanisms involved in the deposition of hydroxyapatite within an organic matrix. For this to occur the $\left( \text{Ca}^{2+} \right) \times \left( \text{PO}_4^{2-} \right)$ solution ion product must be raised locally to levels at which spontaneous precipitation occurs. Two factors are thought to be involved in this, alkaline phosphatase and other enzymes which are found in excess in areas of calcification and thought to be an indicator of active bone metabolism, and calcium binding proteins. The precipitation of these crystals involves the process of nucleation i.e. the formation of the minimum grouping of ions capable of survival and growth. In bone the energy barrier to nucleation is lowered by the presence of a nucleating substrate (Glimcher, 1976) i.e. a substance which creates nucleating sites or removes barriers to these sites.

Collagen is an important nucleation site in bone and Glimcher (Glimcher, 1976) considers there is a close relationship between collagen structure and the form and orientation of the apatite crystals
which may find their place in the hole proposed in the structure of the collagen fibril. Not all electron microscopists accept the presence of the crystals within the collagen fibril although many agree there is an alignment between collagen fibrils and apatite needles. Carbohydrate protein complexes of bone play an important role in the mechanism of calcification and may possibly provide nucleating sites, although they probably play a series of roles which as yet are still not understood.

There is now increasing evidence that the osteoblast and chondrocyte play a crucial role in the initiation of calcification of osteoid and cartilage matrix by being the source of matrix vesicles which provide nucleating sites. These vesicles are membrane invested, extracellular particles serving as initial sites for calcium deposition and were observed in growth plate cartilage (Anderson, 1969) first, but were soon recognised to also occur in bone osteoid tissue (Bonucci, 1971). The vesicles, measuring about 100 nm in diameter, are rich in phosphate, alkaline phosphatase, pyrophosphatase, adenosine triphosphate (ATP) and magnesium. By regulating the Mg, the ATP and possibly ATPase levels, the cell can store or utilise amorphous calcium phosphate granules (Posner et al. 1976-7). The phospho-lipid membrane of the vesicle breaks upon the vesicle entering the matrix, releasing the Mg which inhibits apatite formation and the amorphous phosphate granules either become apatite or meet other factors favouring apatite formation such as calcium binding proteins (Posner, 1978).

There is obviously still a lot to be determined about the precise mechanism of calcification in bone. An exciting aspect of matrix vesicles is that they constitute a common factor in the
mineralisation of diverse tissues and an understanding of vesicle action in one tissue such as bone may well lead to unexpected information relating to the calcification of arteries, tumours etc. (Anderson, 1976).

1.3 Mineral Homeostasis

Bone plays its part in the control of mineral homeostasis in cells and body fluids as the result of action by the metabolites of vitamin D, the parathyroid hormone (PTH) and calcitonin. These hormones are secreted because of changes in the plasma ionised-calcium and phosphate concentration. The major sites of action of vitamin D, parathyroid hormone and calcitonin are shown in fig. 1.6.

Vitamin D

Vitamin D is an important regulatory hormone in calcium metabolism and in excess it causes hypercalcaemia whereas deficiency results in hypocalcaemia and an impaired response to PTH. There is a period of time between the administration of vitamin D and its effects becoming noticeable, during which it forms metabolites e.g. 1,25-dihydroxycholecalciferol. Many such metabolites have been identified, and there may still be unidentified active forms, but their exact mechanisms of control are still not known. Vitamin D acts as a mineralising or bone growth hormone and so both phosphate and calcium deficiency leads to an increase in the synthesis of 1,25-dihydroxycholecalciferol in the kidney. Vitamin D metabolism also depends on the parathyroid status since PTH affects calcium and phosphate transport by the kidney. 1,25 dihydroxycholecalciferol is a direct stimulator of intestinal calcium transport and bone resorption and since the
Fig. 1.6: Major sites of action of vitamin D, parathyroid hormone and calcitonin.
phosphate supply is also increased by these direct effects more mineral is made available for new bone formation. These processes are affected at higher concentrations by other metabolites. Direct effects on matrix formation or mineralisation have been suggested but not yet demonstrated (Raisz, 1965).

Parathyroid Hormone

Its action on bone, kidney and the small intestine increases the concentration of serum calcium. In turn the production of PTH is inhibited by an increase and stimulated by a decrease in serum calcium through direct effects of Ca on the PTH rate of production. This feedback mechanism of regulation of the concentration of Ca in extra-cellular fluid (ECF) is obviously very important. Any tendency towards hypercalcaemia which might be brought about by intestinal malabsorption or by the phosphate retention that occurs in renal failure is counteracted by an increase in secretion of PTH. This increased amount of PTH in blood:

i) acts to increase the rate of dissolution of bone mineral (and matrix), therefore providing increased flow of Ca from bone into the blood,

ii) reduces the clearance of Ca by the kidney returning more of the filtered Ca back into the ECF,

iii) increases the transport of Ca across the intestinal mucosa, resulting in an increased absorption of dietary Ca.

The relative physiological importance of these actions is not completely resolved and it is thought that the effects of PTH on the
gut are mediated through the vitamin D metabolites, mentioned earlier. Its effect on bone appears to be an increase in the numbers of osteoclasts and osteoblasts and a general increase in the remodelling of bone primarily in the direction of dissolution of bone mineral and matrix.

Calcitonin

This hormone is concentrated in the thyroid in man but is also found in the parathyroid and the thymus. Calcitonin secretion is stimulated not only directly by high calcium but also by gastrointestinal hormones, particularly gastrin and its main direct effect is the inhibition of bone resorption although there are effects on renal and intestinal ion transport. This has been proved by using calcitonin to successfully reduce the resorption rate of bone in clinical conditions, such as Paget's disease where bone resorption is very high (Woodhouse et al. 1971). The osteoclastic activity and their number are seen to decrease after treatment with this hormone as would be expected if bone resorption is reduced. The immediate action on the kidney in man is to decrease tubular reabsorption of several electrolytes including \( \text{Ca}^{2+}, \text{Mg}^{2+}, \text{Na}^{+}, \text{K}^{+}, \) and \( \text{PO}_{4}^{3-} \).

However the role of calcitonin in the calcium metabolism of man is still uncertain, partly because there are no recognisable syndromes of under- or over-secretion associated with disturbances in calcium metabolism and also because the concentration of plasma calcitonin, even under conditions of hypercalcaemic stress, is less than the detection limits of the assays in use at the present.
Other hormones, the thyroid, the growth hormone, and the adrenal and gonadal steroids influence skeletal growth and development and have effects on Ca metabolism, but their secretion is determined primarily by factors other than changes in plasma calcium and phosphate.

1.4 Studies of Bone Using Physical Methods of Analysis In-vivo

1.4.1 The hydroxyapatite crystal

The principal components of bone mineral are calcium phosphate and carbonate present as a mixture of hydroxyapatite crystals and amorphous calcium phosphate. The unit cell of the hydroxyapatite crystal is a right rhombic prism given by the formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ which generates by translationally periodic repetitions a simple hexagonal lattice. In size the crystals do not exceed $500 \times 250 \times 100 \, \text{Å}$ and due to this small size an enormous surface area is exposed (Mattar and Siegel, 1979). A layer of water, termed the hydration shell, is bound to the surface of the crystals and ions have to be transferred through this to go to and from the crystal. Much of the bone crystal is inaccessible for exchange with ions in the blood but considerably more is in equilibrium with the extracellular fluid, providing an ion exchange surface of enormous capacity and reactivity (Neuman and Neuman, 1958). It is due to the enormous surface area of the apatite crystals with their high capacity for ion exchange and chemisorption and to the dynamic nature of bone metabolism that bone can be labelled with bone seeking radionuclides.
1.4.2 Use of Radioactive tracers

Physiologically a radioisotope of Ca would be the ideal tracer for the evaluation of regional skeletal mineral metabolism but unfortunately neither of the readily available isotopes are suitable for bone imaging. $^{45}$Ca ($t_\frac{1}{2} = 165$ days) is a pure $\beta^-$ emitter and cannot be detected externally, $^{47}$Ca ($t_\frac{1}{2} = 4.7$ days) does emit several $\gamma$-rays the most abundant being at 1.3 MeV which cannot be readily collimated by nuclear imaging instruments. $^{47}$Ca has however found considerable use in measurements of calcium absorption, retention and turnover using whole body counting techniques. Other alkaline earths such as strontium and barium have been used as analogs of calcium and fluorine too has been used as it exchanges with the $(OH^-)$ ion but the most successful isotopes that are currently used in bone scanning are the technetium labelled phosphate compounds. It is believed that the phosphate compound binds directly with the hydroxyapatite crystal and $^{99}$TcM is a readily available isotope with a short half life ($t_\frac{1}{2} = 6$ hrs.) and an energy of 140 keV, which is ideal for scanning because it produces a low dose to the skeleton. The diphosphonate compounds appear to be the best experimentally and clinically (Hughes et al. 1975) for they are rapidly cleared from the blood giving a high ratio of bone to soft tissue at four hours which is the time of bone scanning. The isotopes are detected using either whole-body rectilinear scanners or by large field $\gamma$ cameras.

The blood supply is obviously an important factor for the labelled compound must travel in the blood through to the capillaries in the bone where they exchange into the hydroxyapatite. It has been suggested that there is a fluid space across which diffusion can occur and that
a membrane exists which normally controls the fluxes of ions to and from the bone substance. From studies of wash-out curves using $^{99}$Tc$^{m}$-MDP (Hughes et al., 1978) it has been suggested that a typical Haversian system consists of four compartments: the capillaries, the perivascular fluid space, the bone fluid and the bone itself see fig. 1.7. This fluid space is obviously important for the diffusion of solutes and possibly for influencing mineral exchange (Khan, 1977). It has also been shown by Hughes et al. (1977) that the transcapillary exchange of solutes in bone is by the process of passive free diffusion, and is dependent on molecular size.

As already stated blood flow plays an important part and it is necessary to distinguish whether increased uptake of the radioisotope is just due to an increase in blood flow or whether there is increased bone turnover. Radioactive microspheres have been used to measure bone blood flow by several investigators (Gross et al., 1979; Morris and Kelly, 1980; Heymann et al., 1977) and proved successful although there are limitations (Tothill and MacPherson, 1980). Using microspheres Lavender et al. (1979) compared the bone blood flow with MDP uptake and concluded that the increased radionuclide uptake in bone scans was greater than the corresponding increase in bone blood flow. Further work has been carried out by McCarthy (McCarthy et al., 1980) to study the effect of flow changes on the transit time and maximum instantaneous extraction.

The use of these radionuclides is now widespread allowing the investigation of a wide range of bone diseases ranging from infections to tumours and metabolic diseases. It is used in both the detection of primary malignant diseases as well as secondary e.g. when meta-
Fig. 1.7: An anatomical 'model' of the four compartments present in bone.
stases are found in the bone from breast cancer. Bone infections that have been examined in this way include e.g. osteomyelitis and septic arthritis whilst metabolic bone diseases studied have been limited but do include osteomalacia and hyperparathyroidism. Fractures and other skeletal injuries are generally diagnosed using clinical and radiographic findings but radionuclide techniques are occasionally of use e.g. as a means of assessing the rate of fracture healing.

Radionuclide scanning is therefore obviously an important technique not only because of its great use diagnostically but also because it may demonstrate exactly what is taking place in bone and establish the vital roles of the skeleton (Hughes, 1980). However it is clear that this accumulation of bone-seeking tracers in the skeleton does not reflect any single physiological phenomenon. The study of these bone compartments is also important to understand the process of the transfer of solutes across the fluid space, and the mechanisms involved in their localisation in bone, for this space may play an important part in bone physiology.

1.4.3 Determination of bone mineral content

Bone loss occurs as man ages, being greater in women than men, and this results in a reduction in the ability of the skeleton to withstand trauma. Therefore it is important that the mechanisms causing the loss should be understood and that quantitative methods be used for the measurement of that loss. A number of physical techniques have been developed therefore for studying mineral content in bones in-vivo.
These techniques all involve the measurement of the transmission or scattering of X-rays or γ-rays passing through the bone or bones under examination. Photodensitometric techniques were carried out using X-ray films and measurements of various indices on the dimensions of bones (Barnett and Nordin, 1961) and a narrow beam gamma transmission method has also been used (Garnett et al. 1973). The third type of measurement is concerned with methods using scattered radiation. Photon scattering has been used to measure bone density using the fact that the macroscopic Compton cross-section depends directly upon the electron density of the scattering material (Webber and Kennett, 1976). Electron densities are changed to mass densities by using the weighted average of atomic number to atomic weight for the material and transmission measurements are carried out to correct for attenuation of the incident and scattered photons. Similarly a Compton scattering method has been used with X-rays using a $^{241}$Am radionuclide source (Olkonnen et al., 1981) and an X-ray tube (Puumalainen et al., 1982) to determine trabecular bone mineral density from measurement of the coherently and Compton scattered photons. Finally X- and gamma-ray transmission computed tomography (CT) have been used to determine bone mineral, one of the main advantages being that the cortical and cancellous bone e.g. in the vertebra can be spatially separated (Cann and Genant, 1980). The principles of CT and its application to the study of bone is discussed more fully in section 3.2.

1.4.4 In-vivo neutron activation analysis (IVNAA)

Two types of IVNAA are generally performed, partial body and total body, to measure the major elements present i.e. Na, Cl, P and
Ca. However with respect to partial body measurements a particular element may be of interest and the irradiation parameters will then be optimised to give its best detection e.g. Se or Cd measurements in the liver (Nicolaou, 1983). With respect to bone, the element of interest is Ca, as the amount of Ca present in the body gives an indication of the bone mass, which is of interest for the reasons given in section 1.4.3. Other elements however have been studied, Spinks et al. (1976) measured the metabolic activity of Na in the hand using neutron activation analysis and found that the rate of loss of Na from the hand was found on average to be greater in patients suffering from metabolic bone disease.

The neutrons used for this purpose may be from a cyclotron or an isotopic source depending upon the facilities available. One of the difficulties in determining total body Ca is the effect of the size and composition of the body (Spinks, 1979) and so corrections must be applied to allow for these (Kennedy et al., 1982). IVNAA has been shown to be of value in the study of various diseases of the skeleton and in determining the successfulness of various treatments. Spinks et al. (1978) studied the effects of the various treatments on Paget's disease, Cushing's disease and osteoporosis and showed that in most patients the total Ca remained stable. However there was a slow upward trend in Paget's disease patients but on average there was no change in either the treated or untreated osteoporotic patients.

Partial body neutron activation analysis in general is carried out on peripheral bone in particular the forearm, for the results obtained can then be compared with those from γ-ray densitometry.
(Tothill et al., 1978). It was found that this technique compared favourably with other methods of monitoring sequential changes of Ca at peripheral sites (Smith and Tothill, 1979) and has proved to be a useful research tool. A technique has been developed for the measurement of Ca in the lumbar spine (Smith and Tothill, 1979) and also calcium measurements of the trunk carried out (Harrison and McNeill, 1982) again to study the effects on bone mass of various diseases. A wide range of conditions are thought to affect the bone mass ranging from renal failure to hyperparathyroidism to osteoporosis, so that information obtained about the Ca content both before or during treatment are obviously of great importance and the technique of IVNAA has proved to be a useful research tool in this field.

1.5 In-Vivo Studies of the Chemical Composition of Bone

As already discussed in section 1.4.1 the principal mineral components of bone are hydroxyapatite and amorphous calcium phosphate and there are smaller quantities of elements such as magnesium, fluorine and sodium as well as the presence of several trace elements. Miller et al. (1980) used extended X-ray absorption fine structure studies on bone and related calcium phosphates to try to provide new information on the actual structure of bone mineral. From their results they concluded that bone mineral is intermediate in structure between an amorphous solid and a perfect crystal rather than simply consisting of hydroxyapatite crystals with distortions. This may therefore explain the variation of the Ca/P ratios found in bone. Hydroxyapatite has a Ca/P molar ratio of 1.67 whereas Woodard (1962) found that the Ca/P molar ratio in bone ranged from 1.37 to 1.71 which therefore can be explained by this difference in structure
just described and by the fact that various elements may substitute into the apatite crystal. Calcium, for example, may be replaced by strontium or barium whereas fluorine and chlorine may exchange with the hydroxyl ion. It is this lack of perfection in bone structure due to the presence of carbonate, sodium and other ions and the deficiency in Ca and OH which combine to make the bone mineral metabolically active (Posner, 1978). It is therefore of great interest to measure the composition of bone and determine the Ca/P ratio as this may be important with respect to the structure and metabolic activity of the bone.

Major elemental composition of bone may be determined by IVNAA (section 1.4.4) but for the determination of any other elements then analysis must be carried out in-vitro. The availability of human samples is therefore limited to samples from either biopsy, amputation or cadavers. Numerous techniques have been developed for the elemental analysis of bone including both chemical and physical methods of analysis. Various chemical methods have been used for the analysis of bone ranging from mass spectrometry to atomic absorption spectrometry (Iyengar et al., 1978). In physical methods using ionising radiations the particular element or isotope is detected due to the interaction of a flux of particles or photons with the material resulting in the emission of characteristic radiation of specific energies. In particular there are two types of particles employed, for trace element analysis of biological materials, protons and neutrons.

Neutron activation analysis is fully described in the next chapter, and has been used to analyse bone with respect to Ca/P ratios.
and in order to determine a larger range of elements. Various neutron energies also have been used depending upon the neutron source available. Neutrons from a cyclotron have been used to analyse small bone biopsies to determine their Ca/P ratio (Batra and Bewley, 1973) and in normal subjects were found to be fairly constant (Chaudry, 1979). A further technique of using 14 MeV neutrons from a neutron generator has also been developed to determine this ratio. Various trace elements and their distribution in the human skeleton have also been determined by neutron activation analysis, usually carried out in a reactor, due to the higher fluxes required to determine the presence of low levels of a particular element. Brätter et al. (1976) examined the concentrations at multiple sites of the skeleton for several trace elements concluding that the element level depended on the actual site with particular reference to the mass ratio of trabecular/compact at that point. This may have important consequences when considering the analysis of a bone biopsy as the results obtained may therefore depend upon the position that the sample was taken from.

Proton interactions, like neutron interactions, are dependent upon energy so that the energy of the proton is decided by taking into account the reaction cross-sections so as to optimise the conditions. PIXE (Proton Induced X-ray Emission) analysis is usually carried out with protons of energies between 2 and 4 MeV to determine a range of elements in bone. Hyvönen-Dabek et al. (1981) used 2.4 MeV protons from a Van de Graaff accelerator to determine the concentrations of nine different trace elements in bone ranging from Cr to Zn. Also PIGE (Proton Induced Gamma Ray) analysis may be
used which is particularly suited for light elements such as fluorine. Hyvönen-Dabek (1981) detected 10 elements in bone using this method. The great advantage of using protons is that spatial information of the elemental concentrations may be obtained. For both PIGE and PIXE a proton microprobe has been used to determine the distribution of different elements in bone. PIGE has been used in particular to study the distribution of F in archaeological bone (Coote and Sparks, 1981; Ryde, 1982) whereas PIXE has been used to study the distribution of lead in the femur from a person exposed to lead in a heavy metal industry (Lindh et al., 1978) and also to investigate the distribution of heavy metals in individual osteons (Lindh, 1980).

Other techniques used for the analysis of bone include X-ray fluorescence e.g. the measurement of Sr content (Snyder and Secord, 1982) and the backscattering of α particles again used to determine Ca/P ratios as well as Ca/O and P/O ratios (Hyvönen-Dabek et al., 1979).
CHAPTER 2 INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS

2.1 Introduction

Activation analysis is used in the determination of elements in a wide range of materials. Lenihan (1969) related the application of activation analysis to the study of the nature of the environment and of man's interaction with it. He chose the components of man's environment to be cosmic, geological, biological, internal biological, artificial and social. Thus studies using activation analysis are found ranging from the study of metallic grains in meteorites (Baedecker, 1982), to the determination of trace elements in cardiovascular diseases (Plantin, 1978), to the measurement of trace elements present in archaeological materials (Mello et al., 1982).

Neutron activation analysis was first performed in 1936 by Hevesy and Levi when they bombarded their samples with neutrons to determine the presence of rare earths. In neutron activation analysis the intensity of the activity induced in an element by irradiating the sample with neutrons is measured to determine the presence of that element, and the activity induced is proportional to the amount of element present. Instrumental Neutron Activation Analysis (INAA) is where no chemical processing prior to irradiation or radiochemical separation following irradiation is performed thus the sample is not destroyed although radiation damage may occur depending on the type of material and the magnitude of neutron flux in which it is irradiated. Also there is less handling of the sample which leads to a reduction in contamination. These are two added advantages of INAA, but in general the advantages of the method are:
i) high sensitivity - for a neutron flux of $\sim 10^{12} \text{ncm}^{-2}\text{s}^{-1}$, concentrations of elements can be determined down to parts per million (e.g. detection limits of 1.1 µg/g for vanadium, 2.4 µg/g for bromine and 1.5 µg/g for manganese for 200 mg samples of soil were found by Kerr (1978)), and sometimes down to ppb (e.g. $^{77}\text{Se}^m$ in blood, Egan et al. 1977);

ii) the elements being measured are independent of their chemical environment;

iii) it is multielemental;

iv) in most cases for the size of samples irradiated 'in-vitro', attenuation of the neutron beam within the sample is negligible and so the whole sample is irradiated in a uniform flux.

For INAA to be used to determine a particular element then that element must have a favourable neutron cross-section and produce upon activation a gamma ray emitting nuclide with a 'suitable' half-life. Another important factor in the process is obviously the magnitude of the neutron flux of the irradiating beam and the energy of the neutrons. The neutrons could be produced from an isotopic source, a neutron generator, an accelerator or a nuclear reactor. It is the nuclear reactor which produces the highest neutron fluxes that are required for many applications. This means that a range of neutron energies is present which must be taken into consideration when examining the reactions taking place upon irradiation. In this work a reactor was used as the source of neutrons to perform instrumental neutron activation analysis.
2.2 Neutron Energy Distribution in a Reactor

In a reactor, the neutron energy range spans some nine decades. Knowledge of the neutron flux and neutron energy spectrum, at the point where a sample is irradiated is important in order to determine the reactions that have taken place. Neutrons may be classified by their energies and Profio (1976) gives the following classification: Fast 0.5 MeV-20 MeV, Intermediate 1 keV-0.5 MeV, Slow 0-1 keV and Thermal 0.025 eV. Neutrons with energies \( \gtrsim 1 \text{ eV} \) are referred to as epithermal (or resonance) neutrons because many resonances in the cross-sections of medium and heavy nuclides are in this range. Finally, neutrons with an energy less than 0.5 eV are absorbed in a thin (\( \sim 1 \text{ mm} \)) sheet of Cadmium so neutrons with energies greater than this are referred to as epicadmium. Cadmium is an element with a large thermal neutron absorption cross-section such that it is effectively opaque to thermal neutrons but transparent to epithermal neutrons above the cadmium cut-off energy \( E_{\text{cd}} \).

In a reactor, fission of uranium produces two types of neutrons:

i) prompt neutrons emitted within \( 10^{-16} \text{s} \) of the initial event with a continuous energy distribution up to several MeV, and of most probable energy of about 2 MeV,

ii) delay neutrons observed after fission - 0.65% of all \( ^{235}\text{U} \) fission events result in the emission of delay neutrons.

Neutrons resulting from fission and which have entered no collisions have a wide energy spectrum (sometimes referred to as the raw fission spectrum) and the distribution of energy can be described by the
empirical expression:

\[ N(E) = \sqrt{\frac{2\pi}{e}} e^{-E \sinh \frac{1}{2E}} \]  

(2.1)

These fissions neutrons are slowed down by elastic collisions with the nuclei in the moderator of the reactor to give the epithermal neutrons which are assumed to have an energy distribution proportional to \( E^{-1} \) per unit energy level, for a medium showing negligible neutron absorption or leakage. Eventually the neutrons lose sufficient energy by these collisions such that they reach thermal equilibrium with the surrounding medium. Thus the thermal neutrons have an energy distribution which follows the Maxwell-Boltzmann distribution:

\[ N(E) dE = \frac{2}{\sqrt{\pi}} \frac{1}{E_T} \left( \frac{E}{E_T} \right)^3 \exp \left( -\frac{E}{E_T} \right) dE \]  

(2.2)

where \( E_T = kT \), with \( k = \) Boltzmann's constant and \( T = \) absolute temperature (°K). At room temperature (\( \approx 293 \) K) the most probable velocity \( v_0 \) is 2200 ms\(^{-1}\) which corresponds to an energy of 0.025 eV.

2.3 Neutron Interactions in Matter

In neutron activation analysis the following three nuclear processes are of interest:

2.3.1 Radiative capture

Thermal neutrons tend to participate most prominently in this type of reaction since the absorption cross-section in this energy region is inversely proportional to the neutron velocity for most
nuclei (the '1/v-law'). Neutrons are uncharged particles thus as they approach the target nucleus there is no Coulomb repulsion. The capture of a neutron contributes approximately 8 MeV of binding energy to the nucleus and so 'prompt' de-excitation of the compound nucleus occurs by the emission of a single γ-ray or a sequence of photons in cascade. The γ-rays emitted have a total energy of the order of 8 MeV since the contribution to the kinetic energy of the excited nucleus by the neutron is negligible.

For many nuclei the absorption cross-section for epithermal neutrons consists of a 1/v slope with sharp resonances superimposed. Neutrons with energies corresponding to these resonances will be absorbed and in some cases this mechanism may predominate over thermal neutron capture.

2.3.2 Transmutation

e.g. (n,p), (n,α) reactions.

Generally fast neutrons are required to initiate these reactions as many of them are threshold reactions where the mass balance between the reactants and the products, the Q value, is such that extra energy is required before the reaction may proceed. Notable exceptions to this are to be found for light nuclei (where the potential barrier is low) e.g. \(^{10}\text{B}(n,\alpha)^{7}\text{Li}\) which has a thermal neutron cross-section of 3900 b.

The threshold energy \(E_T\) for an endoergic reaction is given by:

\[
E_T = - \frac{Q(m_n + M)}{M} \quad (2.3)
\]
where \( m_n \) is the mass of the neutron and \( M \) the mass of the target nucleus. \( E_\gamma \) for such reactions may be of the order of a few MeV, e.g. \( E_\gamma = 3.6 \) MeV for the reaction \(^{23}\text{Na}(n,p)^{23}\text{Ne} \).

In reactions where charged particles are emitted then there must also be sufficient energy for the particle to overcome the Coulombic potential barrier. The energy required to overcome this barrier, \( E_b \), for a given reaction is expressed by:

\[
E_b = -\frac{Z_a Z \alpha e^2}{(R_A + R_a)}
\]  

which represents the magnitude of the potential when the nucleus and emitted particle are just tangential, where \( Z \) and \( R \) are the atomic number and radius of the product nucleus \( A \) and the emitted particle \( a \) respectively and the constant of proportionality depends upon the system of units used.

For such reactions to occur fast neutrons are required except in the case of light nuclei where the binding energy will be small.

### 2.3.3 Inelastic scattering

- e.g. \((n,n')\) and \((n,2n)\) reactions.

In this case the de-excitation of the compound nucleus is performed by the emission of a neutron, the energy difference between the scattered and incident neutron going to form an excited state of the residual nucleus. In the production of radioisotopes the two processes \((n,n')\) and \((n,2n)\) are important:
1) The \((n,n')\) reaction

This is only of interest in activation analysis when the residual nucleus is left in a metastable state i.e. an excited state with a lifetime \(\tau\), long enough to be measured by simple direct timing techniques \((\tau > \sim 10^{-5}\text{s})\). These states occur when de-excitation of the residual nucleus is only possible by a nuclear transition involving large spin changes between levels of small energy difference which is a relatively slow process. Again it is only fast neutrons that are able to undergo this type of reaction since for heavy elements the first excited state is about 100 keV. A metastable isomer may be formed during \((n,\gamma)\) reactions if the compound nucleus is left in a highly excited state and this may be long lived if the de-excitation mode satisfies the above conditions (see Fig. 2.1).

2) \((n,2n)\) reactions

In this case the incident neutron must provide to the target nucleus an energy greater than the binding energy of the least bound neutron i.e. the neutron must have a kinetic energy of the order of 8 MeV, and therefore is only possible with high energy neutrons.

2.4 Reaction Rate

For the irradiation of samples in a reactor the number of events occurring per unit time, the reaction rate is defined as:

\[
R = \int_0^\infty \sigma(E)\phi(E)dE
\]  

(2.5)

where \(\sigma(E)\) is the cross-section at energy \(E\), and \(\phi(E)dE\) is the flux
Fig. 2.1: Distribution of half-lives of all metastable isomers produced by neutron induced reactions (shaded area refers to (n,γ) reactions only).
of neutrons between energies $E$ and $E + dE$. In thermal neutron irradiations the main reaction considered is radiative capture and the reaction rate from raw fission neutrons is small so $R$ is usually expressed as

$$R = \phi_{th} \sigma_0 + \phi_e I$$

(2.6)

where $\phi_{th}$ = conventional flux up to the cadmium cut-off energy $E_{Cd}$

$\sigma_0 = 2200 \text{ ms}^{-1}$ cross-section for a nuclide obeying the '1/\nu' law

$\phi_e$ = epithermal neutron flux per unit logarithmic energy interval

$I = \text{infinitely dilute resonance integral defined by}$

$$I = \int_{E_{Cd}}^{\infty} \sigma(E) \frac{dE}{E}$$

(2.7)

When considering the same material irradiated under cadmium then

$$R_{Cd} = \phi_e I$$

(2.8)

The ratio of the two reaction rates is termed the Cadmium Ratio CR where

$$CR = \frac{\phi_{th} \sigma_0 + \phi_e I}{\phi_e I}$$

(2.9)

Irradiation facilities are usually characterised by the CR of $^{197}$Au as a standard measure of the epithermal flux component. If the CR of Au is known then the CR of any nuclide $x$ for that reactor position can be given by
providing \( \sigma_{ox} \) and \( I_x \) are known and assuming a \( 1/E \) distribution in the intermediate neutron energy region.

For the threshold reactions which occur with the fission neutrons the reaction rate is given by

\[
R = \int_0^\infty \sigma(E)f(E)dE
\]

\[
R = \int_{E_T}^\infty \sigma(E)f(E)dE
\]  

(2.11)

Since \( \sigma(E) = 0 \) for \( E < E_T \), with \( E_T \) the threshold energy, where \( f(E) \) represents the fraction of the fission neutron flux between \( E \) and \( E + dE \).

A 'fission spectrum average cross-section' \( \bar{\sigma} \), and an 'equivalent fast flux' \( \phi_f \) are defined as

\[
\bar{\sigma} = \frac{\int_0^\infty \sigma(E)f(E)dE}{\int f(E)dE}
\]

(2.12)

\[
\phi_f = \int_0^\infty f(E)dE
\]

(2.13)

in which case 2.11 reduces to

\[
R = \bar{\sigma} \phi_f
\]

(2.14)
Tabulated values of $\bar{\sigma}$ are usually calculated with respect to a well defined unperturbed fission neutron spectrum, usually that of $^{235}$U. In practice it is convenient to assume that the reactor fast flux follows this although it is never strictly true.

2.5 The Activation Equation

When considering the production of a radioactive isotope then the number of radioactive nuclei created per unit time is given by

$$R = n\phi\sigma$$

(2.15)

where:
- $n$ = number of target atoms
- $\phi$ = the neutron flux
- $m$ = the mass of the element in the specimen
- $A$ = atomic weight of the element
- $f$ = the fractional isotopic abundance of the target nuclide
- $N_A$ = Avogadro's number
- $\sigma$ = isotopic neutron cross-section, if only thermal activation occurs then $\sigma = \sigma_0$.

However the radioactive product decays during the irradiation thus the growth of activity of the radioactive isotope is the difference between the rate of formation and the rate of decay. The decay of the radionuclide $D$, is given by

$$D = \lambda N$$

where $N$ = the number of radionuclides created in the sample

$\lambda$ = the decay constant of the nuclide.
Thus
\[
\frac{dN}{dt} = R - D
\]
\[
= \frac{\phi \sigma f N}{A} - \lambda N
\]  
(2.16)

The solution of this first order differential equation has been shown by Friedlander and Kennedy (1942) to be:
\[
N(t) = \frac{\phi \sigma f N}{A} (1 - e^{-\lambda t})
\]  
(2.17)

However allowance must also be made for the waiting time \( t_w \), the time between irradiation and counting, and the counting time \( t_c \) during which period the radionuclide decays. Also when considering the detector response \( D_c \) then \( I \), the intensity of the radiation of interest, and \( \epsilon \), the efficiency of the detector for the corresponding energy of the radiation of interest must be taken into account. Thus 2.17 becomes
\[
D_c = \frac{mcIN_{A} \phi f}{A \lambda} (1 - e^{-\lambda t_i}) e^{-\lambda t_i} (1 - e^{-\lambda t_c})
\]  
(2.18)

The most suitable timing parameters can therefore be chosen and the sensitivity of detection of a particular isotope can be estimated. Also since the main constituents of a sample are usually known a rough estimate of the activity expected from the other elements can be made to give an indication of the background. Obviously the timing parameters employed are determined by the half-life of the isotope of interest, so that if a shorter-lived isotope can be used instead of a longer-lived one, (e.g. \(^{77}\)Se \( t_{1/2} = 17\)s, \(^{75}\)Se \( t_{1/2} = 120\)d)
and for which greater detection sensitivity can be obtained, then the time of irradiation and counting required are considerably reduced and thus so is the cost of analysis.

When the detector response for a short-lived radionuclide is to be measured it has been found that it is enhanced by cycling the sample between the source and detector and measuring the cumulative response. Cyclic activation analysis also depresses the signal from longer-lived radionuclides and provides a sensitive, quick analysis on a routine basis of a particular element.

2.6 Cyclic Activation Analysis

Caldwell et al. (1966) first suggested the technique as part of a combination neutron experiment for the remote elemental analysis of linear and planetary surfaces using a pulsed neutron generator. However it was Anders (1960 and '61) who reported the first experiments where the sample was cycled between the irradiation source and detector to enhance the signal to noise ratio (Spyrou, 1981).

Cyclic activation analysis means a sample is irradiated and counted, then irradiated and counted again for a number of cycles, with the residual activity from previous irradiations being counted together with the activity newly produced each time. A cumulative detector response over the experiment can then be obtained by adding all the responses for each cycle.

Let $T$ be the cyclic period where

$$T = t_i + t_w + t_c + t_w'$$
where $t_i$ is the time of irradiation

$t_w$ is the time between the end of irradiation and the start of counting

$t_c$ is the time of counting

$t_w'$ is the time between the end of counting and the start of irradiation.

Thus from 2.18 the detector response for the first-counting period is

\[
D_1 = \frac{I \Phi_0 \nu N_A e^{-\lambda t_i}}{\lambda^2} (1 - e^{-\lambda t_i}) e^{-\lambda t_w} (1 - e^{-\lambda t_c}) \tag{2.19}
\]

where the symbols are defined as before.

For the second counting period

\[
D_2 = D_1 + D_1 e^{-\lambda T}
\]

and therefore for the nth counting period

\[
D_n = D_1 (1 + e^{-\lambda T} + e^{-2\lambda T} + \ldots + e^{-(n-1)\lambda T})
\]

So if the detector responses are summed, to obtain the cumulative detector response $D_c$, then

\[
D_c = \sum_{i=1}^{n} D_i
\]

\[
D_c = D_1 \left[ \frac{n - \frac{e^{-\lambda T}(1-e^{-n\lambda T})}{1-e^{-\lambda T}}}{(1-e^{-\lambda T})^2} \right] \tag{2.20}
\]
D_{c} can then be maximised by the proper selection of timing parameters. It can be shown that for a given total experiment time the maximum value of $D_{c}$ occurs when $t_{w} = t_{w} = 0$ and $t_{i} = t_{c} = T/2$. However the situation is not as simple as that as the background must be taken into consideration. So knowledge of the composition of the sample matrix is important, in order to estimate the contribution that any of the resulting activation products make to the background underlying the signal of interest.

If the total experimental time is $t_{t}$

then $t_{t} = nT = m\tau_{s}$

where $\tau_{s}$ is the half life of the isotope of interest and

$$m = \frac{nT}{\tau_{s}} = \frac{t_{t}}{\tau_{s}} \quad (2.21)$$

Figure 2.2 shows a plot of the detector response for the isotope of interest in both the cyclic and conventional cases, as a function of the total experimental time. This clearly indicates that below a certain value of $m$, conventional activation is preferred. In both cases the effect of $t_{w}$ is to decrease the detector response and to make the cross-over value occur at higher values of $m$. However this does not take into account the background radiation underlying the signal of interest due to isotopes formed from other elements in the sample matrix. Therefore it is the signal-to-noise ratio $\frac{D_{c}}{\sqrt{b_{c}}}$ which must be optimised where

$s_{D_{c}}$ is the cumulative detector response for the isotope of interest and

$b_{D_{c}}$ is the cumulative detector response of the background, underlying the signal from other isotopes in the matrix.
Fig. 2.2: Variation of cyclic and conventional signal with total experiment time for various waiting times.

Fig. 2.3: Variation of cyclic and conventional 'signal-to-noise' ratio with total experiment time for various waiting times.
Figure 2.3 shows the ratio of $\frac{sD_C}{\sqrt{bD_C}}$ plotted against $m$ for different waiting periods expressed in terms of the half-life of the isotope of interest $\tau_x$, as a comparison of cyclic and conventional analyses. The half-life of the major contributing isotope to the underlying background of the signal was taken as $100 \tau_x$. It can be seen that the background plays a significant role in emphasising the difference between cyclic and conventional analyses and that the cross-over point from one to the other has moved to even lower values i.e. to smaller experimental times.

Another feature of cyclic activation analysis is in the determination or confirmation of the half-life of the isotope of interest (Ozek, 1979). Using equation 2.20 and the fact that for large $n$, $(1 - e^{-\lambda T})$ approaches unity then

$$D_C = D_1 \left[ \frac{n}{1-e^{-\lambda T}} - \frac{e^{-\lambda T}}{(1-e^{-\lambda T})^2} \right]$$

and

$$D_C = \frac{D_1}{1-e^{-\lambda T}} \cdot n - \frac{D_1 e^{-\lambda T}}{(1-e^{-\lambda T})^2}$$

which is a linear equation in $D_C$ and $n$, since all other parameters are constant. Therefore we can write

$$D_C = a \cdot n + b$$

where $a = \frac{D_1}{1-e^{-\lambda T}}$ is the slope of the straight line

and $b = \frac{-D_1 e^{-\lambda T}}{(1-e^{-\lambda T})^2}$ is the intercept.
But
\[
\frac{a}{b} = - \frac{(1-e^{-\lambda T})}{e^{-\lambda T}}
\]

and therefore the half life of the isotope of interest,

\[
T_{1/2} = \frac{\ln 2}{\ln(1 - a/b)} \quad (2.23)
\]

This equation is very sensitive to the variations of a and b and so the data must be precise.

If several isotopes emit the same γ-ray energy then determination of half-life may help to confirm the identification of the isotope without requiring further experiments (Adesanmi and Spyrou, 1982).

2.7 The Reactor Facilities

2.7.1 The Reactor

The Consort II reactor at the University of London Reactor Centre was used, this being a swimming pool type which is moderated, reflected cooled and partially shielded by light water. The core contains 24 fuel elements of uranium (80% $^{235}$U) - aluminium alloy clad in high purity aluminium which are placed in a tank of demineralised water. One hundred kilowatts is the maximum operating voltage and at this power the water flow through the core is adjusted to give a 10°C rise in temperature between the inlet and outlet valve, the water being circulated through an external air-cooled radiator to maintain this steady temperature.
Between the fuel elements are four control rods which move vertically in aluminium ducts. There are two 'coarse' and one 'safety' rod which are made of cadmium clad in stainless steel while the 'fine' rod is of stainless steel. In the event of a shutdown these fall by gravity otherwise they are controlled electromagnetically. Concrete is used as the biological shield having a minimum radial thickness of 2.5 m except on two opposite faces where removable blocks allow access to irradiation facilities. Four metres of water and two concrete filled trolleys provide the shielding in the vertical direction. This is illustrated in Fig. 2.4 along with the facilities available (Burholt, 1976).

2.7.2 The Facilities

Figure 2.5 shows a plan of the reactor core and the facilities that were used in this work which were:

1) The In-Core-Irradiation System (ICIS)

The samples are irradiated at the core centre for either a few seconds or up to a few hours and are then transferred rapidly by a pneumatic system to the radiochemical laboratory. Being at the core centre (see fig. 2.5) this facility has the highest thermal flux which is \( \sim 2 \times 10^{12}\text{ncm}^{-2}\text{s}^{-1} \).

2) Core Tubes

These are vertical tubes on the 90° and 270° outer faces of the reactor (see fig. 2.5). They are loaded manually whilst the reactor is shut down and the samples may be irradiated for a day or up to several weeks.
3) The Cyclic Activation System (CAS)

This was installed on the 0° face of the core in 1979 and enables both conventional one shot and cyclic activation to be performed in either a mixed neutron spectrum or an epithermal neutron spectrum. This is due to the presence of a cadmium sleeve which can be lowered to surround the irradiation tube and hence allow epithermal activation analysis to be performed. A full description of the system is given in Burholt (1982), and Fig. 2.6 gives a plan of it. The system has 4 modes of operation:

Mode A - there are always 3 capsules in the system i.e. 1 in the core, 1 being counted and 1 decaying in the directional control valve.

Mode B - the same as mode A but \( t_w \) is set at minimum so there is no sample decaying, hence there are only 2 capsules in the system,

Mode C - only 1 capsule is in the system during a cycle,

Mode D - automatic cyclic activation, with a choice of \( t_i \), \( t_w \), \( t_c \) but \( t_w \) is set at minimum and the number of cycles is preset.

In all cases the number of samples is preset, the magazine taking up to thirty. The minimum \( t_w \) or \( t_c \) is 0.4 seconds, the time taken for the sample to travel from the counting station to the core or vice-versa.
Fig. 2.6

SCHEMATIC DIAGRAM of CYCLIC ACTIVATION SYSTEM
One disadvantage of activation performed in this system is that the polythene capsule may add a significant 'blank' matrix contribution to the irradiated sample. Aluminium in particular is a large contaminant and in October 1981 new capsules were introduced with a lower aluminium concentration than in previous ones. The capsule was found to contain 12 µg of Al (Parry, 1981) and the content was found to be quite reproducible with an estimated error of only 3 µg. Other elements found in only very small quantities are both magnesium and chlorine. Unfortunately there is also a significant background contribution from the reactor itself and the long-lived isotopes of cobalt, manganese, zinc and iron are all detected.

2.8 Gamma-Ray Spectrometry

A typical set-up for a γ-ray spectrometer system is shown in Fig. 2.7.

2.8.1 Detectors

In neutron activation analysis where multielemental samples are being irradiated then the spectrum will contain many different γ-ray energies, some of which may be within a few keV of each other, thus a high resolution detector is required. Therefore the advent of semiconductor detectors in the early 1960's was an important step forward in activation analysis. In this work Ge(Li) detectors only were used.

When the γ-rays impinge on the detector then the interactions that occur will either be the photoelectric effect, Compton scattering or pair production depending upon the incident energy (see Fig.
Fig. 2.7: Schematic Diagram of a Semiconductor Spectrometer System

Fig. 2.8: Relative cross-section of the photoelectric absorption, Compton scattering and pair production processes in Germanium.
2.8. Germanium ($Z = 32$) is used in preference to silicon ($Z = 14$) in γ-ray detectors since the photoelectric absorption coefficient is proportional to $Z^4$ to $Z^5$ of the absorbing material. In the depleted region of the semiconductor the transfer of energy resulting from the interactions excites electrons to the conduction band leaving holes in the valence band. A strong electric field is applied across the diode thus the charge carriers are collected at their respective electrodes giving rise to an output signal proportional to the amount of energy absorbed. The energy required to produce an electron hole pair in the Germanium is only 2.9 eV and so most detectors are kept at liquid nitrogen temperature to reduce the thermal noise, and hence improve the resolution.

In a Ge(Li) detector lithium, an electron donor, is diffused into the p-type germanium to compensate for the acceptor type impurities and this gives rise to a region of intrinsic material which forms the sensitive volume of the detector. This is another reason for maintaining Ge(Li) detectors at liquid nitrogen temperature in order to prevent the lithium from drifting. Detectors, in which the p-type, intrinsic and n-type regions are coaxial, are designed for use with energies above ~ 50 keV and although they are more efficient than a Ge detector in the lower region, the resolution is inferior.

2.8.2 The Spectrometer System

Since several different spectrometer systems were used only the general features as illustrated in Fig. 2.7 will be considered here.
a) The Stabilised High Voltage Supply - This supplies the high voltage of the appropriate polarity necessary for the operation of the detector, typically 1-2 kV.

b) Preamplifier - This is to perform impedance matching between the input from the detector of low capacitance and its output of higher capacitance to the amplifier. It is not designed to give a high gain.

c) Amplifier - This provides a voltage gain, generally \( \sim 100 \), as well as shaping the pulse ready for analysis in the multichannel analyser. To ensure all the charge liberated in the detector and collected by the amplifier is processed, then time shaping constants of \( \sim 6-8 \, \mu s \) are necessary. However this represents a long processing time per event so usually 1-2 \( \mu s \) is used, sacrificing some resolution for the ability to measure higher count rates.

d) Multichannel Analyser (M.C.A.) - This contains the important analog-to-digital converter (A.D.C.) which digitises all pulses according to their height and locates the channel in which the events are to be stored, when the analyser is operating in the pulse height analysis mode. The dead time of an analyser is equivalent to the time taken for the A.D.C. to process a pulse during which time any pulses arriving will be ignored. This dead time usually comprises two components - the processing time of the A.D.C. and the memory storage time. The processing time is generally proportional to the channel number in which the pulse is stored and the processing time/channel is simply the period of the clock oscillator. Therefore the dead-time
where \( v \) is the frequency of the clock oscillator, \( N \) is the channel number in which the pulse is stored and \( B \) is the pulse storage time (Knoll, 1979).

Generally the analyser records the clock time and the live time in the first two channels so that the dead time can be corrected for. Usually a single channel analyser is incorporated in the M.C.A. before the A.D.C. to discriminate against pulses outside the region of interest otherwise they too would contribute to the dead time. For Ge(Li) detectors 4000 channels are generally used to cover an energy range of 0-4 MeV to give a good resolution.

2.8.3 Data analysis

In the analysis of \( \gamma \)-ray spectra the first task is to identify all the photopeaks present. These peaks are produced by two of the interactions of the \( \gamma \)-rays mentioned earlier:

i) the photoelectric effect - in which the photons lose all their incident energy in a single interaction and

ii) pair production - this process yields 2 photons from incident monochromatic photons of energy greater than 1.02 MeV, depending upon whether only one (single escape peak) or both (double escape peak) annihilation quanta escape from the detector.

The other interaction, the Compton effect, contributes to the background continuum under the peaks and therefore makes it more difficult to distinguish peaks of low intensity.
When examining the spectra two other effects must also be
taken into consideration. The first of these is the presence of
sum peaks. If two or more photons interact and deposit all of
their energy in the detector within a time that is short compared
with the response time of the detector and the resolving time of
the electronics, and enough of these events occur, a 'sum peak' will
be observed at an energy corresponding to the sum of the two
individual $\gamma$-ray energies. A continuum of sum events will also occur
at lower amplitudes due to the summation of partial energy loss
interactions. The other effect is due to the interaction of the
primary $\gamma$-rays in the surrounding material giving rise to noticeable
peaks in the recorded spectrum. Generally lead is used to shield
the detector and therefore Pb X-rays are found due to the photo-
electric absorption of the $\gamma$-rays by the lead leading to the
generation of characteristic X-rays.

The isotopes are easily identified, only requiring knowledge of
the energies associated with peaks in the spectrum. The detector is
calibrated using standard isotopic sources and then the peaks
identified from tables of isotopes such as Lederer et al. (1967) or
more usually from tables of $\gamma$-ray energies compiled especially for
activation analysis purposes e.g. MacMahon (1976). However quantit-
avative determination of the elements present requires the determin-
ation of the photopeak area above the background continuum. Simple
hand calculations may be adequate for the determination of only a
few peak areas but for multielemental analysis of large numbers of
complex spectra then a computer based technique is really required.
There are two methods of determining peak areas, the first of which is to simply add together the raw data in the photopeak and then subtract the background continuum. In this case no assumption is made about the shape of the peak or the continuum on either side. Amongst the most popular techniques implementing this particular idea are the Covell method (Covell, 1959) and the Total Peak Area (T.P.A.) method (Yule, 1968). Both of these are simple enough for calculation by hand or are also useful for implementation on a mini-computer.

The second method of determining the peak areas is by the fitting of an analytical function, usually of Gaussian form reflecting the statistical nature of the system noise and the charge collection, to the observed data. The choice of analytical function has been reviewed by several authors (McNelles and Campbell 1975, Takeda et al. 1976) and also the relative merits of fitting and digital methods (Koleta 1973, Baedecker, 1976 Yule and Rook 1976). Most of them agreed that the fitting method, although it has the disadvantage of much larger computing times, gives better precision. It was also found that the most effective, but simple, analytical function for peak fitting consists of a Gaussian distorted by the addition of exponential tails (Routti and Prussin, 1969). At high count-rates tailing on the high energy side of the peak may be caused by pulse pile-up, whilst on the low energy side tailing may be severe due to incomplete charge collection in the detector.

The SAMPO gamma-ray spectrum analysis code written by Routti and Prussin (1969) is based on this function. SAMPO is available as a ULRC package on the CDC 6600 machine at the University of London Computing Centre (ULCC) and has also been implemented
recently (1981) onto the University of Surrey Computer. The
shape of the peak is defined by three parameters in this represent-
ation: the width of the Gaussian and the distances from the centroid
to the junction points with the exponential tails. These parameters
vary smoothly with energy and so their values for any peak in a
spectrum may be found by interpolating between the parameters of
neighbouring peaks. Routti and Prussin (1969) explain fully the
principles of operation of SAMPO whilst Carder (1977) lists the
various options available (e.g. Peakfind - which lists the peaks in
the spectrum and gives their significance, Fitdo - which fits all
the peaks found by Peakfind above a certain significance etc.) and
how to implement them on the computer.

In this project 3 different M.C.A.'s have been used leading
to 2 different methods of analysis of the spectra. As stated earlier
3 types of facility at U.L.R.C. were used, let us consider each of
these in turn:

1) Core Tubes - These samples were transported to the University of
Surrey and counted on a $4 \times 10^4 \text{mm}^3 \text{Ge(Li)}$ detector with a
Nuclear Data 66 M.C.A. Samples were either counted singly and
the data transferred directly onto the Prime (McCuaig, 1982) or
ever 12 samples were placed on the sample changer and all the
data transferred onto a Nova IV Computer (McCuaig, 1982) and
then later transferred across to the Prime (This is because the
line from the PRIME computer cannot be left open for long periods
of time). SAMPO was then used to analyse the data.
2) ICIS - The samples were counted on a Ge(Li) detector connected to a Nuclear Data 6600 system. As well as being a M.C.A. this also has a mini-computer with its own program for fitting peaks. This uses a digital method of fitting single peaks by simply subtracting the background, but fitting is used in the determination of peak areas of multiplets (Nuclear Data Algorithms 1980).

3) CAS - For longer irradiation and counting times (i.e. $t_i$ and $t_c$ > 20s) the CAS may be connected to the NO 6600 system and then analysis is as explained for ICIS. The problem with the Nuclear Data system is that it takes ~10s to write a 4000 channel spectra onto a hard disc so for short timing parameters in cyclic activation this leads to difficulties. Also the clock and live time are only recorded to the nearest second so for short timing parameters this leads to large errors. So in this case the CAS system is connected to the Laben 8000 M.C.A. which records the spectra on magnetic tape which is then sent to ULCC, and analysis is then carried out using SAMPO. The problem of the clock and live time is also resolved since the Laben records time to the nearest tenth of a second.
CHAPTER 3 PHYSICAL METHODS FOR ELEMENTAL DISTRIBUTION

3.1 Introduction

Three techniques have been used to determine information about either the elemental composition or structure of the samples under examination. In chapter 2 a description and review of in-vitro neutron activation analysis, the main technique employed, was given. This chapter describes the main physical principles of two other methods which provide information about elemental distribution. These are electron microprobe analysis and X-ray transmission tomography (Computerised Tomography).

3.2 Electron Microscopy and Microprobe Analysis

Neutron activation analysis allows the determination of the concentration of elements of the whole of a sample whilst to determine the variation of elements within much smaller distances e.g. of the order of microns, other techniques are required. These, for example, make use of charged particle beams which can be focused to small diameters and made to scan the target under examination and include the proton microprobe (as mentioned in 1.5) and the electron probe microanalyser (EPMA). The interaction of the beam of electrons with the sample results in the emission of characteristic X-rays which give information about its elemental composition. An electron microscope gives very high magnifications (up to \(\sim 100,000x\)), showing, therefore, very fine details of morphology and resulting in an improved understanding of the relationship between structure and function in biological specimens. There are two main types of electron microscope, the transmission electron microscope (TEM) and
scanning electron microscope (SEM), both of which may have an associated EPMA. In this work a SEM was mainly used so only this will be described but the principles are similar in both cases.

3.2.1 Electron Interactions

It is due to the interaction of the electron beam within the specimen that the image is formed and that X-rays are produced resulting in information about the elemental composition. Therefore it is very important to understand what interactions may occur. Generally they may be divided into two classes: (1) elastic events, which affect the trajectories of the beam electrons without significantly altering their energy and (2) inelastic events, which result in a transfer of energy to the solid, leading to the generation of secondary electrons, characteristic and continuum X-rays, long-wavelength electromagnetic radiation in the visible, ultraviolet and infra-red regions, electron-hole pairs, lattice vibrations (phonons) and, electron oscillations (plasmons). In principle all of these interactions may be used to derive information about the nature of the specimen e.g. shape, composition, crystal structure.

1) Elastic Scattering: occurs when the electron passes close to a nucleus resulting in a deflection from its incident direction. There are two types:

a) Rutherford scattering: this occurs in the Coulomb field of the nucleus and a single scattering event may result in a large angle of deflection of the electron. The Rutherford cross-section is given by:
\[
\frac{d\sigma(\theta)}{d\Omega} = \frac{e^4(Z-f(\theta))^2}{16E^2\sin^4\left(\frac{\theta}{2}\right)}
\]  
\(3.1\)

where \(Z\) is the atomic number of the nucleus, \(e\) the electron charge, \(E\) is the incident electron energy and \(\theta\) is the angle of deflection of the electron. \(f(\theta)\), is a correction factor allowing for screening by the atomic electrons and is negligible at large angles but dominates at smaller angles. This expression is an approximation and fails for low energy electrons (< 5 keV).

b) Multiple-scattering: consists of many small angle scattering events. When an electron passes through the electron cloud of the atom it is partially shielded from the nucleus, resulting in a small angle scattering event.

2) Inelastic Scattering: during an inelastic scattering event energy is transferred to the target atom and electrons and the kinetic energy of the incident electron decreases. These processes result in the formation of:

a) Secondary Electrons: In an inelastic interaction with the bound electrons energy is transferred from the incident electron to the bound electron which is then ejected leaving a vacant site. If this ejected electron is close enough to the surface of the specimen it may escape and is then referred to as a secondary electron. It is due to the detection of these secondary electrons that an image is formed in the SEM. An important characteristic of secondary electrons is their shallow sampling depth due to the low kinetic energy with which they are generated. Inelastic
scattering has a high probability for low energy electrons, therefore secondary electrons are strongly attenuated by the energy loss from these interactions. Also, the electrons must have sufficient energy to overcome the surface potential barrier (\( \sim \) several eV) therefore the deeper into the sample the electrons are produced the less probability there is that they will escape i.e.

\[
 p = \exp(-z/\lambda) 
\]  
(3.2)

where \( p \) is the probability of escape, \( z \) is the depth below the surface where generation takes place, and \( \lambda \) is the mean free path of the secondary electrons.

b) X-ray Production: X-rays can be formed by two completely different processes resulting in the X-ray continuum and the characteristic X-rays. The X-ray continuum or bremsstrahlung is formed due to the deceleration of the incident electron in the Coulomb field of the nucleus, and this consists of a continuous spectrum of X-ray energies from zero up to the value of the incident electron energy. The intensity of the X-ray continuum \( I_\lambda \), at any wavelength \( \lambda \) has been described by Kramers (1923) as:

\[
 I_\lambda \propto iZ(E_0 - E)/E 
\]  
(3.3)

where \( i \) is the beam current, \( Z \) the average atomic number of the target, \( E \) is the energy corresponding to a particular wavelength of the continuum \( \lambda \) and \( E_0 \) is the initial energy of the electron beam. This continuum forms the background against which characteristic signals must be measured and therefore plays a role in the determination of the minimum detectable level for a particular element.
Characteristic X-rays are produced during the de-excitation of an atom following the ejection of an inner shell electron caused by the interaction of the incident electrons. K, L or M inner shell electrons may be removed if the incident electron has sufficient energy leaving the atom in an ionised or excited state with the K shell having the greatest probability of interaction. In the process of the relaxation of the atom back to its ground state, transitions of electrons occur from one shell to another and excess energy may be lost in quanta of electromagnetic radiation. The photon energy equals the difference in energy between the shells involved in the transitions and therefore lies in the X-ray range of the spectrum for inner shell transitions. Since the electrons are in discrete energy levels, therefore, the characteristic X-rays produced indicate the elemental composition of the sample. However, the transitions may also be non-radiative as in the case of the Auger electron emission process, where the ejected Auger electron has the energy characteristic of the atom. Auger spectroscopy is limited due to the inelastic scattering of the Auger electrons within the material such that only those ejected within 1 mm of the surface can escape carrying their characteristic energy and therefore be useful for analysis.

Characteristic X-rays however, are generated over a substantial fraction of the interaction volume formed by the electron scattering in the solid. The depth from which the X-rays are generated is usually smaller than that of the electrons since X-rays can only be produced at energies above the critical
potential ($E_c$). This X-ray generation range is important when considering the 'sampling volume' for analysis, and this range may also be reduced due to absorption or scattering in the specimen, air and window of the detector. Different materials absorb X-rays to different degrees and are characterised by their mass absorption coefficients. The process is described by the equation:

$$I_T = I_0 e^{-\mu_{abs} \rho x}$$  \hspace{1cm} (3.4)

where $I_T$ is the transmitted intensity and $I_0$ the incident intensity of the X-ray beam, $\rho$ is the density of the absorbing material and $x$ its thickness, $\mu_{abs}$ is the mass absorption coefficient of the sample and is related to the energy of the X-ray being absorbed as well as being dependent on the atomic number $Z$. Due to X-ray absorption a particular atom in the sample will be left in an excited, ionised state which may lead to characteristic X-ray production. This phenomenon is known as X-ray induced fluorescence and may introduce some errors in quantitation since some of the characteristic X-rays measured will not just be from direct electron ionisation but also from this X-ray absorption. Therefore the yield of some characteristic X-rays will be increased but others decreased due to the absorption.

3.2.2 The electron probe microanalyser

This may be used in conjunction with either a scanning or a transmission electron microscope. Generally it has two types of X-ray detection system: (i) a Wavelength Dispersive-crystal
spectrometer (WDS) which uses Bragg's law for the determination of the photon wavelength:

\[ 2d \sin \theta = n \lambda \]  

(3.5)

where \( n \) is an integer, \( \lambda \) is the X-ray wavelength successfully diffracted, \( d \) is the lattice spacing of the diffracting crystal and \( \theta \) is the angle of incidence (and of reflection) of the X-ray beam at the crystal. The photon detector normally used in WDS is a proportional counter; (ii) an energy dispersive solid state detector which generally for this energy range is a Si(Li) detector.

The EPMA may be used in three different modes of operation:

1) Static Probe Analysis: Microareas of interest are analysed one at a time, with the probe remaining on each spot while the X-ray data are collected. Qualitative and quantitative chemical analysis may therefore be performed using this type of analysis.

2) X-ray Dot Maps: If the X-ray signal is used to modulate the brightness of the display tube during rectangular scans then an X-ray emission image is produced. This image will be a map of the distribution of a particular element if the X-ray signal comes from a spectrometer set to that element's particular characteristic photon energy. These images are particularly useful for high concentrations of the element of interest.

3) Line Scans: Again the spectrometer is set for a particular photon energy window but the counting rate is recorded while the probe scans along a line on the specimen. A photograph may be taken superimposing a secondary electron image of the
specimen, a montage of the line scan, and the graph of the intensity of the X-rays along that scan, but no digital data can be extracted. This type of analysis is important if significant variation of the element is expected only in one dimension.

3.2.3 Quantitative analysis

A sample is considered thick when the impinging electrons cannot reach the opposite surface of the sample. These samples therefore should be analysed using the SEM with X-ray detection facilities. In this case most of the electrons are brought to rest by losing their energy chiefly in collisions with the bound electrons of the atoms of the specimen. The concentration $C_x$ of an element $x$ in the specimen compared with a standard of pure element is given by

$$C_x = \frac{I_x}{I_{xs}} C_s$$  \hspace{1cm} (3.6)

where $I_x$, $I_{xs}$ are the intensities of characteristic X-ray emission from the specimen and standard respectively.

However, not all the X-rays reach the detector because of scattering, absorption and fluorescence as already mentioned in section 3.2.1, so that corrections need to be made. Another important factor is the atomic number, for elements with a low atomic number have lower critical ionisation potentials and vice-versa so that the atomic number effect results in a non-linear relation between the generated X-ray and composition. Generally correction factors are required to account for the effects of atomic number, X-ray absorption and X-ray fluorescence, known as the ZAF correction.
These corrections and their magnitudes and applications are discussed fully in Goldstein et al. (1981).

Two other methods of analysing the data reduce the need for these corrections. The first method is based on the use of the ratio between the characteristic and continuum X-ray intensities at the same energy, i.e. the peak to background ratio (P/B). The characteristic and continuum X-rays produced in the same region of the sample are subject to the same absorption and backscatter corrections. The most critical step in the procedure is the accurate measurement of P/B. With well separated peaks this presents little problem and the background can be measured directly on the low energy side of the peak. However when the peaks overlap there is more difficulty (see Goldstein et al. 1981). The ratio P/B for one element can be compared with that of another in the sample and to a first order:

\[
\frac{C_i}{C_j} = k_{ij} \frac{(P/B)_i}{(P/B)_j}
\]  

(3.7)

where \(C_i\) and \(C_j\) are the percent concentrations of elements \(i\) and \(j\) and \(k_{ij}\) is a correction factor which can either be calculated for the two elements concerned or obtained from measurements of standards.

The other method is similar only the continuum produced close to the characteristic X-ray is taken into consideration and

\[
C_i = k_{iB} \frac{I_i}{B}
\]  

(3.8)
where $I_B$ is the continuum produced from the specimen close to the energy $I_1$ and $k_{1B}$ is a constant derived from a standard. The term $I_B$, the continuum is a measure of the electron interaction with the specimen and is proportional to the density and thickness of the specimen. The closeness of the energies $I_1$ and $I_B$ makes absorption corrections unnecessary, and the use of the ratio $I_1/I_B$ removes the need for the atomic number correction. In this case biological samples are being studied and for these characteristic fluorescence is negligible except where a high Z element is present in a low Z-matrix. All of these methods are discussed further in Goldstein et al. (1981).

In biological samples it is also important to remember that the biological material is rarely an ideal sample for surface analysis having variable surface geometry and thickness and may become contaminated during the different techniques of preparation. Also unlike analysis in material sciences, most quantitative biological microanalysis is required to measure the concentration of elements which are thinly distributed in a poorly defined organic matrix. Also X-ray absorption is increased in biological materials compared to other materials due to the greater depth of penetration of the electrons. Finally subsequent attempts to correct for absorption are hampered by an incompletely characterised organic matrix and relatively inaccurate values for the mass absorption coefficients of low-atomic-number elements for the X-rays.

3.3 X-ray Transmission Tomography (CT)

Whereas the electron microscope is used for examining structure at microscopic levels, CT scanning is used to determine structure within much larger samples and in particular in the medical field
this is the human body. The attenuation of a beam of X-rays depends upon the composition of the absorber and X-ray transmission tomography yields images formed by a 'map' of the linear X-ray attenuation coefficients, with reference to a matrix usually water, through a cross-section of the body.

3.3.1 Attenuation coefficients

The attenuation of a beam of photons incident on an absorber of thickness $t$ is given by:

$$I(t) = I_0 e^{-\int_0^t \mu(x) \, dx}$$

where $I_0$ and $I$ are the incident and transmitted intensities respectively. The linear attenuation coefficient $\mu$ is dependent upon the photon energy and the physical properties of the absorber i.e. for a single element of atomic number, $Z$:

$$\mu = n \sigma_a$$

$$= n Z \sigma_e = \rho N_g \sigma_e$$

where $n$ is the number of atoms per unit volume, $\rho$ is the density of the material, $nZ$ is the electron density and $N_g$ is the electron mass density. The cross-section $\sigma_a$ is the total cross-section per atom for all processes and $\sigma_e = \sigma_a/Z$ is the cross-section per electron.

For a material $M$ containing a mixture of $N$ elements the linear attenuation coefficient is given by the mixture rule as

$$\mu_M = \sum_i \left( n_i Z_i \right) \sigma_{e_i} = \rho N_g \sum_i \lambda_i \sigma_{e_i}$$
where
\[ \lambda_i = \left( w_i Z_i / A_i \right) / \sum_{j=1}^{N} \left( w_j Z_j / A_j \right) \]  \hspace{1cm} (3.13)

\[ N_g = \sum_i \left( \lambda_i / A_i \right) = N_A \sum_i \left( w_i Z_i / A_i \right) \]  \hspace{1cm} (3.14)

\( N_A \) is Avogadro's number, \( A_i \) is the atomic weight of the \( i \)-th element and \( w_i \) is the proportion by weight of the \( i \)-th element. For photons with energy \( E \) less than 1 MeV the processes contributing to attenuation are elastic and inelastic scattering and the photoelectric effect. Therefore

\[ a_{\sigma} = a_{\sigma}^{\text{ph}} + a_{\sigma}^{\text{elast.}} + a_{\sigma}^{\text{inelast.}} \]  \hspace{1cm} (3.15)

The atomic cross-section for a single element is often written in the form:

\[ a_{\sigma}(Z,E) = K_{\text{ph}}(E) Z^m + K_{\text{elast.}}(E) Z^n + e_{\sigma,KN}^{\text{KN}}(E) Z \]  \hspace{1cm} (3.16)

where the first term represents not only the photoelectric contribution but also any binding corrections to the inelastic term since \( e_{\sigma,KN}^{\text{KN}} \) is the Klein-Nishina cross-section for scattering from a single, unbound electron. Hence the linear attenuation coefficient of the material \( M \) is now given by

\[ \mu_m(E) = \rho N_g \left( K_{\text{ph}}(E) \tilde{Z}^{m-1} + K_{\text{elast.}}(E) \tilde{Z}^{n-1} + e_{\sigma,KN}^{\text{KN}}(E) \right) \]  \hspace{1cm} (3.17)

with

\[ \tilde{Z} = \left[ \sum_i \lambda_i Z_i^{m-1} \right]^{1/(m-1)}, \quad \tilde{Z} = \left[ \sum_i \lambda_i Z_i^{n-1} \right]^{1/(n-1)} \]  \hspace{1cm} (3.18)

The quantities \( \tilde{Z} \) and \( \tilde{Z} \) are effective atomic numbers. Therefore if the contribution from elastic scattering is small, one effective
atomic number and the electron density may be regarded as the physical parameters which characterise the response of the medium to the transmission of X-rays.

In medical scanners the photon energies are generally in the range 30-150 keV. For water the photo-electric and elastic cross-sections are comparable at 60 keV and for fat they are equivalent at 40 keV and above these energies the elastic cross-section is the largest. Therefore in equations 3.15 and 3.16 all three terms must be retained. It has also been shown (White, 1977) that the exponents m and n depend on both the photon energy and the composition of the material. The preceding analysis fails in this manner due to the incorrect assumption that each separate cross-section can be factorised into a function of E and a function of Z. A more accurate parameterization of the cross-section can be derived from the fundamental quantum theory of photon-electron interactions and is given and discussed more fully in Kouris et al. 1982. It is sufficient here to note that fundamentally the use of effective atomic numbers, photoelectric and Compton coefficients is not justified and may lead to misleading results, for example in deriving the composition of the material.

3.3.2 Image Reconstruction

The image acquired by a CT scanner is a point-by-point representation of the X-ray attenuation in a slice through a 3-dimensional object, formed by reconstruction from projections of that slice. A 3-dimensional image may be obtained by stacking adjacent slices. A view of a slice in a plane perpendicular to the
Z direction is shown in fig. 3.1. The ray-sum $P(r, \phi)$ along OD (ray R) is defined by:

$$P(r, \phi) = \int_{R} \rho(s) ds$$  \hspace{1cm} (3.19)

where $\rho(s)$ is the value of the function to be reconstructed at position $s$ along OD. A set of ray-sums at one angle $\phi$ defines a projection of the slice. Reconstruction provides an estimate of the function $\rho$ throughout the scanned slice given a set of projections for all $\phi$. For transmission CT with monoenergetic X-rays the ray-sum $P(r, \phi)$ is

$$P(r, \phi) = - \log_e(I/I_0)$$  \hspace{1cm} (3.20)

where $I_0$ and $I$ are the incident and transmitted photon flux along ray R. For a well collimated beam 3.9 gives

$$P(r, \phi) = - \log_e \left[ I_0 \int_{R} \exp(-\mu(s) ds)/I_0 \right]$$

$$= \int_{R} \mu(s) ds$$  \hspace{1cm} (3.21)

where $\mu(s)$ is the X-ray linear attenuation coefficient at a distance $s$ along ray R. The function reconstructed for monoenergetic X-rays is therefore identical to the X-ray linear attenuation coefficient. However, it must be remembered that usually in medical applications the source of X-rays is from an X-ray tube and this produces a polychromatic X-ray spectrum. The spectrum quality is dependent on the target material in the tube, the filtration, the maximum or $kV_p$ voltage and the wave form of the applied voltage. The maximum energy
Fig. 3.1: One slice of an object in the (x,y) plane
is equivalent to the maximum voltage. The beam may not be considered as an independent set of monoenergetic beams since the lower energy part of the spectrum is more readily absorbed by the object due to the energy dependence of the linear attenuation coefficient. Therefore the beam hardens as it passes through the object. Also inelastic scattering produces scattered photons of lower energy but these do not contribute to the intensity in the incident direction. Taking these factors into account it is shown in Kouris et al. (1982) that in fact the definition of the ray-sum for a polychromatic beam does reduce to the correct limit for a monochromatic beam.

The image of the scanned slice is presented as an array of square elements or 'pixels', in most digital reconstruction techniques, with a single value being assigned to the reconstructed function at each pixel, and this function is assumed to be constant within each pixel. There are a finite number of ray-sums and reconstruction is a problem of matrix inversion. The ray-sums, \( P_R \), are given by

\[
P_R = \sum_i a_{Ri} p_i \tag{3.22}
\]

where \( a_{Ri} \) is the length of ray \( R \) within pixel \( i \) and \( p_i \) is the value of the reconstructed function at pixel \( i \). Reconstruction tries to solve these simultaneous equations and provide values of \( p_i \). The unit of reconstructed values of attenuation in CT is generally the Hounsfield unit (H) defined by

\[
H(E) = 1000 \left\{ \frac{\mu(E) - \mu_w(E)}{\mu_w(E)} \right\} \tag{3.23}
\]
where $\mu(E)$ is the linear attenuation coefficient of the scanned material and $\mu_w(E)$ is the linear attenuation coefficient of water, at energy $E$, for monoenergetic X-rays. For the definition of the Hounsfield number for a polyenergetic beam see Hawkes (1982).

There have been many different methods of reconstruction which can be divided into 2 main categories, analytic and iterative. In both groups there are many different methods and a review of these is given by Brooks and Di Chiro (1976).

3.3.3 Bone mineral determination by CT

Loss of bone mineral is an important parameter to measure as already discussed in 1.4.3 and the measurement of bone mineral content at various skeletal sites is an important application of quantitative CT. Trabecular bone is claimed to show changes in the mineralisation faster than cortical bone so that CT has the potential advantage over other methods described in 1.4.3 in that it is able to quantify trabecular bone separately from the superimposed compact bone and soft tissue. Bone mineralisation has been correlated with Hounsfield numbers by several workers (e.g. Ruegsegger et al. 1981, Pullan and Roberts 1978, Revak, 1980). One consideration is the dose received by the patient during the scan so that repeated measurements during a course of therapy are not usually feasible. Another difficulty encountered is reproducibility particularly for trabecular bone measurements. Ruegsegger et al. (1981) devised an instrument using a $^{125}\text{I}$ source ($E_\gamma = 28$ keV, $t_\gamma = 60$ days) for application at peripheral measuring sites, its main advantages being the monoenergetic photon beam and the low dose given to the patient. It should also be remembered that there is an unknown and variable fat content in
bone mixture and ignoring this may introduce substantial error in the calculated concentration of bone. However the error due to this unknown amount of fat depends also upon the beam energy. Therefore with a low energy source such as $^{125}$I, (mean energy 27 keV) used by Rüegsegger et al., the photon attenuation by mineral is accentuated relative to fat and soft tissue due to the $Z$ dependence of the P.E. attenuation process which dominates at this low energy, giving this method an added advantage.

To eliminate this error due to variable fat content the dual energy CT method is applied. Also although good reproducibility may exist information on both density and mineral content cannot be separated by a single measurement. Reliable measurements of mineral content, independent of density variations and fat replacement, requires measurement of the reconstructed values at two X-ray energies. However somewhat disappointing results have been obtained in attempts to characterise tissue by dual energy CT which again may be due to inaccurate parametrisation of the cross-sections as discussed earlier in this section.

An important relationship in CT is that between the linear attenuation coefficients of a mixture of compounds and the attenuation coefficients and concentrations of each of the compounds. If $\mu_i(E)$ ($i = 1$ to $n$) are the linear attenuation coefficients at energy $E$ of the corresponding compounds, $\rho_i$ are the physical densities and $C_i$ are the concentrations then

$$\mu(E) = \sum_{i=1}^{n} \frac{\mu_i(E)}{\rho_i} C_i$$

(3.24)
Also for a given volume

\[ l = \sum_{i=1}^{n} \frac{C_i}{\rho_i} \quad (3.25) \]

CT measurement of the linear attenuation coefficients of the mixture at \((n - 1)\) energies in a region of interest yields \(n\) simultaneous equations which can be solved for any of the concentrations. The linear attenuation coefficients of the compounds must be linearly independent. Since the linear attenuation coefficient at any diagnostic energy is a (non-linear) function of the energy, electron density and effective number, only 2 CT measurements are required to form the \(n\) linear equations. This mixture rule may be applied to both the single and dual energy methods to derive the corresponding relationships for the 2 methods which is done fully in Genant and Boyd (1977).

The use of CT for bone mineral determinations is therefore of great potential value, but its application requires further development. In particular higher precision and accuracy are required for bone mineral densitometric analyses. For serial studies repositioning is still a problem that has to be overcome as well as variations in the effective energy of the X-ray beam.
CHAPTER 4 ANALYSIS OF ROMAN HAIR

4.1 Introduction

Contemporary hair has been the subject of many studies of trace element analysis for a wide range of reasons, in particular to determine whether it acts as an environmental monitor (Ryabukhin, 1980) or even perhaps as an epidemiological monitor (Lodge Rees and Campbell, 1975). A unique opportunity to study hair from a different population compared to a contemporary one arose when samples of hair were found preserved at a Romano-British cemetery at Dorchester. Samples of this hair were analysed using both neutron activation analysis and electron microprobe analysis to determine their elemental composition and the results obtained were compared with the concentrations of trace elements in contemporary hair to see if these indicated any differences in dietary or environmental factors.

The hair was made available by the Natural History Museum in London and there the styling and condition of the hair had been carefully examined. From this examination several questions arose as to the state of the hair for example, whether some had been henna'd or whether another sample had been bleached. From the elemental analysis of the hair it was hoped that answers would be found to some of these questions.

4.2 Trace Element Analysis of Contemporary Hair

Hair, relative to other biological tissues, may be obtained from a living population with ease and this has led to it being analysed in many different studies in order to examine:
i) its ability to act as an environmental monitor e.g. the measurement of As levels in the hair of people living close to a gold smelter (Jervis and Tiefenbach, 1978), and the measurement of Hg in hair (Al-Shahristani and Al-Haddad, 1973).

ii) whether it can serve as an epidemiological monitor of disease e.g. Ca levels were found to be higher in the hair of breast cancer patients (Othman and Spyrou, 1980). In another study patterns of trace minerals in the hair have been related to the clinical states of the patients (Lodge-Rees and Campbell, 1975).

iii) the elemental levels in hair of different populations (Othman and Spyrou, 1978 and Clemente et al. 1978) with respect to dietary and environmental factors.

In order to determine the significance of the results in such studies it is important to consider the sources of trace elements in hair.

4.2.1 Sources of trace elements in hair

According to Hoppes (1977) there are three ways in which trace elements are incorporated into the hair - in the histogenesis of the hair, from endogenous and from exogenous sources.

A hair is a slender keratinous filament that develops from a cluster of matrix cells that form the bulb shaped follicle found in the dermis. These cells display intense metabolic activity during the growth phase of the hair cycle, producing 0.2-0.5 mm per day, and throughout this period the developing hair is exposed to the
metabolic milieu of the matrix cells, including circulating blood, lymph and extracellular fluids. As the growing hair approaches the skin layer the outer layer of the hair hardens and becomes relatively impermeable thus preserving the metabolic products accumulated during the formation of the hair. Hairs do not grow continuously since the follicles that produce them have controlled periods of growth and rest, collectively known as hair-growth cycles, and the follicles grow and rest independently.

There are four internal or endogenous sources of trace elements which must be considered:

1) the sebaceous gland - the sebaceous secretion is derived from body tissues and varies in composition depending upon where it is secreted. Generally it consists of fatty acids and unsaponified matter including cholesterol and waxes.

2) the eccrine sweat glands - the quantity and concentration of eccrine sweat varies considerably depending upon the individual and his environmental circumstances. Water and salts of Na and K are the principal ingredients although urea, amino acids, lactic and pyruvic acids are also present. Other elements present in small but significant amounts include N, Ca and P and the trace elements Cu, Mn, Mg and Fe.

3) the apocrine glands - these are only found in axial and perineal regions, not the human scalp, and their secretion is a milky fluid rich in lipids.

4) the epidermis - this is continually desquamating (skin contains a wide range of trace elements from Ag to Zn (Iyengar et al., 1978))
and so when incorporated in sebum and/or secretions from sweat glands it may come into contact with the hair, but Hopps (1977) considers it to be only a minor potential source of trace elements.

The external or exogenous sources of trace elements in hair are materials to which the hair may be exposed such as the air, water, soap, shampoo, lacquer, dye, medication etc. For example Gordus (1973) observed that persons who swam often in a pool where the water was brominated developed Br levels in scalp hair 1-13 times normal. He also reported that application of selenium-containing scalp medication increased the level of Se in scalp hair by 20-40 times.

Other factors that must be taken into consideration are age, sex, race and location e.g. Sakurai et al. (1978) found that several elements were higher in concentration in females than males in a Japanese population and Othman and Spyrou (1978) measured the Hg concentrations in Kenyan samples of hair to be 4-5 times higher than in N. American hair. Hence a number of studies have been carried out to determine elemental concentrations in hair for populations from different geographical locations (Clemente et al. 1978, Al-Shahrastani et al. 1978). Also for a particular person the concentrations will vary depending upon where the hair was taken from and the part of the hair that is analysed. It has been found for scalp hair that composition is affected by the position of the hair on the scalp (Cornelis, 1973) and also variations in concentrations of elements along the length of a hair may be as high as an order of magnitude (Valkovic, 1977). It would therefore be desirable
to collect hair samples of the same length and close to the root as well as sampling from several areas of the scalp.

Therefore, there are a number of important factors which must be taken into consideration when analysing the trace element content of hair, before any conclusions may be reached as to whether these concentrations reflect the total body concentrations. Hambridge et al. (1972) studied chromium and found it was not affected by external factors so that the concentration of Cr in hair gives an indication of the Cr nutritional state of the individual. Similarly zinc was found to be related to diet intake and low levels of Zn in hair indicate a low level of Zn in the body (Klevay, 1970).

4.2.2 The structure of hair

The structure of the hair itself may also be an important factor when considering trace element analysis. Fig. 4.1 shows an active follicle growing straight, pigmented and medullated hair. One parameter to vary considerably with the region of the body is the diameter of the hair. There is also a variation among hairs from the same region among different individuals and to a lesser extent within the same region of the same individual.

Fig. 4.2 shows the three distinct regions present in hair: the medulla, cortex and cuticle. In the centre of all hair shafts, except the extremely fine ones, is the medulla composed of large loosely connected keratinised cells. Sometimes the medulla may be only one or two cells in diameter. Keratin is a group of proteins which contains disulphide bonds and which are resistant to enzymatic digestion and all but the strongest chemicals. A typical keratin
Fig. 4.1: A Human Hair

Fig. 4.2: Schematic view of a mature hair
molecule is a two (or three) stranded cable of highly oriented polypeptide chains wound into a helix with secondary folds or distortions associated with a relatively unorganized matrix. The cells in the cortex, which forms the main constituent of the hair shaft, are heavily keratinised and also carry most of the pigment. However the cuticle which is single layered, is non-pigmented and translucent, and the individual cells are so elongated that many overlap at certain places and form stacks. The arrangement and shape of these cells or scales form patterns that vary between types of hair and between species.

In particular the colour of hair may be important as to which elements are present. Othman (1979) used a transmission electron microscope to study cross-sections of hair and in particular to look at the elements present in the pigments of different coloured hairs. He found that blonde hair had lower concentrations of Zn and Ca, which agreed with the results of Schroeder et al. (1969) that Zn and Mn concentrations are generally lower in blonde hair than in black hair. Hair pigments seem to be more rich in some elements than the matrix but generally the reason for the variation in elemental concentrations with colour is not known.

4.3 The Burial Site and Preservation of the Roman Hair

In 1971 during some construction work in Dorchester, a late Roman Cemetery of the 3rd-4th centuries A.D. was discovered. From this Romano-British excavation site a unique set of samples of hair and bone, from a number of individuals including children and adults of both sexes have been obtained. These samples provide a way of
studying the levels and distribution of bulk and trace elements in a Romano-British population. However in order to study dietary and environmental factors it is necessary to establish the various routes of contamination of the elements of interest due to the varying burial conditions present i.e., in gypsum, lead-lined coffins, wooden coffins etc. The hair samples were found on bodies which had been packed in gypsum inside lead-lined coffins. Gypsum is a hydrated sulphate of calcium which is soluble in water forming a weak acid. The presence of this gypsum has doubtlessly contributed to the preservation of the hair especially of those lead coffin burials which have remained well sealed and hence dry. It has been suggested (Molleson, 1982) that the idea of covering the bodies in gypsum is an imitation of the method of embalming in Egypt and Nubia in early Christian times, where the corpse was packed in a large quantity of common salt and then wrapped in a coarse cloth.

In Dorchester only a few hair samples in fact were preserved but sufficient quantities were found to allow hair styling and hair care in those times to be studied, before small samples were removed for analysis.

4.4 The Hair Samples

As already stated only a few hair samples were preserved and Table 4.1 lists these together with some of the background information surrounding them which should be considered. Samples 1 and 2 were found in the same coffin, sample 1 coming from a loose tress which it is thought may have been used to cover a bald patch and so may have been a false piece, whilst sample 2 was the head hair.
Table 4.1 The Roman Hair Samples

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Sex of individual</th>
<th>and approx. age at death</th>
<th>Is the false piece (1) from the same individual as the head hair (2)?</th>
<th>Is the hair henna'd?</th>
<th>3 stages of preservation of a hair coil?</th>
<th>Hair colour dark blonde.</th>
<th>Head hair</th>
<th>Six-strand plait</th>
<th>Five strand plait</th>
<th>hair colour</th>
<th>light brown</th>
<th>Blonde hair, bleached?</th>
<th>Brown, naturally wavy hair</th>
<th>Curly dark hair</th>
<th>Hair from coil, dark brown (wife to No. 1).</th>
</tr>
</thead>
</table>
This hair also has a reddish hue and it has been suggested (Molleson, 1982) that the hair may have been henna'd. Figure 4.3 shows these samples and the gypsum that was used to pack around the bodies. Samples 7, 8 and 17 (see Fig. 4.4) were also from the same individual, 8 and 17 being six and five stranded plaits, respectively, which were also found in the coffin along with the head hair, sample 7. Molleson (1982) suggested that the two plaits again may have been false pieces since false hair and wigs are known to have been used at this time. An interesting aspect of sample 10 is that its condition and colour suggest it may have been bleached, at that time a combination of goats grease and wood-ash, known as sapo, was used for this purpose. The hair of samples 4, 5 and 6 varied considerably in colour even though taken from the same coil at the back of the head of a 25-28 year old woman. This hair was not in good condition and it is possible that the hair may have become discoloured since burial, as its condition deteriorated.

4.5 Preparation of the Hair Samples for Analysis

Prior to analysis the hair required cleaning and great care needed to be taken since in the washing of biological samples of any kind the procedure may affect trace element levels.

4.5.1 The effect of different washing procedures on the elemental concentrations

In the analysis of human head hair the elements of interest are generally those from endogenous sources which have become incorporated into the hair, so that any dirt or surface contamination on the hair needs to be removed. Many different methods of
Fig. 4.3: The 'henna'd' hair found packed in gypsum

Fig. 4.4: Samples of head hair and five and six stranded plaits
washing have been proposed ranging from distilled water and detergents to mineral acids, however the effect of washing procedures on trace element concentrations in hair do differ, and therefore this must be taken into consideration when comparing results from different laboratories. It would be useful if cleaning procedures were stated in the literature which at present they are not.

The merits and demerits of various washing procedures for hair have been extensively evaluated by investigators. Bate (1965) recommended that for the analysis of hair by activation analysis a non-ionic detergent was the most effective as it almost completely removed the sodium and bromine. Assarian et al. (1977) studied the effect on Zn, Cu and Mg using detergent, hexane and ethanol, acetone and ether and detergent. They found a significant decrease in the concentration of these elements in all cases except for Zn after the hexane wash and also that the Cu content showed the greatest variability after washing by the different methods, whereas Mg showed the least. Salmela et al. (1981) also consider that the number of successive washes is also important for their results indicate that for each element there is a level below which the concentration cannot be reduced by further washing. However the level for each element is strongly dependent upon the washing procedure used.

Othman (1979) examined the surface of the hair using a Scanning Electron Microscope to determine whether all contamination had been removed during three different washes. He found that a short wash in detergent, followed by agitation in an ultrasonic bath for one hour was the most effective.
4.5.2 Preparation of the Roman Hair for I.N.A.A. and E.M.P.A.

In previous studies in the group (Sanders and Spyrou 1980, Othman and Spyrou, 1978 and 1980) hair has been cleaned by placing the samples in double deionised water in an ultrasonic bath for one hour and then dried overnight in a laminar flow cabinet. It was decided to use the same method, taking into consideration the effects of different washing procedures mentioned in 4.5.1, in order that the results of the Roman Hair could be compared with those of contemporary populations.

For analysis on the electron microprobe, the samples were mounted onto perspex stubs using double-sided sellotape and then coated with a thin layer of carbon. Initially the S.E.M. images were examined to determine whether all the exogenous material had successfully been removed from the hair in the case of the washed samples. Figures 4.5 and 4.6 show washed and unwashed samples respectively and the cleaning has been successful in removing the exogenous material from the hair. However, upon examination of different samples it appeared that hair scales were only apparent on some of the hair, most appeared brittle and damaged (Fig. 4.7).

Therefore it was thought that the cleaning may have been too rigorous for such fragile hair, so the process was repeated and this time the hair was only agitated for 10 minutes in the ultrasonic bath. Similar results were obtained however, with scales only being visible on some of the hair (see Figs. 4.8 and 4.9). Upon further investigation it was observed that scale patterns were best preserved only in samples 1 and 2. This could be an indication that
Fig 4.5: A washed Romano-British hair

Fig. 4.6: An unwashed sample showing the contamination
Fig. 4.7: A brittle and not very well preserved hair
Fig. 4.8: A well preserved hair

Fig. 4.9: A damaged hair
the hair had been henna'd and that this had played a role in the preservation of the hair.

Washed and unwashed samples were examined by both INAA and EMPA to give an indication of the effect of washing on the elemental concentration and also to examine the routes of contamination of the hair.

4.6 INAA of the Roman Hair

4.6.1 The irradiation procedures used

The irradiation procedures were performed on the samples to determine both the shorter and longer lived isotopes:

i) An irradiation of 20 minutes in ICIS, a waiting time of approximately 90s, followed by a 20 minute count on the Ge(Li) detector connected to the ND 6600 system.

ii) The samples were placed in the core tubes of the reactor for a week, left to decay over the weekend, then counted for 3000 or 4000s on the Ge(Li) detector connected to the ND 66 multichannel analyser.

In both cases the samples were transferred into clean pre-weighed capsules after the irradiation and before counting and then the samples reweighed when the activity had died away. Standard reference materials were irradiated in the same conditions as the samples so that analysis could be performed as described below.
4.6.2 Standard Reference Materials

Two methods are available to determine quantitative information when using INAA:

a) The absolute Method: this may be used when the neutron flux and the nuclear and atomic data of the irradiated element are known accurately. Then, the measurement of the absolute disintegration rates of the activated product by a calibrated γ-ray spectrometer will be proportional to the concentration of that element.

b) The Comparator Method: this is more commonly used in neutron activation analysis and removes the need for accurate nuclear data. Usually a multielemental standard containing known amounts of the elements of interest is used. This may be prepared by evaporating standard chemical solutions onto filter paper, or else a Standard Reference Material (SRM) may be chosen which contains many elements homogeneously distributed throughout the sample. SRMs may also be used to test the precision and the accuracy of either the analytical laboratories or the technique.

For the analysis of biological materials the most commonly used SRMs are Orchard Leaves and Bovine Liver, prepared by the United States National Bureau of Standards, and Bowen's Kale. The International Atomic Energy Agency also produces a number of references materials including dried animal blood, calcined bone, dried milk etc. The SRMs used in this work were Bowen's Kale, Orchard Leaves and Bovine Liver.
4.6.3 Detection Limits

The fundamental limit on the detection of a peak is due to the statistical fluctuations in the background continuum. If it is assumed that the counts in the background (B) underlying a peak are Poisson distributed, the standard deviation is ±VB. Therefore the smallest signal, S, detectable above the background may be expressed as a multiple of the standard deviation i.e. S = f VB. The background is defined as the continuum counts underlying the peak full width at tenth maximum. These limits enclose ~ 97% of a Gaussian total peak area. Generally in this group f is chosen to be 2 for the detection limit however for the peak search routine on the ND 6600 this has been set to 3.

4.6.4 The results obtained from INAA

Initially the results were examined to determine the effect of washing upon the elemental concentrations of the hair. Fig. 4.10 shows a plot of the variation in concentration against the different elements for washed and unwashed hair for two different hair samples. This illustrates that Na, Cl and Br are significantly reduced by the washing. Bate (1965) as discussed in section 4.5.1, also found that Na and Br values were reduced by washing.

The problem of the 'false pieces' of hair was examined next to determine whether the elemental concentrations either confirmed or refuted that they were false. Figure 4.11 illustrates the variation in concentrations of the different elements for the washed hair samples. First of all let us consider samples 7, 8 and 17 which were the two plaits and the head hair found in the same coffin.
Fig. 4.10: Comparison of elemental concentrations for washed and unwashed hair.

- washed
- unwashed

<table>
<thead>
<tr>
<th>Rel. Varn. in Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>ND</td>
</tr>
<tr>
<td>1</td>
</tr>
</tbody>
</table>

Hair Sample No 8

Hair Sample No 7

Mn  Ca  Cu  Na  Cl  Br  I  ND
Fig. 4.11: Comparison of elemental concentrations in Romano-British hair.
From Fig. 4.11 it can be seen that the profiles of the three different samples follow each other very closely within experimental errors and expected hair to hair variations indicating that the hair is all from the same person and that neither of the plaits are false pieces. The profiles of samples 1 and 2, however, are not the same but the marked difference is only in the Na and Cl concentrations. This could have been due to ineffective washing for some of the hair was matted and so may not have been completely cleaned. These were the samples which it is thought may have been henna'd and this may have affected the hair. Henna is derived from the powdered leaves of Lawsonia which is mainly cultivated on the South Mediterranean coast. When in acid solution it penetrates the hair keratin and the colour is deposited in the hair shaft rather than on the surface. The active ingredient is organic (2 - hydroxy - 1, 4 - naphthaquinone) but since henna is derived from a plant, trace elements found in the plant could be present. If henna had been applied in an acid solution to this hair then this may have leached some of the other elements from the hair and could explain the somewhat lower concentrations of Br and I found in these two samples.

The top three profiles in Fig. 4.11 are for samples 10, 15 and 16 and it can be seen that sample 10, which it is thought may have been bleached, has a particularly high concentration of Mn. Usually naturally blonde hair has a low concentration of Mn, so this could be an indication that the hair is naturally dark and has been bleached. This sample also has a particularly high concentration of Na which is also shown in the next diagram, fig. 4.12, which gives a plot of the variation in concentration against sample number for each element.
Fig. 4.12: Relative variation of elemental concentration in the Romano-British Hair

- Cl
- I
- Br x 10^{-1}

- Cu
- Ca
- Na
- V

Rel. Var. in Conc.
From Fig. 4.12 it may be observed that the vanadium concentration of the hair does not vary greatly except for samples 16, 1 and 2. It is interesting to note that samples 1 and 2 were from the gentleman found buried next to the lady of sample 16 and they are thought to have been man and wife. Br also does not vary significantly, except for the case of the child's hair which contains a considerably greater concentration. Due to the nature of burial it is difficult to assess the importance of the Ca concentrations as they may have been affected by contamination from the gypsum. There does not seem to be a great variation for different hair samples and the actual concentrations are high compared with a normal contemporary population as seen in Table 4.2.

In this table a comparison is given of the ranges of concentrations for different elements for this study compared with an ancient Peruvian hair study (Benfer et al., 1978), a contemporary normal African population (Othman and Spyrou, 1978) and a compilation of values for hair published by Iyengar et al. (1978). One notable difference is that some extremely high concentrations of Au are found in the Romano-British hair compared with contemporary populations. In all contemporary 'normal' populations the level of Cu is fairly constant, however the Roman hair has significantly higher concentrations. Several elements are listed in Table 4.2 which have not been plotted in the previous graphs. This is either because that particular element was not detected in all the samples or large errors are associated with the values of their concentrations. One of the difficulties was that due to the rarity of the specimens only small amounts of the hair were released for analysis.
# Table 4.2 Comparison of Trace Element Concentrations in Hair

<table>
<thead>
<tr>
<th>Element</th>
<th>Romano-British samples</th>
<th>Ancient Peruvian hair</th>
<th>Contemp. African pop.</th>
<th>Contemp. Populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au</td>
<td>0.077-2.6</td>
<td>NQ</td>
<td>0.004-0.006</td>
<td>0.0017-1.25</td>
</tr>
<tr>
<td>Br</td>
<td>3-88</td>
<td>NQ</td>
<td>0.9-19</td>
<td>0.65-53.3</td>
</tr>
<tr>
<td>Ca</td>
<td>0.56-2.2%</td>
<td>0.07-6.5%</td>
<td>0.029-0.129%</td>
<td>146-3190</td>
</tr>
<tr>
<td>Cl</td>
<td>63-391</td>
<td>NQ</td>
<td>110-794</td>
<td>950-4805</td>
</tr>
<tr>
<td>Cu</td>
<td>11-175</td>
<td>8.5-39.5</td>
<td>12-27</td>
<td>11-34</td>
</tr>
<tr>
<td>I</td>
<td>3.6-16</td>
<td>NQ</td>
<td>0.28-3</td>
<td>0.085-15.1</td>
</tr>
<tr>
<td>Mg</td>
<td>280-1942</td>
<td>68-8422</td>
<td>140-775</td>
<td>19-163</td>
</tr>
<tr>
<td>Mn</td>
<td>1-21</td>
<td>0.6-380</td>
<td>2-83</td>
<td>0.25-5.7</td>
</tr>
<tr>
<td>Na</td>
<td>14-243</td>
<td>5.3-222</td>
<td>4-46</td>
<td>18-1720</td>
</tr>
<tr>
<td>Sb</td>
<td>1-42</td>
<td>NQ</td>
<td>NQ</td>
<td>0.09-3.0</td>
</tr>
<tr>
<td>Sr</td>
<td>29-270</td>
<td>0.4-73.7</td>
<td>10-20</td>
<td>0.046-0.92</td>
</tr>
<tr>
<td>V</td>
<td>0.6-6.7</td>
<td>NQ</td>
<td>0.04-0.44</td>
<td>0.0045-0.53</td>
</tr>
</tbody>
</table>

All values in μg/g unless otherwise stated.

1) Benfer et al. (1978)
2) Othman and Spyrou (1978)
3) Iyengar et al. (1978)

NQ - Not Quoted
ND - Not Detected
so the typical sample size was ~10 mg whereas in contemporary studies > 50 mg would be used. This gives rise to increased detection limits which is why some of the elements may have been detected in some samples and not others. Table 4.3 gives a list of detection limits for the Roman samples compared with a contemporary population (Othman, 1979). However it must be pointed out that for Othman's results $t_i$ is only 600s, which is why some of the detection limits are higher than the Romano-British where $t_i$ is 1200s. Other elements detected include Ag and Sb, however these are found in the lead lining the coffins so they may be due to contamination. Mo, K, Co and Fe were also detected but only in very few of the samples and there were large errors associated with their values.

Samples 4, 5 and 6, which were suggested may have been from three stages of preservation of a hair coil, have not been included in the results so far because they proved to be difficult to analyse by INAA. Upon washing No. 4 appeared to disappear into a cloudy solution and so few hairs were present that there was an insufficient amount to analyse by INAA. An unwashed sample was irradiated but this became very active as it appeared to contain large amounts of Na and Cl, however other elements such as Au, Br, Ca and Cu were detected indicating the presence of small amounts of hair. There was also only a very small amount of sample 6 so this was not activated but it was analysed on the electron microprobe (see section 4.7). Sample 5 was analysed using INAA and appeared different from the other hair samples in that it had much higher concentrations of most elements in particular for Cu the concentration was ~2700 µg/g.
Table 4.3  Comparison of detection limits in hair of the Romano-British compared to a contemporary population

Detection Limits in µg/g

<table>
<thead>
<tr>
<th></th>
<th>Romano-British hair (6.7mg)</th>
<th>Contemporary hair (43 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Br</td>
<td>0.9</td>
<td>3</td>
</tr>
<tr>
<td>Ca</td>
<td>336</td>
<td>280</td>
</tr>
<tr>
<td>Cl</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>Cu</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>I</td>
<td>0.9</td>
<td>0.06</td>
</tr>
<tr>
<td>Mg</td>
<td>134</td>
<td>430</td>
</tr>
<tr>
<td>Mn</td>
<td>0.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Na</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>Sr</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>V</td>
<td>0.18</td>
<td>0.002</td>
</tr>
</tbody>
</table>

1) \( t_i = t_c = 1200s \quad t_w \approx 90s \)

2) \( t_i = t_c = 600s \quad t_w = 90s \) Othman (1979).
4.7 Results Obtained Using EMPA

Further analysis of the hair samples was performed using the electron microprobe attached to a scanning electron microscope as described in section 3.2. A selected area of the hair surface was scanned using a low current \((2 \times 10^{-10} \text{A})\) with an accelerating voltage of 25 kV. Each spectrum was recorded on floppy disc and also plotted using an x-y plotter. It was found that the number of detected elements varied from sample to sample, S and Ca being the only two elements to be detected in every sample. In contemporary hair, although Ca may be detected it is not usually found so consistently (Othman, 1979) so that this would indicate that the high levels measured by activation analysis are largely due to contamination from the gypsum. Lead, undoubtedly contamination from the coffin, was also detected on many of the washed hairs, but not all of them, as was Fe. Other elements detected included S, Cu, P and Si. A typical plot is shown in Fig. 4.13 of a washed sample and in Fig. 4.14 of an unwashed sample where in particular an area of contamination was being examined. This illustrates clearly that Pb, Ca, Fe and P are present in large amounts in the unwashed hair so that any of the concentrations of these elements in the clean hair could have been affected by contamination.

Further work carried out by Forbes (1982) confirmed this and the results are given in Table 4.4. Quantitative results are difficult to obtain, as discussed in section 3.2.3, however since sulphur is one of the main constituents of hair the sulphur peak of each spectrum gives an indication of the amount of hair in the interaction area of the electron beam. Therefore if the peak area of the element of
Fig. 4.13: Spectrum obtained for a washed hair sample using electron microprobe analysis.

1 --- S
2 --- Ca
Fig. 4.14: Examination of contamination on an unwashed hair sample

1 --- Al  5 --- K
2 --- Si  6 --- Ca
3 --- P  7 --- Fe
4 --- S  8 --- Pb
Table 4.4 Results of Electron Microprobe Analysis of Romano-British Hair

<table>
<thead>
<tr>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0.16</td>
<td>0.25</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>0.16</td>
<td>0.21</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.02</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.17</td>
<td>0.15</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
<td>0.05</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>5</td>
<td>0.46</td>
<td>0.29</td>
<td>0.04</td>
<td>0.04</td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td>0.18</td>
<td>0.44</td>
<td>ND</td>
<td>ND</td>
<td>0.02</td>
<td>0.03</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>7</td>
<td>0.07</td>
<td>0.13</td>
<td>ND</td>
<td>ND</td>
<td>0.2</td>
<td>0.1</td>
<td>0.0</td>
<td>0.01</td>
</tr>
<tr>
<td>8</td>
<td>0.13</td>
<td>0.20</td>
<td>ND</td>
<td>ND</td>
<td>0.01</td>
<td>0.02</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>10</td>
<td>0.58</td>
<td>0.60</td>
<td>0.16</td>
<td>0.17</td>
<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>14</td>
<td>0.20</td>
<td>0.33</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>0.23</td>
<td>0.31</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>16</td>
<td>0.24</td>
<td>0.29</td>
<td>ND</td>
<td>ND</td>
<td>0.02</td>
<td>0.02</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Examination of Surface Contamination on Unwashed Samples

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Calcium</th>
<th>Phosphorous</th>
<th>Iron</th>
<th>Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>5.78</td>
<td>3.0</td>
<td>2.65</td>
<td>1.37</td>
</tr>
<tr>
<td>14</td>
<td>102.27</td>
<td>5.3</td>
<td>33.86</td>
<td>1.77</td>
</tr>
</tbody>
</table>

NQ - Not Quoted
ND - Not Detected
interest is divided by the sulphur peak this gives an indication of the proportion of the element in the hair. Similarly the peak area may be divided by an area of the bremsstrahlung continuum, as described in section 3.2.3, to give an indication of the amount of that element present. This is how the results in Table 4.4 have been presented and clearly in the unwashed samples large amounts of Ca, P, Fe and Pb are present, therefore confirming that these elements could have been introduced into the hair due to contamination. It also may be observed that samples 1, 2 and 15 only contain Ca, and in 2 also Pb, thus indicating either that these were the best sealed coffins, or in the case of 1, 2 the henna may have again protected the hair from contamination.

4.8 Conclusions

The elemental profiles confirm that the two plaits are indeed the same hair as the head hair found in that coffin. For samples 1 and 2, the loose tress and head hair, although the elemental profiles are similar there are marked differences for Na and Cl indicating that they may be different. The henna'ing of the hair appears to have preserved the hair in two ways: i) the actual structure of the hair is well preserved i.e. the scales are well preserved and ii) EMPA shows the hair has been less contaminated than the other samples.

To compare the results of elemental concentrations of archaeological samples with contemporary ones then the routes of contamination need to be investigated. In this instance the electron microprobe proved a valuable tool for analysing surface contamination indicating that values of concentrations for Ca, Pb, Fe and P levels
could all have been affected by contamination. Therefore with respect to contemporary populations the most marked differences in the concentrations of elements are for Au and Cu both of which appear to be considerably higher for the Romano-British hair.
CHAPTER 5  ELEMENTAL ANALYSIS OF ROMAN BONE

5.1  Introduction

The analysis of hair samples from an excavation site at Dorchester has been discussed in the previous chapter. At this site a cemetery was found containing a large number of skeletons, from bodies from a wide range of ages at death. It therefore provided the opportunity to study in a Romano-British population the composition of bone and its elemental distribution in comparison with a contemporary population.

A preliminary study was undertaken to examine the elemental composition of a range of bone specimens, in particular tibiae, and to compare the results obtained with those for fresh samples which are discussed in chapter 6. The values of elemental concentrations found would also allow investigation of the effects of burial. For example the concentrations of Ca, P and Pb were found to be high in the hair samples analysed because the bodies had been packed in gypsum and buried in lead coffins. The exchange of elements and their penetration in bone would provide evidence to confirm whether the high concentrations of elements were due to 'contamination' or had been present at death.

5.2  The Preservation of Archaeological Bone

The greatest alteration to structure of archaeological bone due to 'weathering' has been found to be chemical in nature (Race et al. 1966) and Haversian systems are often observable even in those samples in which considerable chemical replacement has occurred.
In hydroxyapatite the calcium, hydroxyl and phosphate ions are highly subject to isomorphous substitution (with yttrium, fluoride and carbonate respectively) and during the fossilization process the geometric structure is essentially preserved (Posner, 1969).

The only major organic component that remains is collagen, although occasionally other structures such as blood vessels and erythrocytes may be observed and there have been some attempts to apply biochemical methods to determine the antiquity of bone. There is a striking similarity in average amino acid composition of insoluble proteins, from fossil bone of different ages and environments. This suggests that an insoluble product of the degradation of collagen is formed that is relatively unaffected by time or diagenic conditions (Matter and Miller, 1972).

Since the histological structure is well preserved histology has also become a popular research method for the study of ancient remains. However the paleohistologist must be able to recognise post-mortem changes from features that normally occur in an age series of modern bone. It has been suggested that histological techniques may be useful for identification of individuals amongst mixed skeletons (Stout and Gehlert, 1979) and in particular for estimating the age of skeletal remains (Stout and Gehlert, 1980).

However in this study the focus of our interest is towards the elemental concentrations of bone. Hydroxyapatite, when biologically formed, always contains significant amounts of several minor elements in addition to the essential elements of the structure. The concentration of these minor elements differs between different
species and also between individuals and populations of the same species, due to various biological and environmental factors. However in the case of archaeological bones it is necessary to separate the diagenic effects from the biological factors.

As has already been stated skeletons undergo chemical changes after burial and rates of diagenic change differ enormously from place to place and from tissue type to tissue type. Parker and Toots (1973) consider very few elements to be unaffected and discuss which elements are added and which are lost. They consider that enrichment during fossilization is characteristic of F, Si, Mn, Fe, Y as well as many other elements, whereas depleted elements include Na, Mg, Cl, and K. The enrichment of both F and U due to exchange with the hydroxyl and calcium ions respectively has led workers to suggest these may be used for dating bones (Seitz and Taylor 1974, Oakley, 1963). The enriched elements may be divided into two groups: those that become combined in the crystal structure of the hydroxyapatite e.g. $F^-$ and $Y^{2+}$ preferentially replace $OH^-$ and $Ca^{2+}$, and those that are present in minerals filling voids in the tissue e.g. Mn is present as various simple and complex oxides. Toots and Voorhies (1965) found one element not to be affected was strontium. They examined bones from a single quarry site so that the background level of Sr was probably uniform. Fossils of the same species were tested to check whether age of the individual has any influence on Sr and also different parts of the skeleton were compared. From this it was found that the Sr content only showed minimal variation within a species but they did find small, but highly significant differences between species. Therefore when studying the results
of elemental concentrations in archaeological bone samples it is important to allow for these effects before any comparison can be made with contemporary populations.

5.3 The Samples and Their Preparation for Analysis

The samples analysed were taken mainly from tibiae but in some cases where different states of preservation within the same skeleton could be observed, samples from various sites e.g. the skull and rib were taken. It was hoped that this would enable some indication to be given of the effects of 'weathering' so that allowance could be made for the diagenic effects when considering the elemental concentrations of the bone.

When taking samples from the tibia great care had to be taken in order not to alter the length of the bone so that only 'cores' of bone could be removed from along the length of the bone. In order to do this a stainless steel tank cutter was made for use with a machine drill for coring the bone along the shaft. Great care had to be taken to ensure that the force of the drill did not shatter the bone.

The samples were cleaned by placing them in double deionised water in an ultrasonic bath for an hour, rinsed in double deionised water then placed in a laminar flow cabinet overnight to dry. Some samples were not washed and these were just placed in polythene capsules ready for irradiation in the reactor.
5.4 Elemental Analysis of Romano-British Skeletons

For the analysis of these archaeological bone samples a variety of irradiation procedures were used in order to maximise the number of elements determined. These included:

1) A 60s irradiation in ICIS followed by ~60s wait, then a counting time of 300s on the Ge(Li) detector connected to the ND6600 system.

2) A 600s irradiation under Cd on the CAS system with a minimum waiting time (typically 0.4s) followed by a 550s count on the Ge(Li) detector.

3) An irradiation of ~30 hrs. followed by ~72 hrs. decay and then the samples counted for 4000s on the Ge(Li) detector connected to the ND66 system.

These irradiation procedures gave rise to the detection of the following elements in the bone samples: Au, Br, Cl, Ca, I, Mn, Na, P, Sb, V and Zn. Other elements detected but which had large associated errors include Ag, Co, Cu, Fe, Mg, La and Sr.

At this Romano-British cemetery a variety of burial conditions were found: wooden coffins, lead-lined coffins and sometimes the bodies were buried without coffins in chalk pits. It was decided initially to examine bones found under the same type of burial condition as the hair so that the elemental concentrations obtained could also be compared with those from the hair samples. These skeletons therefore were found packed in gypsum inside lead-lined coffins and some had been preserved better than others. Figure 5.1(a)
Fig. 5.1: (a) One of the less well preserved skeletons
(b) Skeleton found in a lead-lined coffin.
shows the skeleton from grave number 376 which was not very well preserved, although it can be seen that this is one grave where hair was preserved, corresponding to sample 10 in Chapter 4. The skeleton (No. 1012) in Fig. 5.1(b) however is generally in much better condition apart from the skull which has become very black. This appears to occur where the coffin was found to be damaged and water would have seeped into it. Due to the different states of preservation present in these two skeletons various samples were taken, as described in Table 5.1, in order to try to ascertain the diagenic effects caused by this type of burial.

However, a range of samples were first taken and analysed to discover the effect of the washing procedures. The samples were taken from the particular piece of bone, one being washed and the other remaining unwashed, and irradiated in order to determine a range of elements. These results are given in Table 5.3 and in Table 5.2 the detection limits are given for these elements for a sample of typical size of 30 mg. Generally it can be seen from Table 5.2 that the elemental concentrations are, within experimental error, very similar for the unwashed and washed samples. Vanadium, particularly for the tibia of sample 852, does appear to have an increased concentration for the unwashed compared to the washed. Similarly Zn in several cases appears to be slightly higher for the washed but the differences here do fall within one standard deviation. Therefore, except for V, the elemental concentrations determined appear to be unaffected by washing so that any contamination from these elements present on the outer surface of the bone is not easily removed by washing.
Table 5.1 Romano-British Bone Samples

<table>
<thead>
<tr>
<th>Grave No.</th>
<th>Bone Type</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1012</td>
<td>Skull</td>
<td>Blackened and appears crumbly</td>
</tr>
<tr>
<td>1012</td>
<td>Rib</td>
<td>Normal light brown</td>
</tr>
<tr>
<td>376</td>
<td>Rib</td>
<td>Blackened</td>
</tr>
<tr>
<td>&quot;</td>
<td>Radius</td>
<td>Extremely crumbly</td>
</tr>
<tr>
<td>&quot;</td>
<td>Finger</td>
<td>Dark grey brown, whitish on outside</td>
</tr>
<tr>
<td>&quot;</td>
<td>Tibia</td>
<td>Discoloured and fragile</td>
</tr>
</tbody>
</table>

Table 5.2 Detection Limits in Romano-British Bone

<table>
<thead>
<tr>
<th>Short-lived Isotopes</th>
<th>D.L. (in µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>173</td>
</tr>
<tr>
<td>I</td>
<td>6.8</td>
</tr>
<tr>
<td>Mn</td>
<td>7.4</td>
</tr>
<tr>
<td>V</td>
<td>0.55</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Long-lived Isotopes</th>
<th>D.L. (in µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au</td>
<td>0.008</td>
</tr>
<tr>
<td>Br</td>
<td>0.47</td>
</tr>
<tr>
<td>Na</td>
<td>5.5</td>
</tr>
<tr>
<td>Sb</td>
<td>0.39</td>
</tr>
<tr>
<td>Zn</td>
<td>131</td>
</tr>
</tbody>
</table>

All values are quoted for a sample size ~ 30 mg.
<table>
<thead>
<tr>
<th></th>
<th>Au</th>
<th>Sb</th>
<th>Zn</th>
<th>Br</th>
<th>Na</th>
<th>Cl</th>
<th>I</th>
<th>V</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>1012</td>
<td>N.D.</td>
<td>1.03</td>
<td>332</td>
<td>18.7</td>
<td>3355</td>
<td>530.5</td>
<td>Det. Large</td>
<td>0.84</td>
<td>31.4</td>
</tr>
<tr>
<td>Rib U.W.</td>
<td>± 8.5%</td>
<td>± 10%</td>
<td>± 6.2%</td>
<td>± 15%</td>
<td>± 19%</td>
<td>Error</td>
<td>± 16%</td>
<td>± 12.5%</td>
<td></td>
</tr>
<tr>
<td>1012</td>
<td>0.069</td>
<td>1.20</td>
<td>552</td>
<td>18.6</td>
<td>3918</td>
<td>550.2</td>
<td>Det. Large</td>
<td>0.73</td>
<td>54.4</td>
</tr>
<tr>
<td>Rib W.</td>
<td>± 25%</td>
<td>± 8.5%</td>
<td>± 10%</td>
<td>± 6.3%</td>
<td>± 15%</td>
<td>± 19%</td>
<td>Error</td>
<td>± 25%</td>
<td>± 12.5%</td>
</tr>
<tr>
<td>1012</td>
<td>N.D.</td>
<td>0.164</td>
<td>175</td>
<td>5.31</td>
<td>2376</td>
<td>605.6</td>
<td>N.D.</td>
<td>N.D.</td>
<td>12.5%</td>
</tr>
<tr>
<td>Skull U.W.</td>
<td>± 17.5%</td>
<td>± 23%</td>
<td>± 7.6%</td>
<td>± 15%</td>
<td>± 19%</td>
<td>Error</td>
<td>N.D.</td>
<td>12.5%</td>
<td></td>
</tr>
<tr>
<td>1012</td>
<td>N.D.</td>
<td>0.275</td>
<td>147</td>
<td>7.35</td>
<td>2788</td>
<td>618.1</td>
<td>Det. Large</td>
<td>N.D.</td>
<td>3.99</td>
</tr>
<tr>
<td>Skull W.</td>
<td>± 14%</td>
<td>± 15%</td>
<td>± 7%</td>
<td>± 15%</td>
<td>± 19%</td>
<td>Error</td>
<td>N.D.</td>
<td>± 60%</td>
<td></td>
</tr>
<tr>
<td>376</td>
<td>12.98</td>
<td>13.55</td>
<td>864</td>
<td>6.09</td>
<td>5735</td>
<td>1108</td>
<td>N.D.</td>
<td>0.66</td>
<td>86.7</td>
</tr>
<tr>
<td>Rib U.W.</td>
<td>± 8%</td>
<td>± 8%</td>
<td>± 10%</td>
<td>± 13.9%</td>
<td>± 15%</td>
<td>± 19%</td>
<td>N.D.</td>
<td>± 35%</td>
<td>± 12.5%</td>
</tr>
<tr>
<td>376</td>
<td>2.16</td>
<td>10.5</td>
<td>782</td>
<td>3.82</td>
<td>6186</td>
<td>1227</td>
<td>N.D.</td>
<td>Detected</td>
<td>61.0</td>
</tr>
<tr>
<td>Rib W.</td>
<td>± 8.5%</td>
<td>± 8%</td>
<td>± 12.8%</td>
<td>± 12.2%</td>
<td>± 15%</td>
<td>± 19%</td>
<td>100% error</td>
<td>± 12.5%</td>
<td></td>
</tr>
<tr>
<td>862</td>
<td>0.069</td>
<td>2.0</td>
<td>1200</td>
<td>130.1</td>
<td>3644</td>
<td>N.D.</td>
<td>Det. Large</td>
<td>5.2</td>
<td>150.8</td>
</tr>
<tr>
<td>Trab. U.W.</td>
<td>± 23%</td>
<td>± 16%</td>
<td>± 30%</td>
<td>± 8.7%</td>
<td>± 15%</td>
<td>Error</td>
<td>± 10%</td>
<td>± 12.5%</td>
<td></td>
</tr>
<tr>
<td>862</td>
<td>0.056</td>
<td>3.5</td>
<td>1127</td>
<td>75.4</td>
<td>14,493</td>
<td>N.D.</td>
<td>Det. Large</td>
<td>0.77</td>
<td>109.3</td>
</tr>
<tr>
<td>Trab. W.</td>
<td>± 23%</td>
<td>± 16%</td>
<td>± 37%</td>
<td>± 15.7%</td>
<td>± 15%</td>
<td>Error</td>
<td>± 75%</td>
<td>± 12.5%</td>
<td></td>
</tr>
<tr>
<td>376</td>
<td>0.127</td>
<td>64.0</td>
<td>1678</td>
<td>41.6</td>
<td>4097</td>
<td>2197</td>
<td>Det. Large</td>
<td>N.D.</td>
<td>13.0</td>
</tr>
<tr>
<td>Knee U.W.</td>
<td>± 9.5%</td>
<td>± 8%</td>
<td>± 10%</td>
<td>± 6.3%</td>
<td>± 15%</td>
<td>± 19%</td>
<td>Error</td>
<td>N.D.</td>
<td>± 12.5%</td>
</tr>
<tr>
<td>376</td>
<td>0.171</td>
<td>65.8</td>
<td>1436</td>
<td>57.6</td>
<td>3520</td>
<td>4275</td>
<td>Det. Large</td>
<td>N.D.</td>
<td>18.1</td>
</tr>
<tr>
<td>Knee W.</td>
<td>± 9.5%</td>
<td>± 8%</td>
<td>± 10%</td>
<td>± 6.3%</td>
<td>± 15%</td>
<td>± 19%</td>
<td>Error</td>
<td>N.D.</td>
<td>± 12.5%</td>
</tr>
</tbody>
</table>
Table 5.4 lists the results of the analysis of washed samples from grave Nos. 376 and 1012 listed in table 5.1. The skull from 1012, which was blackened, appears to be depleted of elements compared to the other samples e.g. Au, Mn and Cu are not detected. Therefore if water has entered the coffin it appears to have leached elements from the bone. One possible explanation may be that the water has formed weak sulphuric acid due to the presence of the gypsum (this has the chemical formula 8 \( \text{CaSO}_4 \cdot 2\text{H}_2\text{O} \)) and this has leached the elements out. In all samples from 376 the concentrations of Sb and Zn are high compared to contemporary values and therefore may be due to contamination from the method of burial. As discussed in Chapter 4 Sb is found in lead, and as it was also found in the hair, this is probably a strong indication that in bone the high Sb concentrations are also due to the presence of the lead. However Zn was not detected in the hair, whereas it always is in contemporary hair and is of importance to studies related to diet intake (see 4.2.1), therefore there could not have been large quantities due to contamination. This would seem to indicate therefore that either

1) these Zn levels do reflect the bone levels at that time which are considerably higher than for contemporary populations - a range of 50-190 \( \mu g/g \) is given by Iyengar et al. (1978), or

2) due to the crystalline nature of bone mineral the Zn has exchanged into the matrix whereas in hair no such exchange or bonding was possible.
<table>
<thead>
<tr>
<th></th>
<th>Au</th>
<th>Sb</th>
<th>Zn</th>
<th>Br</th>
<th>I</th>
<th>Cl</th>
<th>Na</th>
<th>Mn</th>
<th>Y</th>
<th>Cu</th>
<th>Ca%</th>
<th>P%</th>
<th>Ca/P Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>376</td>
<td>0.171</td>
<td>65.8</td>
<td>1436</td>
<td>57.6</td>
<td>22.4</td>
<td>4275</td>
<td>3520</td>
<td>18.1</td>
<td>N.D.</td>
<td>0.206</td>
<td>22.2</td>
<td>7.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Knee</td>
<td>±9.5%</td>
<td>±8%</td>
<td>±10.9%</td>
<td>±7%</td>
<td>±20%</td>
<td>±19%</td>
<td>±15%</td>
<td>±22%</td>
<td>N.D.</td>
<td>±10%</td>
<td>±4.7%</td>
<td>±4%</td>
<td></td>
</tr>
<tr>
<td>376</td>
<td>0.113</td>
<td>42.0</td>
<td>896.7</td>
<td>5.5</td>
<td>N.D.</td>
<td>1707</td>
<td>3018</td>
<td>17.1</td>
<td>N.D.</td>
<td>N.D.</td>
<td>30.2</td>
<td>9.0</td>
<td>3.36</td>
</tr>
<tr>
<td>Shaft</td>
<td>±10%</td>
<td>±8%</td>
<td>±11%</td>
<td>±9.4%</td>
<td>N.D.</td>
<td>±19%</td>
<td>±15%</td>
<td>±10%</td>
<td>N.D.</td>
<td>N.D.</td>
<td>±4.7%</td>
<td>±4%</td>
<td></td>
</tr>
<tr>
<td>376</td>
<td>0.044</td>
<td>127.1</td>
<td>808.5</td>
<td>13.5</td>
<td>N.D.</td>
<td>983.9</td>
<td>4406</td>
<td>N.D.</td>
<td>0.05</td>
<td>37.1</td>
<td>10.8</td>
<td></td>
<td>3.44</td>
</tr>
<tr>
<td>Ankle</td>
<td>±9.5%</td>
<td>±8.7%</td>
<td>±21%</td>
<td>±8.4%</td>
<td>N.D.</td>
<td>±19%</td>
<td>±15%</td>
<td>±10%</td>
<td>N.D.</td>
<td>N.D.</td>
<td>±35%</td>
<td>±4.7%</td>
<td>±4%</td>
</tr>
<tr>
<td>376</td>
<td>0.067</td>
<td>27.7</td>
<td>2389</td>
<td>2.6</td>
<td>N.D.</td>
<td>275.8</td>
<td>3664</td>
<td>40.2</td>
<td>N.D.</td>
<td>0.236</td>
<td>35.1</td>
<td>10.0</td>
<td>3.51</td>
</tr>
<tr>
<td>Finger</td>
<td>±14%</td>
<td>±8.5%</td>
<td>±10%</td>
<td>±17.5%</td>
<td>N.D.</td>
<td>±35%</td>
<td>±15%</td>
<td>±13%</td>
<td>N.D.</td>
<td>±12%</td>
<td>±4.7%</td>
<td>±4%</td>
<td></td>
</tr>
<tr>
<td>376</td>
<td>0.144</td>
<td>134.7</td>
<td>1470</td>
<td>10.9</td>
<td>N.D.</td>
<td>1780</td>
<td>5023</td>
<td>54.6</td>
<td>0.2</td>
<td>0.059</td>
<td>36.0</td>
<td>9.9</td>
<td>3.64</td>
</tr>
<tr>
<td>Radius</td>
<td>±10%</td>
<td>±8%</td>
<td>±14.8%</td>
<td>±7.2%</td>
<td>N.D.</td>
<td>±19%</td>
<td>±15%</td>
<td>±12%</td>
<td>N.D.</td>
<td>±4.7%</td>
<td>±4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>376</td>
<td>0.500</td>
<td>45.5</td>
<td>2261</td>
<td>5.5</td>
<td>N.D.</td>
<td>1227</td>
<td>6186</td>
<td>61.0</td>
<td>0.3</td>
<td>0.000</td>
<td>33.0</td>
<td>9.99</td>
<td>3.30</td>
</tr>
<tr>
<td>Ribs</td>
<td>±9.5%</td>
<td>±8.5%</td>
<td>±10.6%</td>
<td>±13%</td>
<td>N.D.</td>
<td>±19%</td>
<td>±15%</td>
<td>±12%</td>
<td>N.D.</td>
<td>±4.7%</td>
<td>±4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1012</td>
<td>0.069</td>
<td>1.2</td>
<td>552</td>
<td>18.6</td>
<td>4.9</td>
<td>606.7</td>
<td>3232</td>
<td>85.3</td>
<td>0.85</td>
<td>N.D.</td>
<td>23.9</td>
<td>7.48</td>
<td>3.20</td>
</tr>
<tr>
<td>Ribs</td>
<td>±25%</td>
<td>±8.5%</td>
<td>±10%</td>
<td>±6.3%</td>
<td>±63%</td>
<td>±20%</td>
<td>±15%</td>
<td>±10%</td>
<td>±34%</td>
<td>N.D.</td>
<td>±4.7%</td>
<td>±4%</td>
<td></td>
</tr>
<tr>
<td>1012</td>
<td>0.275</td>
<td>147</td>
<td>7.35</td>
<td>N.D.</td>
<td>734.6</td>
<td>2985</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>36.1</td>
<td>9.99</td>
<td>3.61</td>
</tr>
<tr>
<td>Skull</td>
<td>±14%</td>
<td>±15%</td>
<td>±7%</td>
<td>±20%</td>
<td>±15%</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>±4.7%</td>
<td>±4%</td>
<td></td>
</tr>
</tbody>
</table>

All concentrations are in μg/g unless otherwise stated.  
N.D. = Not Detected.
Similarly the Au concentrations are also much higher than for contemporary populations for bone and indeed this was found to be true for the hair. It must be noted that concentrations will be higher than for 'fresh' bone since no organic matter is present which has the effect of making the elements appear 'more concentrated'. However the differences for Au and Zn are large and in some cases orders of magnitude greater and so can not be explained by this factor.

Another factor to be taken into consideration is the packing of the bodies in gypsum which contains large amounts of Ca. This may therefore have led to increased values for Ca and to the higher values found for the Ca/P ratio (3.64) compared to the contemporary values (2.8-3.2, see Chapter 6). Therefore to study the variation of Ca and P and their ratio it would probably be desirable to examine samples from a different type of burial e.g. wooden coffins.

5.5 Elemental Analysis of Romano-British Tibiae

The left tibia from grave 529 was taken and, using the tank cutter, cores of bone were drilled from five places along the shaft. Each core was split into two halves such that concentrations from the outer and inner part of the shaft could be determined, and in one core there was sufficient trabecular bone for it to be separated and analysed separately. This should then give an indication as to whether elements had been leached from the outer surface or migrated into the bone.

The concentrations obtained for the elements producing shorter lived isotopes on neutron irradiation are given in table 5.5
<table>
<thead>
<tr>
<th></th>
<th>Knee</th>
<th></th>
<th>Ankle</th>
<th>Arithmetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2A</td>
<td>2B</td>
<td>3A</td>
<td>3B</td>
</tr>
<tr>
<td>Ca%</td>
<td>28.59</td>
<td>30.23</td>
<td>31.39</td>
<td>29.19</td>
</tr>
<tr>
<td></td>
<td>±3%</td>
<td>±4%</td>
<td>±3%</td>
<td>±3%</td>
</tr>
<tr>
<td>Na</td>
<td>3348</td>
<td>4267</td>
<td>3718</td>
<td>3496</td>
</tr>
<tr>
<td></td>
<td>±7%</td>
<td>±9%</td>
<td>±9%</td>
<td>±8%</td>
</tr>
<tr>
<td>Cl</td>
<td>930</td>
<td>1094</td>
<td>795</td>
<td>308</td>
</tr>
<tr>
<td></td>
<td>±18%</td>
<td>±22%</td>
<td>±25%</td>
<td>±5%</td>
</tr>
<tr>
<td>Br</td>
<td>N.D.</td>
<td>38.1</td>
<td>82.1</td>
<td>36.0</td>
</tr>
<tr>
<td></td>
<td>±19.4%</td>
<td>±10.4%</td>
<td>±21.1%</td>
<td>±24.3%</td>
</tr>
<tr>
<td>I</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>±49%</td>
<td>±47%</td>
<td>±57%</td>
<td>±57%</td>
</tr>
<tr>
<td>V</td>
<td>3.03</td>
<td>2.30</td>
<td>4.12</td>
<td>3.27</td>
</tr>
<tr>
<td></td>
<td>±15%</td>
<td>±24%</td>
<td>±11%</td>
<td>±15%</td>
</tr>
<tr>
<td>Sr</td>
<td>N.D.</td>
<td>818</td>
<td>467</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D. - Not Detected
A - outer
B - inner
T - trabecular
along with the mean and standard deviation and the ranges of concentrations of the elements. Generally there does not consist-
ently appear to be one element which is higher in the outer (A) and lower in the inner (B) part of the bone or vice-versa. Vanadium, however does seem to be higher in the outer part of the bone except for the last sample, (taken from the ankle end of the shaft), which may indicate migration of V into the bone. It must also be noted that in section 5.4 V was found to be higher in the unwashed samples. This indicates that V is one element which is present as contamination in this type of burial and is taken up by the bone. For bromine, also, this appeared to be the case except for the knee end, the first sample taken, Br was higher in the inner part of the bone but for the remainder of the samples it was higher on the outside of the shaft. However due to the quite large errors on the values for these two elements it is difficult to determine any statistical significance of these two observations. Therefore if the variation of elements could be plotted in going from the outer to the inner part of the shaft this would suggest what migration of elements takes place, in and out of the bone and the variation may plateau somewhere inside the bone where the 'original' elemental concentrations of the skeleton have not been affected by diagenic effects. It is not possible to do this using neutron activation analysis, since even if sufficiently thin slices of bone could be cut (which would be difficult due to the brittleness of bone) there would then be insufficient material to analyse. Therefore other techniques are required and one particularly promising one is the proton microprobe which may be used to induce either X or γ-rays (see section 5.6).
The variations of the elemental concentrations along the bone for sample 529 (listed in table 5.5) were also examined and the only two elements which showed any variation were Cl and I. It was particularly noticeable that I was only detected in the middle of the shaft, and for C1 there seemed to be a general decrease in concentration in going down the shaft and it was not detected at all in the sample closest to the ankle end of the tibia. For the remaining elements no definite variation occurred and within experimental error the values appeared relatively constant.

In total, four tibiae were analysed and the results are tabulated in table 5.6 where for 529 results are only given for the knee and ankle ends, and for the middle of the shaft. It is particularly noticeable that for 376, which was not very well preserved (see table 5.1), the Au, Sb, Zn concentrations are higher than for the other tibiae and V was not detected whereas it has been in all the other samples. The Zn is also high in concentration for 478 (the child whose hair was sample No. 14) where again the bone was not very well preserved, and in the trabecular bone from sample 862 which also was 'crumbly' and 'fragile'. This indicates, then, that an uptake of Zn occurs in this type of burial condition when the bones are not well preserved. Sb as stated before is most likely taken up from the lead in which it is found. Since the Au concentrations are also increased in the poorly preserved samples this may be an indication that Au is taken up in some form by the bone and explains the higher concentrations found in the hair compared to contemporary populations. Although Cu has only been detected in a few samples its concentration is considerably lower
Table 5.6 Elemental Analysis of Roman-British Tibiae

<table>
<thead>
<tr>
<th>Sample Location</th>
<th>Ca</th>
<th>V</th>
<th>Mn</th>
<th>Ti</th>
<th>Br</th>
<th>Cl</th>
<th>Mn</th>
<th>Au</th>
<th>Sb</th>
<th>Zn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical Error</td>
<td>&lt;3%</td>
<td>&lt;15%</td>
<td>&lt;12%</td>
<td>&lt;30%</td>
<td>&lt;25%</td>
<td>&lt;20%</td>
<td>&lt;12%</td>
<td>&lt;10%</td>
<td>&lt;10%</td>
<td>&lt;10%</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>476 Shaft</td>
<td>33.98%</td>
<td>1.45</td>
<td>88.4</td>
<td>29.5</td>
<td>91</td>
<td>474</td>
<td>2286</td>
<td>0.027</td>
<td>0.63</td>
<td>1147</td>
<td>N.D.</td>
</tr>
<tr>
<td>862 Shaft</td>
<td>29.06%</td>
<td>2.75</td>
<td>141</td>
<td>19.5</td>
<td>127</td>
<td>282</td>
<td>4282</td>
<td>0.053</td>
<td>353</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>862 Trab.</td>
<td>28.11%</td>
<td>0.77</td>
<td>109.3</td>
<td>56</td>
<td>75.4</td>
<td>N.D.</td>
<td>14,493</td>
<td>0.056</td>
<td>3.5</td>
<td>1127</td>
<td>N.D.</td>
</tr>
<tr>
<td>376 Knee</td>
<td>22.21%</td>
<td>N.D.</td>
<td>18.1</td>
<td>22.4</td>
<td>57.6</td>
<td>4275</td>
<td>3520</td>
<td>0.171</td>
<td>65.8</td>
<td>1438</td>
<td>0.206</td>
</tr>
<tr>
<td>376 Midshaft</td>
<td>39.2%</td>
<td>N.D.</td>
<td>17.1</td>
<td>N.D.</td>
<td>5.5</td>
<td>1707</td>
<td>3018</td>
<td>0.113</td>
<td>42.0</td>
<td>897</td>
<td>N.D.</td>
</tr>
<tr>
<td>376 Ankle</td>
<td>27.1%</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>13.5</td>
<td>983.9</td>
<td>4486</td>
<td>0.044</td>
<td>127</td>
<td>829</td>
<td>0.65</td>
</tr>
<tr>
<td>529 Knee</td>
<td>29.9%</td>
<td>3.03</td>
<td>N.D.</td>
<td>34.6</td>
<td>900</td>
<td>3348</td>
<td>0.0039</td>
<td>0.623</td>
<td>112</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>529 Midshaft</td>
<td>29.3%</td>
<td>4.11</td>
<td>61.5</td>
<td>11.2</td>
<td>32.5</td>
<td>449</td>
<td>4100</td>
<td>0.0135</td>
<td>0.75</td>
<td>276</td>
<td>N.D.</td>
</tr>
<tr>
<td>529 Ankle</td>
<td>28.6%</td>
<td>3.15</td>
<td>16.7</td>
<td>N.D.</td>
<td>79.4</td>
<td>N.D.</td>
<td>4051</td>
<td>0.0015</td>
<td>0.388</td>
<td>134</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D. - Not Detected

The samples listed as knee and ankle are taken from the knee and ankle ends respectively of the shaft of the tibia.
than for contemporary populations (see table 5.7) whereas in hair it was found to be relatively higher. Vanadium appears to be of the same order of magnitude as for contemporary populations and apart from 376 the samples appear to be very similar in concentration. This was the case for 529 too whereas for the hair samples V was found to be much higher in concentration in this particular sample (along with the sample from the lady (530) buried next to him) than the other samples.

Range of V concentration in bone    Range of V concentration in hair
0.77-4.11 µg/g                   0.6-6.7 µg/g
excluding 529 and 530
       0.6-1.86 µg/g

Generally the values for Na do appear to be reduced compared to a contemporary population which is in agreement with the results of Parker and Toots. However the values of Mn do not appear to have been increased or for Cl decreased compared to a contemporary population. This may be due to the skeletons being found in lead-lined coffins for the effect on elemental concentrations due to the soil will not have been as great but different problems have been introduced due to the presence of the lead and gypsum.

5.6 Other Techniques for Analysis of Roman Bone

PIXE analysis using a microprobe could prove to be an important tool for examining profiles of elements in bones, since variations of elements can be measured down to microns. Tolson (1982) carried out some preliminary work to examine what elements could be detected
### Table 5.7 Comparison of Elemental Concentrations of Romano-British Bone with Contemporary Populations and an Ancient Population

<table>
<thead>
<tr>
<th>Element</th>
<th>Ancient Population 1)</th>
<th>Contemp. Population 2)</th>
<th>Tibiae from Romano-British Population</th>
<th>Range</th>
<th>Mean ± std. deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au</td>
<td>N.Q.</td>
<td>~0.016</td>
<td></td>
<td>0.0015-0.171</td>
<td>0.054 ± 0.056</td>
</tr>
<tr>
<td>Br</td>
<td>7.38 ± 0.96</td>
<td>38</td>
<td></td>
<td>5.5-127</td>
<td>50.7 ± 40.5</td>
</tr>
<tr>
<td>Ca</td>
<td>18.8% ± 0.4%</td>
<td>10.8-39%</td>
<td></td>
<td>22.2-37.1%</td>
<td>29.8 ± 4.1%</td>
</tr>
<tr>
<td>Cl</td>
<td>N.Q.</td>
<td>632</td>
<td></td>
<td>282-4275</td>
<td>1300 ± 1396</td>
</tr>
<tr>
<td>Cu</td>
<td>N.Q.</td>
<td>1-25.7</td>
<td></td>
<td>0.05-0.206</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>N.Q.</td>
<td>15</td>
<td></td>
<td>11.2-56</td>
<td>25.9 ± 17.4</td>
</tr>
<tr>
<td>Mn</td>
<td>104 ± 26</td>
<td>0.19-116</td>
<td></td>
<td>16.7-141</td>
<td>64.6 ± 50</td>
</tr>
<tr>
<td>Na</td>
<td>5100 ± 230</td>
<td>5600-14100</td>
<td></td>
<td>2285-14,493</td>
<td>4834 ± 3686</td>
</tr>
<tr>
<td>P</td>
<td>N.Q.</td>
<td>5-17.4%</td>
<td></td>
<td>7.4-10.8%</td>
<td>9.16 ± 1.22%</td>
</tr>
<tr>
<td>Sb</td>
<td>0.25 ± 0.04</td>
<td>0.01-1.5</td>
<td></td>
<td>0.62-127</td>
<td>30.1 ± 46.2</td>
</tr>
<tr>
<td>V</td>
<td>N.Q.</td>
<td>0.87-17.6</td>
<td></td>
<td>0.77-4.11</td>
<td>2.54 ± 1.22</td>
</tr>
<tr>
<td>Zn</td>
<td>159.9 ± 7.2</td>
<td>50-190</td>
<td></td>
<td>112-1436</td>
<td>691 ± 500</td>
</tr>
</tbody>
</table>

All values quoted in µg/g unless otherwise stated.

1) Bratter et al. (1976)
2) Iyengar et al. (1978)

N.Q. = Not Quoted.
The results are shown in Fig. 5.2 and it can be seen that Ca, Cr, Fe, Ni, Zn, Pb, Br and Sr can all be measured where Pb is due to contamination from the lead lined coffins.

PIGE (Proton Induced Gamma Ray Emission) analysis has also been carried out using the same microprobe facilities in order to measure fluorine profiles in the bone (Ryde, 1982). Two MeV protons were used to bombard the sample and the resulting γ-rays were measured using a NaI(Tl) detector in preference to a Ge(Li) detector because of its superior efficiency at the high energies involved (6129 keV). Using IAEA Calcined Bone as a standard, values ranging from 765-2111 ppm were found for the concentrations of F in the archaeological bone. Profiles were also plotted for cores of bone taken from tibia and one such profile is shown for 530 in Fig. 5.3. The profile left to right indicates the yield from the outer to inner surface. It can be seen that there is a general decrease in going from the outer to inner surface. This was found to be the case for several of the bone samples but for some the opposite was true (see Fig. 5.4) i.e. there was an increase in concentration in going from the outer to the inner bone surface. The important thing to note is the change in fluorine concentrations e.g. in one case the concentration changed by a factor of roughly 3 in 2 mm. Therefore there seems to be a large variation dependent upon the position of the sample in the bone which obviously needs further investigation.

From these two preliminary studies PIXE and PIGE microprobe analysis appear to be an important step forward in the study of these Romano-British samples. Profiles of a range of elements in
Fig. 5.2: Spectrum obtained using PIXE analysis for a sample of unwashed Roman bone.
Fig. 5.3: Radial concentration profile for fibre for a washed sample from bone no. 53D.

Fig. 5.4: Radial concentration profile for fluoride for a washed sample from bone no. 376.
bone samples could be plotted to further investigate their uptake and loss due to diagenic effects.

Scanning Electron Microprobe Analysis was not performed for these samples since the bones were very rough on the surface and this would have caused great problems due to scattering. To overcome this the samples would have had to be set in resin and sections cut from this.

5.7 Conclusions

Instrumental neutron activation analysis has been used to determine a wide range of elements in bone samples from skeletons packed in gypsum and buried in lead-lined coffins. Studies were undertaken to ascertain the effects of burial and in particular the concentrations of Au, Sb, and Zn were found to be increased in the samples that were not well preserved. Preliminary work suggests that PIXE and PIGE microprobe analysis will prove a useful method for further examination of diagenic effects.

Samples were taken from along the shafts of human tibiae but variations only occurred for Cl which decreased going down the shaft towards the ankle and I which was generally only found in the middle of the shaft. Br and V both appeared to decrease in going from the outer to the inner surface of the bone and again PIXE could be used to further verify this in order to then determine the 'original' concentrations of these elements in the bone.
CHAPTER 6  ELEMENTAL COMPOSITION AND DISTRIBUTION OF BONE

6.1  Introduction

Bone scanning using $^{99}$Tc$^{m}$ labelled diphosphonates is now a routine clinical investigation in orthopaedics as well as a diagnostic tool for cancer. As discussed in section 1.4.2 the uptake and wash-out of such labelled compounds has been studied by many workers some of whom have suggested 'models' in order to explain the mechanisms of distribution of the compounds in normal bone and to determine the difference in these measurable parameters when 'abnormal' bone (lesions, fractures etc.) is involved. It has been suggested (Khan, 1977) that cortical bone consists of four bone compartments (see 1.4.2) through which minerals introduced into the blood stream pass from the capillaries to the bone crystal. Bone appears to have an increased avidity for certain elements and compounds which it is speculated may depend on the structure of the hydroxyapatite crystals.

Many 'in-vivo' techniques have been developed to measure bone mineral and Ca/P ratios (see section 1.4) but in order to determine the presence of other elements, which may appear in trace quantities, and investigate their concentration levels in bone then 'in-vitro' methods are required. Here several physical techniques have been used to examine elemental composition and distribution in human tibiae.

One 'in-vivo' technique widely used to determine bone mineral content is CT scanning particularly using the dual energy method (see section 3.3.3). Therefore before any elemental analysis was
carried out the tibiae were scanned to see if any changes found later by subsequent 'in-vitro' methods could be related to any differences found by an 'in-vivo' technique.

6.2 The Samples and Their Preparation for Analysis

For this series of experiments the samples used were human tibiae removed from elderly people with no known metabolic bone disease. The bones were removed at the Princess Margaret-Rose Orthopaedic Hospital of the University of Edinburgh and were split into two, cleaned of any organic matter, then frozen which was the condition in which they were received. Each tibia consisted mainly of the shaft with the distal and proximal metaphyses cut away. Three types of experiment were carried out: Computerised Tomography (CT), 'in-vitro' neutron activation analysis and electron microprobe analysis, each requiring different preparation of the samples.

The first method to be applied was X-ray transmission tomography for which little preparation was required. Each half of the bones in turn was placed in a phantom of double deionised water and nine scans were taken along the length of each bone as will be described in section 6.5.

Then one half of each bone was sectioned using a Macrotome II with a diamond tipped rotary blade. Two sections each 2 mm thick were taken at each of the nine intervals at which CT scans were obtained and for the first bone, TIB 1A, these sections were labelled K-S and for the other bone TIB 2A they were sections B-J. At the knee end of each bone two extra sections of thickness 1 mm were also taken. All the sections were soaked in Analar acetone for several
hours to remove any excess organic material and were finally rinsed in double deionised water.

For the electron microprobe analysis the sections were mounted separately onto perspex stubs using double-sided tape. Since bone is not a conducting material the sections were coated with a thin layer of carbon to conduct away the current carried by the electron beam and so prevent a build-up of charge. For the in-vitro n.a.a. each section was split into smaller pieces as required, see section 6.4, and then placed into polyethylene containers.

It had been hoped that the other half of each bone would be analysed using 'in-vivo' neutron activation analysis to study the variation of the distribution of the major elements along the bones using this technique. However it was found that the variations could not be obtained with sufficient precision (Spyrou and Matthews, 1981).

6.3 Electron Microprobe Analysis

The prepared sections were analysed on the scanning electron microscope with microprobe analyser, the electron beam energy being 25 keV. As described in section 3.2 the EMPA may operate in three different modes and in this case all the modes were used:

1) X-ray spectra were collected for 100s live time each.

2) Calcium and phosphorous X-ray dot maps were recorded.

3) Line scans were also traced for calcium and phosphorous variations across the bone.
Analysis of the X-ray spectra collected revealed, as would be expected, that Ca and P were always detected, but otherwise little else was found, with only Cl being detected occasionally. From these results the ratio of Ca to P was calculated, for as discussed in section 1.5 this parameter may be important with respect to the structure and metabolic activity of the bone. The photopeak counts were obtained from the irradiation of three separate areas of each section which were subsequently averaged. The results obtained in this manner for TIB 2A are given in Table 6.1. The variation in the photopeak count ratio for the two elements is fairly constant, lying between 2.61 and 2.72, with a mean and standard deviation of 2.67 ± 0.03. This indicates therefore that the relative composition of cortical bone in terms of P and Ca is generally constant over the whole tibia. By correcting for detector efficiency and for the X-ray yield then this value can be converted into the molar ratio. The mean value for this is 2.18 ± 0.025 and does not include any allowance for errors in the efficiency and yield. For hydroxyapatite the molar ratio is 1.67 and is therefore lower than the results obtained. However in measuring the characteristic X-rays emitted by Ca and P not all the X-rays reach the detector because of scattering, absorption and fluorescence (see section 3.2.1) and the magnitude of these effects will depend upon the individual energies of the X-rays. Also elements with a low atomic number have lower critical ionisation potentials and vice-versa so that the atomic number effect results in a non-linear relation between the generated X-ray and composition. Therefore these effects may give rise to higher values in the molar ratio.
Table 5.1  Ratio of Calcium to Phosphorous Measured Using the Electron Microprobe

<table>
<thead>
<tr>
<th>Section</th>
<th>Mean Ratio of Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>2.61 ± 0.02</td>
</tr>
<tr>
<td>C1</td>
<td>2.65 ± 0.03</td>
</tr>
<tr>
<td>D1</td>
<td>2.64 ± 0.04</td>
</tr>
<tr>
<td>E1</td>
<td>2.70 ± 0.01</td>
</tr>
<tr>
<td>F1</td>
<td>2.67 ± 0.02</td>
</tr>
<tr>
<td>G1</td>
<td>2.68 ± 0.04</td>
</tr>
<tr>
<td>H1</td>
<td>2.72 ± 0.03</td>
</tr>
<tr>
<td>I1</td>
<td>2.65 ± 0.01</td>
</tr>
<tr>
<td>J1A</td>
<td>2.67 ± 0.03</td>
</tr>
<tr>
<td>J1B</td>
<td>2.67 ± 0.02</td>
</tr>
</tbody>
</table>

Mean value = 2.67 ± 0.03

Correcting for detector efficiency and X-ray yields
Then Molar Ratio = 2.18 ± 0.025.
In figures 6.1(a) and 6.1(b) the X-ray dot maps for phosphorous and calcium, respectively, are shown for the same section. Apart from the clearly higher density of points in the calcium X-ray map, the two scans indicate a fairly similar distribution for the two elements. Instruments do exist for pattern recognition, which are becoming less expensive with the use of microprocessors, but none are available within the department to allow closer study of these particular results in order to quantify the similarity in the distribution of the two elements and whether any physical characteristics emerge. Figures 6.1(c), (d), (e) show scans taken along the line going through the middle of each photograph with the Ca variation being represented above and the P variation below this line. Figure 6.1(c) shows the outer edge of a section of bone whilst 6.1(d) represents an area of cortical bone, where the Haversian canals can be seen. An area on the inner edge of the bone is shown in Fig. 6.1(e) and it can be clearly seen that the bone is becoming less structured and more like trabecular than cortical bone. In this case the large variation in distribution along the line scan cannot be interpreted with any confidence as actual differences in composition since the interaction of the electron beam is not with a uniform volume in the same plane and significant areas of the bone structure contain holes.

Finally some samples were prepared for analysis on the transmission electron microscope with microprobe analyser, for which very thin sections are required i.e. ~1 μs thick. Bone is very hard and difficult to cut particularly for such thin sections therefore it has to be embedded before it is cut (Page, Bancroft and Stevens, 1977)
Ca and P Variations in bone

Fig. 6.1(d)

Fig. 6.1(e)
give a full discussion on bone and the preparation of bone sections). In this case Spurr's resin (Spurr, 1969) was used as the embedding medium. The procedure given in Table 6.2 was followed (Chescoe, 1980) using small pieces of bone (\(\sim 1 \text{ mm}^3\)) and the thin sections were then cut using a microtome. Upon analysis the results yielded only the detection of Ca and P again. Therefore such a lengthy procedure to prepare the samples for analysis may seem unnecessary, at first, when these two elements may be determined using the SEM with EMPA. However with the TEM much smaller areas may be examined due to the larger magnifications available (\(\sim 100,000x\)) and this could be important in looking for variations at the cellular level under properly controlled conditions for trace element analysis. In this case the bones had not been 'fixed' and therefore no cellular structure remained to be examined. Ten percent neutral or buffered formalin is generally suitable for all but certain specialised techniques. The main disadvantage of this technique, apart from the lengthy period of preparation, is that great care needs to be taken to ensure that elements are not added or removed due to either the fixing or the embedding of the specimen in the resin and no suitable technique exists in the field to date. However there is one obvious advantage, quantitative results could be obtained using the TEM, since the volume of interaction of the electron beam within the sample is well defined. This allows accurate correction to be made for attenuation within the specimen of the X-rays emitted from this volume as well as to express concentrations in terms of the volume.
Table 6.2 Procedure to Embed Bone for Analysis Using the Transmission Electron Microscope

Place each small piece of bone (~1 mm³) in the following solutions in turn:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Length of Time</th>
<th>No. of Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>30% Ethanol</td>
<td>10 min</td>
<td>2</td>
</tr>
<tr>
<td>60% Ethanol</td>
<td>10 min</td>
<td>2</td>
</tr>
<tr>
<td>90% Ethanol</td>
<td>10 min</td>
<td>2</td>
</tr>
<tr>
<td>Absolute Ethanol</td>
<td>10 min</td>
<td>2</td>
</tr>
<tr>
<td>Propylene Oxide</td>
<td>20 min</td>
<td>2</td>
</tr>
<tr>
<td>Propylene Oxide/Spurr</td>
<td>30 min</td>
<td>1</td>
</tr>
<tr>
<td>Resin: 50:50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Finally place in Spurr's resin and polymerise at 70°C for 9 hours.
6.4 In-Vitro Neutron Activation Analysis (IVNAA)

In order to determine a range of elements present in bone by IVNAA, several different irradiation and counting modes must be used to determine both the short and long-lived isotopes resulting from neutron reactions. The procedures used included:

1) A 60s irradiation of the samples in ICIS, then after a waiting period of approximately 60s they were counted for 300s on the Ge(Li) detector connected to the ND6600 system.

2) A 60s irradiation of the samples under cadmium in CAS. There was a waiting time of 1s and the samples were then counted for 100s on the Ge(Li) detector connected to the Laben 8000 series MCA.

3) The samples were placed in Core Tubes and irradiated for 20-30 hours. After several days, they were counted for periods of between 1500 and 3000s, again on a Ge(Li) detector connected to the Nuclear Data system.

The remaining sections of TIB 2A (the first set having been analysed by the EPMA as already described in section 6.3) were first analysed. Each of these sections was split into two parts, since large masses of bone would become very active and present considerable dead-time problems, in some of the irradiations where the shorter-lived isotopes were to be determined, by completely masking peaks of interest and distorting the peak shapes. Then the samples were analysed using the different procedures listed above.
Elements that were found in all sections were: O, Na, Mg, Cl, P, Ca, Zn and Br. Other elements detected in the samples, but which did not occur in all the sections or were associated with large errors were: F, K, Mn, and Sr. It should be noted that the total length of the tibia was 244 mm and inspection of the specimen did suggest that section J was closer to the 'knee' than section B was to the 'ankle'. Sections J2A and J2B were alternate sections interleaved by the sections used in electron microprobe analysis and therefore the separation was 2 mm.

First of all consider the two parts of each section where part 1 corresponds to the posterior and part 2 to the anterior of the bone. In fig. 6.2 two graphs are plotted showing the relative variations of Ca and Mg along the bone for the two different parts. These indicate that the variation was similar for each part and inspection of the results indicated this to be generally the case for the other elements, too. It is also observable that the concentrations of both Ca and Mg are greater in the part 2 than the part 1 indicating higher concentrations of the elements in the anterior compared to the posterior of the tibia. Straight lines have been fitted to the Ca variations giving gradients of 0.009 and 0.007 for parts 1 and 2 respectively. However for Mg there seemed to be no definite variation and it was not possible to fit a straight line or a curve to either the part 1 or 2 values with any statistical precision.

In Table 6.3 the results are tabulated for the anterior half of each section i.e. parts B22, C22 etc., for the elements which were found in all sections. This gives an indication of the range of
Relative variation in Mg concentration

- --- anterior
  \[ l = 2730 \pm 500 \mu g/g \]
- --- posterior
  \[ l = 2400 \pm 480 \mu g/g \]

Relative variation in Ca concentration in % by weight

- --- anterior
  \[ \text{grad.} = 0.007 \text{ for ant.} \]
  \[ l = 23.3 \pm 1 \% \]
- --- posterior
  \[ \text{grad.} = 0.009 \text{ for post.} \]
  \[ l = 19.4 \pm 0.9 \% \]

grad is in conc./cm.
Table 6.3 Elemental Concentrations for TIB2A

<table>
<thead>
<tr>
<th>Section - B2</th>
<th>C2</th>
<th>D2</th>
<th>E2</th>
<th>F2</th>
<th>G2</th>
<th>H2</th>
<th>I2</th>
<th>J2A</th>
<th>J2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td>Ca</td>
<td>P</td>
<td>Mg</td>
<td>O</td>
<td>Na</td>
<td>Cl</td>
<td>Zn</td>
<td>Br</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.042</td>
<td>0.923</td>
<td>1.116</td>
<td>0.802</td>
<td>1.042</td>
<td>1.941</td>
<td>0.816</td>
<td>0.919</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.025</td>
<td>1.006</td>
<td>0.991</td>
<td>0.994</td>
<td>1.010</td>
<td>1.990</td>
<td>0.593</td>
<td>0.675</td>
<td></td>
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<tr>
<td></td>
<td>0.912</td>
<td>0.772</td>
<td>0.904</td>
<td>0.945</td>
<td>0.886</td>
<td>1.355</td>
<td>0.511</td>
<td>0.572</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.008</td>
<td>0.899</td>
<td>1.209</td>
<td>0.910</td>
<td>1.015</td>
<td>1.970</td>
<td>0.557</td>
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<tr>
<td></td>
<td>0.844</td>
<td>0.839</td>
<td>0.878</td>
<td>0.930</td>
<td>0.875</td>
<td>2.010</td>
<td>0.620</td>
<td>0.572</td>
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<tr>
<td></td>
<td>0.834</td>
<td>0.839</td>
<td>0.897</td>
<td>0.861</td>
<td>0.847</td>
<td>2.043</td>
<td>0.713</td>
<td>0.389</td>
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<tr>
<td></td>
<td>0.926</td>
<td>0.847</td>
<td>0.991</td>
<td>0.908</td>
<td>0.960</td>
<td>2.955</td>
<td>0.775</td>
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<tr>
<td></td>
<td>0.843</td>
<td>0.950</td>
<td>0.757</td>
<td>0.834</td>
<td>0.832</td>
<td>2.211</td>
<td>0.745</td>
<td>0.290</td>
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<tr>
<td></td>
<td>0.951</td>
<td>0.901</td>
<td>0.757</td>
<td>0.932</td>
<td>0.941</td>
<td>2.211</td>
<td>0.745</td>
<td>0.785</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.009</td>
<td>0.017</td>
<td>0.128</td>
<td>0.141</td>
<td>0.028</td>
<td>0.211</td>
<td>0.121</td>
<td>0.157</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.014</td>
<td>0.024</td>
<td>0.175</td>
<td>0.113</td>
<td>0.042</td>
<td>0.476</td>
<td>0.112</td>
<td>0.169</td>
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<tr>
<td></td>
<td>0.013</td>
<td>0.025</td>
<td>0.161</td>
<td>0.141</td>
<td>0.040</td>
<td>0.474</td>
<td>0.103</td>
<td>0.167</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.012</td>
<td>0.023</td>
<td>0.196</td>
<td>0.134</td>
<td>0.036</td>
<td>0.474</td>
<td>0.103</td>
<td>0.128</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.014</td>
<td>0.023</td>
<td>0.175</td>
<td>0.132</td>
<td>0.041</td>
<td>0.477</td>
<td>0.107</td>
<td>0.122</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.009</td>
<td>0.022</td>
<td>0.165</td>
<td>0.129</td>
<td>0.035</td>
<td>0.311</td>
<td>0.088</td>
<td>0.122</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.015</td>
<td>0.023</td>
<td>0.164</td>
<td>0.128</td>
<td>0.038</td>
<td>0.325</td>
<td>0.087</td>
<td>0.108</td>
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</tr>
<tr>
<td></td>
<td>0.014</td>
<td>0.023</td>
<td>0.157</td>
<td>0.122</td>
<td>0.039</td>
<td>0.683</td>
<td>0.094</td>
<td>0.249</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Detection Limit</td>
<td>144 μg</td>
<td>41 μg</td>
<td>71 μg</td>
<td>33 μg</td>
<td>12.5 μg</td>
<td>8 μg</td>
<td>0.06 μg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conc. for B2</td>
<td>23.95 %</td>
<td>8.27 %</td>
<td>0.27 %</td>
<td>0.31 %</td>
<td>17.54 %</td>
<td>120.3 %</td>
<td>0.057 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arithmetic Mean Value</td>
<td>±1.18 %</td>
<td>±0.43 %</td>
<td>±0.05 %</td>
<td>±4.2 %</td>
<td>±1.12 %</td>
<td>±35 %</td>
<td>±0.15 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>22.48 %</td>
<td>7.76 %</td>
<td>0.268 %</td>
<td>0.31 %</td>
<td>16.0 %</td>
<td>84.1 %</td>
<td>0.56 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>±1.89</td>
<td>±0.42</td>
<td>±0.039</td>
<td>±1.12</td>
<td>±1.75</td>
<td>±1.39</td>
<td>±0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.0 - 25.0</td>
<td>6.94 - 8.32</td>
<td>0.204 - 0.326</td>
<td>0.579 - 0.725</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
concentrations of the various elements and of their corresponding errors. A typical sample mass was 120 mg and the detection limits for the elements are quoted in terms of $3\sqrt{B}$ where B is the background underlying the photopeak of interest, for this mass.

Figure 6.3 shows a plot of the relative variation of the concentrations of the 3 major elements forming the hydroxyapatite matrix i.e. Ca, P and O along with the ratio of Ca and P. Straight lines have been fitted to the Ca and P variations with the gradient of the Ca variation being approximately twice that of the P straight line. It was not possible to fit any distribution to the O values and their corresponding errors, but within experimental error it can be seen to follow the P distribution as would be expected due to the majority of the O being linked to P as the phosphate molecule within the hydroxyapatite matrix. The mean ratio for the P to O concentrations was found to be $0.49 \pm 0.04$, and for Ca to O was found to be $1.41 \pm 0.15$. The ratio of Ca to P varies from 3.18 to a minimum of 2.5, the mean value being $2.82 \pm 0.24$. In this case a 2nd order polynomial gives the best fit to the data although with this there is still a 10% probability that the values do not lie within this fit. The fit is of the form:

$$y = 2.67(\pm 0.12) + 1.45 \times 10^{-2}x - 4.37 \times 10^{-5}x^2$$

Therefore there appears to be an increase of approximately 1.5% in the Ca to P ratio every 2.5 cms in going from the knee down towards the ankle.

In Fig. 6.4 the relative variations of Mg, Na, Cl, Zn and Br are plotted. As already stated it was not possible to fit any
Rel. varn. in Concns. in % by weight, grad. in conc./cm.

**Ca Variation**

\[ \text{grad} = 7.3 \times 10^{-3} \pm 5.5 \times 10^{-4} \]
\[ I = 23.3 \pm 0.9 \% \]

**P Variation**

\[ \text{grad} = 3.7 \times 10^{-3} \pm 1.0 \times 10^{-3} \]
\[ I = 8.27 \pm 0.5 \% \]

**O Variation**

\[ I = 17.5 \pm 4.2 \% \]

**Ca/P Variation**

KNEE to ANKLE
Fig. 6.4

Mg variation

1 = 2730 ± 500 µg/g

Na variation

1 = 6960 ± 120 µg/g grad = 0.066 ± 0.016

Cl variation

grad = -0.03 ± 0.01
1 = 280 ± 59 µg/g

Zn variation

grad = 0.004
1 = 120 ± 10 µg/g

Br variation
1 = 0.972 ± 0.16 µg/g
grad = 0.028 ± 0.007

KNEE

ANKLE
distribution to the Mg values and their associated errors but it is particularly noticeable that the Mg values are considerably higher in the middle of the shaft. If the Ca to Mg ratio is calculated it is found to be at a minimum in the middle. This is of interest since Mg and Ca both belong to the same periodic group and also since it is thought that the presence of Mg inhibits the formation of hydroxyapatite as already discussed in section 1.2.4. Sodium can be seen to mimic the distribution of Ca very closely and within the fitting error the gradient of the fitted straight line agrees with that found for Ca. Chlorine, however, although there were large statistical errors associated with its measurement, appears to be the only element measured which decreases in concentration in going from the knee down towards the ankle. The concentration of Br again appeared to increase in going down the shaft as did Zn although for this straight line fit there was an 8% probability that the values would not fall within these values. Examination of the Zn values reveals that concentration increases towards the knee and ankle ends and the same could be surmised for the Br concentration. If specimens of bone further towards the ankle and knee ends were available for analysis this may have been confirmed.

For the analysis of TIB 1A there were two sets of sections labelled K-S to be examined using 'in-vitro' neutron activation analysis since neither set had been used for analysis by other methods. First of all one set of sections was taken and analysed as for the sections from TIB 2A with each section being split into two parts again, part 1 being the posterior and part 2 the anterior. Concentrations for the same range of elements as for TIB 2A were
determined since similar irradiation procedures had been performed, and again the variations of the elements along the bone were examined. These results again showed that the concentrations of the elements were higher in the anterior rather than the posterior part of the tibia. This is illustrated in Fig. 6.5 where the relative variations of Ca for both parts of each section are plotted for the two tibiae. This also shows that for both tibiae there is a general increase in concentration of the Ca in going down the shaft from the knee towards the ankle. Further examination of these results confirmed the variation of elements found with TIB 2A.

Since there appeared to be a variation in elemental concentration transversely in each section it was decided to examine this more closely. For the second set of sections K-S each one was split into seven parts see Fig. 6.6. This gave rise to a fairly large number of samples so it was decided, initially, to only carry out the short irradiations, to determine the shorter-lived isotopes such as Na, Ca, Mg etc. and to study their variation.

The results obtained were plotted and examined for variations transversely in the sections. Some results are shown in Fig. 6.7 for Ca and it can be seen that for sections 0 and R there is a general decrease in concentration in going from 1 to 7 i.e. from the anterior to posterior and in fact within experimental error both sections have the same gradient ~ -5.5. However for K due to the low value for 1 this gives the impression of an increase from 1 to 7. This was generally found to be the case; although some sections confirmed the decrease in concentration of the elements in the posterior compared to the anterior there were also a few cases where
Fig. 6.5: The variation in Ca for TIB1A and TIB2A.

concs. in % by weight, grads. in conc./cm.

TIB1A

ant. grad = 0.003+ 0.0003
I = 22.3+ 0.8 %
pst. grad = 0.009+ 0.0002
I = 19.8+ 0.8 %

TIB2A

ant. grad = 0.007+ 0.0005
I = 23.3+ 1.0 %
pst. grad = 0.009+ 0.0003
I = 19.4+ 0.9 %
Fig. 6.6: Sample taken from the knee end of the bone, showing the orientation and how the bone was split.
Section K
grad = 4.6 ± 0.57

grads. in rel. concn./cm.

Section 0
grad = -5.21 ± 0.57

Section R
grad = -5.79 ± 0.76
the opposite was shown. Therefore there was no absolute confirmation that in all the sections the concentrations of elements such as Ca, Na, Mg and P were higher in the anterior than the posterior, although generally this would appear to be true.

In Table 6.4 the results of the mean concentrations of the whole range of elements determined are compared with the range of values given for bone by Iyengar et al. (1978). All of the values fall well within the ranges given except for Br where the value is much lower. However, Iyengar only quotes a value which is for one sample only so that a range is not available with which to compare these values.

**Table 6.4  Comparison of Elemental Concentrations in Bone**

<table>
<thead>
<tr>
<th>Mean Value for TIB 2A</th>
<th>Values from Iyengar et al. (1978)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ca</strong></td>
<td>22.48 ± 1.9 %</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>7.76 ± 0.4 %</td>
</tr>
<tr>
<td><strong>Mg</strong></td>
<td>0.268 ± 0.04 %</td>
</tr>
<tr>
<td><strong>O</strong></td>
<td>16.0 ± 1.1 %</td>
</tr>
<tr>
<td><strong>Na</strong></td>
<td>0.655 ± 0.05 %</td>
</tr>
<tr>
<td><strong>Cl</strong></td>
<td>501.2 ± 164.6 µg/g</td>
</tr>
<tr>
<td><strong>Zn</strong></td>
<td>84.1 ± 1.39 µg/g</td>
</tr>
<tr>
<td><strong>Br</strong></td>
<td>0.56 ± 0.27 µg/g</td>
</tr>
</tbody>
</table>

NQ - Not quoted.
6.5 CT Analysis

As already mentioned in section 6.2 the CT analysis was performed before any sectioning of the bones took place and each half of the bones was placed in a phantom of double deionised water. The measurements were taken with the whole body EMICT5005 scanner at King Edward VII Hospital, Midhurst at nine intervals along the longitudinal axis of the bone. The appropriate aluminium connection wedges were used to give a field of view of 320 mm in diameter and a slice thickness of 13 mm was obtained per scan. Reconstructed images with $320 \times 320$ picture elements (1 pixel = 1 mm$^2$) each assigned a Hounsfield number, $H$ (see section 3.2.2) were obtained. From these results two types of analysis may be carried out:

1) qualitative analysis to determine the areas of cortical, trabecular and integral bone,

2) quantitative analysis which makes use of the dual energy technique (see section 3.2.3) and here was used in the determination of bone density and bone characterisation in terms of a Ca/P ratio.

First let us consider the qualitative analysis which requires the measurement of the number of pixels within each region of bone. The two main problems here are in deciding the boundaries of each type of bone and the errors incurred due to the partial volume effect. In this experimental set-up the volume element is $1 \text{ mm} \times 1 \text{ mm} \times 13 \text{ mm}$ so that the CT number representative of the average linear attenuation coefficient of the material in this volume may be for a combination of the two types of bone or of bone and water.
A method was developed by Spyrou et al. (1982) to overcome the uncertainty due to this effect and to locate the boundaries between the different regions. Using this method analysis of the results showed that for each bone there were two distinct populations of H-values taken to be equivalent to cortical and trabecular bone. The areas for each type of bone and the ratio of cortical to trabecular could then be calculated. Figure 6.8 shows the area of the two regions and their ratio plotted at each of the nine intervals along the bone for one of the halves of the bones TIB 2A, where mean values of H were 698 ± 18 for cortical and 219 ± 13 for trabecular at an X-ray tube setting of 140 kV. The curves are as expected since it is known that the trabecular volume increases as the knee and ankle are approached whereas there is mainly cortical bone in the shaft of the tibia. An intermediate region was also found between the cortical and trabecular whose area was of the same order of magnitude as the area of trabecular bone and this forms an interface region between the two types of bone.

The quantitative analysis using the dual energy method was performed by a fellow research student and the theory used is covered clearly in his thesis (Nicolaou, 1983). The principle involved is that the attenuation coefficient of soft tissues and bone may be reproduced by hypothetical mixtures of water and some reference material (Hawkes, 1982). The tissue's linear attenuation coefficient at a particular energy \( \mu(E) \) may then be given as

\[
\mu(E) = \rho_w \cdot (m_w \cdot \mu_w(E) + m_r \cdot \mu_r(E)) \tag{6.1}
\]
Fig. 6.8: The variation of the areas of the cortical and trabecular bone and their ratio.
where \( \rho_w \) is the density of water

\( u_w \) and \( u_r \) are the mass attenuation coefficients for water and the reference material

and \( m_w \) and \( m_r \) are the mass fractions of water and the reference material multiplied by the specific gravity of the hypothetical mixture.

The reference material chosen for its similarity of attenuation properties to bone mineral is \( \text{CaCl}_2 \), so various solutions were made up and scanned under the same conditions as for the bones. Using the composition of hydroxyapatite the total mass attenuation coefficients of bone at 72 keV (\( \pm 100 \text{ kV}_p \)) and 79 keV (\( \pm 140 \text{ kV}_p \)) were calculated and then substituted in 6.1 to give a pair of simultaneous equations. Hypothetical aqueous solutions of \( \text{CaCl}_2 \) were then fitted to the sample using the equations:

\[
\begin{align*}
\nu_{\text{apatite}}(72 \text{ keV}) &= \alpha_w u_w(72 \text{ keV}) + \alpha_r u_r(72 \text{ keV}) \\
\nu_{\text{apatite}}(79 \text{ keV}) &= \alpha_w u_w(79 \text{ keV}) + \alpha_r u_r(79 \text{ keV})
\end{align*}
\]

where \( \alpha_w \), \( \alpha_r \) are the mass fractions of water and \( \text{CaCl}_2 \) and \( u_w(E) \), \( u_r(E) \), \( u_{\text{apatite}} \) are the mass attenuation coefficients for water, \( \text{CaCl}_2 \) and hydroxyapatite at the equivalent energy \( E \). For a \( \text{Ca/P} \) ratio of 2.15 (i.e. as in the hydroxyapatite matrix) the solution of this system gives \( \alpha \equiv 0.6 \).

Using these equations hypothetical aqueous solutions of \( \text{CaCl}_2 \) were fitted to the bone sample TIB 2A. Figure 6.9 shows the results obtained where the variation of \( \alpha_r \) along the bone is plotted for
Fig. 6.9: Comparison of Ca/P ratios obtained using IVNAA and CT scanning.

Results obtained from CT Analysis

\[
\begin{align*}
\text{Ca/P} &= 3.00 \\
\text{Ca/P} &= 2.15 \\
\text{Ca/P} &= 1.00
\end{align*}
\]

Grads.
\[
\begin{align*}
c &= 4.54 \times 10^{-2} \\
l &= 8.75 \times 10^{-2} \\
t &= 1.75 \times 10^{-2}
\end{align*}
\]

Scanning position along bone

Results obtained from IVNAA

\[
\begin{align*}
\text{Ca/P} &= 3.2 \\
\text{Ca/P} &= 2.8 \\
\text{Ca/P} &= 2.4
\end{align*}
\]

Grad = 3.63 \times 10^{-2}

Scanning position along bone

Grads. are in cm\(^{-1}\).
cortical, trabecular and integral bone and the slopes of the straight lines through these points are given. The coefficients $a$, for different Ca/P ratios were calculated and are also given here. These results have been compared with those obtained using INAA that have already been discussed in section 6.4. It can be seen that both techniques show a similar trend indicating an increase in the ratio towards the ankle. It is important to remember that the volume analysed by the CT is much larger than that analysed by INAA. Also the bone sections analysed by INAA contained both cortical and trabecular although some of the trabecular bone may have been lost during cutting. However the CT has provided information about the trabecular and cortical bone separately and it can be seen that the slope of the Ca/P ratio for INAA is similar to that predicted by CT for the cortical region, indicating that the sample did contain mainly cortical bone.

Differences in photon mass attenuation coefficients within a material therefore give information about variations in elemental concentrations and the method developed by Nicolaou (1983) described above shows that the variation of the Ca/P ratios within bone can be measured by CT and agree with results obtained by INAA. This therefore gives rise to the question of how other elements within the matrix affect the attenuation coefficient and hence whether measurable variations in the photon mass attenuation coefficient could be detected due to changes in the concentrations of certain elements.

Kouris and Spyrou (1977) derived an equation for the calculation of a quantity termed "minimum detectable fraction" relating the mass attenuation coefficient of the element of interest and that of the
bulk matrix (in their case water). This equation is derived from the mixture rule which gives that:

\[
\left( \frac{\mu}{\rho} \right)_M = \sum_{i=1}^{n} p_i \left( \frac{\mu}{\rho} \right)_i, \quad \sum_{i=1}^{n} p_i = 1 \tag{6.2}
\]

where \( p_i \) is the fractional abundance by weight of the \( i \)th constituent element and \( (\mu/\rho)_i \) the corresponding mass attenuation coefficient. The introduction of an additional element denoted by \( n + 1 \) into the matrix will change the attenuation coefficient to \( \left( \frac{\mu}{\rho} \right)' \) where

\[
\left( \frac{\mu}{\rho} \right)' = p_{n+1} \left( \frac{\mu}{\rho} \right) + \sum_{i=1}^{n} p_i \left( \frac{\mu}{\rho} \right)_i \tag{6.3}
\]

where \( p_i' \) is the new fractional abundance of the \( i \)th constituent element and

\[
p_{n+1} + \sum_{i=1}^{n} p_i' = 1 \tag{6.4}
\]

If the fractional change \( f \) in the mass attenuation coefficient is defined as

\[
f = \left[ \left( \frac{\mu}{\rho} \right)' - \left( \frac{\mu}{\rho} \right)_M \right] \left( \frac{\mu}{\rho} \right)_M \tag{6.5}
\]

than it can be shown that

\[
p_{n+1} = \frac{(\mu/\rho)_M}{(\mu/\rho)_{n+1} - (\mu/\rho)_M} f. \tag{6.6}
\]

where \( p_{n+1} \) is called the minimum detectable mass fraction.
For bone using the formula for hydroxyapatite then the mass attenuation coefficient can be calculated for the two energies (72 keV and 79 keV) that were used experimentally, using tabulated mass attenuation coefficients of the individual elements (Storm and Israel, 1970). The detectable mass fractions were then calculated for a range of elements that are found in bone (for the two photon energies) for \( f = 0.01 \) i.e. for a 1% change in the mass attenuation coefficient, but can be multiplied by an appropriate factor if a different value of \( f \) is required.

The values calculated are listed in Table 6.5 along with the ranges quoted for the corresponding element in Iyengar et al. (1978). It can be seen that for most elements there would have to be abnormal concentrations present in order to affect the mass attenuation coefficient significantly. One element which seems to be the exception is fluorine for which some high concentrations are quoted. However the concentration is found to vary depending upon whether the bone is cortical and trabecular and more importantly with the age of the patient. Wix and Mohamedally (1980) measured fluorine levels in samples of iliac crest bone ash and found a linear relationship of F concentration with age which therefore may be an important factor to note.

Taking this a stage further the mass attenuation coefficient was calculated for bone using the mean values obtained for TIB 2A of Ca, P and O and this is shown in Table 6.6. Then allowance has been made for the presence of other elements and the two values compared. As can be seen from the table the presence of the other elements only gave rise to a change \( \sim 0.7\% \) of the mass attenuation coefficient.
<table>
<thead>
<tr>
<th>Element</th>
<th>Minimum Detectable Mass Fraction in Hydroxyapatite for 1% change</th>
<th>Values from Iyengar et al. (1978)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>2.3 % 2.6 %</td>
<td>0.06 - 2.8 %</td>
</tr>
<tr>
<td>Na</td>
<td>2.7 % 3.1 %</td>
<td>0.56 - 1.4 %</td>
</tr>
<tr>
<td>Mg</td>
<td>3.3 % 3.8 %</td>
<td>700 - 9370 µg/g</td>
</tr>
<tr>
<td>Cl</td>
<td>18.2 % 69.8 %</td>
<td>632 µg/g</td>
</tr>
<tr>
<td>K</td>
<td>3.2 % 4.25 %</td>
<td>1470 µg/g</td>
</tr>
<tr>
<td>Sc</td>
<td>2 % 2.4 %</td>
<td>4.6 µg/g</td>
</tr>
<tr>
<td>V</td>
<td>1.2 % 1.5 %</td>
<td>0.87 - 17.6 µg/g</td>
</tr>
<tr>
<td>Mn</td>
<td>7740 µg/g 9570 µg/g</td>
<td>0.5 - 116 µg/g</td>
</tr>
<tr>
<td>Fe</td>
<td>6360 µg/g 7740 µg/g</td>
<td>3 - 2040 µg/g</td>
</tr>
<tr>
<td>Cu</td>
<td>4320 µg/g 5310 µg/g</td>
<td>1 - 25.7 µg/g</td>
</tr>
<tr>
<td>Zn</td>
<td>3620 µg/g 4480 µg/g</td>
<td>50 - 190 µg/g</td>
</tr>
<tr>
<td>Br</td>
<td>2260 µg/g 2750 µg/g</td>
<td>38 µg/g</td>
</tr>
<tr>
<td>Sr</td>
<td>1760 µg/g 2120 µg/g</td>
<td>42.6 - 237 µg/g</td>
</tr>
<tr>
<td></td>
<td>72 keV</td>
<td>79 keV</td>
</tr>
<tr>
<td>---</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>1) Assuming a hydroxyapatite crystal:</td>
<td>$0.3057 \times 10^2$</td>
<td>$0.2683 \times 10^2$</td>
</tr>
<tr>
<td>2) Assuming Ca + P + O (contribution from H negligible)</td>
<td>$0.3288 \times 10^2$</td>
<td>$0.2854 \times 10^2$</td>
</tr>
<tr>
<td>values obtained using INAA:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) Assuming Ca + P + O + trace elements measured using INAA:</td>
<td>$0.3264 \times 10^2$</td>
<td>$0.2835 \times 10^2$</td>
</tr>
<tr>
<td>4) % difference due to presence of trace elements</td>
<td>0.73%</td>
<td>0.66%</td>
</tr>
<tr>
<td>5) Values obtained from CT Nos. for i) cortical</td>
<td>$0.3566 \times 10^2$</td>
<td>$0.3122 \times 10^2$</td>
</tr>
<tr>
<td>ii) trabecular</td>
<td>$0.2379 \times 10^2$</td>
<td>$0.2238 \times 10^2$</td>
</tr>
</tbody>
</table>
Therefore unless abnormally high values of trace elements are present then this would not alter the CT number significantly from the contribution from the Ca, P and O. Therefore CT cannot be used as a technique for measuring trace element variation within the bone matrix so that at present no 'in-vivo' method is available for the study of elemental composition and bone disease, except at the gross level.

To examine the variation of trace elements within bone within microns (in-vitro) further methods are required and preliminary work has been carried out (Tolson, 1982) using a PIXE (Proton Induced X-ray Emission) microprobe. In this case several elements not detected using IVNAA were found including Cu, Fe, Pb, Sr (see Fig. 6.10) and this may prove to be an important complementary technique particularly for studying the variation of elements with much smaller distances and structures e.g. within Haversian systems.
Fig. 6.10: Spectrum for human bone obtained using PIXE analysis.
CHAPTER 7 CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

The elemental composition and distribution of human tibiae have been examined using several physical methods of analysis. Neutron activation analysis, using a range of irradiation and counting procedures, proved to be a most useful method of analysis allowing the determination of a range of elements: Ca, P, O, Mg, Na, Cl, Zn and Br. Several other elements, F, K, Mn and Sr, were detected but had large associated errors.

The results obtained using INAA are listed and the variations along the bone plotted, in section 6.4. First of all let us consider the variation of the minor and trace elements along the tibia. Magnesium did vary along the length of the bone but a function could not be fitted to the results with any statistical accuracy. The Mg values were found to be considerably higher in the middle of the shaft and the Ca/Mg ratio at a minimum at this part of the bone. As discussed in section 1.2.4 Magnesium is thought to inhibit the formation of hydroxyapatite and here Mg is found to be increased where there is mainly cortical bone, but occurs in lower concentrations where there is more trabecular bone present and hence greater bone turnover occurs. However it is not possible to discern whether the levels of Mg thus play a role in controlling the formation of apatite or whether the Mg concentrations are determined at different positions in the bone by the differing rates of turnover.

The concentrations of zinc and bromine were found to be increased towards the ankle and knee ends of the tibia. This agrees with the results of Brätter et al. (1976) who found Zn and Br behaved in a similar manner for the humerus of skeletons from an ancient population.
Since both Zn and Br concentrations appeared to have been affected by burial conditions, it was not possible to determine whether this occurred for the Romano-British tibiae analysed. These two elements therefore seem to occur in higher concentrations where greater mineral activity occurs and at lower concentrations throughout the whole bone mineral indicating that they are 'volume' seekers rather than 'surface' seekers of bone.

Sodium however mimicked the Ca distribution and within the fitting error the gradient of the fitted straight line agreed with that found for Ca, and for both there was a decrease in concentration in going from the knee down towards the ankle. The Na in bone may be assumed to be extracellular and to be distributed between the true extracellular fluid (ECF), the hydration layer of the bone crystals, and the bone crystals themselves (Widdowson and Dickerson, 1964). For practical purposes it is impossible to distinguish between the first two of these fractions, for it is probable that the Na in the hydration layer of the crystals is in equilibrium with the Na in the true ECF. The Na of the bone crystals is thought to be adsorbed on the crystal surfaces in association with carbonate, probably in the form of Ca-O-CO$_2$-Na (Widdowson and Dickerson, 1964). Therefore this explains the very similar distribution found for the Ca and Na if they are bonded through the carbonate ion. However, chlorine although it is present in ECF does not behave in a similar manner to the Na and Ca, and this has been found to be true by other workers (Widdowson and Dickerson, 1964). For the tibiae, although large statistical errors were associated with its measurement, chlorine, was in fact found to decrease in concentration in going from the knee down towards the ankle.
Phosphorus, as would be expected, followed a similar distribution to Ca and when a straight line was fitted to the results its gradient was found to be approximately half the value of the gradient for Ca. Since in hydroxyapatite the ratio of Ca to P is 2.15 this indicates that there is an increasing amount of bone mineral present in going down the shaft from the knee towards the ankle. For oxygen it was not possible to fit a distribution to the results but within experimental error it could be seen to follow a similar distribution to P, as would be expected since in hydroxyapatite the majority of the O is bonded to P in the phosphate ion. Further experiments carried out on the scanning electron microscope with a microprobe analyser allowed the study of the Ca/P variations within much smaller areas. These results indicated that the Ca/P ratio remained fairly constant for cortical bone throughout the tibiae suggesting that any variations in the Ca/P ratio are due to differences in the trabecular bone present.

The results discussed so far have been from 'in-vitro' methods of analysis and in order to compare them with a widely used 'in-vivo' method for examining bone, the tibiae prior to sectioning were examined using 'X-ray transmission' tomography. From the resulting images obtained two types of analysis were then carried out. First of all the relative areas of trabecular and cortical bone were obtained and as would be expected the shaft consisted mainly of cortical bone and the trabecular volume increased as the knee and ankle were approached. An interface region was also found between the two types of bone whose area was generally of the same order of magnitude for the trabecular bone. Secondly quantitative analysis was performed
using the dual energy method to determine the Ca/P ratios along the bone so that these could then be compared with the 'in-vitro' results. Both techniques showed a similar trend with an increase in the Ca/P ratio in going down the shaft towards the ankle.

Calculations were then carried out to determine the effect on the photon mass attenuation coefficients due to the presence of other elements in bone. These showed that for the normal concentrations of other elements present in bone then no detectable change would be produced. One exception may be F since for a 1% change in the mass attenuation coefficient for photons of 72 keV 2.3% F must be present in the hydroxyapatite and Iyengar et al. (1978) quote a range of 0.06-2.8% for F in bone. However, generally, unless abnormally high values of minor and trace elements are present in bone then there will be no noticeable effect on the mass attenuation coefficient. Therefore there is no 'in-vivo' method capable of indicating the trace element content in bone and so 'in-vitro' methods are required. Clinically this means that biopsy samples are the only possible way of obtaining information about the elemental composition and this introduces the difficulty of sampling. Assuming that only one sample can be taken, it may not be representative, for the above results show that there are significant differences depending on the position of the sampling site. Since generally biopsy samples are taken from the iliac crest then it is also important to consider the relative amounts of cortical and trabecular bone present in the sample as trace element concentrations were found to be greater in trabecular compared to cortical bone. CT images may be an important method in helping to select a
representative area of the bone and in studying the relative amounts of trabecular and cortical bone present in this area.

The elemental concentration and distribution has also been examined in skeletons found at a Romano-British cemetery in Dorchester. Hair samples preserved by a certain type of burial were analysed and for this preliminary study the bones from the same type of grave were examined. In archaeological samples it is important to establish whether elements have been depleted or increased in concentration due to burial, before trace element concentrations can be compared with contemporary populations. In this case, both Sb and Zn in bones appeared to be higher in concentration due to uptake from the surroundings, the Sb coming from the lead-lined coffin. However Zn was not detected in hair, whereas it is usually determined quite easily in the hair of contemporary populations and therefore the hair does not seem to have been contaminated by external sources with which it came into contact. This suggests that Zn concentrations are higher in bone of the Romano-British period than for contemporary populations. In the less well preserved bones Au was also found to be increased in concentration and in general the hair samples had greater concentrations of this element than contemporary populations again indicating uptake of this element during burial. Where water had entered the coffin then the opposite appeared to have occurred in that there was a depletion of elements possibly due to the water and gypsum reacting to give a weak sulphuric acid.
In particular tibiae were examined so that the results could be compared with the contemporary values. Cores of bone taken from along the shaft were split into two and then analysed using INAA. Both V and Br appeared to be increased on the outer part indicating an uptake of these two elements from the surroundings, the concentration for V is of the same order as for contemporary populations whereas for Br it is greater than for contemporary populations. Variations along the bone were also examined but for most elements within experimental error there appeared to be little change except for Cl and I. I was only found in the middle of the shaft and Cl, as for contemporary bone, was found to decrease in going from the knee down towards the ankle. In order to study Ca and P variations then it would be necessary to examine bones from a different burial condition since the gypsum, a Ca containing compound, may have affected the concentrations as indeed for the hair much larger values than for contemporary populations were found.

Contamination of the hair samples was examined more closely by use of the electron microprobe analyser. Unwashed samples upon further examination indicated that Ca, P, Fe and Pb were all increased in concentration due to diagenic effects. Samples 1 and 2 which it was suggested may have been henna'd were particularly interesting since they were the best preserved structurally (i.e. the scales were still observable) and also under examination on the EMPA the contamination was only from Ca and Pb. Henna is a compound which was used in the Middle East well before the time of this Romano-British settlement and is in fact still used in shampoos today for 'reddening' and 'conditioning' the hair.
In contemporary populations Zn and Cu are usually fairly constant but in these samples Cu was generally higher in concentration whereas Zn was not detected. For the bone concentrations the reverse was true and Cu was lower whereas Zn was higher than for contemporary populations. At present there is still serious uncertainty as to the meaningful interpretation of hair mineral data due to a lack of knowledge about the quantitative relationships if any, between hair mineral concentrations and internal body burdens. It has been suggested by Rabinowitz et al. (1973) from work on Pb concentrations in hair that a 'pool' model may relate hair trace elements within the tissues to ingestion from the environment. Chittleborough and Steel (1980) have proposed a generalised pool model in which hair is a sink for endogenous elements and the quantities of these elements deposited in hair depends upon factors such as the size of the pool, amount ingested and the exchange coefficients between pools and/or hair itself. Therefore the skeleton may act as such a 'pool' for certain elements explaining the differences obtained in values for the hair and bone. However there were not sufficient hair samples preserved to enable a detailed study to be carried out to investigate this further.

Suggestions for further work

The skeleton participates in many metabolic processes e.g. in the maintenance of mineral homeostasis, yet the knowledge of trace element distribution in the different bone fractions and the whole skeleton is not complete. Here trace element distributions in human tibiae have been examined but there is still a considerable amount of data required to be determined about other bones for both
normal and abnormal states. Biopsy samples are generally analyzed for Ca and P, and their ratio, in order to give an indication of the bone mineral status, whereas using analytical techniques such as INAA more information could be obtained about the total elemental composition. Sampling site is also important and the variation composition due to varying amounts of trabecular and cortical bone requires further investigation. Computerised Tomography may be a useful method in selecting the areas from which to extract the biopsy sample. Also from the analysis of the tibiae there was found to be an intermediate region of bone between the trabecular and cortical bone, whose area was of the same order of magnitude as that of the trabecular bone. This requires further investigation as to whether this is a normal transition area from the trabecular to cortical bone or whether it is an indicator of some type of abnormality e.g. since these samples were from elderly patients they may have been slightly osteoporotic.

Clinically there are a range of techniques available for measuring various parameters in bone: nuclear medicine, computerised tomography, Compton scattering measurements for density, in-vivo neutron activation analysis. Therefore there is a continuous turn-over of data on bone from patients with a wide range of conditions and diseases from which more information could be extracted. The provision of a data bank would allow the storage of this data so that intercomparison of techniques could occur and also a much more detailed study of the data could take place.

The development of the proton microprobe may be an important way forward in the analysis of bones. From the measurement of X and
γ-rays emitted, due to the interaction of the protons within the sample, the variation of elements within distances of microns may be examined with significantly greater sensitivity than electron microprobe analysis. Therefore the role of elements, other than Ca and P (which may be studied using the EMPA), in areas of remodelling and mineralisation could be studied.

The proton microprobe may also prove to be a useful tool for examining diagenic effects in samples of archaeological or forensic bone. Where uptake of elements has occurred e.g. V, Br then profiles of these could be measured through the bone so that if a plateau is found within the sample, this will represent the original concentration of that element. This may also be important to be taken into consideration in methods of dating e.g. by F, since if diagenic effects can be separated from normal concentrations then this would make the technique more accurate.

Here samples of bone analysed were from one particular type of burial condition although there were several other types of burial at this cemetery. If the other types were also studied this would give information about the exchange of elements, in different conditions, with the bone. Through comparison of results obtained from the different burial conditions clarification in each case of the magnitude of diagenic effects may be determined.

As mentioned in Chapter 5 due to the presence of the gypsum, it was difficult to examine Ca, P and their ratio (in the archaeological samples) and this would have to be done with samples from other burial conditions, and the results compared with contemporary
populations. Due to the large size of this cemetery there is still a great deal of information to be obtained about the trace element composition of bone for this population.
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