BORON AND OTHER TRACE ELEMENTS

IN HUMAN TISSUES AND FLUIDS

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by

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ABSTRACT

The multielemental technique of inductively coupled plasma source mass spectrometry (ICP-MS) was used to measure the elemental concentration of boron and 28 other elements within blood and its fractions, saliva, urine, hair, nails, synovial fluid, brain and bone samples. A number of biological standard reference materials were also analysed: IAEA A13 animal blood and H4 animal muscle, NIST 1577A bovine liver and SRM909 human serum, and SINR 0920 Chinese hair. Boron levels were found to be in the order of 30-40ng/mL in saliva and synovial fluid, 150ng/mL in blood, 1μg/mL in urine, 2-4μg/g in hair, bone and brain, and 10μg/g in nails. Other elements ranged in concentration from sub-nanogram/gram (or mL) to percentage (%w/w) levels. Associations of boron with other trace elements, such as boron and calcium in blood, were also investigated in the various tissues and fluids, as were variations due to age and sex.

Frequency distribution curves were plotted for trace elements and micronutrients in all tissues and fluids. Since most elements demonstrated a symmetrical distribution in blood, this was thought to be indicative of the amount of homeostatic regulation, rather than the essentiality of the element. The distribution of elements among the various sample matrices, and occurrences of elemental or matrix interdependence, were investigated. Rubidium and caesium, also calcium

and strontium, for example, were consistently found to behave in similar ways among the clinical specimens. In addition, alterations in elemental levels due to factors such as age and sex were assessed in the range of tissues and fluids.

Full descriptions of the elemental content of brain, bone and synovial fluid samples were obtained. Comparisons were made between rheumatoid arthritic (RA) and control subjects for brain and bone, and between RA and osteoarthritic (OA) patients for the synovial fluid. Boron was shown to be "very highly significantly" depleted in RA bone (at the 0.1% level). Other elements showing variations in bone and brain were lithium and iron. Iron was also at lower levels in RA synovial fluid compared to OA, in contrast to phosphorus, copper and zinc, where increases were seen. Elemental levels were linked with other clinical parameters in synovial fluid, such as crystal content and white blood cell count (a measure of the extent of inflammation), to assess any possible variations. Fluid containing crystals displayed elevated levels of scandium, strontium and caesium. Several elements showed a positive correlation with white blood cell count; these were caesium, magnesium, phosphorus, copper, zinc and rubidium.

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1. INTRODUCTION

1.1. Introduction to Trace Elements

1.1.1. Essentiality

Most, if not all, elements in the Periodic Table are found within the body at various levels of concentration. The major, structural elements comprise carbon, hydrogen, nitrogen and oxygen; also, calcium and phosphorus in hard tissue. Minor elements, which maintain the electrolyte balance, consist of calcium, chlorine, potassium, magnesium, phosphorus and sodium. All other elements within the body can be designated trace elements, accounting for less than 1% of the total (1). The word "trace" originates from the fact that early workers were unable to measure the precise concentration using the analytical methods then available (2).

Among the trace elements, 15 are currently thought to be essential: arsenic, chromium, cobalt, copper, fluorine, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium and zinc. Cotzias (3) gives a very full description of essentiality. He maintains that for an element to be essential, it must meet the following criteria:-

i) it is present in all healthy tissue of all living things.

ii) its concentration from one animal to the next is fairly constant.

iii) its withdrawal from the body induces reproducibly the same physiological and structural abnormalities regardless of the species studied.

iv) its addition either reverses or prevents these abnormalities.

v) the abnormalitues induced by deficiency are always accompanied by pertinent, specific biochemical changes.

vi) these biochemical changes can be prevented or cured when the deficiency is prevented or cured.

Numerous other elements are found in the body, which do not meet the above criteria, for example bromine, caesium, rubidium, strontium and titanium. They are believed to be acquired by the animal body as environmental contaminants and to reflect the contact of the organism with its environment. These non-essential elements have been shown to follow skewed (log-normal) distribution patterns of concentration within human organs, while essential elements are normally or symmetrically distributed (4,5). Some elements, including lead, mercury and cadmium, have been placed into a third classification of "toxic" elements since they impair health at even minute levels (1). It must be remembered, however, that all trace elements are toxic

if ingested or inhaled at sufficiently high levels and for long enough periods (2).

1.1.2. Discovery of trace elements

Essentiality of iron to life has been known since the 17th century. Similarly, the role of iodine as an essential part of human health has been recognised since 1850. Most discoveries linked with trace elements, however, have been in this present century. During the first 25 years, further work was carried out on iron and iodine (6). Moreover, the advent of emission spectrography allowed simultaneous estimation of approximately 20 elements at low concentrations, thus permitting extensive investigation of soils, plants, animal and human tissues (7,8,9).

In the 1920's, first attempts were made to use purified diets in animal studies, where the levels of certain elements were strictly controlled (10,11). These were largely unsuccessful due to deficiencies in other nutrients, particularly vitamins. However, within the next few years, a group used rats fed a special diet to show that supplementary copper, in addition to iron, was necessary for growth and haemoglobin formation. The same group followed this by using special and purified diets to prove the essentiality of manganese and zinc in rodents and other species (12-15).

During the 1930's, a large number of nutritional disorders of man and livestock were found to be caused by deficient or excess intakes of trace elements from the natural environment. By discovering such deficiencies, the essentiality of cobalt was shown (16,17), and further confirmation was given for elements such as manganese and copper (18-22). Symptoms caused by excessive intakes of fluoride, selenium and molybdenum were also noted (23-28). While devising practical means of prevention and control of the disorders just described, it became evident that, in addition to the acute conditions first noticed, there were a series of minor maladies affecting more animals in a greater number of regions. It was also shown that the severity of these deficiency or toxicity states was altered by presence or absence of other elements or nutrients. An example of this is the three-way interaction of copper, molybdenum and inorganic sulphate discovered in the early 1950's. Also in that decade, the purified diet technique was used to identify molybdenum, selenium and chromium as essential (29-33).

In the following years, advances in analytical methods continued with the development of atomic absorption and neutron activation techniqes, and the use of radioactive isotopes to follow metabolic movements and kinetics. Due to such research, many metalloproteins with enzymatic activity were discovered, for example, the role of copper in elastin

biosyntheses (34). In the 1970's, six more elements were identified as having an essential role: tin, vanadium, fluorine, silicon, nickel and arsenic. This became possible by confining animals, on purified diets, to a plastic "isolator", free from any metal, glass or rubber components, and from the contaminating effects of dust (35). Since 1977, no further significant advances have taken place, and no additions have been made to the fifteen trace elements.

1.1.3. Role of chemical elements

Despite their low concentrations, the essential elements are of great importance for growth and development (2). In some cases, they act as constituents of vital biological molecules, for example, iron in haemoglobin and iodine in thyroid hormones. In other situations, they are either part of an enzyme system, or act as cofactors for various reactions mediated by enzymes. Table 1 summarizes the biological roles of all known trace elements.

There are narrow limits within which the characteristic concentrations and functional forms of the trace elements must be maintained. Otherwise, the functional and structural integrity of the tissues and the growth, health and fertility of the animals (or humans) may be

Table 1: Mode of action of the essential trace elements (1)

Element	Some functions and deficiency aspects
Iron (17th century)	Involved in oxygen and electron transport Deficiency results in anemia, which is widespread on a global scale Excess is dangerous in hemochromatosis
Iodine (1850)	Constituent of thyroid hormones Deficiency results in goiter and depression of thyroid function Excess leads to thyrotoxicosis
Copper (1928)	Linked to oxidative enzymes, interacts with iron Essential for cross-linking of elastin (e.g., connective tissue) Deficiency results in anemia and changes in ossification and is widespread in malnourished populations and in patients receiving total parenteral nutrition (TPN); pregnant women are easily susceptible to deficiency
Manganese (1931)	Participates in mucopolysaccharide metabolism and is connected with superoxide dismutase No deficiency effects in humans are known Toxic if inhaled and excess results in neurological disorders
Zinc (1934)	Constituent of over 100 enzymes involved in a variety of fundamental metabolic processes Deficiency has grave consequences and is reflected in growth depression, sexual immaturity, skin lesions, depression of immunocompetence, taste acuity, etc. Deficiency in several parts of the world, including Turkey, is suspected, proven in Iran and Egypt, and risked by vegetarians, malnourished populations, patients on TPN, and those suffering from certain genetic disorders of metal transport; subjects under traumatic stress are seriously affected by the imbalance
Cobalt (1935)	Part of vitamin B-12 whose deficiency in humans is established Low intake from vegetarian diets seriously affects some sections of the population
Molybdenum (1953)	Linked to xanthine, aldehyde, and sulfide oxidases Deficiency symptoms in humans are not known Excessive exposure results in gout-like syndrome (seen in U.S.S.R.)
Selenium (1957)	Component of glutathione peroxidase and interacts with heavy metals Known disease in humans is cardiomyopathy (Keshan disease) in China, conditioned by selenium deficiency, completely cured by selenium administration Toxic effects well recognized in animals
Chromium (1959)	Believed to activate insulin Deficiency associated with impaired glucose tolerance and elevated serum lipids, and suspected link with heart disease; deficiency proven in malnourished conditions, in aging, in patients under TPN, and in subjects consuming highly refined foods; also known for occupational hazards, chromium allergy, eczema, and cancer
Tin (1970)	Believed to be essential for growth in animals Metabolic interactions largely unknown
Vanadium (1971)	Believed to be essential for growth in animals Metabolic interactions largely unknown At high concentrations, interferes with iron absorption
Fluorine (1971)	Essential for the structure of teeth, possibly of bones, possibly growth effects Deficiency in humans observed through increased incidence of caries; possibly risk factor for osteoporosis Toxic at high concentrations; fluorosis demonstrated in some populations
Silicon (1972)	Connected with calcification and possibly in connective tissue formation Deficiency aspects largely unknown and probably unlikely
Nickel (1976)	Interferes with iron absorption; other metabolic interactions largely unknown Excess exposure causes eczema and cancer
Arsenic (1977)	Believed to be essential for growth in animals Metabolic interactions largely unknown

Note: Figures in parentheses indicate year that essentiality of respective element was discovered.

impaired (2). The body can utilise protective mechanisms which delay or minimise the effects of changes brought about by deficient, imbalanced or toxic quantities of elements in the diet or the environment. Ultimately, however, changes will be seen in the health of the animal; the extent and form of these will depend on a number of factors, including age, sex, animal species and degree of dietary imbalance.

1.1.4. Needs for elemental analysis

There is a need for elemental analysis of biological systems in many branches of the biological, environmental and medical sciences. In the first of these, there is very little current knowledge on the exact role(s) that various elements play in biochemical processes. Precise identification and quantification is necessary so that metabolic investigations can produce meaningful results. Among environmental scientists, and even the general public, there is an increasing awareness of the dangers to public health from pollution. It is therefore important to carry out monitoring and surveillance activities; plant-life, soils and dusts can be important sources of imformation. In medicine and related areas of work, elemental analysis is necessary for a number of situations. Examples of this are documentation of clinical problems from deficiency or toxicity states, preparations of diets and total parenteral nutrition, and

identification of inherited disorders of human trace metal metabolism. It is of vital importance to have a reliable set of reference values for essential and toxic elements in various tissues and fluids, particularly the readily accessible clinical specimens so that valid comparisons can be made and therapeutic action begun. Other areas of study involve clinical monitoring of patients undergoing dialysis, or those with replacement or restorative surgery.

In addition, information is required about the daily elemental requirements of humans, availability from the diet, and so on, particularly since trace element supplements are now readily available for purchase (1,36). The present literature is sparse for many elements, for example, boron and germanium, and for "new" analytical techniques, such as ICP-MS. As analytical methodology improves, it is important that reference information and data-bases be continually updated.

1.2. Analytical Methodology

1.2.1. Analysis techniques

Some of the principal methods by which the elemental contents of biological tissues and fluids can be measured are described in this

section. Table 2 compares the detection limits of a number of these techniques.

1.2.1.1. Atomic absorption spectrometry (AAS)

AAS allows measurement of the concentration of a species in an atomic vapour which absorbs radiation at characteristic, element-specific, optical wavelengths. The energy is provided for vapourisation and atomisation by a flame or by an electrothermal method, where structures such as carbon rods or graphite furnaces are subjected to resistive heating. It is essentially a single elemental technique, although in recent years, some attempts have been made to adapt this method so it can be used in a simultaneous, multi-element mode (37,38).

Flame AAS requires sample introduction via a nebuliser and spray chamber, which is a relatively inefficient method; it is a simple technique to use, however, and interferences or systematic errors are relatively low (39). Flameless AAS, of which graphite furnace (GF) AAS is most frequently used, utilises considerably smaller sample volumes, in the range of 5-50μL. This method is particularly suited to the requirements of biological trace metal analyses, since it provides an in-built dry ashing procedure which can free the determination from the influence of the organic matrix (40). The details of this three-

Table 2: Comparison of detection limits for different analytical techniques (41): ng/mL

Element	XRF	FAAS	GFAAS	ICP-AES	ICP-MS	NAA
Li	-	-	-	2	0.01	-
Be	-	-	0.003	0.001	0.01	-
Mg	-	-	0.0002	0.003	0.01	50
Al	-	30	0.1	0.8	0.01	30
P	-	-	-	15	0.1	-
K	3.5	-	0.004	200	10	20
Ca	4.5	-	0.05	0.01	10	500
V	5	40	0.1	0.06	0.01	0.5
Cr	5	2	0.004	0.8	0.01	0.5
Mn	6	1	0.0005	0.02	0.01	0.1
Fe	5	3	0.01	0.09	0.1	10
Co	3	6	0.08	3	0.001	0.5
Ni	3	4	0.05	0.1	0.01	0.6
Cu	-	1	0.005	0.6	0.01	3
Zn	4.5	0.8	0.001	0.01	0.01	5
As	5	-	0.08	2	0.01	1
Mo	-	30	-	0.1	0.01	0.2
Sn	-	-	0.03	3	0.01	30
Cd	-	0.5	0.0002	0.2	0.01	5
Ba	12	8	0.04	0.01	0.01	10
Au	-	6	-	40	0.001	0.3
Hg	-	-	0.2	10	0.01	1
Pb	3	10	0.07	1	0.01	-
Bi	-	20	-	50	0.001	-
U	6	-	-	30	0.001	2

stage heating program of drying, ashing and atomisation must be carefully chosen, however, requiring highly skilled operation. Serious matrix effects have been troublesome in this technique, but significant progress has been made in the reduction of these systematic errors by the introduction of the Zeeman background correction, treatment of graphite furnace surfaces and adaptation of tube geometry.

Both flame and flameless techniques are well established, and equipment is relatively inexpensive to purchase and run. Detection limits for GFAAS are about 100 times smaller than those of FAAS. The main disadvantage is the fact that neither technique is routinely capable of multielemental analysis.

1.2.1.2. X-ray fluorescence (XRF)

This method is based on the observation that when a specimen is irradiated with a beam of sufficiently short-wavelength X-radiation, characteristic X-ray spectra are generated (41). Other excitation sources include electrons, gamma-rays, and more recently, charged particles. Simultaneous multielement detection is possible; detection limits range from several µg/g in favourable cases (particularly around atomic number 30) to approximately 100% by weight (39,41).

Accuracy and precision is good, although matrix effects in biological or environmental samples may cause difficulties.

Recent developments in this area include PIXE (particle induced X-ray emission). The absolute detection limit of PIXE can be below the picogram level, if special procedures are used; more importantly, however, the proton beam can be focussed to about 1μm in diameter (1,39).

1.2.1.3. Atomic emission spectroscopy (AES)

The principle of this technique involves the coupling of enough energy to a small sample, to result in vapourisation and emission of light, as excited atoms return to lower energy states. The characteristic spectra of individual elements may be identified and their concentration related to the intensity of the lines.

Various excitation sources have been used, including a high voltage, electric arc, high-temperature flame, direct current plasma (DCP), microwave-induced plasma (MIP) or inductively coupled plasma (ICP). Of these, the ICP has been the most successful for multielement trace analysis, with detection limits of 0.01-50ng/mL. The method is relatively free from matrix effects although spectra can be very

complex. Stray light and background recombination may cause some difficulties, when using ICP-AES (1,41).

1.2.1.4. Neutron activation analysis (NAA)

This technique involves inducing radioactivity in the sample material, followed by measurement of the radiation emitted by these radioactive nuclides. Neutrons are generally chosen as the irradiation source; gamma-rays are the usual choice for quantification of induced activity, as they exhibit discrete energies and are not subject to significant absorption within the sample (1,41). NAA has good detection limits of 1-50ng/g, depending on the irradiation conditions used, and allows simultaneous determination of many elements (42,43). It is a non-destructive technique; in addition, solid samples require little or no sample preparation which reduces contamination problems. However, liquids must not be irradiated directly because of the leakage of radioactive material. Disadvantages of this technique also include expense, since access is required to a suitably equipped nuclear reactor. In addition, several elements cannot be routinely determined, such as boron and lithium, while others demand excessive counting times, including cobalt, zinc and iron.

Radiochemical NAA may be used in the determination of the shorter-lived isotopes ($T_{1/2}$<1 week) or to isolate low radioactivities of

long-lived low-energetic isotopes which are otherwise indistinguishable from the compton background. Elements such as vanadium, chromium, manganese and others, found at and below the ng/mL level in serum, have been successfully measured using this technique (77). The main advantage about this technique is that the risk of contamination to the sample is minute, provided sample collection techniques are good. The manipulation required of a sample, before irradiation, is minimal e.g. separation of serum from whole blood and freeze-drying. After irradiation, any contamination introduced in digestion and separation procedures is not important, since the elements added are not radioactive (77).

1.2.1.5. Mass spectrometry (MS)

This method of analysis involves the ionisation of sample material with subsequent ion separation on the basis of charge/mass ratio. The numerous designs of mass spectrometer include spark source, isotope dilution and secondary ion MS. Of these, until recently, only spark source has been of major importance for simultaneous multielement trace analysis (44-52). For this technique, excellent detection limits in the range 1-10ng/g are achieved. Sample preparation is difficult; extreme care must be taken to ensure homogeneity of the sample material since only a fraction of the total sample is utilised in

sparking. Spectra are difficult to interpret and sample throughput is small at low levels of concentration.

1.2.2. ICP-MS and biological systems

In the last decade, the technique of ICP-MS has been developed; this combines an inductively coupled plasma source with a quadrupole mass spectrometer to give a rapid, sensitive, multielement technique. The high sensitivity of the mass spectrometer and low background levels give detection limits in the order of 0.01-1 ng/mL in aqueous solution. Only a few elements cannot be measured by this technique, while spectral interferences result in a small number of problems. Other advantages include simple spectra and a wide dynamic range. The equipment is, unfortunately, quite expensive to purchase and run, when compared with techniques such as AAS; high sample turnover is possible, with more than 90% of the elements in the Periodic Table being covered. A thorough assessment of the analytical capabilities of ICP-MS has recently been completed (53).

It is still a relatively new technique, but the number of papers being published on various applications of its use is rapidly growing. Some of the more recent developments and reports will be described here.

Because of its low detection limits and multielemental capability, ICP-MS is ideal in obtaining information on elemental composition of various samples. These include more "unusual" samples such as human oviduct fluid and cerebrospinal fluid as well as the more usual serum (54,55). Some studies investigate various health problems, such as the composition of seminal fluid in male infertility (56), brain tissue of human stillbirths (57) and zinc supplementation to anorexics (58). Analysis has also been carried out on both human milk and commercial infant formulas (59,60).

Determination of isotope ratios is also possible with ICP-MS. Isotopes of zinc in faeces and whole blood (61), and of iron in faeces and erythrocytes (62,63) have been considered in bioavailability and tracer studies. Selenium isotope ratios have also been measured, using human urine after a dose of isotopically enriched selenium had been taken orally (64,65). Tracer studies have been reported for strontium (66) and blood lead isotope ratios have been measured following environmental exposure (67). In addition, iron isotope ratios, in small samples of blood, have been carried out using electrothermal volatilisation as an alternative to solution nebulisation (68). This method of sample introduction could be of significant importance in medical studies where a limited quantity of sample material is available. The other major alternative to solution nebulisation is laser ablation of solids. This method is still in the development

stage but has proved to be successful in the analysis of several biological reference materials (41). Suitable standardisation can present some difficulties with this technique.

Finally, ICP-MS has been used to good advantage when coupled with other analytical techniques. For example, it has been used as an on-line detector for the analysis of the metalloproteins ferritin and metallothionein in liquid chromatography eluates (69). Moreover, the speciation of cadmium in pig kidney has been investigated using size exclusion chromatography interfaced directly with ICP-MS (70).

1.2.3. Choice of analytical methodology

The development of such a wide range of analytical techniques, as described in the previous sections, indicates that no single technique can meet the needs of all the elements in the periodic table, for all types of sample under investigation. Two main factors which must be considered when planning a trace element study are the extent of elemental coverage and the order of magnitude of the instrument's detection limits for the range of elements.

It is clear that within any given multielement technique, there are some elements which are capable of being measured to a high degree of accuracy and precision, while others are less suitable, thus requiring

some degree of compromise when making a choice. For example, aluminium is readily measured by ICP-MS, and by the single-elemental GFAAS. If NAA is used, however, there is a danger of interference from the reaction of fast neutrons with the phosphorus in the sample. Another example is calcium, which can be evaluated using NAA and FAAS. In ICP-MS, use of the main isotope of calcium ^{40}Ca is impossible because of the presence of the ^{40}Ar plasma gas peak i.e. other isotopes must be used instead e.g. ^{44}Ca with an abundance of 2%. Fortunately, the levels of calcium in most biological tissues and fluids are sufficiently high to allow determination using ICP-MS.

1.2.4. Aims of this study

The main objectives of this work involve the further application of solution ICP-MS to the measurement of trace elements within biological specimens which are readily available within a clinical setting. In particular, the suitability of this technique is assessed with reference to the element boron. Since a certain amount of interest has been shown in whether or not it is essential for animal life, its levels and associations with other elements within certain tissues and fluids are investigated.

The multielement capabilities of ICP-MS are utilised in order to obtain information about a wide range of elements, such as lithium,

germanium and lanthanum, in human tissues and fluids and in standard reference materials. In order to investigate patterns of elemental behaviour in various biological samples, the observed elemental levels, and correlations between elements and matrices are assessed.

Finally, ICP-MS is used as a technique for evaluating how elemental levels may become altered in cases of human disease; in this instance, the disorder of rheumatoid arthritis is investigated.

2. METHODS OF ANALYSIS

2.1. Instrumentation

2.1.1. Inductively coupled plasma source mass spectrometry

Inductively coupled plasma source mass spectrometry (ICP-MS), is a comparatively new analytical technique: some of its applications to the measurement of biological samples have already been summarised. In this section, the principles of operation will be described.

The design of the VG Elemental Ltd (Winsford, Cheshire) Plasmaquad used in this project is shown schematically in Figure 1. Basically, samples are introduced by means of a pneumatic nebuliser and ionised in the argon plasma. A portion of this ionised gas is introduced to the quadrupole mass analyser, which scans the entire mass/charge range. Beyond this, the ion detector is connected to the multi-channel analyser (MCA) and computer. In this chapter, each important step in this process will be outlined in detail.

2.1.1.1. Inductively coupled plasma

The ion source consists of an electrodeless argon plasma operated at atmospheric pressure, sustained by inductive coupling. Figure 2 shows

Figure 1: Schematic of complete ICP-MS system (41)

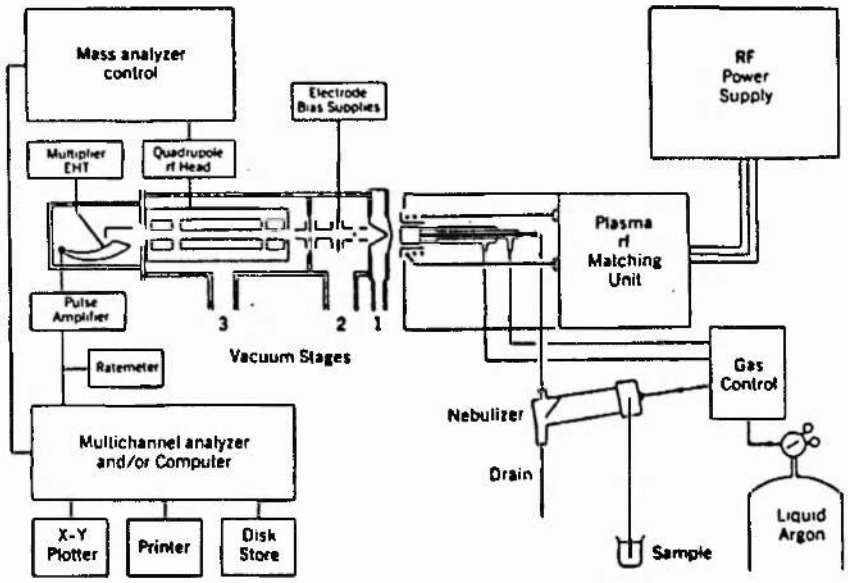
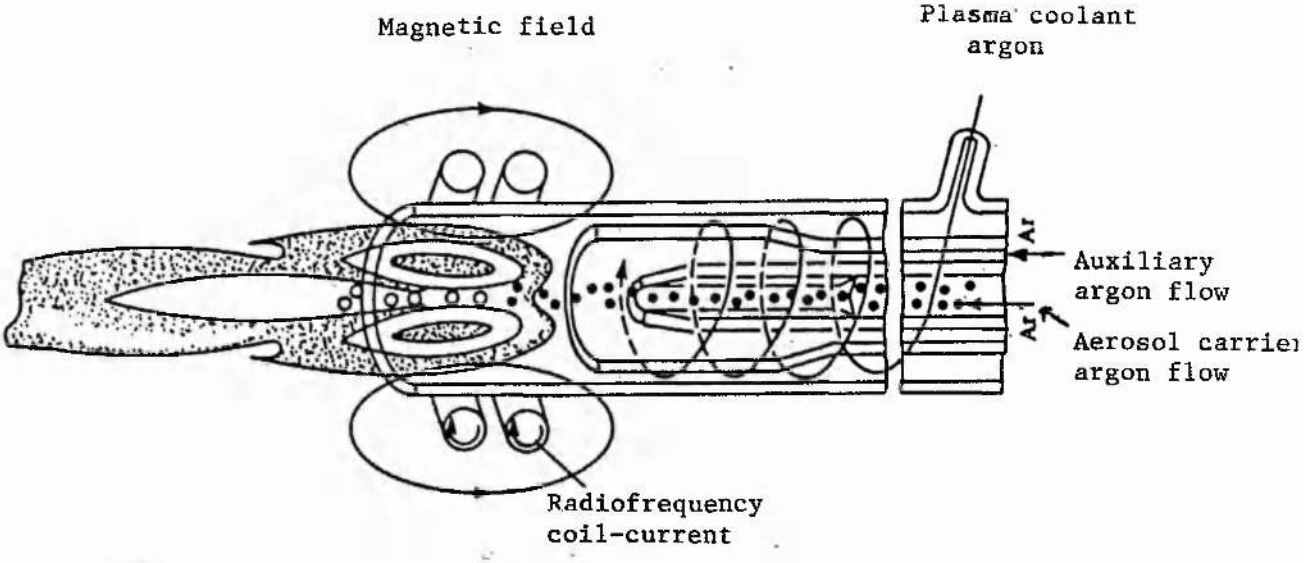


Figure 2: The ICP torch (41)



the inductively coupled plasma (ICP) torch schematically. Two to three turns of a water-cooled copper "load" coil surround the tip of the quartz torch, and are coupled to a radio-frequency power supply (operating frequency 27.12 MHz, power output 1-2.5 kW).Argon is introduced tangentially to both the inner tube (1 L/min) and the annular region (10-15 L/min), thus producing a spiral flow. Since argon gas is initially neutral, a spark is required to produce an stream of electrons, which pick up energy from the load coil. A plasma fireball is formed in the mouth of the torch, and kept off the walls by the coolant flow of gas.

Samples are introduced into the torch via a jet of cold argon (0.5-1 L/min) which flows through the central injector tube, narrowing to a capillary at its end. The resulting high velocity jet forms a central cooler channel through the fire-ball, surrounded by an annulus of hot gas at approximately 10,000K. The plasma is self-sustaining because the annulus of hot gas couples to the load coil. The central channel in this region reaches a temperature of almost 8000K, but this falls rapidly as the carrier gas leaves the torch mouth induction region, and enters the tail flame area. Figure 3 demonstrates the temperature profile of the ICP. Samples require sufficient time in the hottest part of the plasma to ensure that complete vapourisation and dissociation may take place i.e. complete release of elements from within the matrix microparticulates. Considering an optimised carrier

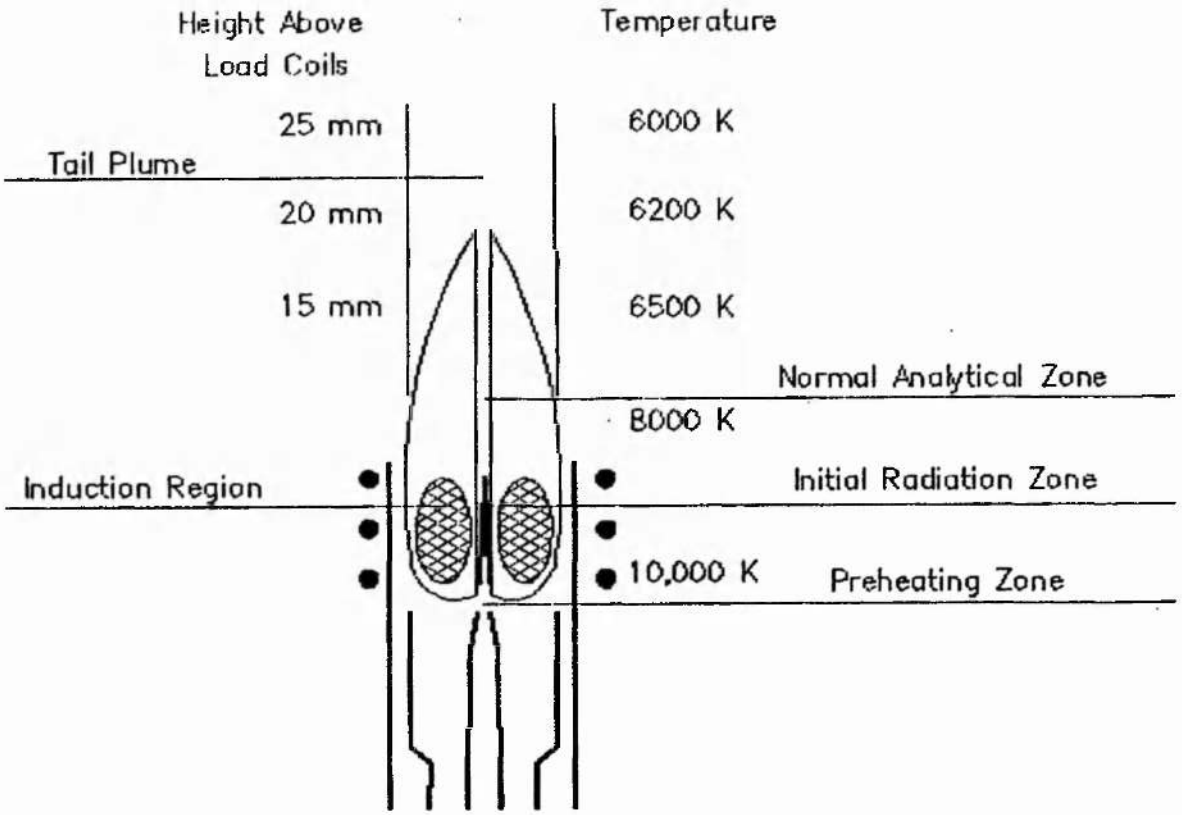


Figure 3: Temperature profile in the ICP (53)

gas flow, and the length of the fireball, the sample may be said to reside within the plasma for approximately 2ms (53).

A pneumatic nebuliser is used to introduce the sample to the plasma, using a peristaltic pump at a typical rate of 1mL/min. Much of the sample taken up this way is wasted, because the gas stream is only able to support droplets below about 4μm diameter. These particles are rapidly vapourised on entry into the plasma; particles up to approximately 1μm can be vapourised by the plasma described here.

The usefulness of the plasma as an ion source depends on the efficiency of ionisation of analyte atoms in the ion plume. The Saha equation can be used to measure the degree of ionisation, α, of a species:-

Equation 1. $$\alpha = \frac{n_i}{n_t}$$ where n_i is the ion population, n_t is the total population

$$= \frac{M^+}{M^+ + M}$$ for singly charged ions

The Saha equation is as follows:-

Equation 2. $$\log S_n = \frac{3}{2}\log T_i - \frac{5040 E_i}{T_i} + \log \frac{Z_i}{Z_a} + 15.684$$

where $$S_n = \frac{\alpha}{1-\alpha} n_e$$

T_i is the ionisation temperature, E_i is the ionisation energy for the species, Z_i, Z_a are partition coefficients for ions and atoms, and n_e is the electron population (71).

Figure 4: Calculated values for degree of ionisation of M^+

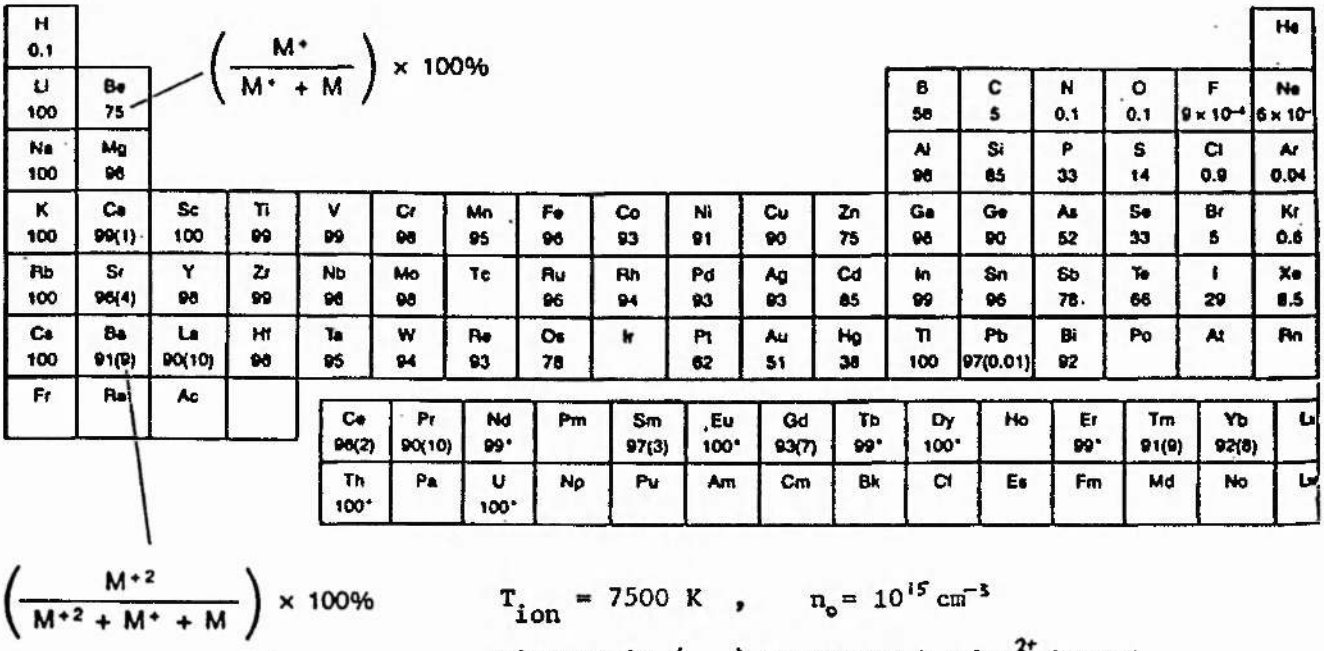


Figure 4 shows values calculated from this equation with an ionisation temperature of 7500K. Many elements have an efficiency of more than 90% for formation of singly charged ions, and even metalloid or non-metallic elements can be ionised. Doubly charged ions should be negligible - the highest proportion are found with barium, lanthanum and several other rare earths.

2.1.1.2. Source/analyser interface

In this stage, ions must be extracted from the plasma, which is at atmospheric pressure, and transferred to the mass analyser, in high vacuum, without altering the ionic composition of the sample. Figure 5 is a schematic of a typical ion extraction interface, showing two of the three stages of vacuum used.

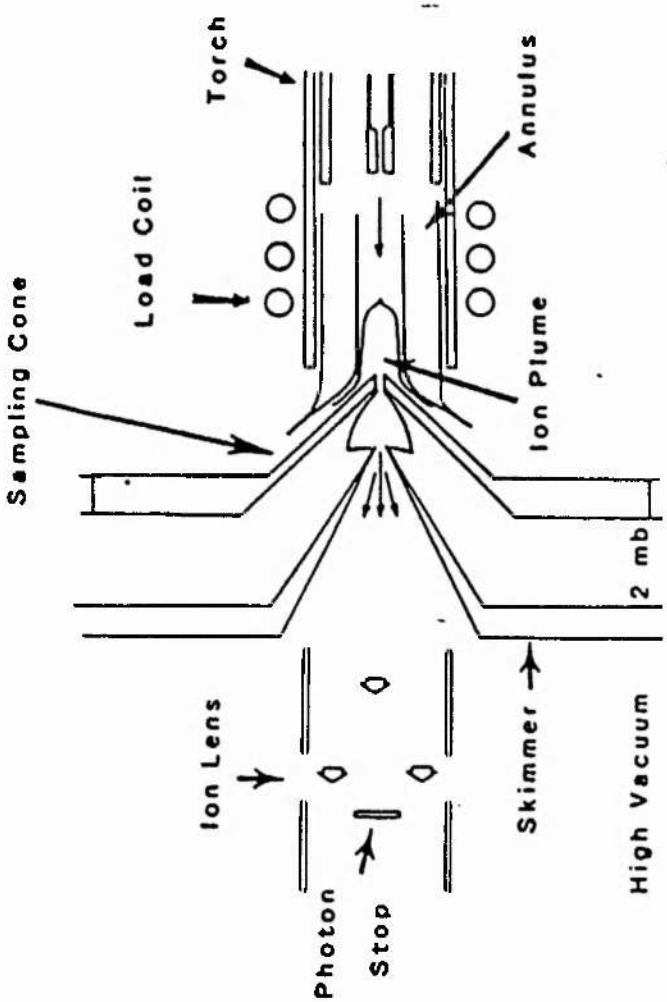


Figure 5: Source/analyser interface

Both the skimmer and sampling cone are typically made of nickel, which has a good thermal conductivity and chemical resistance. A 0.5-1.0 mm aperture is drilled in the tip of the cone; the minimum would be approximately 150μm, or 100 times the mean free path of atoms in the plasma. In practice, larger sizes are used, to minimise the problem of clogging due to sample condensation around the aperture. This also results in a very large volume of gas being admitted; hence the intermediate expansion stage, maintained at a pressure of 1-3 mbar by a simple rotating pump. The steep pressure gradient thus formed in the aperture drives a jet of gas from the centre of the plasma into the first stage of vacuum, with a rapid drop in temperature of approximately 6000K to 200K in a few microseconds. Thus the composition of the sample is effectively "frozen" i.e. only reactions which occur in <1-5μs can take place, for example, the formation of some polyatomic ions from argon gas and sample matrix ions. Behind the extraction cone, the sharply-angled skimmer (external angle 57°), extracts ions from the centre of the expanding jet of gas in stage 1, and passes then to the second stage. The mean free path is now in the same order as the skimmer aperture, at about 1mm, with the result that many of the species collide with the walls of the skimmer. In stage 2, the pressure is maintained at about 10^{-3}-10^{-4}mbar by a diffusion pump; at this level of vaccuum, the gas flow becomes fully molecular. Several electrostatic lenses are used to separate the neutral species and the ions. The latter are then focussed into the mass analyser in

the third stage of vaccuum, past a metal baffle which is used to reduce noise due to detected light photons (53,71).

2.1.1.3. Mass analysis and ion detection

The quadrupole mass analyser is situated in the third stage of vaccuum where the pressure is approximately 2 x 10^{-6} mbar. Figure 6 shows this part of the system schematically. A quadrupole analyser consists of four electrically conducting, parallel rods, typically manufactured from stainless steel or molybdenum and mounted in ceramics. The VG Plasmaquad, in addition to the normal analysing rods shown in Figure 7, has a set of short rods (25mm long), mounted before and after them. These are supplied by a.c. potentials only and act to ensure that low-energy ions may enter. Opposite rods are connected together; a d.c. voltage and a radio-frequency (RF) alternating voltage ($U + V\cos 2\pi ft$) is applied to one pair while an equal and opposite voltage ($-U - V\cos 2\pi ft$) is applied to the other pair. For a given RF potential and frequency, only one mass/charge (m/z) ratio can pass through the analyser and be detected. Lighter elements will oscillate with increasing amplitudes, while heavier ions will drift slowly towards the electrodes; both groups strike the rods and are neutralised. The

Figure 6: Schematic of lens system and mass spectrometer

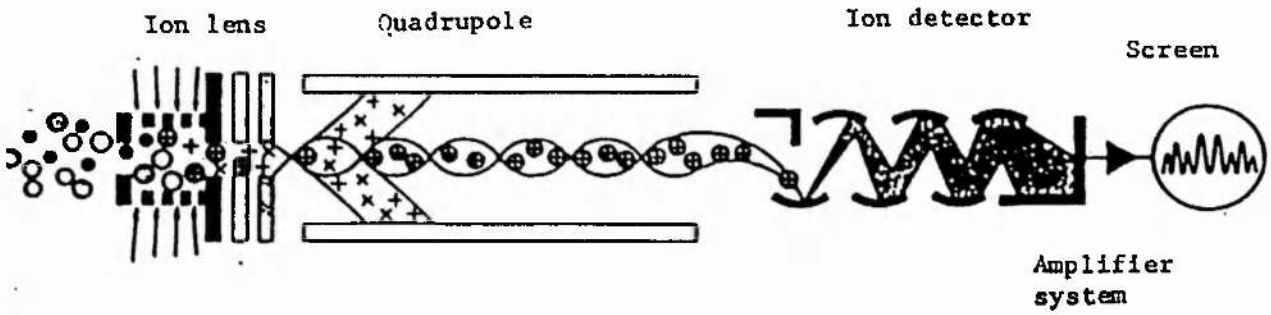
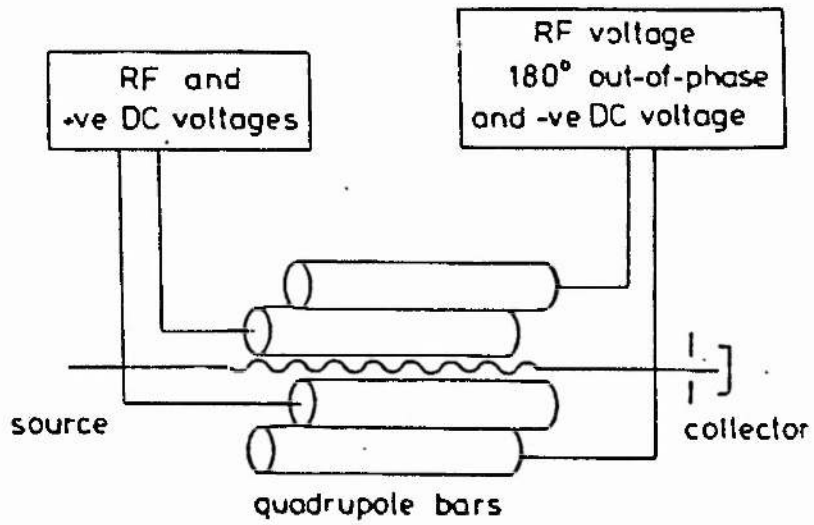


Figure 7: Detail of quadrupole mass spectrometer



quadrupole parameters determining which will have a stable path are shown in equation 3 :-

Equation 3 $$\frac{m}{z} = \frac{C.V}{f^2 r_o^2}$$

where C = constant
r_o = radius of the inner surface of the rods
f,V = frequency, magnitude of a.c. voltage

By scanning d.c. and RF voltages through their ranges, multi-element measurements can be carried out. Ions will then be transmitted and detected in an order which depends on their m/z ratio (53,72).

The resolution of the analyser, or its ability to separate ions of different masses, is controlled by the U/V ratio, which can be varied electrically. A useful definition of resolution uses any given peak in the mass spectrum (71):-

$$\frac{M}{\Delta M}$$

where M is the mass of the peak
ΔM is the peak width at a peak height of 5%

The peaks produced by the quadrupole mass analyser are approximately triangular; imperfections in peak shape ("tailing") occur due to lack of symmetry in rod geometry, a reduced number of RF cycles during ion flight, and energy spread among the ions which enter the quadrupole, for example.

2.1.1.4. Ion detection and data handling

Ion detection is carried out by a channel electron multiplier (CEM) in a pulse counting mode. This is able to detect individual ions, and thus a high level of sensitivity is achieved. Within the CEM, a gain of about 10^8 takes place; the resulting pulse passes via an amplifier and discriminator to a ratemeter and a multichannel analyser (MCA), in which any given channel corresponds to a unique m/z ratio. This is done by synchronously sweeping the mass scan of the analyser and the channel addresses of the MCA. A spectrum over a specified mass range can thus be built up with successive sweeps; it is then downloaded to a storage medium, such as a floppy disk, via a computer.

2.1.1.5. Practical considerations

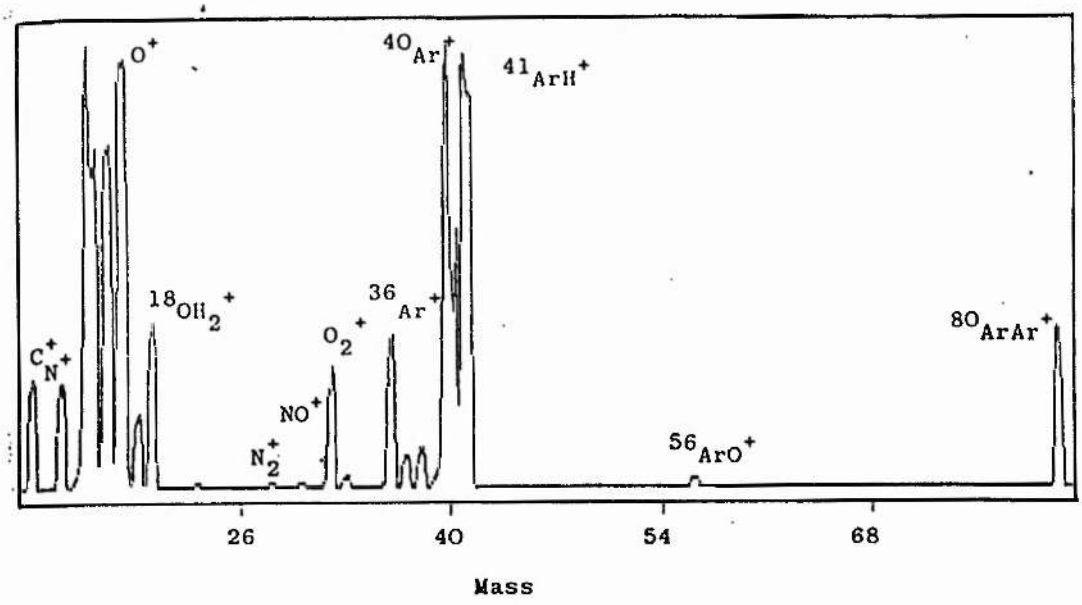
Theoretically, ICP-MS has detection limits superior to many other analytical techniques (see Table 2, section 1.2.1.). In practice, however, these levels are rarely obtained when working with "real" samples, especially those with high levels of matrix, or small sample volumes and high dilutions. A number of factors are involved in this degradation, both physical and spectroscopic (53,71).

One factor which was mentioned earlier was extraction-cone blockage. This is caused by the condensation of solid during the rapid cooling of the gas, and usually occurs at high levels of dissolved solids, particularly refractory matrices. To avoid this, it is important to consider the total dissolved solids levels of samples during preparation, although care must be taken not to "over-dilute" samples to beneath the detection limits. There is also the possibility of general analyte signal suppression in the presence of matrix elements.

The main reason for degradation in detection limit levels, however, are spectroscopic interferences with the isotope(s) of interest. Polyatomic interferences are a result of ion-molecule reactions between major species in the plasma. These occur during the extraction process at the cone, or the expansion stage immediately after this. The major elements present in solvent acids, or occasionally in the sample matrix, such as nitrogen, chlorine, phosphorus or sulphur, give rise to awkward interfering species when combined with argon, oxygen and hydrogen, for example. Most of these interferences take place below mass 80 (53); see Figure 8 .

The number of polyatomic interferences created is a very large factor in the choice of mineral acid used in sample digestion and dissolution, and in multi-element standard preparation. Table 3 compares nitric, hydrochloric and sulphuric acids at the 1% level (53). An

Figure 8: Blank spectrum of 1% HNO3 showing "water" and "gas" peaks and associated polyatomic ions (53)



important consideration in this is that nitrogen has a higher ionisation potential than chlorine and sulphur, and thus nitrogen-containing peaks will tend to be smaller than those containing chlorine or sulphur (71).

These factors lead to the decision to use nitric acid alone in sample preparations (see section 2.2.3). It is important that acid concentrations are kept below approximately 5%, to avoid corrosion of the nickel cone and skimmer, and contamination of the first stage pump. Despite the careful choice of acid, interferences may still occur if elements such as phosphorus, chlorine and sulphur are found at high concentrations in the analyte matrix. One such interference is $^{35}Cl^{16}O^+$ on ^{51}V, but a correction technique has been devised to deal with this, utilising various isotopes of chlorine and chromium (53).

Table 3: Relative concentrations of some mineral acid polyatomic ions (53)

1% Nitric Acid

Polyatomic ion	Mass (u)	Conc.
ArN	54	23.1
ArO	56	232
ArAr	80	2580

1% Hydrochloric Acid

Polyatomic ion	Mass (u)	Conc.
ClO	51	1500
ClO or ClOH	52	24.5
ClO	53	497
ArN	54	52.5
ArO	56	365
ArCl	75	58.5
ArAr	80	4840

1% Sulphuric Acid

Polyatomic ion	Mass (u)	Conc.
SO	48	4750
SO or SOH	49	65.1
SO or SOH	50	258
SO or SOH	52	3.38
ArN	54	23.8
ArO	56	265
S_2 or SO_2	64	1130
S_2 or SO_2	65	59.4
S_2 or SO_2	66	56.4
ArAr	80	2800

Equivalent concentrations, ng.mL^{-1} (ref. 1µg.mL^{-1} ^{59}Co)
Extraction cone 0.7mm, Skimmer 1mm.

Another form of interference is due to the formation of analyte oxide ions, from incomplete dissociation in the plasma, recombination in the cooler boundary layer which forms over the sample cone, or from ion-molecule reactions at the cone aperture. The plasma operating parameters are of importance in determining their levels (53). The rare earth elements, thorium and uranium are particularly susceptible to oxide formation; the size of these peaks are normally less than 1% of the element peak. Another example of an oxide interference is $^{48}Ti^{16}O$ on ^{64}Zn; in this case, there are other isotopes of Zn which are available for use (masses 66 and 68).

Finally, doubly charged ions of analyte or matrix species may cause some interferences. Figure 4 shows the degree of double ionisation predicted by the Saha equation. This indicates that barium and some rare earth metals will exhibit some degree of double ionisation. Only elements with a second ionisation potential below the first ionisation energy of argon can form these species and this number is limited to about thirty. Thus, doubly charged ions tend to be a minor cause of interference, at levels of <1% of the total response (53).

2.2. Pre-analysis Treatment of Samples

2.2.1. Sample population

In this study, the elemental content of a wide range of human tissues and fluids are analysed. These samples are taken from a number of subgroups within the British population; details of sex, age, number of population and tissue-type are summarised in Table 4.

2.2.2. Sample collection

2.2.2.1. Blood and blood fractions

Whole blood was collected by insertion of a stainless steel needle, pre-washed by alcohol to remove surface contaminants, into the vein of the right arm. Approximately 4-5mL of blood was withdrawn through a "butterfly" connection into a plastic collection tube, coated with lithium heparin anticoagulant. The tube was slowly turned, but not shaken, to ensure thorough mixing of the anti-coagulant without causing sample clot disruption. A further 4-5mL of blood was then withdrawn into a non-coagulated blood serum tube, allowed to stand for 10-15 minutes at room temperature until the clot had formed, and spun at 2500 rpm for 10 minutes. The two fractions were separated into blood serum and clot, and stored in polypropylene containers. A third

Table 4: Sections of British population used in this study

a) Adults (n=215)

Geographical areas	Number of subjects M[a]	Number of subjects F[b]	Subject age M[a]	Subject age F[b]	Sample type	Health status
S.W.England	24	12	38±18	29±18	whole blood,saliva urine,hair,nails	healthy
Aylesbury	8	54	63±13	54±10	whole blood,urine hair,nails	various cancers
Sheffield	4	9	59±6	59±10	whole blood,serum clot,packed cells	healthy
Manchester	34	29	47±19	54±22	synovial fluid	various arthritic disorders
London	18	18	67±6	68±8	brain	rheumatoid arthritis/ controls
	4	1	76±4	48	bone	as above

b) Children (n=70)

Geographical areas	Number of subjects M[a]	Number of subjects F[b]	Subject age M[a]	Subject age F[b]	Sample type	Health status
Midlands	-	14	-	17±1	saliva,urine,hair	healthy
Sussex/ Hampshire	9	6	6±4	5±3	urine,hair	healthy
Bucks/ Berkshire	34	7	9q6	6±4	saliva,urine hair,nails	healthy

a: male b: female

aliquot of blood was drawn into a coagulated tube and spun in order to collect plasma and packed cell samples. However, due to some contamination in the separation of these two fractions, the blood plasma samples were discarded. All blood fractions were stored at -20°C within 30 minutes of blood collection or subsequent separation.

2.2.2.2. Saliva

Samples were collected between 8 and 9 a.m., after overnight fasting, to reduce variations due to dietary intake or diurnal changes. Saliva was collected directly from the subject into a sterile 5mL capacity polystyrene tube. Samples were labelled and stored at -20°C in 2-4 hours.

2.2.2.3. Urine

Urine samples were collected between 8-9 a.m. for the same reasons as saliva. Specimens were taken in mid-flow, directly into sterile 25mL polystyrene bottles. Storage, prior to analysis, was at -20°C and was achieved within 2-4 hours of sample collection.

2.2.2.4. Hair and nails

Samples were taken using standard stainless steel scissors and clippers. Hair was removed from a specifically designated region (for reproducibility reasons), namely the nape of the neck (midway between the shoulders), and cut as close to the scalp as possible. The "bundle" of hair was placed in a small plastic bag and sealed firmly. Nail clippings were taken from each finger and toe; finger-nails and toe-nails were stored in separate, sealable, plastic bags.

2.2.2.5. Synovial fluid

Synovial fluid samples were collected from the knees of patients. Before this, skin was disinfected with either 70% isopropyl alcohol, 15% chlorohexidane or an aqueous solution of iodine. A sterile polypropylene hyperdermic syringe, lubricated with a silicone gel, was inserted into the knee joint. Then, liquid was drawn through this by a conventional stainless steel needle and ejected into a sterile 20mL polystyrene bottle. Prior to storage, the samples were divided into cellular and acellular subgroups. Half of the original sample was poured into a smaller capacity bottle; this was the cellular synovial fluid. The remaining sample was spun down in the original bottle (1000 rpm for 20 minutes) and a sterile glass pipette was used to remove the

supernatant i.e. the acellular synovial fluid. All synovial fluid, with or without cells, was stored within three hours of collection.

2.2.2.6. Brain and bone samples

Brain and bone tissue was collected from autopsy cases using a tantalum knife and a titanium saw respectively. All bone samples were well rinsed with doubly distilled deionised water (DDW) to remove blood or soft tissue particles which may have adhered during collection. Brain and bone samples were preserved, prior to sample digestion and analysis, at -20°C in plastic containers.

2.2.3. Sample preparation

The ICP-MS instrument used in this study, with its pneumatic nebuliser, requires samples to be introduced in a liquid form. Various types of digestion techniques were used, depending on the nature of the sample. These can be divided into the main groups of dry- and wet-digestions, which will be described in turn. At the time of sample preparation, "reagent" blanks and standard reference materials of a similar matrix were also prepared. Reagent blanks are samples made by carrying out all steps in any given sample digestion, for example, volume of reagent, length and manner of heating, further dilution and storage conditions, but without the addition of any biological sample

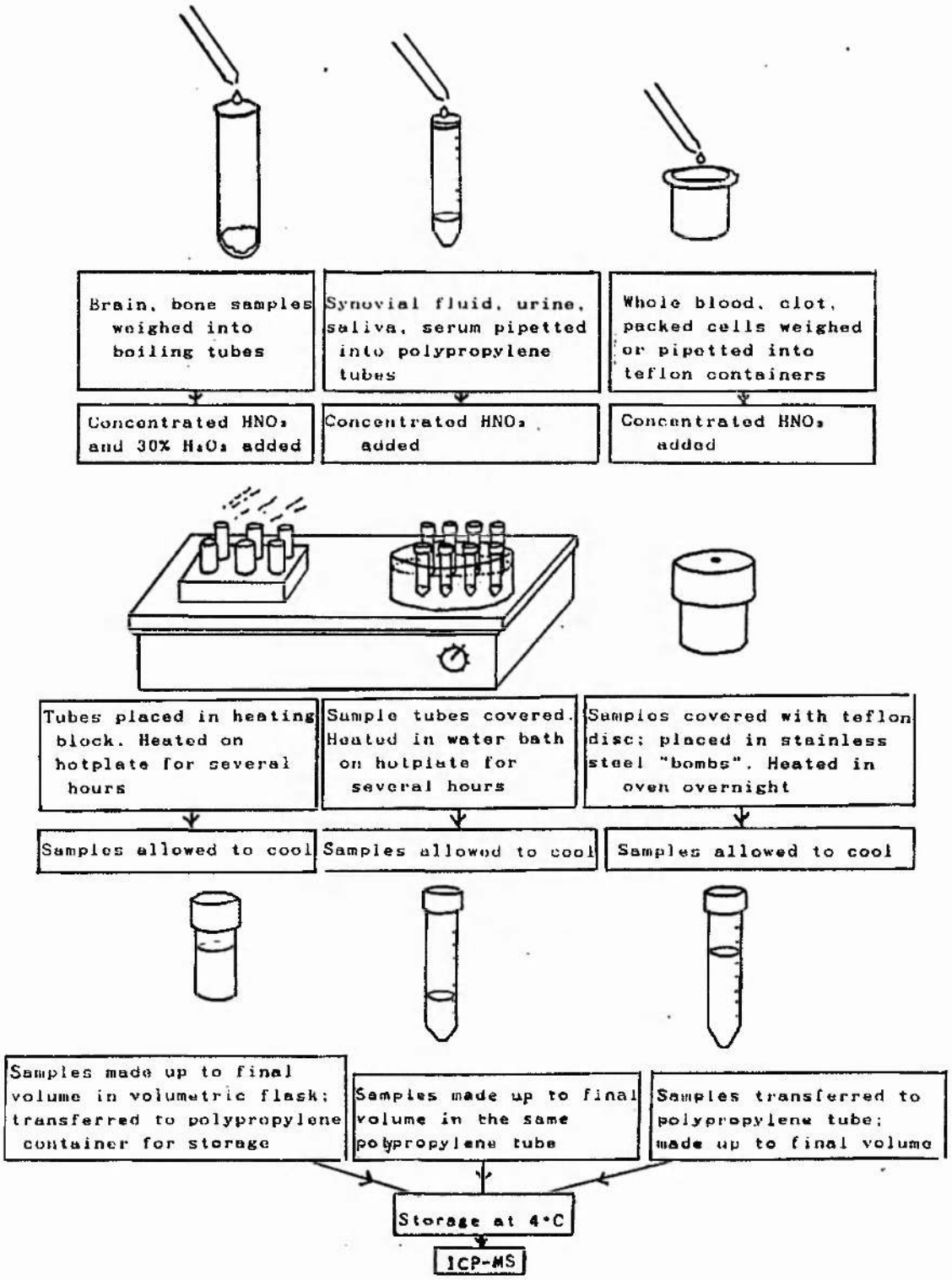
material. The same digestion techniques were also used to prepare standard reference materials which were as close as possible to the actual sample matrix. By definition, reference materials are substances of which one or more properties are sufficiently well established to be used for the calibration of an apparatus or for the verification of a measurement (73). Standard reference materials (SRMs; sometimes also known as certified reference materials or CRMs) are reference materials accompanied by a certificate stating the property value(s) concerned. Agencies such as the National Institute of Standards and Technology (NIST) or International Atomic Energy Agency (IAEA) provide the reference materials and the certification; in some cases, additional "information" values are provided. These are probable levels that are not sufficiently qualified for certification or recommendation (1). Utilisation of such materials provides a check on all stages of sample digestion and analysis.

2.2.3.1. Wet digestion

These were carried out on blood fractions, saliva, urine, synovial fluid, brain and bone samples; the procedure is summarised in Figure 9.

Brain (0.2-0.6g) and bone (0.5-1.1g) samples were digested using 5mL concentrated nitric acid (BDH Chemicals Analar Grade Reagent™) and

Figure 9: Flow diagram of wet digestion preparative techniques used in this study



5mL of 30% hydrogen peroxide (BDH Chemicals Analar Grade Reagent™) in quartz boiling tubes. These were heated on a hot plate at approximately 90°C for 1-2 hours. The "clear" solutions obtained were transferred to volumetric flasks with copious rinsings and made up to 50mL with DDW. Final storage was in polypropylene containers at 4°C.

Whole blood, clots and packed cells (1mL or equivalent) were placed in teflon containers with 3mL concentrated nitric acid (BDH Chemicals Aristar Grade Reagent™), covered with teflon discs, and then enclosed in stainless steel digestion "bombs". These were placed in an oven at approximately 80°C and then allowed to digest for approximately 20 hours. Samples were allowed to cool, transferred to polypropylene containers and made up to 10mL.

Serum, synovial fluid, saliva and urine samples were digested in closed polypropylene tubes in a water bath. Wherever possible, 1mL of sample was pipetted into the digestion vessel (samples with volumes <0.4mL were discarded).Then, 0.5mL (for saliva and urine) or 0.8mL (for serum and synovial fluid) of concentrated nitric acid (BDH Chemicals Aristar Grade Reagent™) was added. Samples were allowed to digest for 3-4 hours; after cooling, synovial fluids were diluted to 3mL and the remaining sample groups to 5mL.

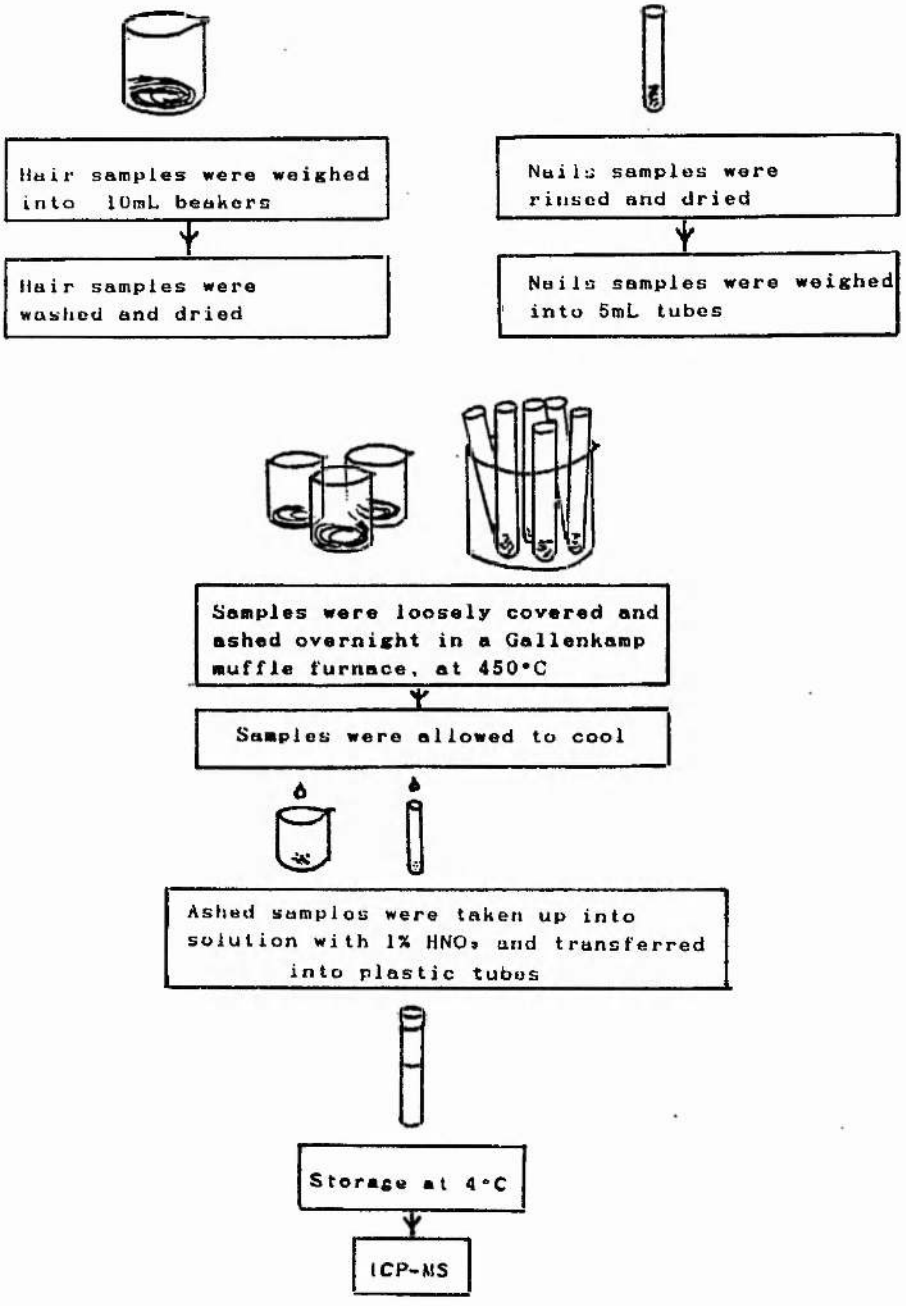
Wherever possible, samples were diluted to final volume and stored without being transferred from the digestion vessel. This was in order to reduce the possible areas of contamination or errors due to sample loss during transfer. If this was impossible, because digestion vessels were to be re-used, the vessels were repeatedly rinsed with DDW to minimise such losses. All reusable containers were washed in 15% Decon (FSA Laboratory) made up in boiling distilled water, rinsed and boiled in 50% HNO_3 for at least four hours and finally, rinsed thoroughly with DDW. All samples were stored at 4°C until analysis was carried out.

2.2.3.2. Dry digestion

This type of digestion was used for scalp hair and finger- and toe-nails; the procedure is summarised in Figure 10.

Unlike the tissues and fluids in the previous section, scalp hair and nails were washed before analysis. The decision of whether or not to wash such tissues is utterly dependent on the type of analysis being undertaken. If it is important to discover the effect of the external environment e.g. the workplace on the elemental levels of hair, washing may not be necessary. In this study, however, the intrinsic trace element content of hair and nails was of interest, and so it was necessary to remove external contaminants by washing (74). In

Figure 10: Flow diagram of dry digestion preparative techniques used in this study



addition, a single washing technique applied to all samples is important in an attempt at standardisation. This is particularly important for hair, since there are a wide range of shampoos and other hair products available on the market, each with a different composition and a different effect on the hair (75).

Hair samples were weighed into beakers; typical weights were 50-300mg. Each individual sample was sonicated for 20 minutes with 1% Triton-X-100 detergent (BDH Chemicals) ; this procedure was repeated twice with fresh aliquots of detergent, and followed by thorough rinsing with DDW. The samples were dried before placing in a Gallenkamp muffle furnace. Nail samples were individually rinsed in acetone before being weighed into 4mL glass sample tubes, and were then placed in the muffle furnace. All samples were loosely covered in the furnace to prevent any contamination from dust or degradation of the furnace walls. The temperature used was 450°C; this was chosen to ensure thorough ashing but to minimise volatilisation of certain elements. Samples were allowed to ash for at least 8 hours; generally this took place overnight. Following digestion, ashed samples were transferred from the digestion beakers or tubes into plastic containers for storage; this was achieved by dissolving the ash in 1% HNO_3. Successive rinsings were carried out to ensure efficient transfer, and samples were made up to a final volume of 3-5mL.

All digestion vessels were cleaned before reuse by first soaking, and then boiling in 50% HNO_3. These were dried and then stored in sealed plastic bags until required again.

2.3. Analysis of Samples by ICP-MS

2.3.1. Standard preparation

One multi-element standard was used for all analysis by ICP-MS. This was used for calibration of the instrument, and also to monitor any drift in the instrumental performance during the period of analysis. The latter was carried out by measuring the standard solution at various stages throughout the analytical period. The various elemental concentrations within the standard were chosen for a number of purposes, requiring a certain degree of compromise. The concentrations needed to be sufficiently high to ensure reasonable counting statistics, but low enough to minimise the possibility of memory effects within the system. In addition, a spread of concentrations was required which would approximately conform to the concentrations expected within the biological specimens. The following levels were used in the stock multielement standard solution:-

4μg/mL: Li,B,Al,Sc,V,Cr,Mn,Co,Ge,As,
Mo,Cd,Sn,Sb,Cs,Ba,La,Ce,Hg,Pb

10μg/mL: Fe,Ni,Cu,Zn,Rb,Sr

50μg/mL: Mg,P,Ca

Solutions were made up in plastic volumetric flasks. The flasks were cleaned prior to use by thorough rinsing, followed by standing for at least 12 hours while containing 10% HNO_3. After further rinsing, the flasks were filled with 1% HNO_3 and allowed to stand for at least five days before thorough rinsing and use.

Standards were made using proprietary single element 1000µg/mL stock solutions (BDH Chemicals) and 1% HNO_3. Nitric acid was used for improved stability and also to match the samples which were always digested in nitric acid. In addition, nitric acid is recommended for use in ICP-MS work because of the comparatively few polyatomic peaks it produces compared with other acids (see Section 2.1.1.5.). Whenever standard solutions were prepared, a "standard blank" would also be made. This involved using an identical plastic volumetric flask and 1% HNO_3. It was stored, with the multi-element stock solution, at 4°C.

For analysis, dilution of the above multi-element standard and accompanying blank solutions was required. A hundred-fold dilution, also in 1% HNO_3, was carried out, giving final elemental concentrations of 40-500ng/mL.

2.3.2. Instrumental operating conditions and procedures

The same operating conditions were used for all the separate analyses carried out in this study, and are summarised in Table 5.

The VG Plasmaquad was completely under computer control during analysis. Therefore, in order that the analyses should be carried out according to specifications, an "element menu" and "run procedure" was written by the analyst and stored on the hard disk of the computer. The element menu defines the elements and their isotopes which are to be measured in a given scan, passing over areas in the Periodic Table where extremely large "gas" or "water" peaks are found. The run procedure defines the order in which samples are to measured; a critical factor in good analysis. At the start of any given "run", several readings of the blank were taken, followed by measurement of the standard solution in order to calibrate the instrument. Next, the reagent blank solution was measured, and finally, the "real" samples were introduced to the system. At various times during the run procedure and after the last biological sample, measurement of the multi-element standard was repeated so that instrumental drift could be monitored. Between each sample (blank, standard or "real"), the system was washed out for a period of 2-3 minutes using 1-2% HNO_3.

Table 5. ICP-MS operating conditions

Plasma power-reflected	1300/<10W
Nebuliser pressure	40 psi
Coolant flow	14 L/min
Sample pump rate	1.5 mL/min
Skimmer type, aperture diameter	Ni, 0.7 mm
Cone type, aperture diameter	Ni, 1.0 mm
Distance torch/cone	10 mm
Expansion pressure	2.7x100 mbar
Intermediate pressure	$<1x10^{-4}$
Analyser pressure	$2x10^{-8}$
Mass range	5-206 amu
Number of channels	1024
Number of scan sweeps	120
Dwell time	500 µs
Skipped mass regions	12.5-21.5 39.6-41.5

All data acquired was stored automatically on a floppy disk, and a hard copy printed out while the run continued. This enabled any anomalies to be seen and dealt with immediately.

2.3.3. Data calculation

The data accumulated during the course of a run is in the form of "raw" counts; before statistical analysis of such data can take place, it must be transformed from counts to concentrations i.e. ng or μg of a given element per g or mL of sample. A computer program, entitled NEWPQ (76), was written to facilitate this manipulation of data.

The principle of this calculation involves comparing the counts obtained by measuring a sample with those from the multielement standard, where each element is at a specified concentration. "Standard" and "reagent" blanks are subtracted; in addition, changes in sensitivity are taken into account by using a corrected standard value for each sample (assuming a linear variation between consecutive sensitivity standards). Mass and dilution factors must also be considered.

Other systematic errors can be introduced by interferences from polyatomic ions, or other spectral sources. Included in the NEWPQ programme is a correction for the chlorine interference on vanadium (51) using chromium (52 and 53). This method is only accurate, however, in the absence of significant carbon content, which would otherwise lead to interefence with ^{52}Cr from $^{40}Ar^{12}C$.

2.4. Accuracy and Precision Measurements

2.4.1. Standard reference materials

Whenever biological samples were prepared for analysis by ICP-MS, standard reference materials were digested alongside these tissues and fluids. The reference materials used were as follows:-

IAEA A13 Animal blood: n=7; 0.16-0.29g dry weight

IAEA H4 Animal muscle: n=4; 0.13-0.29g dry weight

NIST SRM909 Human serum: n=12; 0.5-1.0mL wet

NIST 1577A Bovine liver: n=15; 0.19-0.35g dry weight

SINR 0920 Chinese hair: n=12; 0.04-0.12g dry weight

The Chinese reference hair, SINR 0920, was used whenever hair and nails samples were digested. Therefore samples, in the mass range described, were ashed at 450°C in a Gallenkamp muffle furnace and made up to 5mL with 1%v/v HNO_3. NIST SRM909 was used with blood serum, synovial fluid, urine and saliva, while NIST 1577A bovine liver, IAEA A13 animal blood and H4 animal muscle were used in conjunction with brain, bone, whole blood, clot and packed cell samples. Thus, wet digestions were carried out using concentrated nitric acid; heating took place on a hotplate or in an oven. Duplicate analyses of each sample were carried out; the number of separate preparations is listed above.

The results obtained for these analyses are listed in Tables 6 to 10, with certified or information values where available. The reference materials, therefore, acted as a monitor of the digestion and analytical procedures used in this study.

For most elements, in each reference material, there appears to be good agreement between measured and certified or information values. It is unfortunate that, for a number of elements, no such comparison was possible due to the lack of reference data. On the whole, however, good accuracy was achieved. For some elements in certain reference materials, however, the precision was slightly less good in the measured data than in the certified or information values quoted i.e. there was a larger spread around the mean value than expected. This was most noticeable in AIEA H4 animal muscle; the precision is very much better in AIEA A13, for example. There have been some difficulties involved in the certification of AIEA H4, possibly due to lack of homogeneity of the material; recently, the certificate has been revised, with several certified and information values being altered (77). In the main, however, accuracy and precision, as demonstrated by the measurement of these five standard reference materials, were maintained at a satisfactory standard throughout this study.

Table 6: Analysis of biological reference material IAEA A13 animal blood: ng/g (dry weight) unless stated

Element	This study		Certified[b]/ Information[c]	
	Mean	s.d.[d]	Mean	s.d.[d]
Li	100	54	—	—
B	1815	1531	—	—
Mg[a]	96	7	99[c]	29
Al	511	93	—	—
P[a]	843	704	940[c]	216
Ca[a]	257	44	286[b]	54
Sc	108	19	—	—
Fe[a]	2142	334	2400[b]	151
Mn	636	494	—	—
Ni	1260	157	1000[c]	400
Co	84	74	—	—
Cu[a]	4.2	0.4	4.3[b]	0,6
Zn[a]	12.5	0.2	13[c]	1
Ge	196	60	—	—
Rb	2.0	0.3	2.3[b]	0.7
Sr	317	123	—	—
Mo	1256	917	—	—
Cd	54	4	—	—
Sn	33	27	—	—
Sb	48	38	—	—
Cs	28	19	—	—
Ba	91	18	—	—
La	20	15	—	—
Ce	16	13	—	—
Hg	32	27	—	—
Pb	163	35	180[c]	79

a: µg/g (dry weight)
d: standard deviation

Table 7 : Analysis of biological reference material IAEA H4 animal muscle: ng/g (dry weight) unless stated

	This study		Certified[b]/ Information[c]	
	Mean	s.d.[d]	Mean	s.d.[d]
Li	639	456	-	-
B	852	285	-	-
Mg[a]	1019	167	1050[b]	59
Al	343	235	10000[c]	-
P[a]	10446	1206	-	-
Ca[a]	213	27	188[b]	24
Sc	240	321	-	-
Fe[a]	47	9	49[b]	2
Mn	520	147	466[b]	42
Ni	35	25	64[c]	37
Co	<6	-	2.7[c]	1.0
Cu[a]	3.7	0.2	3.65[b]	0.35
Zn[a]	85	2	86[b]	3
Ge	26	16	-	-
Rb[a]	18	2	19[b]	1
Sr	1127	750	-	-
Mo	148	110	40.9[c]	2.8
Cd	2	1	4.9[c]	1.0
Sn	77	55	-	-
Sb	17	13	-	-
Cs	111	24	120[b]	14
Ba	<3	-	-	-
La	<0.4	-	-	-
Ce	<0.3	-	2.3[c]	-
Hg	18	16	6.8[b]	0.4
Pb	<0.1	-	-	-

a: µg/g (dry weight)
d: standard deviation

Table 8: Analysis of biological reference material NIST SRM 909 human serum: ng/mL (wet) unless stated

Element	This study		Certified	
	Mean	s.d.[b]	Mean	s.d.[b]
Li[a]	9.0	1.3	11.4	0.3
B	1111	738	—	—
Mg[a]	25.5	2.7	29.4	0.7
Al	118	114	—	—
P	128	25	—	—
Ca[a]	115	19	121	4
Sc	14	14	—	—
Fe[a]	1.8	0.3	1.98	0.27
Mn	65	7	—	—
Ni	39	15	—	—
Co	1.0	0.8	—	—
Cu[a]	1.0	0.3	1.1	0.1
Zn[a]	1.4	0.4	—	—
Ge	1.0	0.9	—	—
Rb	5.5	4.2	—	—
Sr	39	5	—	—
Mo	6.4	4.2	—	—
Cd	1.1	0.8	1.24	0.10
Sn	1.5	1.3	—	—
Sb	12	6	—	—
Cs	0.5	0.4	—	—
Ba	308	170	—	—
La	3.2	2.9	—	—
Ce	0.1	0.1	—	—
Hg	1.3	1.1	—	—
Pb	15.5	2.9	20	2.5

a: µg/mL (wet)
b: standard deviation

Table 9: Analysis of biological reference material NIST 1577a bovine liver: ng/g (dry weight) unless stated

Element	This study		Certified[b]/ Information[c]	
	Mean	s.d.[d]	Mean	s.d.[d]
Li	157	49	176[c]	5
B	571	257	590[c]	80
Mg[a]	592	74	600[b]	15
Al	2733	521	2000[c]	
P[a]	11597	647	11000[b]	396
Ca[a]	126	38	120[b]	7
Sc	61	43	-	-
Fe[a]	184	78	194[b]	20
Mn[a]	8.4	1.0	9.9[b]	0.8
Ni	232	129	-	-
Co	163	49	210[b]	50
Cu[a]	153	18	158[b]	7
Zn[a]	122	9	123[b]	8
Ge	28	17	-	-
Rb[a]	13.1	3.1	12.5[b]	0.1
Sr	111	38	138[b]	3
Mo[a]	3.1	0.9	3.5[b]	0.5
Cd	290	85	444[b]	62
Sn	130	102	-	-
Sb	25	19	3[c]	-
Cs	19	11	-	-
Ba	374	249	-	-
La	29	21	-	-
Ce	24	19	-	-
Hg	10	8	4[b]	2
Pb	155	80	135[b]	15

a: µg/g (dry weight)
d: standard deviation

Table 10: Analysis of biological reference material SINR 0920 Chinese hair: ng/g (dry weight) unless stated

Element	This study		Certified
	Mean	s.d.[b]	Mean
Li	184	256	-
B	1441	504	-
Mg[a]	85.5	7.3	75
Al[a]	9.4	1.8	13.3
P[a]	154	30	184
Ca[a]	875	49	790
Sc	6.2	9.2	-
V	106	94	-
Cr[a]	3.7	1.8	4.8
Fe[a]	45	11	-
Mn[a]	2.2	0.2	2.9
Ni[a]	2.6	0.8	3.2
Co	122	112	-
Cu[a]	20.1	1.6	23
Zn[a]	165	13	189
Ge	15	10	-
Rb	4.1	5.0	-
Sr[a]	4.0	0.3	4.19
Mo	2039	714	-
Cd	71	40	-
Sn	540	622	-
Sb	279	153	-
Cs	4.8	7.4	-
Ba[a]	4.5	0.5	5.4
La	16	9	-
Ce	29	8	-
Pb[a]	6.0	1.0	7.2

a: µg/g (dry weight)
b: standard deviation

2.4.2. Detection Limits

The term "detection limit" describes the "least value which can be distinguished from zero" (244) for a given element being measured by a particular analytical technique. It is calculated using the standard deviation (σ) of the blank sample multiplied by a specific factor, usually three. For each run procedure (i.e. group of samples) carried out by ICP-MS, detection limits were calculated for the entire range of elements, using 3σ of the reagent blank. The mean value of detection limit was calculated, and is shown in Table 11.

To investigate this theme further, a standard solution was repeatedly diluted so that a range of concentrations was obtained:-

0.16-16ng/mL: Li,B,Al,Sc,V,Cr,Mn,Co,Ge,As
Mo,Cd,Sn,Sb,Cs,Ba,La,Ce,Hg,Pb

0.4-4ng/mL: Fe,Ni,Cu,Zn,Rb,Sr

2-200ng/mL: Mg,P,Ca

These samples were analysed, by ICP-MS, in ascending and descending orders of concentration, separated by a sensitivity standard (40,100 or 500ng/mL). The results obtained by this are shown in Figure 11 a) to f), where measured concentration is plotted against nominal concentration. The data obtained is also displayed in Table 11, alongside the calculated detection limits. For some elements, including aluminium, phosphorus and molybdenum, only a part of the

Table 11: Detection limits for ICP-MS (ng/mL 3σ blank and standard dilution comparison)

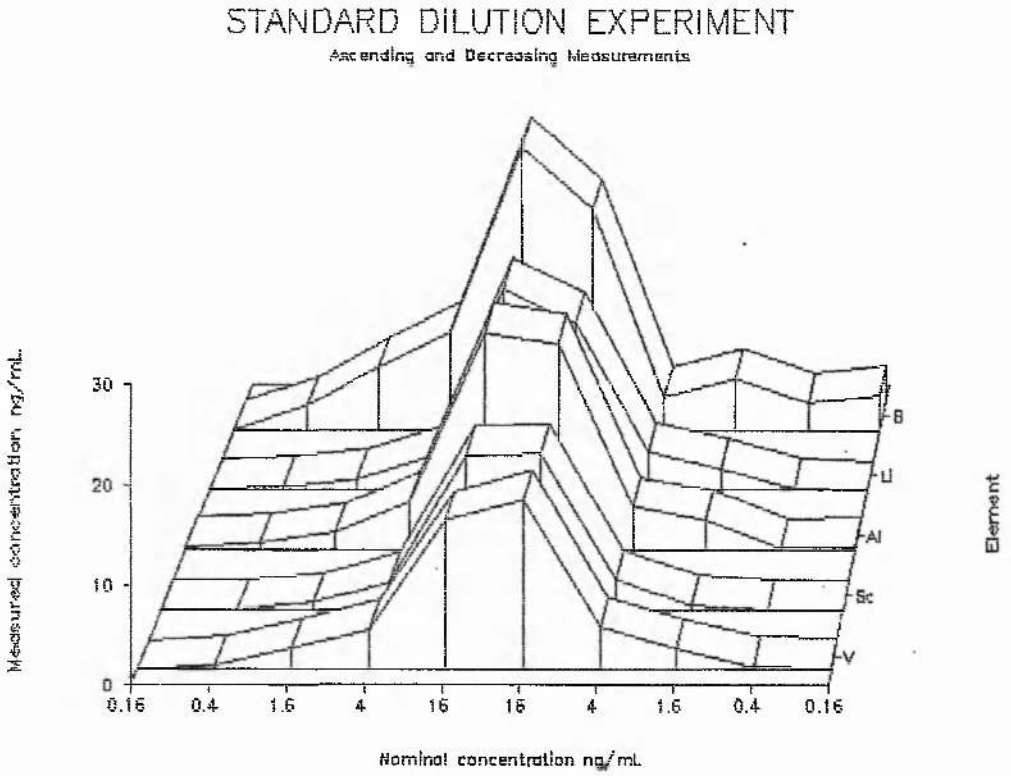
Element	Detection limit ng/mL	
	3σ blank[a]	Standard dilution[b]
Li	0.12	<0.2
B	2.6	<4
Mg	0.1	-
Al	0.12	<0.3
P	30	<30
Ca	15	<15
Sc	0.03	<0.2
V	0.29	<0.3
Cr	0.98	<1.5
Fe	0.03	-
Mn	0.03	-
Ni	0.32	-
Co	0.6	-
Cu	0.3	-
Zn	0.96	<1.0
Ge	0.22	<0.3
As	0.7	<0.5
Rb	0.13	-
Sr	0.11	-
Mo	0.3	<2.0
Cd	0.29	<0.3
Sn	0.18	<0.2
Sb	0.08	<0.4
Cs	0.09	-
Ba	0.3	<0.2
La	0.04	<0.2
Ce	0.03	<0.2
Hg	0.23	<1.0
Pb	0.01	-

a: σ is the average standard deviation of the reagent blanks

b: value below which subsequent standard dilutions could not be measured

Figure 11: Measurement of standard solutions by ICP-MS

a) B, Li, Al, Sc, V.



b) Cr, Mn, Co, Ge, As.

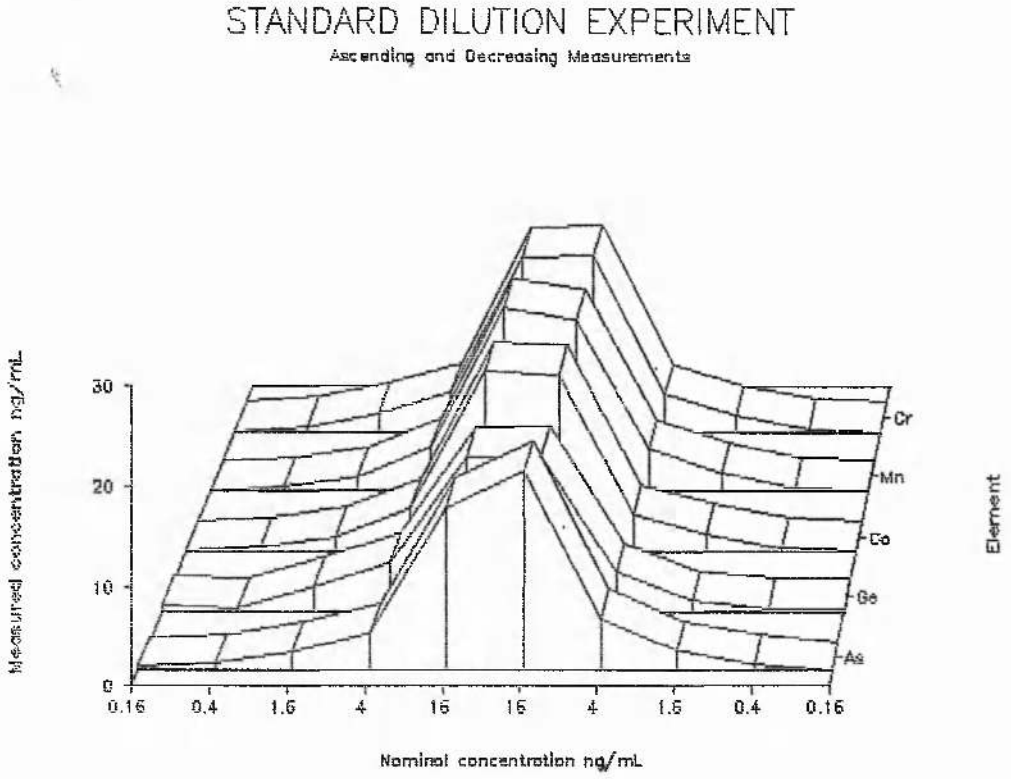
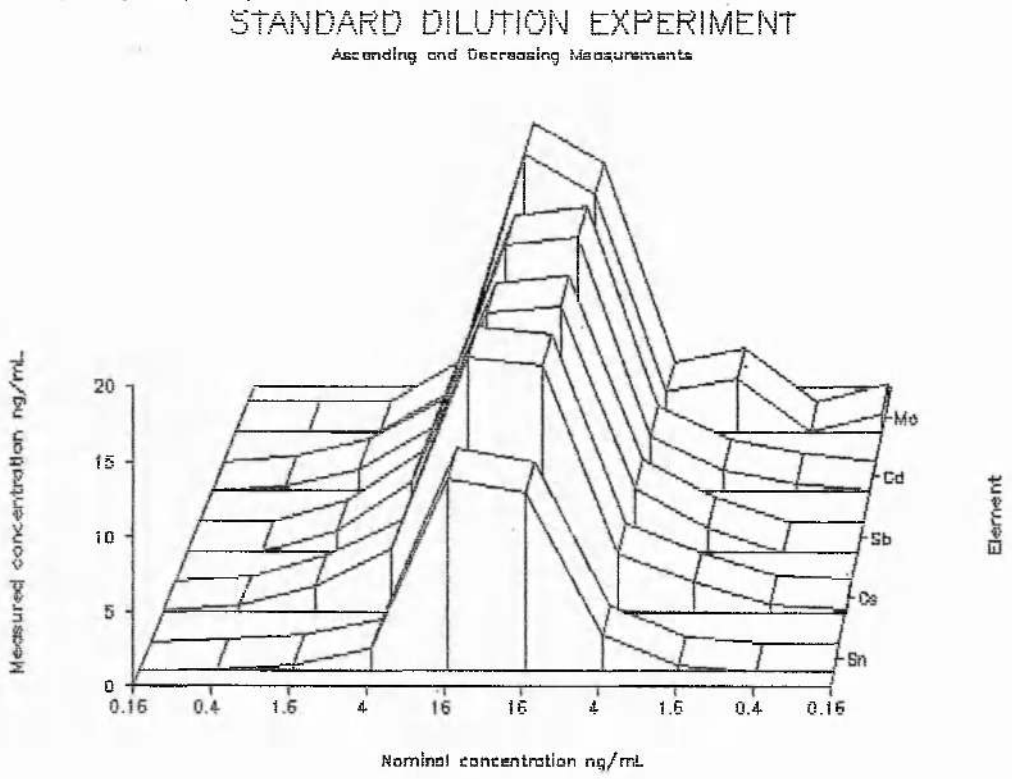


Figure 11 (con.): Measurement of standard solutions by ICP-MS

c) Mo, Cd, Sb, Cs, Sn



d) Ba, La, Ce, Hg, Pb.

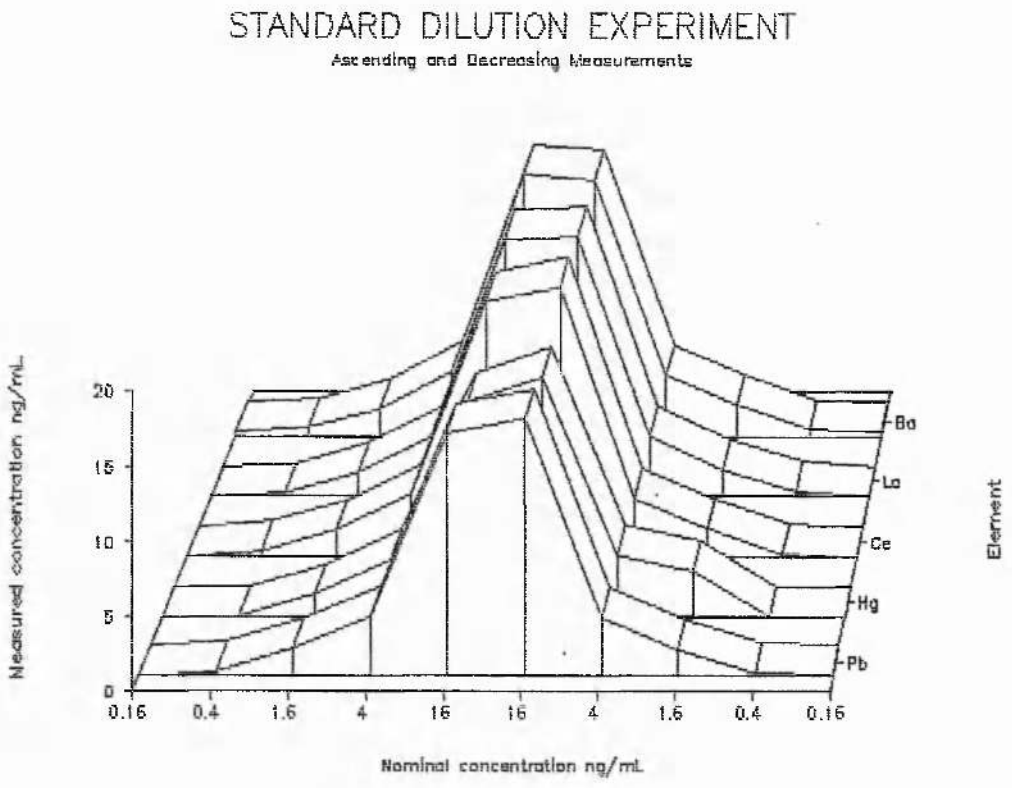
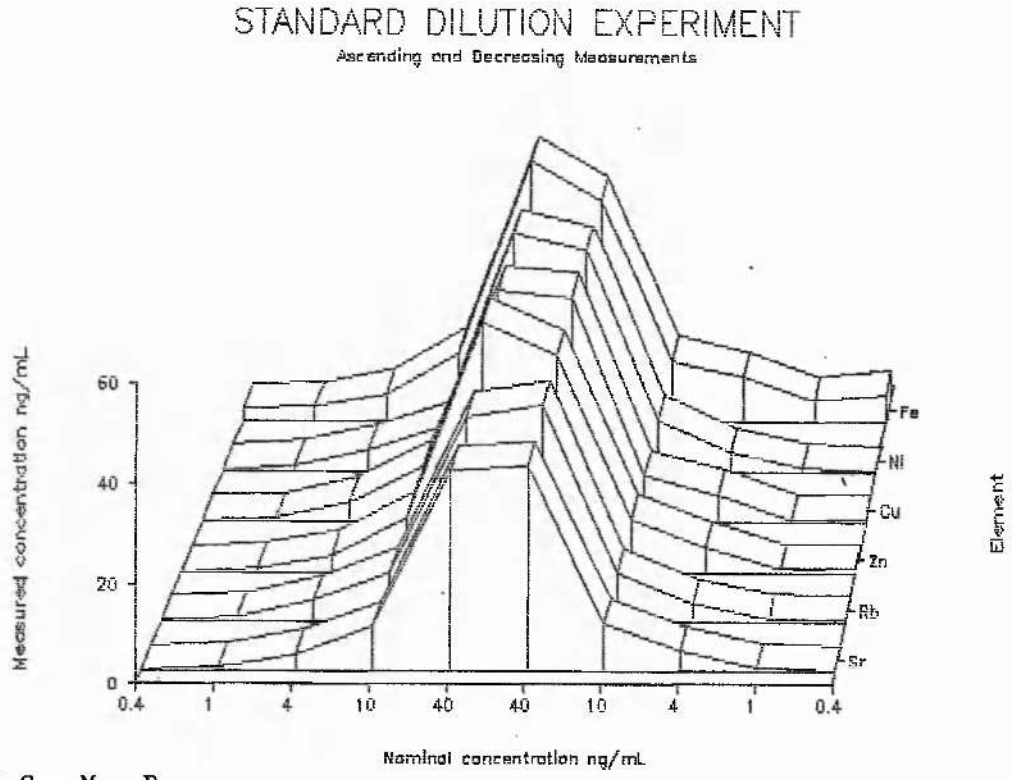
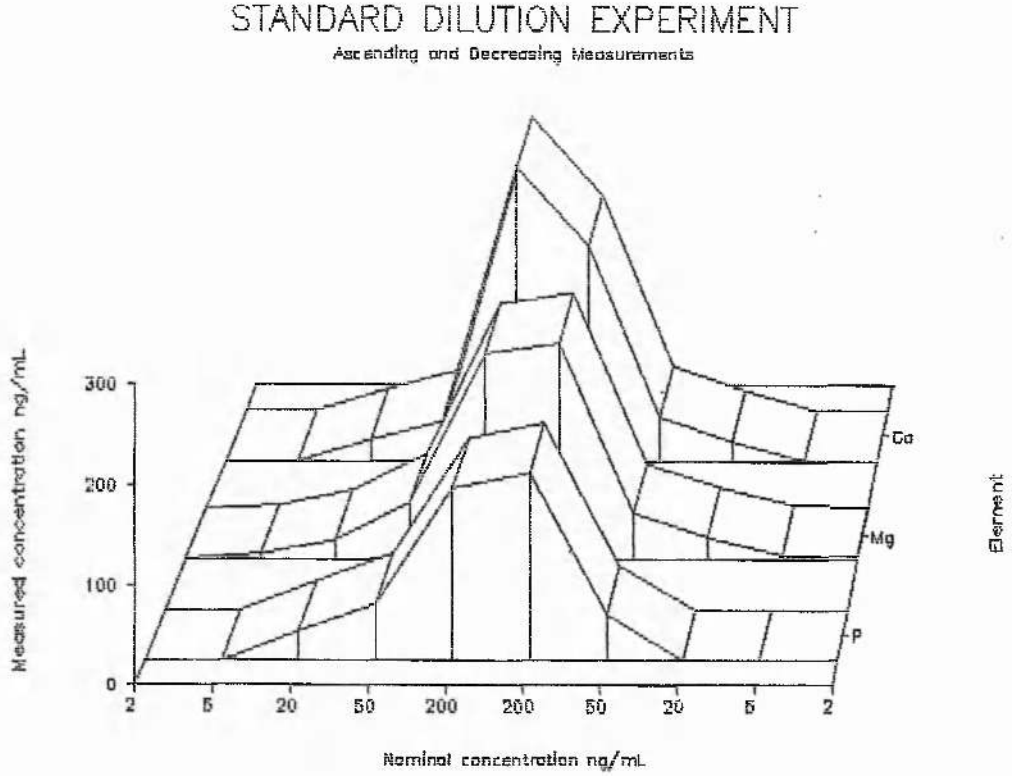


Figure 11 (con.): Measurement of standard solutions by ICP-MS

e) Fe, Ni, Cu, Zn, Rb, Sr



f) Ca, Mg, P



full range of concentrations can be distinguished from the blank. For others, notably boron and iron, erroneous values of concentration were obtained in the decreasing sample sequence, reflecting a slight increase in the background level of the element. This is probably due to memory effects caused by adhesion of elements to the plastic tubing and glassware of the spray chamber etc. Therefore, in Table 11, a figure is given for those elements, below which, if values are obtained, data must be treated with caution. In most cases, this figure is very close to the detection limit calculated using 3σ of the reagent blank.

2.4.3. Standard addition experiments

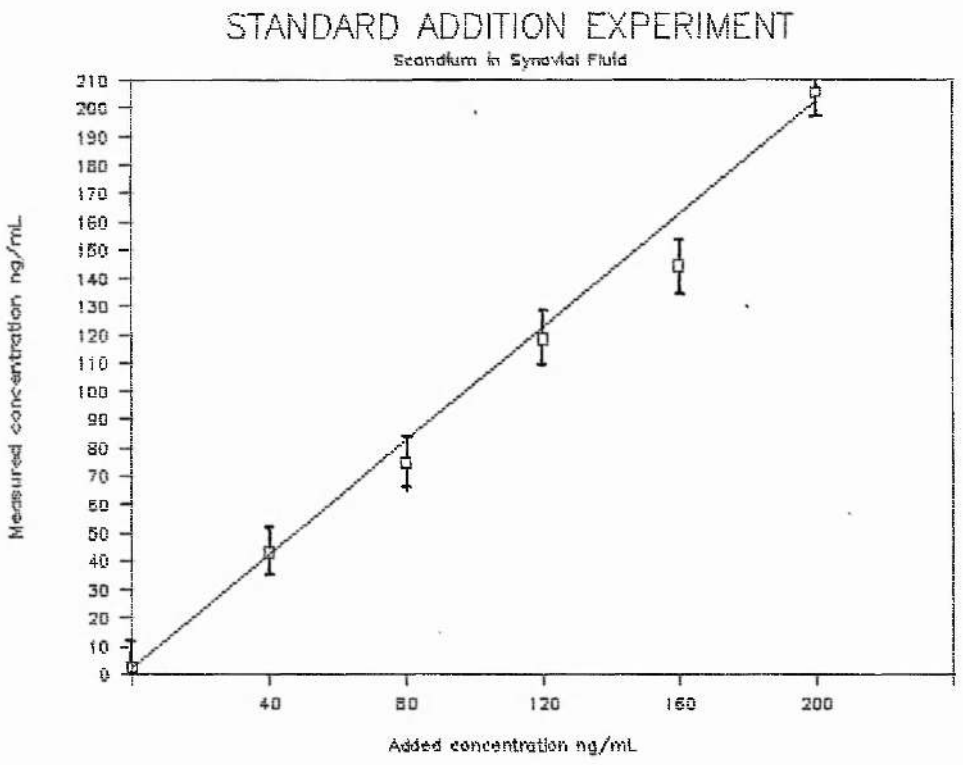
The suitability of the two main types of sample digestion used i.e. wet and dry ashing, was investigated. This was done by the method of matrix addition; that is, by adding various aliquots of multi-element solutions to a number of hair and synovial fluids before digestion. A large sample of scalp hair was chopped finely and well mixed, while a large-volume sample of synovial fluid was repeatedly inverted, in order to attain homogeneity as far as possible. These samples were then ashed, diluted and analysed by ICP-MS, along with other, more routine samples.

Figures 12 and 13 show the results obtained for lead, copper and phosphorus for both matrices, scandium in synovial fluid and tin in hair; in addition, the data for boron, magnesium and manganese are displayed in Section 3.2.1.4. Error bars are drawn on the graphs; these are based on the variations in elemental concentration between three, separately digested aliquots of hair or synovial fluid, without sample addition.

The elements shown in Figures 12 and 13 are representative of the remaining elements. In general, there is little deviation from the expected values of concentration, induced during the sample digestion procedures; for example, there seem to be no major systematic elemental losses or gains. It can be seen that the largest variations in data occur when the "addition" of standard solution is relatively close to the intrinsic concentration of that element. This is demonstrated by phosphorus, with additions of approximately 40% and 10% to synovial fluid and hair respectively, compared with additions of 1000% to scandium in synovial fluid and 100% to lead in hair. Therefore, the inter-sample variations of phosphorus are clearly evident but those of scandium and lead are completely hidden by the comparatively large standard addition.

Figure 12: Standard addition to synovial fluid

a) Scandium



b) Lead

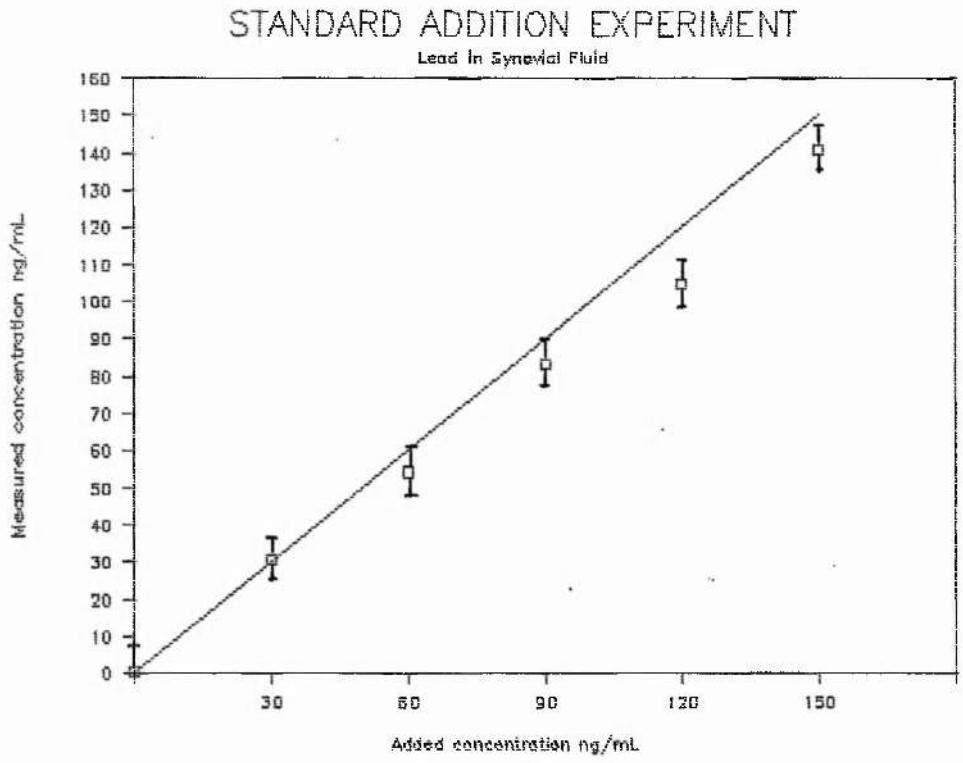
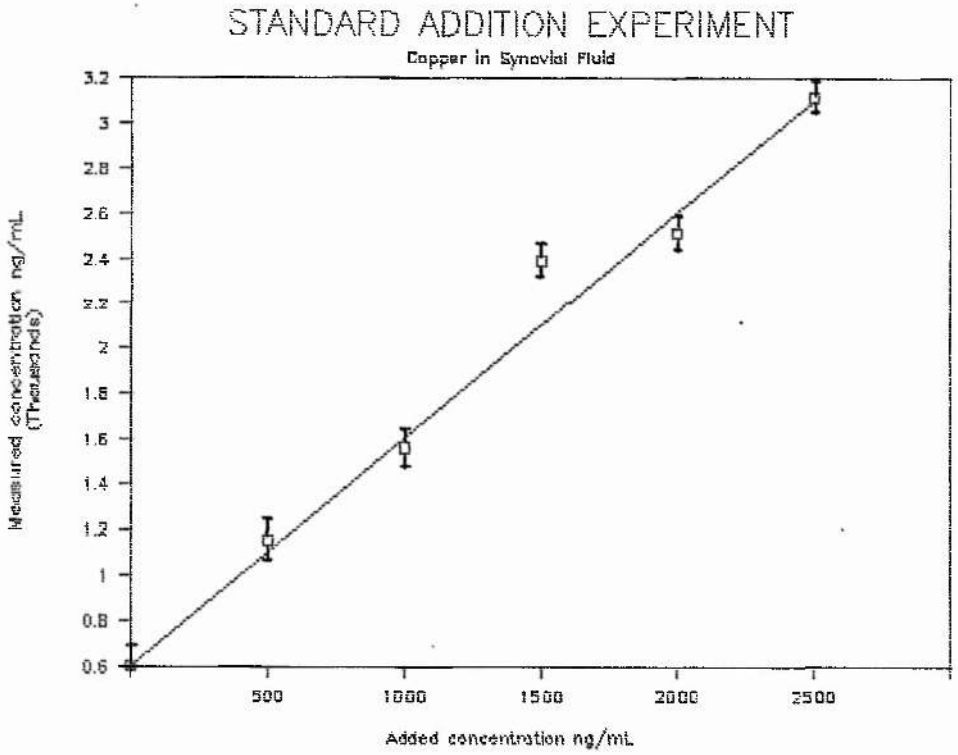


Figure 12 (con.): Standard addition to synovial fluid

c) Copper



d) Phosphorus

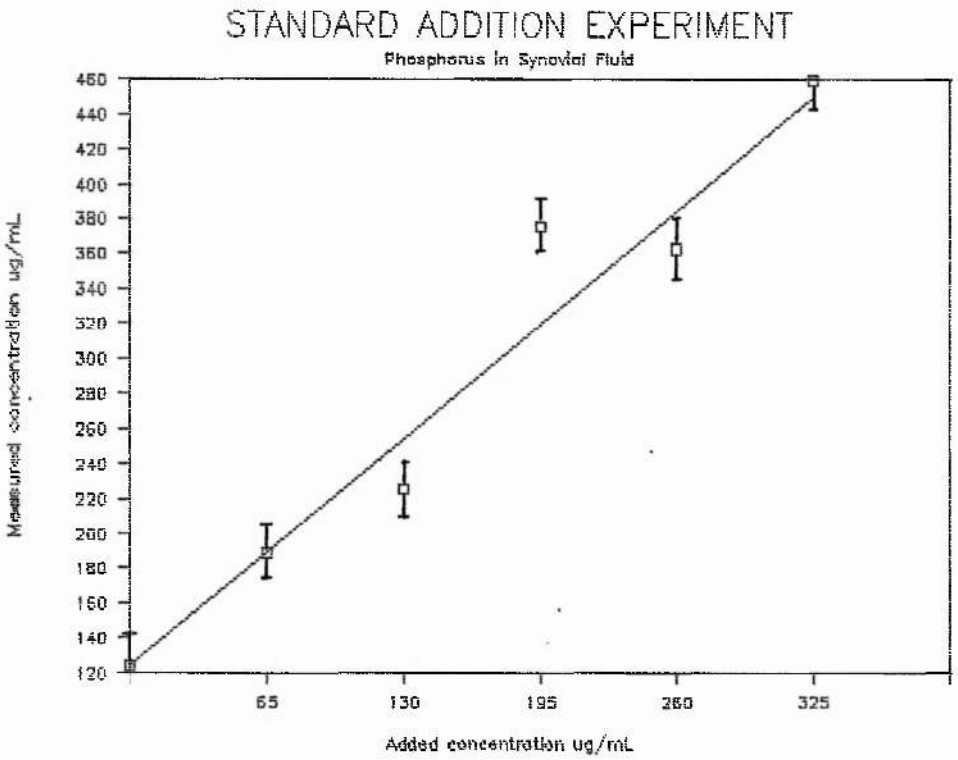
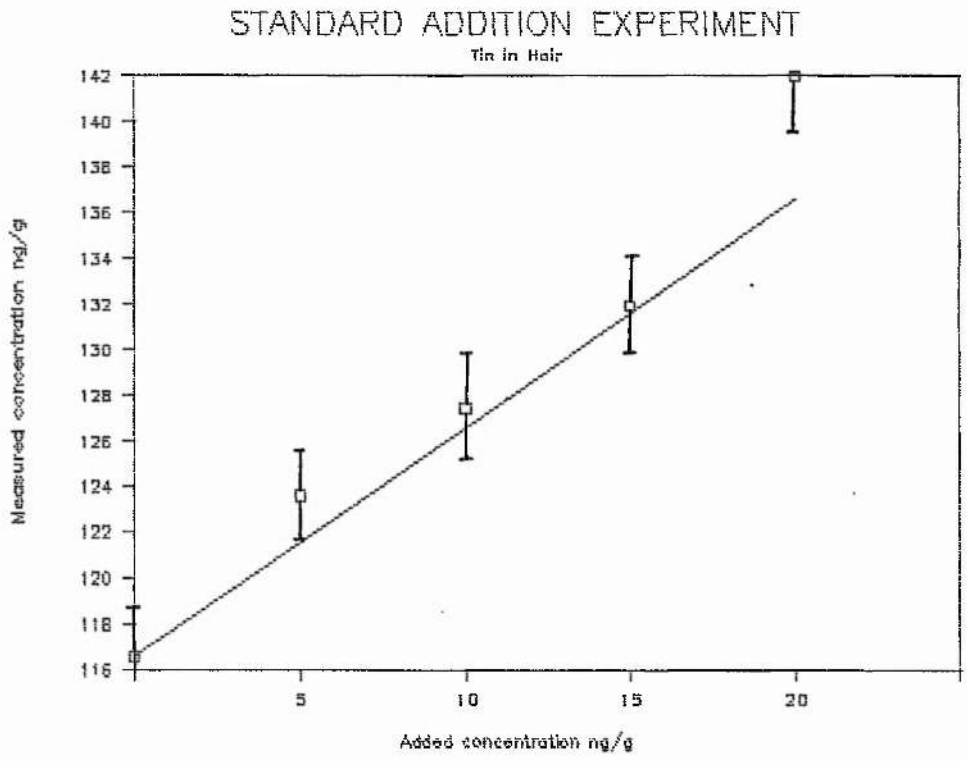


Figure 13: Standard addition to hair

a) Tin



b) Lead

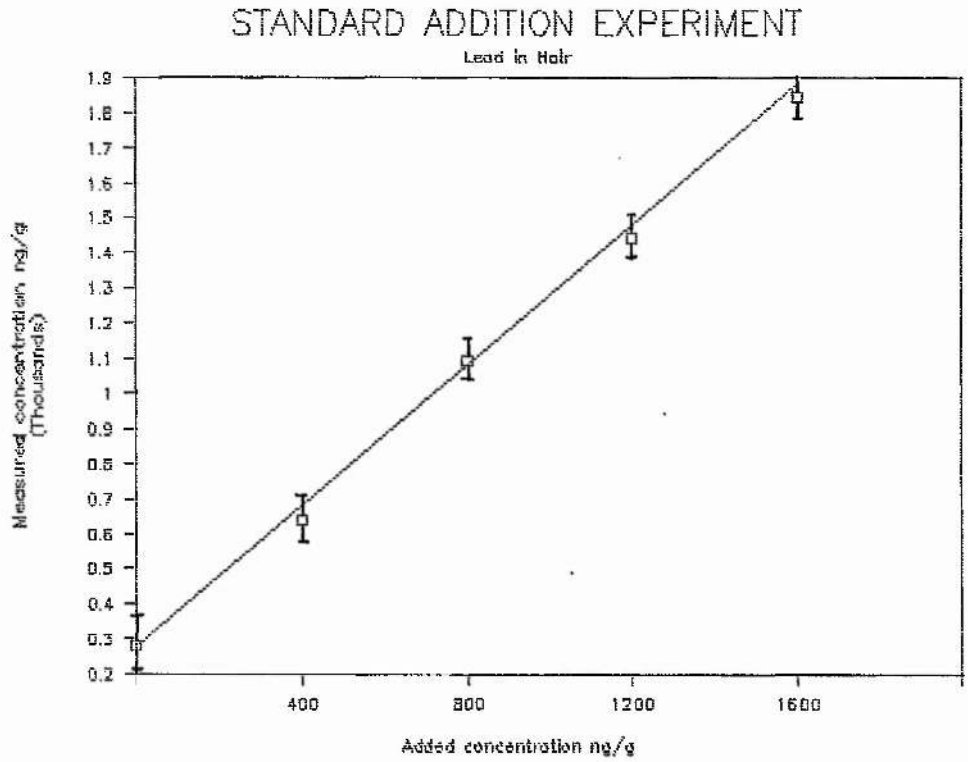
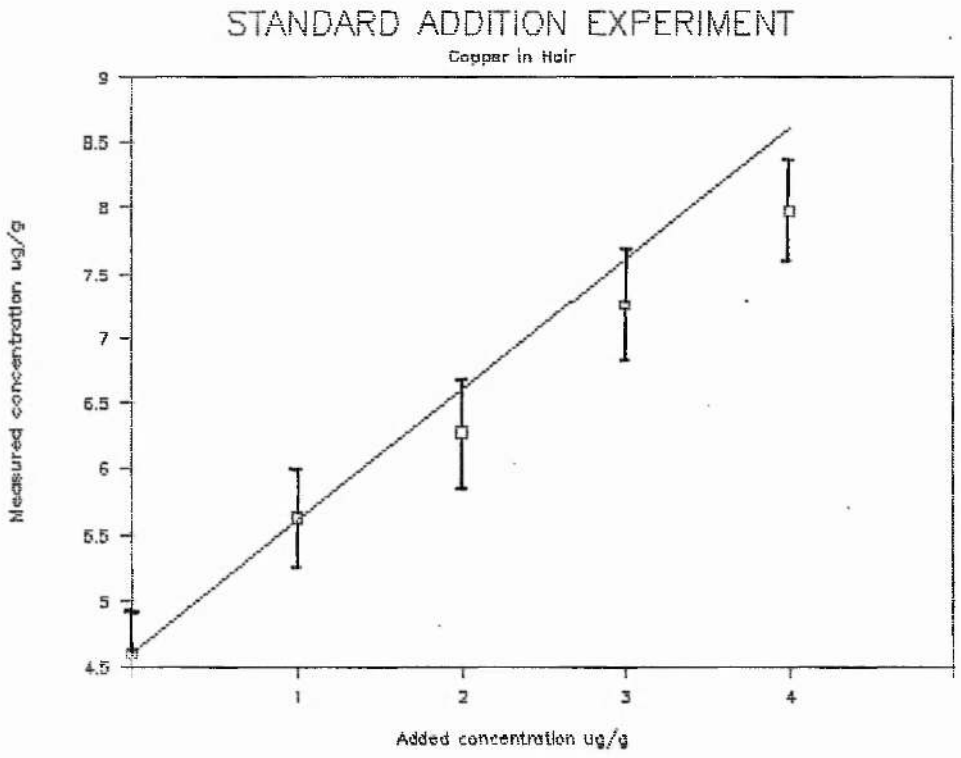
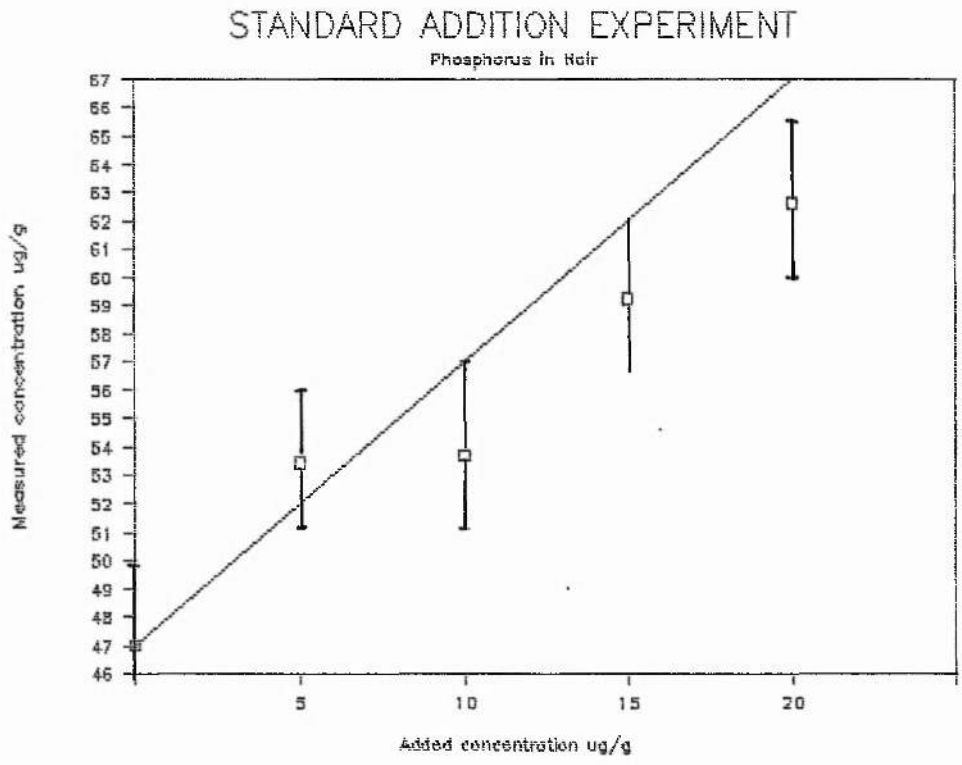


Figure 13 (con.): Standard addition to hair

c) Copper



d) Phosphorus



3. BORON

3.1. Introduction to Boron

3.1.1. Physical and chemical properties

Boron is in the second period of group IIIA in the Periodic Table, and can best be classified as a metalloid element. Natural boron consists of two isotopes; one of atomic mass 10, with an abundance of 18.9% and the other of atomic mass 11, with 79.1%. It is quite a rare element, being found at approximately 0.001% of the earth's crust (78). Much of it is localised in evaporated lake beds in south western parts of the USA in the form of borax ($Na_2B_4O_7.10H_2O$) and kernite ($Na_2B_4O_7.4H_2O$), hydrated sodium salts of tetraboric acid (79). Other naturally occuring minerals containing boron are colemanite ($Ca_2B_6O_{11}.5H_2O$) and boracite ($Mg_7Cl_2B_{16}O_{30}$) (80). These commercial deposits are thought to have resulted originally fron spring water in volcanic areas. As borates are soluble in water, no deposits now remain in regions which have rainfall and adequate drainage to the sea (81).

Boron is often found in soils as boric acid or as a borate. Boric acid is a Lewis acid i.e. an electron acceptor. It is a very weak acid, and also acts as a mildly acidic antiseptic.

Equation 3. $H_3BO_3 + H_2O \rightleftharpoons H_4BO_4^- + H^+$

The solid acid melts and loses water at very high temperatures, and gives a melt of boric oxide, which becomes a glass-like solid. Boric acid is used in fabrication of borosilicate glass, which shows considerable resistance to heat.

It is difficult to obtain elemental boron to any degree of purity. When boric acid is heated with magnesium, boron is seen as a dark brown or black powder. Purer samples can be obtained by passing a mixture of hydrogen and purified boron trichloride over a tungsten or tantalum wire at 1300-1400°C, when semi-crystalline boron is deposited on a wire (81). The structure of elemental boron seems to have certain unique characteristics among the elements. Its three valence electrons are too localised for it to be metallic, but insufficient in number to form a simple covalent structure. The three allotropic modifications, whose structures are known, contain groups of twelve boron atoms in an icosahedral array as the major structural unit, as shown in Figure 14. These units are strongly linked to each other to form very stable three-dimensional networks, of which the α-rhombohedral is the simplest (78).

Boron forms an interesting range of compounds; these include boranes (B_nH_{n+4} or B_nH_{n+6}; $n>1$), and carboranes ($B_mC_2H_{m+2}$), along with all four trihalides which are also Lewis acids. Boron and nitrogen form

some compounds that closely resemble the carbon analogues, because the B-N bond is isoelectronic with C-C; see Figure 15. If borazine is heated, it forms boron nitride which can exist in two modifications, somewhat similar to carbon i.e. a hexagonal form like graphite, and a cubic form, borazon, like diamond. Elemental boron, and boron carbide ($B_{12}C_3$) also approach diamond in their hardness (81).

Like aluminium and germanium, boron forms complexes with organic groups containing hydroxyl (OH) groups. This is the case, particularly when the hydroxyl groups are on adjacent carbon atoms and on the same side of the molecule (cis). Reactions increase in intensity as the number of adjacent hydroxyl groups increases. For example, the strength of reaction with the following alcohols is as follows: glycerol<erythritol<adonitol<arabitol<mannitol (82).Complexes may also be formed with compounds containing certain trans 1,2-diol groups, provided that the angle of the adjacent hydroxyl group relative to the carbon-carbon axis is suitable, for example, in several ring configurations (83). The ability of boric acid and the borate ion to react with the hydroxyl groups means that complexing may take place with a whole range of biologically important compounds, for example, polysaccharides and sugars, adenosine-5-phosphate, pyridoxine, riboflavin and others (82,84). These complexes are thought to be of the form I to III, as shown in Figure 16. Phenylboric acid (IV) also complexes with diols, and has been known to substitute for boric acid

Figure 14: Icosahedral array of boron

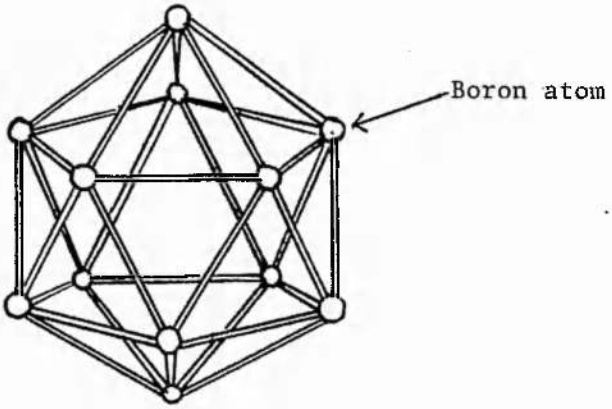
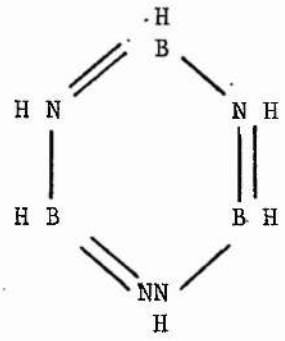
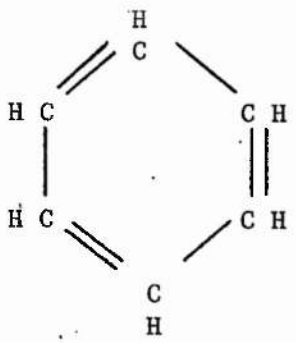


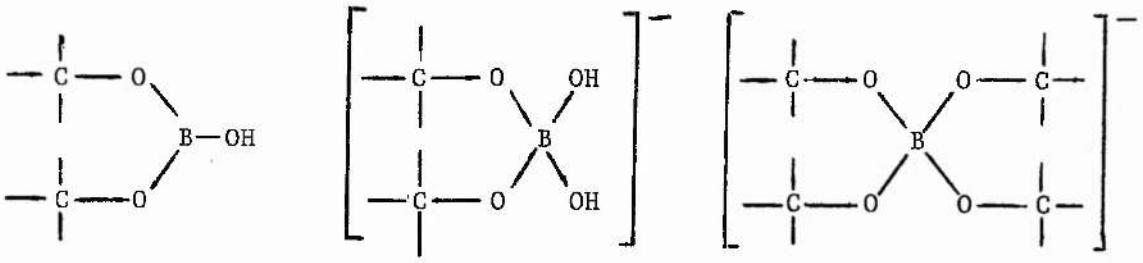
Figure 15: Boron-nitrogen and carbon analogues



Borazine $B_3N_3H_6$



Benzene C_6H_6



I. II. III.

IV. V.

Figure 16: Complexes of boron

in a plant (the diatom Cylindrothea fusformis), whereas derivatives such as tetraphenyl borate (V) do not substitute (85). The structure of the only biologically synthesized metabolites known to contain boron show the use of the structure III. These are aplasmomycin and boromycin, both antibiotics, isolated from Streptomyces griseus and Streptomyces antibioticus respectively (86,87).

3.1.2. Distribution and availability of boron

Apart from a number of commercial deposits, boron is found at low levels in most plant and animal life, soils, rocks and water. It has been known that boron is essential for higher plants for the last seventy years (88), although there has been much debate over the precise role of the element. The question of boron essentiality in animals and humans is still open. In this light, it is important to know the distribution of boron in soil and plant-life and its availability in food and from other sources.

The range of boron concentrations in plants required for "normal" growth (between deficiency and toxicity) is quite narrow. Levels of boron within plants or crops are highly dependent on soil type; deficiency is more common in low acid igneous rocks, as shown in Table 12. Here, borate is easily lost in leaching, and lime can induce a deficiency, which is normally seen as abnormal or retarded growth with brittle leaves or stem (89). There is also some seasonal behaviour; this is probably an impairment in boron mobility in the soil medium during dry weather. Crops particularly sensitive to boron deficiencies are sugar beet and celery, whereas apples, pears and grapes are comparatively insensitive. Boron toxicity is more usually associated with marine sediments, arid or semi-arid regions. It is shown by yellowing of the leaf tip and progressive necrosis, until

Table 12: Boron concentrations in rock, soil and plant-life (89)

Substance	Concentration µg/g
Igneous rock (granite)	15
Sedimentary rock (shale)	200
Soil	7-80
Plant ash	30-300
Trees/shrubs ash	50-500

finally the plant takes on a scorched appearance and dies. Grapes and peaches are very sensitive to high boron levels, while barley, maize, potato and tobacco are slightly more resistant.

Figure 17 shows how plants and animals can obtain boron. Its levels in natural water are normally very low (90). The soil quality, therefore, is the major consideration here. Enthusiastic use of NPK fertilisers has resulted in low availability from the soil (91). While attempting to rectify this deficiency, farmers have encountered some toxicity problems (92).

In the main, animals and humans obtain boron from foodstuffs, although it may be taken in other forms, for example, eyewash containing boric

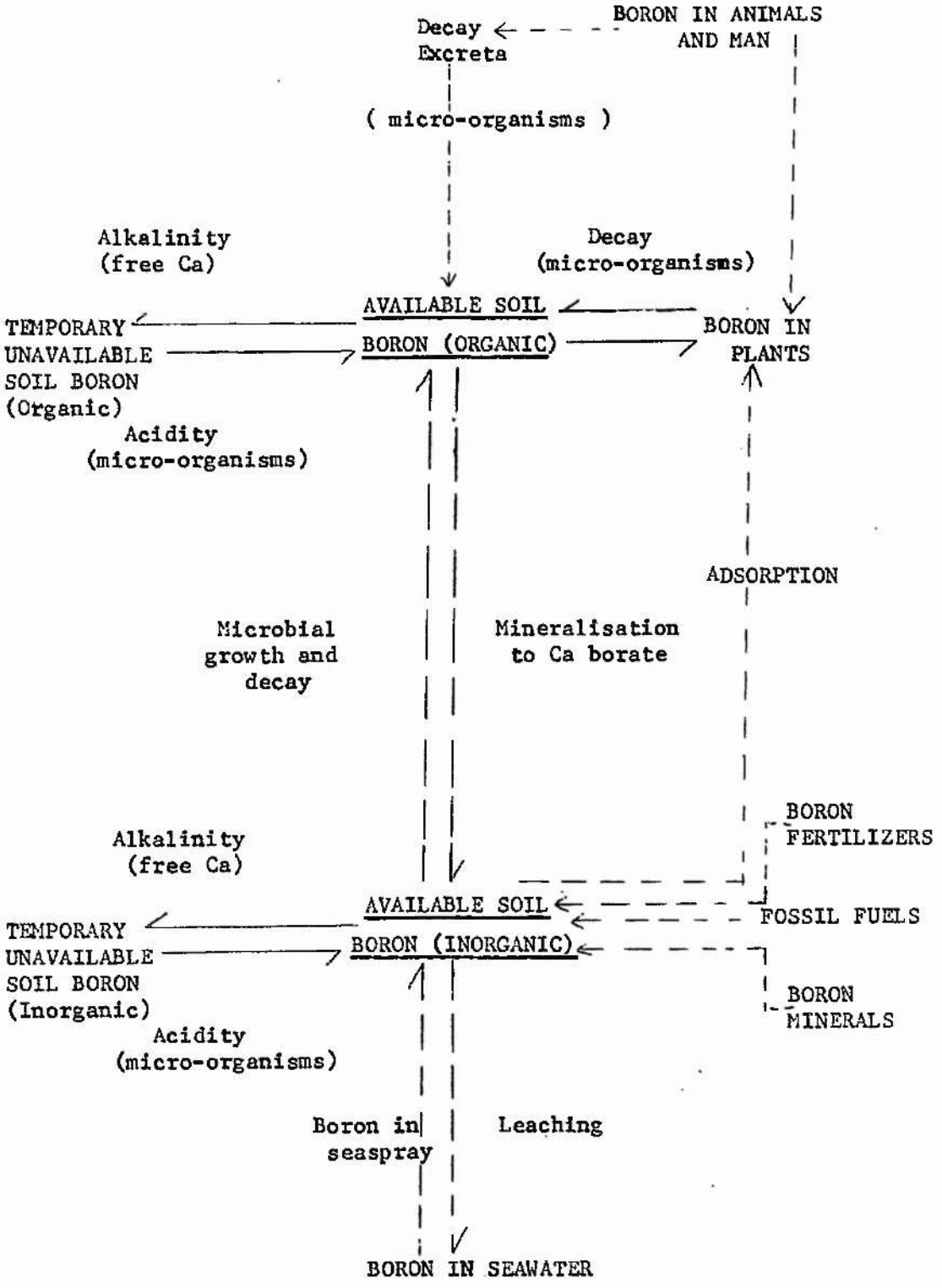


Figure 17: The boron cycle (243)

acid, borax in shampoo and bathsalts, and also in soaps and detergents (81). In general, plant foods such as vegetables and fruit are good sources of the element in the diet, while animal and fish products have a lower boron level. Boron is particularly high in nuts, dried fruit and honey (93). There is considerable variation between produce from tropical and sub-tropical regions (94). Recent studies of Finnish food and some English foodstuffs are shown in Tables 13 and 14. Boron concentrations are quoted in different units (dry compared to fresh weight) and so direct comparison is difficult. It is thought, however, that cooking or processing of vegetables and fruits leads to a considerable loss of mineral content (95). The Finnish study gave a high dietary intake of boron as 1.7mg/day (96). This compares quite well with 3.1 mg/day in US diets (97), and 2.8±1.5 mg/day for British diets (98).

The boron in food and any supplementation in the form of sodium borate or boric acid is quickly and almost completely absorbed and excreted. this latter process takes place mainly in the urine (99-101). There have been some cases of toxicity from boron; symptoms of poisoning include anorexia, nausea and vomiting, cardiac weakness, convulsions, diarrhoea and a skin eruption (80). Most examples of toxic effects have been after treatment of large burns with boric acid (102,103), and it seems that boron has a low order of toxicity when given orally.

Table 13: Average boron content (μg/g dry weight) in Finnish food groups (96)

Food group	Boron μg/g (d.w.)
Cereals	0.92
Meats	0.16
Fish	0.36
Dairy products	1.1
Vegetable foods	13
Other	2.6

Table 14: Boron concentration in English foodstuffs (104) (μg/g solids, μg/mL liquids fresh weights)

Foodstuffs	Boron μg/g or mL (f.w.)
Breakfast cereals	1.65
Bread	0.55
Cereals	1.55
Whole milk	0.03
Cheese, cheddar	0.27
Eggs, raw	0.07
Beef, roast	0.17
Chicken	1.06
Vegetables, cooked	1.44
Fruit, fresh	2.61
Fruit, tinned	0.42
Coffee, powdered	10.6
Sugar, white	0.04

3.1.3. Role of boron in plants

One of the reasons for the confusion about the exact role of boron in plants is that even within a particular "family" of plants, a wide range of symptoms are shown when they are deficient in boron. These signs include inhibition of root growth, breakdown of root and shoot apical meristems and malfunction of the reproductive systems. Over a longer time period, uncontrolled cell expansion takes place, producing giant, thin-walled cells which collapse. These regions become necrotic, with a resulting black or brown slime (105). These symptoms are probably secondary effects of a boron deficiency, rather than the primary action, which explains their divergence, along with the long time-scale over which these symptoms can arise.

Researchers in this field, therefore, have attempted to assess boron's role during the onset of deficiency, to try to identify the mechanism of the primary action (106). In the last thirty years, experimentation has been carried out into a number of plant biochemical systems, considering the propensity of boron to form compounds containing cis-hydroxyl groups. In attempting to connect these disparate areas of work, Parr and Loughman (106) have suggested that impaired membrane function, caused by lack of boron, may be that link.

Some electrochemical experiments have given credence to the view that boron is involved in membrane transport; the presence of borate caused an alteration in the selective permeability of the plasmalemma (membrane at junction of cell wall and cytoplasm) to sodium and potassium ions relative to chlorine ions, without changing the cation distribution (107). Tanada found that the boron of mung bean seedlings was localised in the membranes (108). A reduction in capacity for ion transport (for example, phosphate uptake) of 25% has been seen just five hours after boron was removed from the culture solution; a similar 20% drop in rubidium uptake was also shown, while the anion uptake seemed unaffected (109). These results, so soon after the onset of deficiency, are important because they imply that the primary role of boron may be involved in the maintenance of membrane integrity (106).

Other important findings involve interactions with auxins (growth hormones) and phenols, both of which are known to affect membrane transport, usually by inhibition of absorption at the root (110). There is, however, no evidence that the phenol levels, for example, alter sufficiently during boron deficency to initiate the changes in membrane function. It has been suggested that the central role for boron is to act as a control on the levels of these two types of compound (111), but it is possible that boron has a more direct part to play in membrane function than this (106). Some recent NMR work

(105) measured ^{31}P in control roots and boron deficient roots and showed that the distribution of inorganic phosphate between cytoplasm and vacuole had been altered. This suggested that a deficiency of boron had in some way affected the transport capacity of the tonoplast (the membrane at the junction of vacuole and cytoplasm). It was also clear that the growth of boron deficient roots was impaired.

To summarise, it appears that the early effects of removal of boron from the root environment of many species are found to be directed at a membrane site. The physical symptoms which arise later are probably as a result of interference with processes which depend on the close control of metabolites moving between and within cells (106).

3.1.4. Boron in animals and humans

Following the early discovery of the essentiality of boron to plants in 1923 (88), several attempts were made during 1939-44 to induce a boron deficiency in rats. These were unsuccessful, despite low dietary levels of 155-163 ng/g. In 1945, one study reported that potassium deficient rats, fed a boron supplement, showed enhanced survival, maintenance of body fat and elevated liver glycogen levels. A further report was unable to confirm these findings, when rats were given a different diet containing an unknown amount of boron, and various

levels of boron as supplements (112). After these disappointing results, the assumption was made that boron had no role to play in animal metabolism, and no further experimentation was carried out until the early 1980's. In 1981, Hunt and Nielsen (113) reported that boron might be essential for chicks; deprivation of this element depressed growth and elevated plasma alkaline phosphatase activity in chicks fed adequate cholecalciferol (a component of vitamin D, necessary for calcium and phosphorus activity and linked with the parathyroid). Further experiments confirmed that cholecalciferol deficiency indirectly enhanced the body's need for boron; hence the possibility of an involvement of boron in the action of the parathyroid hormone, and thus in the metabolism of calcium, potassium and magnesium (114). More work in this field (115-8) has indicated that rats respond to changes in dietary boron in a way that is dependent on levels of dietary methionine (an essential amino acid), potassium, magnesium, cholecalciferol, aluminium and calcium.

Other workers have independently carried out animal studies linking boron and parathyroid activity. Elsair et al. (119,120) found that high levels of boron in the diet partially alleviated the secondary hyperparathyroidism symptoms of hypercalcemia, hypophosphatemia and depressed renal absorption of phosphorus, induced by fluoride given to rabbits. Seffner and co-workers (121,122) noted a reduction in the

fluoride-induced thickening of the cortices of the long bones, and an alteration in the histology of the parathyroid gland when borate was administered to pigs. Moreover, Baer et al. (123) reported findings in which boron corrected radiographic and histologic changes caused by fluoride toxicity in bone.

Neilsen considered that the animal studies carried out by himself and co-workers indicated that when diet was manipulated in some way, leading, in particular, to a possible alteration in cellular membrane integrity (potassium or magnesium deficiencies) or in hormone responsiveness (magnesium or cholecalciferol deficiencies, aluminium toxicity), many responses were shown by the animals to a change in dietary boron (124). This was not seen to the same extent when animals were fed an apparently "optimal" diet i.e. no known nutritional deficiences. This suggested that the need for boron was not crucial under normal conditions, but became enhanced under metabolic or nutritional stress (83).

These findings have led to a number of human studies, again by Nielsen and co-workers, directed at groups of people in situations of the previously described stress. Research was carried out, for example, under conditions of low magnesium intake or when hormonal changes were occuring, for example, during the menopausal period with the resultant increased loss of calcium from bone. In one such study (125), twelve

post-menopausal women consumed a conventional diet (with boron of 2.5 mg/day) for 119 days, and were given a supplement of 3 mg boron/day for the following 48 days. Boron supplementation reduced calcium excretion in urine, and increased the serum concentration of ionised calcium and estradiol-17β; all these changes were most noticeable when the dietary magnesium was low.

Another recent project by the same group involved four men (aged 45 years and above), nine postmenopausal women, of which five were on estrogen therapy and four were not, and one premenopausal woman (93). Again, a period of boron supplementation followed one of a "conventional" diet; the latter contained 0.23 mg boron/2000kcal and was also low in magnesium. Several indicators of calcium status showed a variation between depletion and repletion regimes; in particular, plasma ionised calcium and serum 2,5-hydroxycholecalciferol were higher, while serum osteocalcin, calcitonin and glucose were lower during boron supplementation. Moreover, after this supplementation, the values in the groups not receiving estrogen therapy moved closer to those that were. This suggested that boron has a role in calcium metabolism, and thus in the maintenance of healthy bones; also, this role became more evident when calcium loss from the bone was likely. Other experiments have shown that dietary boron also affects copper metabolism in humans (126), and that under conditions of low dietary

magnesium, boron can influence the brain function of healthy adult men and women, measured by elecroencephalogram (EEG) (127).

How boron is acting in the body is still unknown. However, the knowledge that it has the ability to complex with organic compounds containing hydroxyl groups is significant. Boron deficiency has been shown to exacerbate symptoms of cholecalciferol deprivation, including abnormal bone function and poor growth, and it has been suggested that boron may be required for a hydroxylation step to create the active species of cholecalciferol. Moreover, the formation of estradiol-17β from precursors involves the creation of hydroxyl groups on the steroid structure. If boron is involved in the hydroxylation of biological substances in the body, it may have a role to play in numerous other disorders, for example, rheumatoid arthritis (RA). Corticosteroids, which are frequently used to alleviate symptoms of RA, involve the formation of hydroxyl groups during synthesis (124). This is particularly interesting since claims have been made that boron supplementation can play an important therapeutic role in the treatment of RA (91).

In addition to the investigations of the role of boron in animals and humans, a limited number of studies have been carried out to identify the levels of boron within various tissues and fluids. A recent example is the analysis of bone and teeth in rheumatoid arthritic and

control subjects using prompt gamma ray activation analysis (128), in which there appeared to be a reduced level of bone boron in arthritic subjects. Table 15 gives a summary of the work carried out on boron in animals and humans.

3.1.5. Analysis of boron

Analysis of boron in the past has involved the use of various techniques, including spectrochemistry (235), colorimetry (236) and atomic absorption spectrometry (237-40). The relative difficulty in these analytical processes, in both sample preparation and measurement, has resulted in very few attempts to investigate boron and a possible role in animal and human health (128).

In recent years a new neutron irradiation/gamma-ray spectroscopic procedure has been devised which is a significant advance on the previously available methods (128). In addition to this, ICP-MS, with both solution and solid sample introduction, seems to provide an opportunity to extend still further the knowledge of boron in biological systems. The postulated detection limit of boron in solution ICP-MS, calculated from "ideal" solutions indicates that this technique would be suitable for the elemental levels expected in most biological samples. This value, and those from some other techniques, are given in Table 16. Of course, measurement of "real" samples

Table 15: Summary of research on boron in animals and humans

Year	Research work
1923	Essentiality in plants (88)
1939-44	Unsuccessful attempts to induce B-deficiency in rats (112)
1945	Unconfirmed reports of enhanced survival of K-deficient rats when given B-supplementation (112)
1953	Values of B in hair and nails of 5 and 33µg/g, measured by AES[a] (129)
1963	Values of B in blood and urine measured by AES/FES[a]: 104 and 919 ng/mL respectively (130)
1980	Boron reported as an antidote to acute fluoride intoxication in rabbits: reference to F and Ca/P metabolism (119)
1981	B first suggested as a cure for arthritis (91)
1981	Suggested interaction between B and cholecalciferol in chicks; possible role in Ca and P metabolism (113)
1983	B as an antidote in experimental fluorosis in pigs (121)
1985	Interactions suggested among dietary Al, B, Mg, and methionine in the rat (115)
1986	Effects of B, Al and Mg on major mineral metabolism were studied in post-menopausal women were studied; reduction in urine excretion of Ca and Mg (124)
1987	B in bone of RA subjects found to be low (128)
1989	Dietary B found to affect Cu metabolism (126)

a: AES/FES arc/flame emission spectrometry

Table 16. Detection limits of boron ng/mL (3σ blank)

Technique	ICP-MS[a] (244)	FAAS[b] (244)	LA-ICP-MS[c] (41)	PG-NAA[d] (128)
Detection limit	0.1	700	3000	50

a: solution inductively coupled plasma source mass spectrometry
b: flame atomic absorption spectroscopy
c: laser ablation ICP-MS
d: prompt gamma ray neutron activation analysis

generally results in a less than "ideal" analytical situation, with an accompanying increase in detection limits.

3.2. Results and Discussion

Standard reference materials and a range of human tissues and fluids were prepared and analysed by ICP-MS (as outlined in Chapter 2) to provide information on their elemental levels of boron, and the accuracy and precision with which they can be measured. The results are presented in the following section.

3.2.1. Analytical quality control

3.2.1.1.Standard reference materials

A range of standard reference materials was analysed for the element boron; details of sample preparation are given in Chapter 2. The results are presented, in Table 17, as the mean boron concentration (ng/mL or ng/g), with its associated standard deviation. Certified or literature values are presented for comparison, where available. NIST 1577A bovine liver shows quite good agreement with the literature data, although the precision of the data is less good. The boron levels of the remaining standard reference materials are presented for future comparison and confirmation.

3.2.1.2. Detection limits

The detection limit for boron was calculated from 3σ (where σ denotes standard deviation) of the blank value. After each run procedure, detection limits were evaluated using the reagent blank. From this data, a mean value of 2.6 ± 0.8 ng/mL was calculated. This, however, does not take into account the varying dilution factors of the different biological samples under analysis. Therefore, when samples are found to fall below this level they are quoted in tables or

Table 17: Concentration of boron in standard reference materials

Standard Reference Material	Boron concentration Mean ± s.d.	Certified[a]/ Information[b] data
IAEA SRM909[c] Human serum	1110 ± 738	
NIST SRM 1577A[d] Bovine liver	571 ± 257	590 ± 80[b]
IAEA A13[d] Animal blood	1650 ± 1450	
IAEA H4[d] Animal muscle	500 ± 170	
SINR 0920[d] Chinese hair	1370 ± 530	

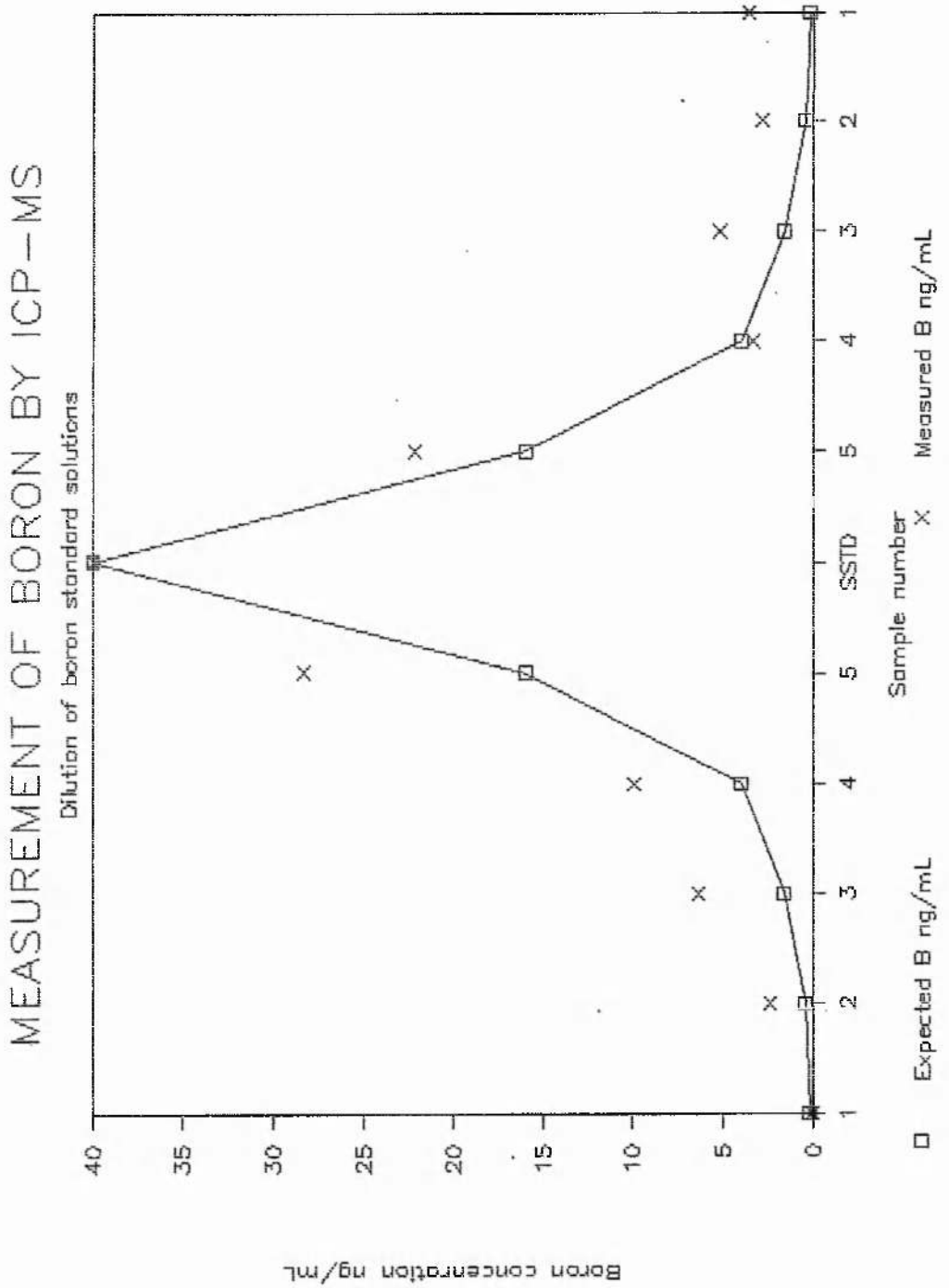
c: ng/mL
d: ng/g dry weight

figures as being "<X ng/mL or ng/g", where X is the product of the dilution factor and the detection limit of 2.6ng/mL.

3.2.1.3. Standard dilution experiment

A standard solution containing boron was repeatedly diluted until the boron was at levels close to the calculated detection limit i.e. at concentrations of 0.16, 0.4, 1.6, 4 and 16 ng/mL (see section 2.4.2.). These samples were then analysed, by ICP-MS, in both ascending and descending order of concentration, separated by a sensitivity standard at 40 ng/mL; the results are shown in Figure 18. It is interesting to note that while the increasing steps of concentration are well-defined, the downward trend is rather less clear. This is probably due to a slight increase in the background levels of boron following the measurement of the higher concentration samples. Boron seems to cling to the plastic of the nebuliser tubing and to linger in the spray chamber even after extensive washing. At levels of below approximately 4 ng/mL, according to this experiment, it becomes difficult to distinguish between samples. This tallies quite closely with the detection limit calculated from 3σ of the blank, given in section 3.2.1.2..

Figure 18: Measurement of boron standard solution by ICP-MS



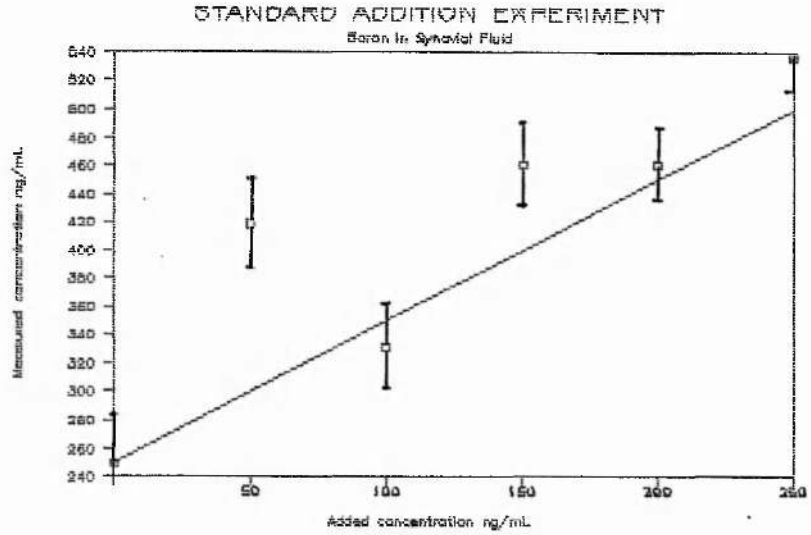
3.2.1.4. Standard addition experiment

The detection limit is basically an instrumental factor; it is also important to consider whether boron levels will be affected in any way by pre-analytical sample preparation. To study this in more detail, matrix addition was carried out; various aliquots of multielement standard solution (including boron) were added to hair and synovial fluid before digestion, as described in section 2.4.3.. Scalp hair was a representative of the dry-ashing technique, and synovial fluid of the wet-ashing method. The samples were then digested, along with other more routine samples, and measured by ICP-MS.

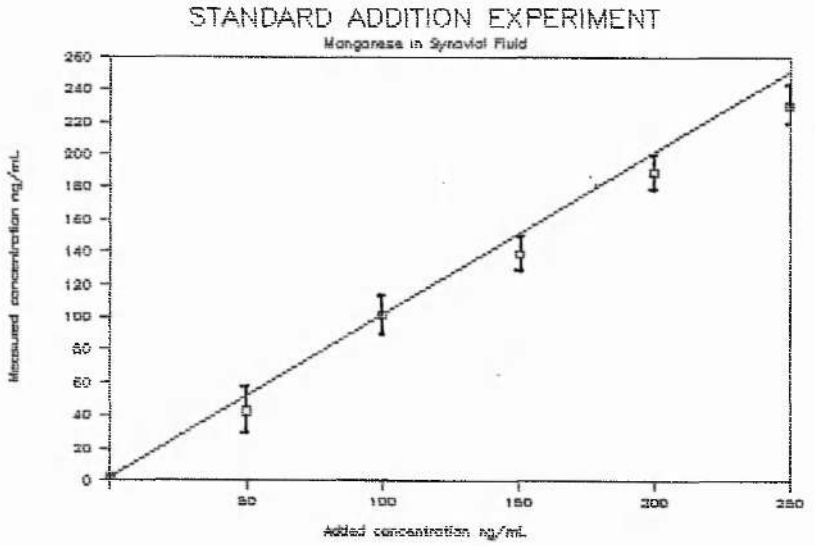
The results for boron are shown in Figures 19 and 20, with those for magnesium and manganese presented as comparisons. Error bars shown on the graphs are based on variations in elemental concentration between three, separately digested aliquots of hair or synovial fluid, without additions. At first glance, it appears from these graphs that analysis of boron is subject to larger experimental errors than the other elements. However, the "additions" of boron to synovial fluid were approximately 20% of the intrinsic boron concentration, compared to 200% for magnesium and 4000% for manganese. It is easy to see, therefore, that small inter-sample variations are masked by the large "additions" for both magnesium and manganese, while they remain visible for boron. Similarly, "additions" of boron to hair were

Figure 19: Standard addition of boron to synovial fluid, with comparisons

a) Boron



b) Manganese



c) Magnesium

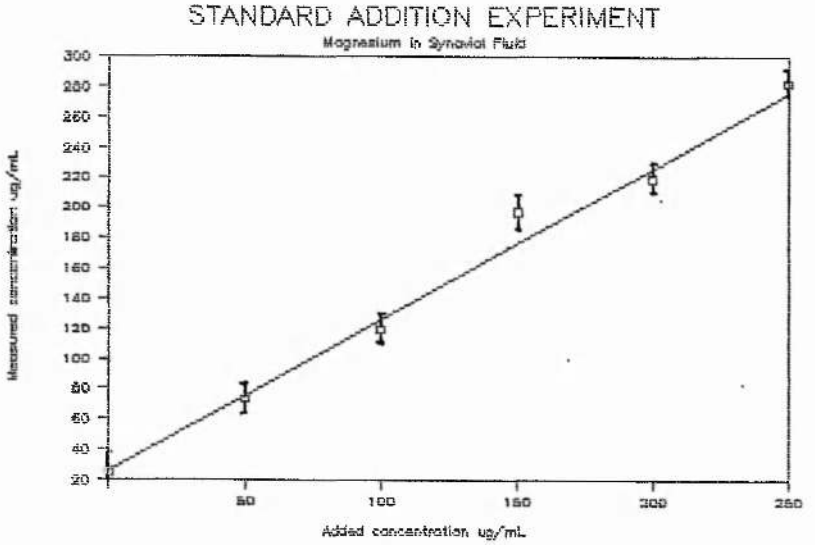
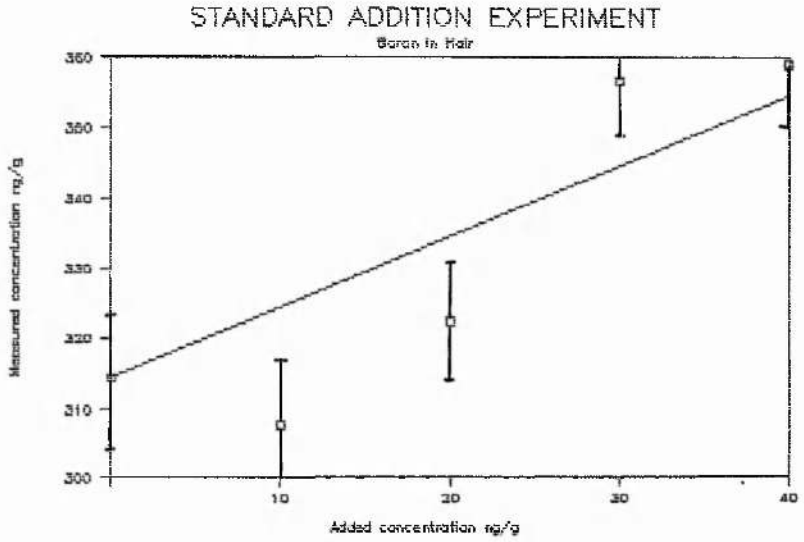
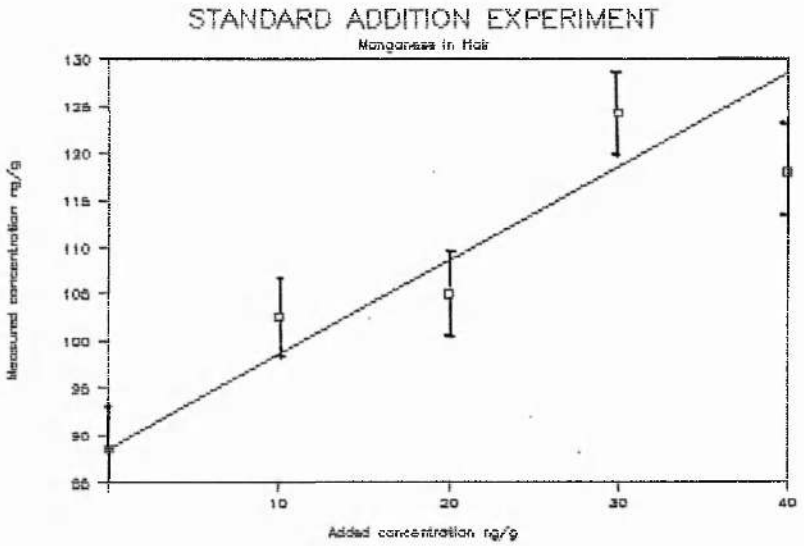


Figure 20: Standard addition of boron to hair, with comparisons

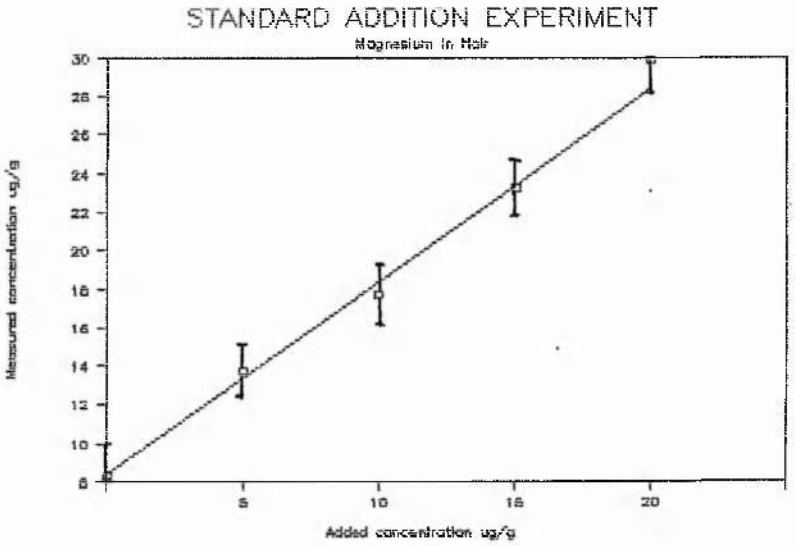
a) Boron



b) Manganese



c) Magnesium



approximately 3% of the intrinsic levels, with 11% for manganese and 63% for magnesium, so the wider spread of the boron values was not surprising.

In summary, the detection limits for boron measurement by ICP-MS were determined, and adequate quality control was ensured by use of standard reference materials and checks on the digestion techniques. A wide range of biological tissues and fluids were then analysed in order to characterise their boron content. The results of these analyses will be presented in the next section.

3.2.2. Boron in tissues and fluids

3.2.2.1. Elemental levels

The levels of boron found in a range of blood fractions, in synovial fluid, urine, hair, nails, bone and brain are presented in Table 18. As the terms "mean" and "standard deviation" are usually used to describe a normal distribution of data, it was felt that the median, interquartile range and the full sample range would provide a better description of the data.

Table 18: Concentration of boron (ng/mL or ng/g fresh weight) in various tissues and fluids

Tissue/fluid	Median	Lower Quartile	Upper Quartile	Minimum	Maximum
Whole blood[a]	156.6	131.3	259.2	50.1	730.5
Blood serum[a]	161.6	140.2	428.6	45.5	795.6
Packed cell volume[b]	196.0	155.8	215.2	122.7	237.8
Clot[b]	288.5	244.6	335.0	159.3	375.8
Synovial fluid[a]	30.3	7.9	105.2	<7.8	529.5
Saliva[a]	41.4	21.4	120.9	<13.0	588.2
Urine[a]	1079	637.4	2021	163.8	8374
Hair[b]	2573	677	6580	<13	32710
Nails[b]	12269	6312	19991	<13	82738
Bone[b]	2130	1123	4648	150	9380
Brain[b]	4263	811	8015	<60	19756

a: ng/mL
b: ng/g (fresh weight)

In general, fluids such as blood, synovial fluid or saliva have relatively low levels of boron. The values for blood and urine are quite close to the values quoted in Table 15. Urine is known to be the route by which most ingested boron is excreted, and thus has quite a high boron content, as expected. Nails and hair can also be described as "excretory pathways"; because of their slow growth, boron can accumulate there over a long period of time. In addition, the low water content of these matrices makes high levels of boron quite likely. Similar concentrations of the element in the epidermal (and dermal) layers have been noted in the literature (131), for example, hair and hoof of mammal, feathers of birds. The results also show considerable accumulation of boron in the bone and brain tissues; this tendency has been reported by other authors for both bone and tooth enamel (80). The range of values quoted for brain tissue is somewhat higher than the rather limited data in the literature. If a high dose of boron is ingested, however, the brain may show a particularly high increase in its boron level, compared to other tissues (103).

3.2.2.2. Distribution of boron data

It has been suggested that essentiality can be defined by investigating the shape of the distribution curve, in tissue, of any given trace element. That is, essential elements should have a symmetrical or normal distribution, whereas environmental

contaminants should result in a skewed pattern (4,5). Samples of hair, nail, saliva, urine and blood from the different groups ("control" or "normal" subjects only) within the British population were brought together, giving the values in Table 18. The distribution of these data have also been demonstrated in Figure 21, showing the number of samples within certain concentration ranges. Boron in blood is closest to a normal distribution; the remaining graphs all have similar shapes, approximately log-normal. For some of matrices, part of the upward slope of the distribution is masked by the closeness of the data to the detection limits.

It is interesting to compare the results of hair and nails analysed from one individual with the population as a whole. Samples were collected from a man who had been taking a boron supplement of 5mg/day over a period of years; boron concentrations were 5.8 μg/g in hair and 42.5 μg/g in nails. If these values are compared with Table 18 and Figure 21d and e, it is clear that the levels of boron are quite high in this individual. The value for hair is near the upper quartile in that distribution, while that for nails is between the upper quartile and the maximum. This again suggests that boron may accumulate in these tissues; attempts to minimise the possibility of external contamination, by washing prior to digestion, were carried out, as described in section 2.2.3.2..

Figure 21: Frequency distributions of boron in clinical specimens

a) Whole blood

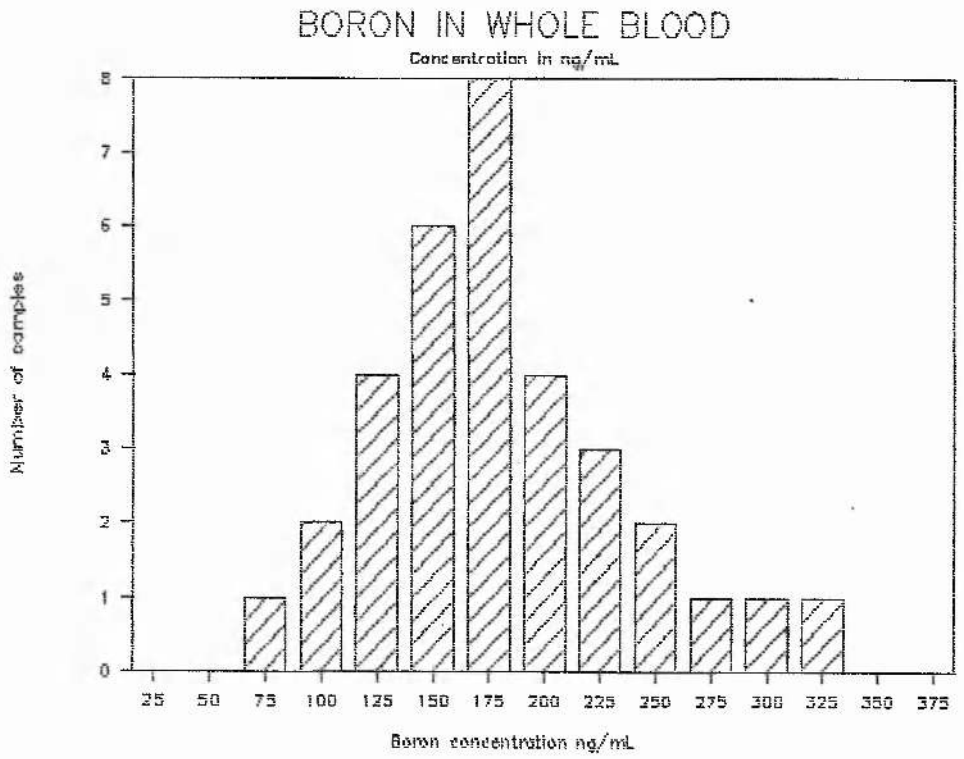
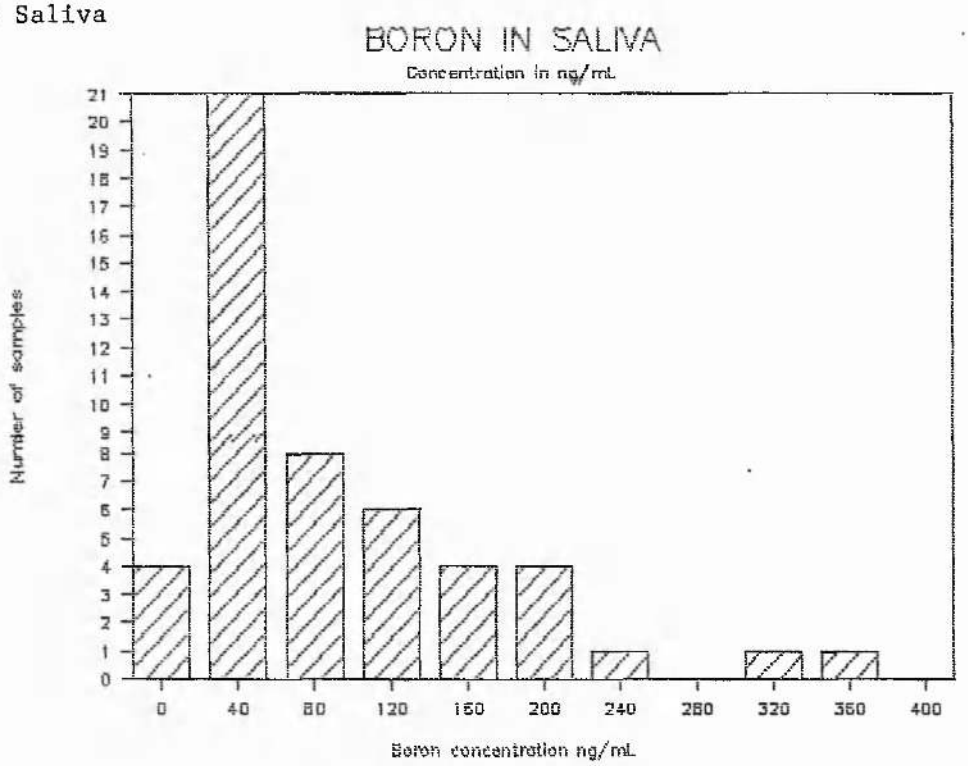


Figure 21 (con.): Frequency distributions of boron in clinical specimens

b) Saliva



c) Urine

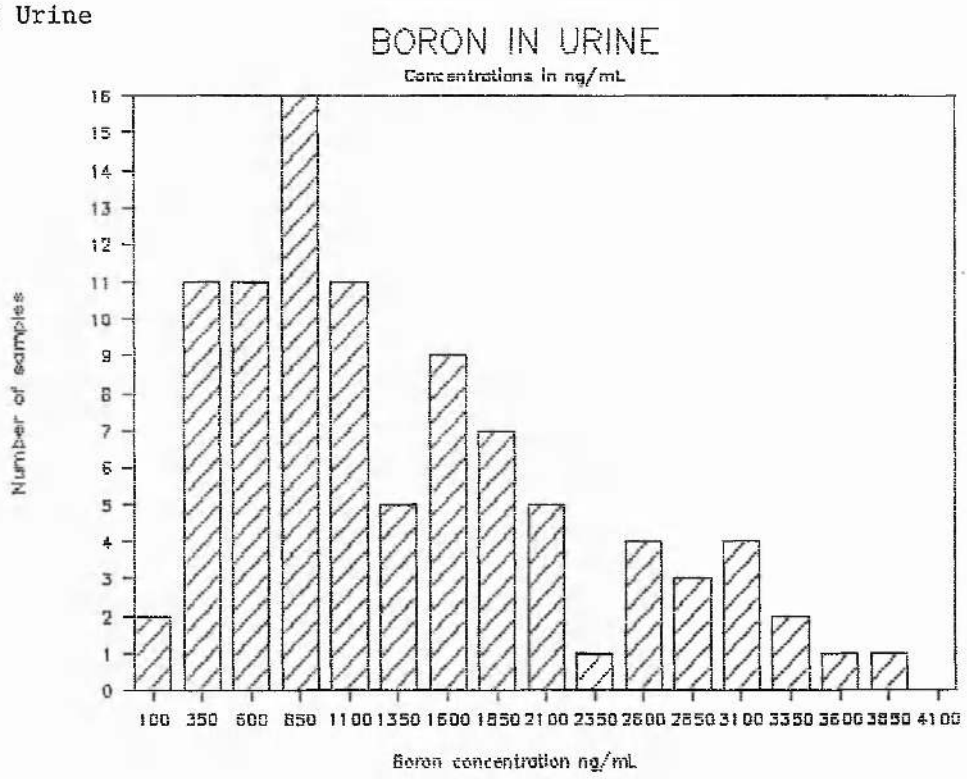
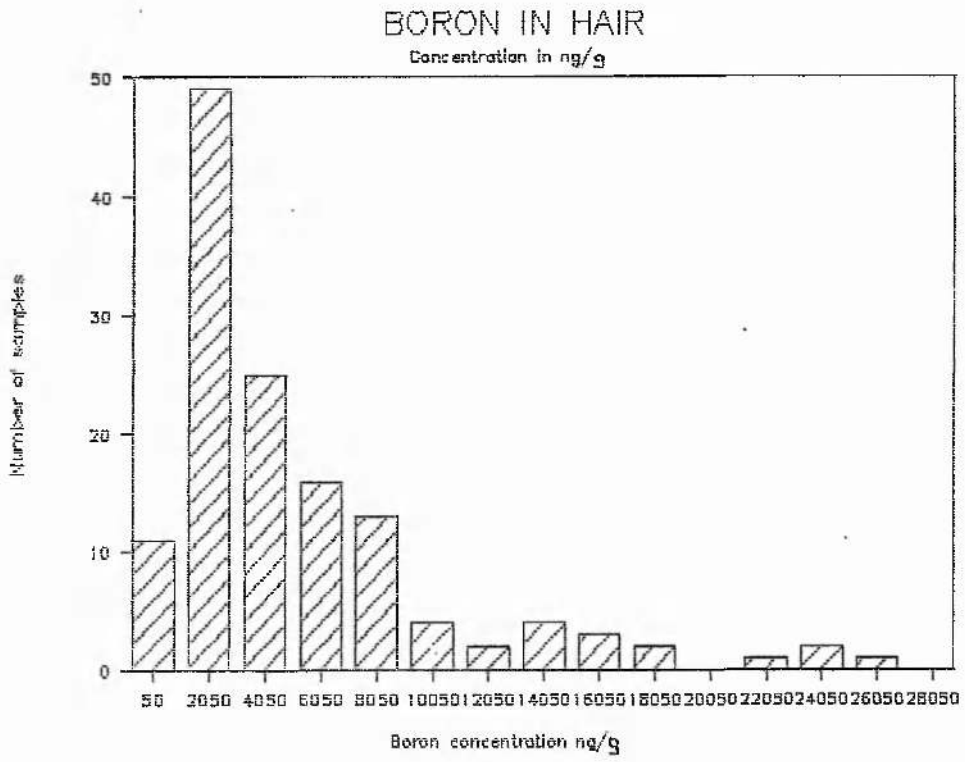
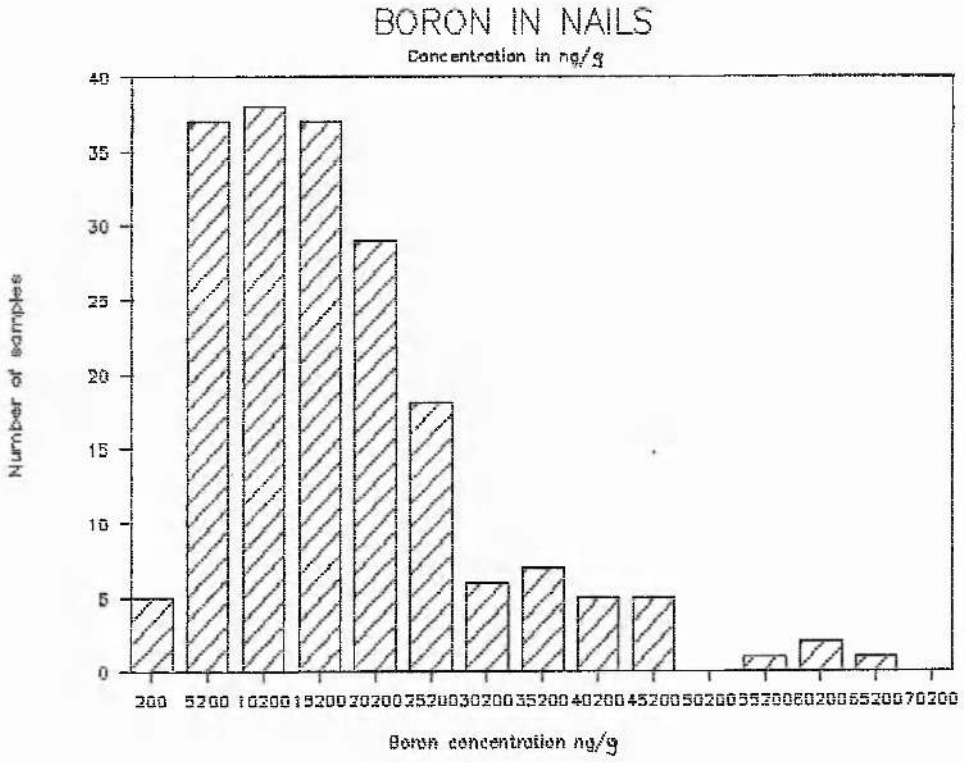


Figure 21 (con.): Frequency distributions of boron in clinical specimens

d) Hair



e) Nails

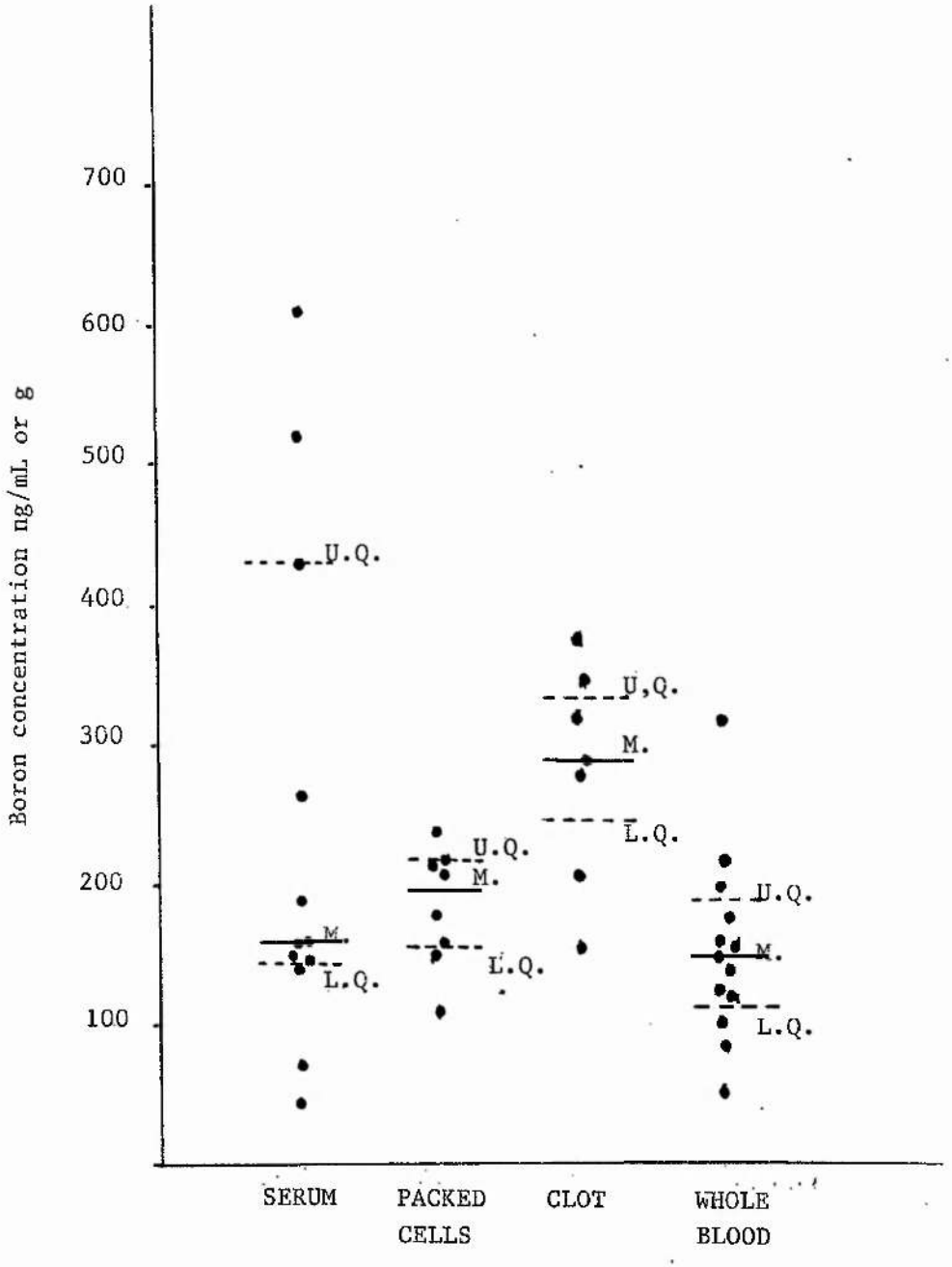


3.2.2.3. Boron in blood

After considering the approximately normal distribution of boron in whole blood, it was interesting to investigate various components of the blood. Within the large sample population, a group from Sheffield gave blood. This was collected in anticoagulated tubes, giving whole blood and the packed cell volume (plasma was discarded because of contamination problems), and in untreated tubes, giving serum and its associated clot. The data obtained from analysis of these samples are presented in Table 18, and graphically in Figure 22.

The clot has the highest median value, followed by the packed cell volume, serum and whole blood; all values are quite close in magnitude, however. It is thought that contamination to plasma may have taken place when the "spun" plasma was being removed from the packed cell volume since the use of anti-coagulant does not seem to have affected concentrations in whole blood or packed cells. If this is the case, this may also explain why the serum samples have a comparatively large range, unlike the remaining blood fractions. Similar contamination, but to a lesser extent, may have taken place to serum when being separated from the clot.

Figure 22: Boron in various blood fractions



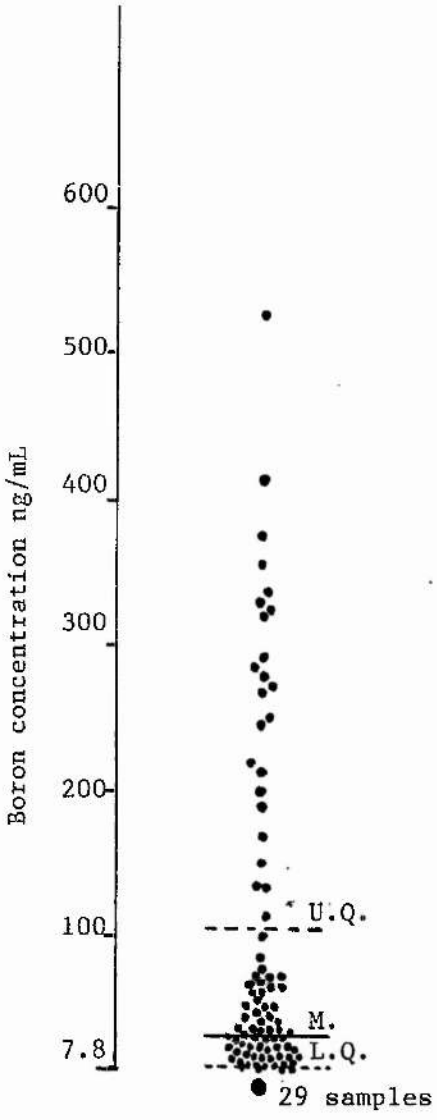
U.Q.: Upper quartile
M.: Median
L.Q.: Lower quartile

3.2.2.4. Boron in synovial fluid

The synovial fluid samples analysed in this study were obtained from subjects suffering from a number of different arthritic disorders, and could therefore not be designated a "normal" population. This fluid was measured in order to investigate if boron showed any significant patterns of behaviour between the different disorders; boron has been linked with rheumatoid arthritis (91,128). The difficulties inherent in trying to obtain synovial fluid from healthy individuals are obvious; in addition, after death, the composition of the fluid is known to change (132) with the result that postmortem samples are not particularly useful.

The distribution of boron concentrations in synovial fluid samples are shown in Figure 23. The pattern of the distribution is unclear because a sizeable proportion of the samples fell below the detection limit of 7.8 ng/mL. It is interesting to note that the median boron level in synovial fluid is somewhat lower than those reported for both serum and plasma, in this study and in the literature (80,133). Synovial fluid is similar in composition to both these fluids; it is a dialysate of plasma. In general, the protein content of synovial fluid is rather lower, consisting mainly of smaller molecules e.g. albumin. This changes in the case of most inflammatory disorders; for example, fibrinogen (a large molecule involved in clotting processes) is absent

Figure 23: Boron in synovial fluid



U.Q.: Upper quartile
M.: Median
L.Q.: Lower quartile

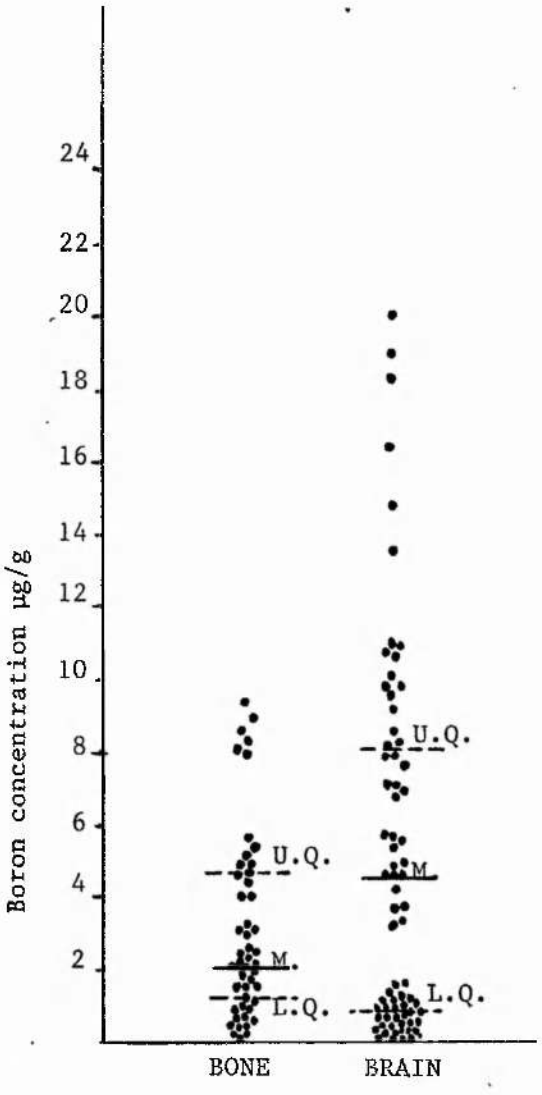
in "healthy" or "non-inflammatory" synovial fluid but may be present in fluid taken from sufferers of rheumatoid arthritis.

Such possible variations in protein content may account for the wide range of boron levels, as may the possible presence of small fragments of cartilage, ligament and bone. Moreover, some arthritic disorders result in the formation of crystals within the fluid; these may be bone-derived, monosodium urate or calcium pyrophoshate, for example. All these factors will have a bearing on the general trace element composition of the synovial fluid, as well as the boron content, and will be discussed in more detail in Chapter 5.

3.2.2.5. Boron in brain and bone.

Figure 24 shows the sample distribution obtained by the analysis of boron in brain and bone samples. In the case of both tissues, the data seem to be clustered, rather than being evenly spread. The reasons for this are unclear, but neither brain or bone are particularly homogenous materials. In the brain, the regions from which samples are taken may be important in trace element analsis (1). On this basis, the student's t-test was used to distinguish between data from the hippocampus and the basal ganglia regions; no significant variations in boron content were found. In the case of bone, it is felt that the difference between the rheumatoid arthritic and control samples

Figure 24: Boron in bone and brain



U.Q.: Upper quartile
M.: Median
L.Q.: Lower quartile

(significant at the 0.1% level) does not completely explain the observed "clustering". It is likely that the effect would probably have been alleviated if a larger number of samples had been available for analysis, thus reducing the effects of tissue inhomogeneity.

3.2.3. Consideration of age/sex parameters

The sample groups of urine, hair, nails, saliva, whole blood, brain and synovial fluid were analysed statistically to investigate the importance of an individual's age and sex on the boron concentration within their tissues. It was felt that the size of the bone and remaining blood fractions sample groups were too small to permit such statistical manipulation. The student's t-test (two-tailed) was used to compare the elemental status of males and females. The influence of age on boron levels was investigated by using regression analysis. The values of the correlation coefficient, r, thus obtained, were substituted in the following equation (134) to produce a "t-value":-

Equation 4. $$t_{n-2} = |r| \frac{\sqrt{(n-2)}}{\sqrt{(1-r^2)}}$$ where n is the number of samples

In the above case, the null hypothesis is that there is no correlation between age and boron concentration. If the calculated value of t is greater than the tabulated critical value, this hypothesis is rejected i.e. a significant correlation does exist. The data obtained were also used to plot graphs, with a regression line superimposed on the measured boron levels.

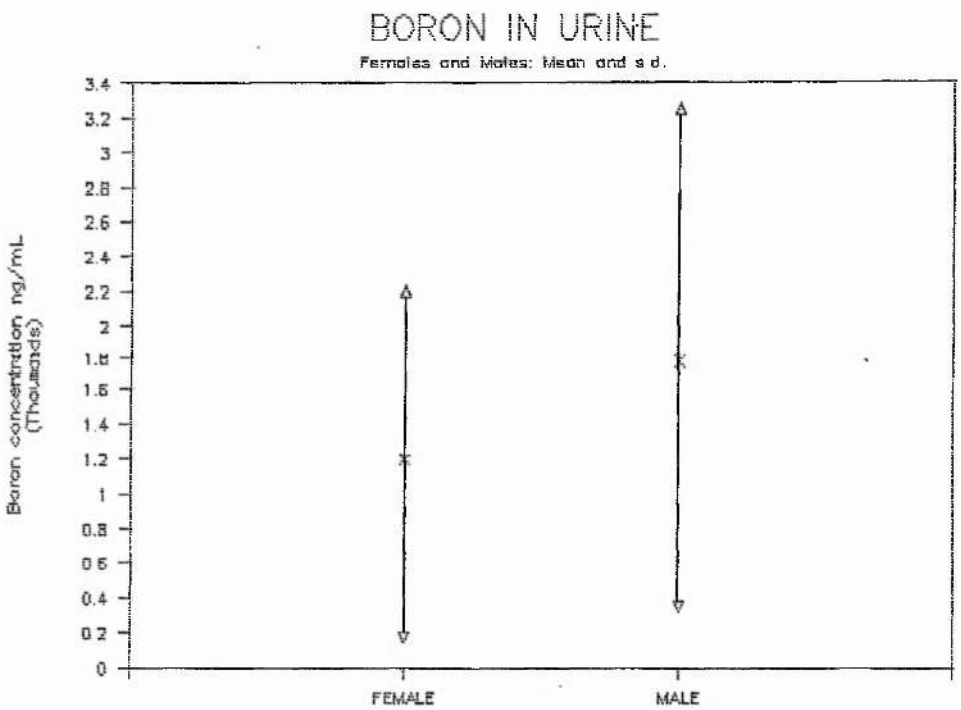
3.2.3.1. Differences due to sex

Most of the matrices analysed, that is, hair, whole blood, synovial fluid, brain, nails and saliva showed no relationship between the sex of the individual from which samples were taken and the boron content of these samples. Urine was the only matrix in which such a dependance was seen; the concentration of boron in the urine of males was "significantly" greater than in females (i.e. at the 5% level). This is shown graphically in Figure 25. It has been found that most ingested boron is excreted in the urine (80); it follows from this that the amount of boron excreted is proportional to the quantity ingested. On average, males have a slightly greater calorific intake than females; if more food is being eaten, a marginally greater amount of boron may be ingested, with the resultant slight increase in urinary boron.

3.2.3.2. Differences due to age

For hair, synovial fluid and brain samples there appeared to be no relation of the boron levels to the age of the individual involved. Nails showed a significant relationship, S (at the 5% level), urine a highly significant relationship, S* (at the 1% level), while saliva and whole blood both demonstrated a very highly significant relationship, S** (at the 0.1% level). Figures 26 to 29 show these

Figure 25: Boron in urine: variation due to sex



results graphically. In Figure 26, a simple dependence of boron in nails on age can be seen. There appears to be a slight accumulation in nails as the individual increases in age. Figure 27 shows the slightly more complicated regression curve obtained when investigating how boron in urine varies in age. It suggests that the levels are slightly lower at the youngest ages, reaching a maximum in the late teens and early twenties, with a gradual decline as the individual reaches the middle years and old age. As with the variation between males and females, this may be related to average food consumption at various stages in life, with a resulting alteration in excretion. It should be noted that there are a number of exceptionally high values at just

Figure 26: Boron in nails: variation with age

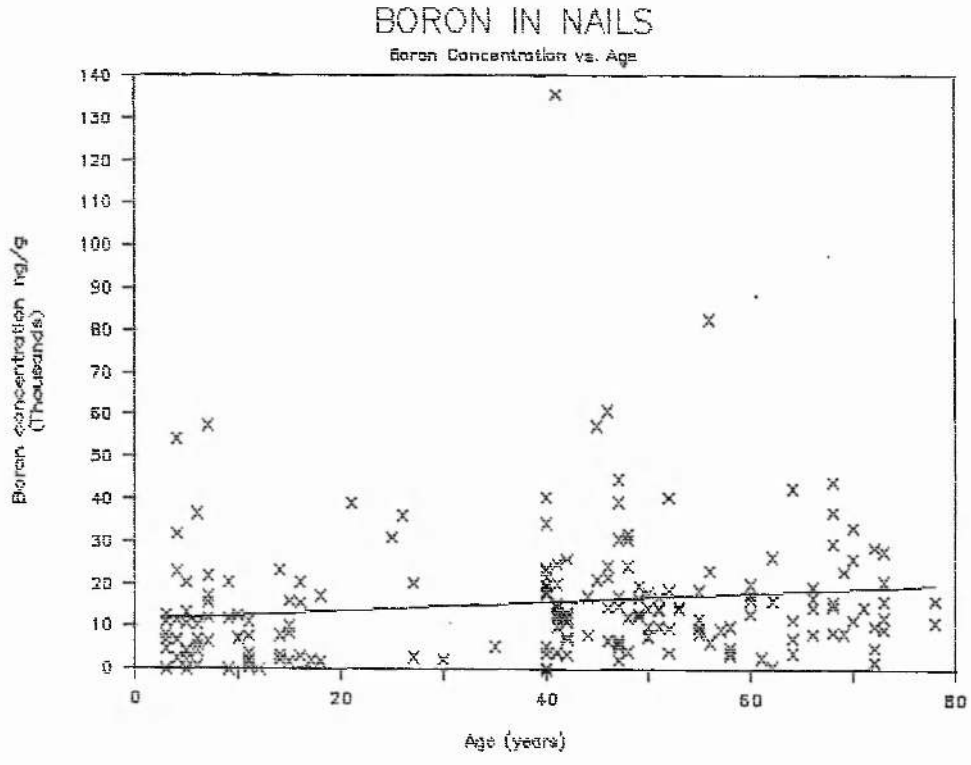


Figure 27: Boron in urine: variation with age

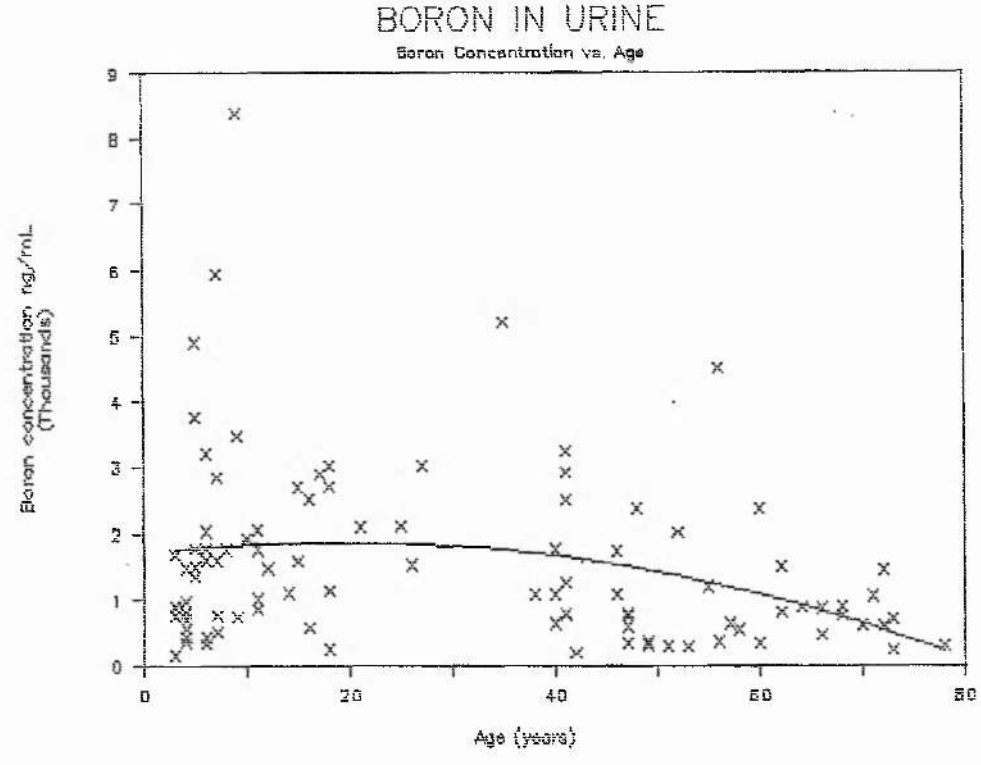


Figure 28: Boron in saliva: variation with age

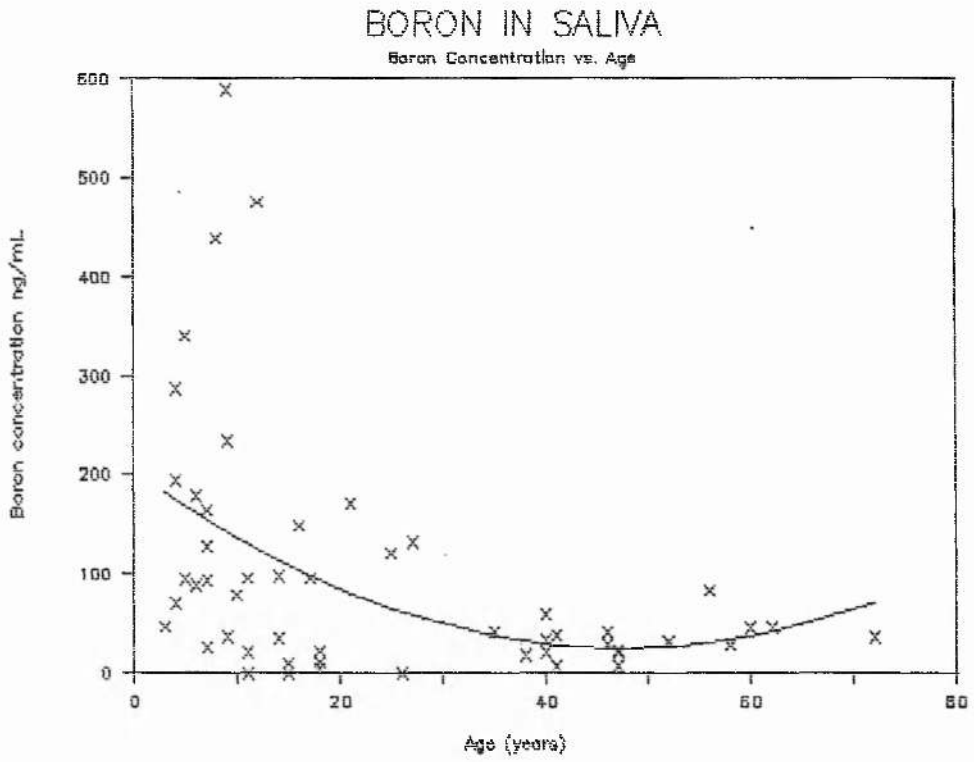
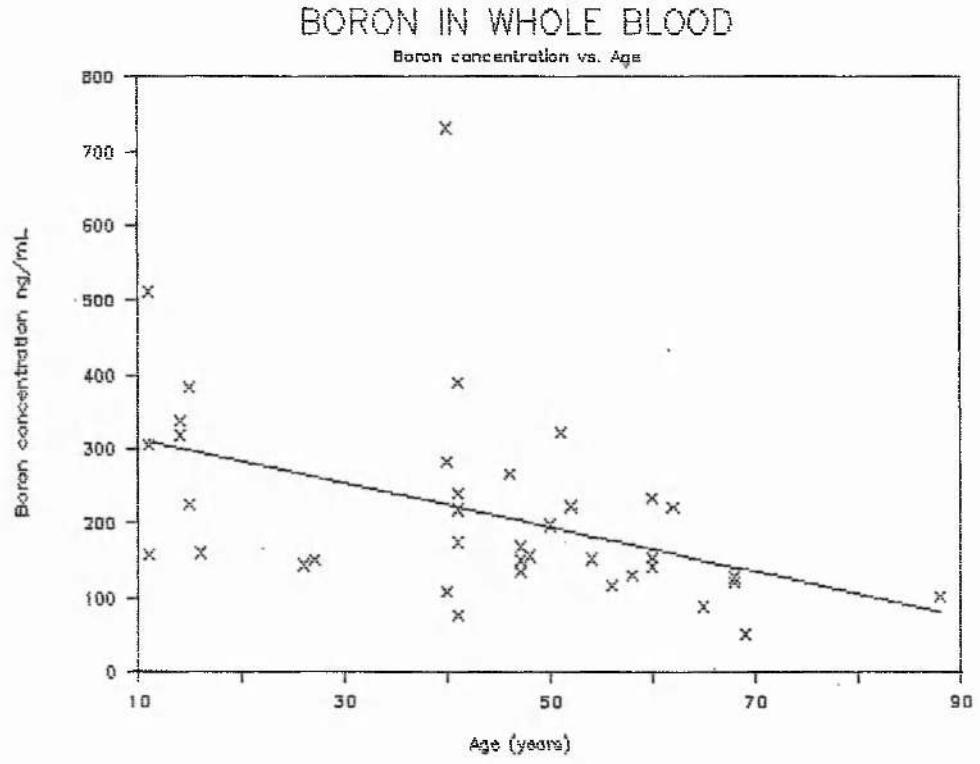


Figure 29: Boron in whole blood: variation with age



below ten years of age; these tally closely with several high values obtained for saliva shown in Figure 28. In fact, children and adolescents seem to have the highest levels of boron in saliva, with a general decrease through the middle and senior years. The values of boron in whole blood also show a tendency to decline as the individual's age increases, as demonstrated in Figure 29.

3.2.4. Associations of boron with other elements

Linear regression analyses were carried out on the different tissues and fluids, comparing the boron content with the levels of the other trace elements within each matrix. Values of regression coefficient, r, were calculated and substituted into equation 4 as in the previous section. The results obtained from these processes are presented in Table 19.

3.2.4.1. Blood and blood fractions

Blood serum, plasma, packed cell volume, clot and whole blood data all underwent regression analysis. The first four of these groupings were rather limited in sample number; the small number of associations recorded for serum, plasma and packed cell volume was therefore unsurprising. A number of associations significant at the 5% level (S) were seen for the clot. Prominent among these was the element calcium;

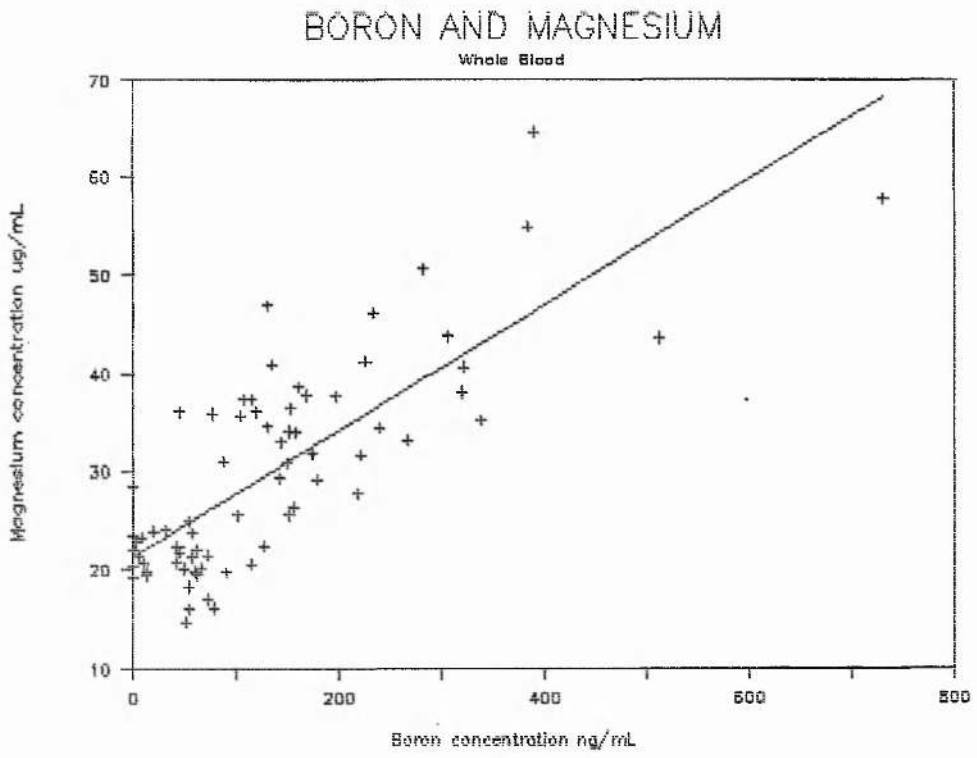
Table 19: The statistical significance of associations between boron and other elements

Tissue/fluid	0.01<p<0.05 (S)	0.001<p<0.01 (S*)	p<0.001 (S**)
Blood serum	-	Sr	-
Packed cell volume	-	-	-
Clot	Ca,Ge, Sb	La	-
Whole blood	-	Sc,Rb,Hg,Pb	Mg,P,Ca,Fe Cu,Zn,Sr,Cs
Hair	Al,Cr,As,Mo	Li,Rb	Zn
Nails	As,Mn,Zn	Ca,Cr,Rb	Li,Mg,P,Sc
Saliva	Al,Pb	-	Ba,Ce
Urine	Al,Cr,Mn,Ni Cd	Ca,Sr	Li,Mg,P,Zn Rb,Mo,Cs
Synovial fluid	Cr	Mo	P
Bone	Li,Sr,Sb	Co,La	Sc,Ni,Cs,Ce
Brain	-	Ca,	Li,Al,Sc,Ni Sr

a very highly significant (S**:0.1%) association was also shown between boron and this element in whole blood, shown in Figure 30c. Among other very highly significant relationships are boron with magnesium, phosphorus and copper (see Figure 30 a,b,d). These are all essential elements named by Nielsen in his work on dietary boron and mineral metabolism. He has suggested that boron may affect the action of the parathyroid hormone, and therefore be involved in the metabolism of calcium and magnesium (114). Along with Neilsen, other authors have linked boron with calcium and phosphorus, and with the maintenance of healthy bones (83,93). Other experiments have shown that dietary boron affects copper metabolism in humans (126). Other essential elements with which boron was seen to be strongly associated, in blood, were zinc and iron; to date, no dietary studies have been carried out investigating boron and the metabolism of these elements. However, these elements are known to interact within the body. Boron levels also appeared to be related to scandium, strontium, caesium, rubidium, mercury and lead. None of these elements are essential, although they are found in most tissues and fluids. Calcium and strontium have showed a certain amount of interactive behaviour, as have rubidium and caesium (with the essential element potassium) (2). Mercury and lead are known to be toxic, even at very low levels.

Figure 30: Boron associations in whole blood

a) Magnesium



b) Phosphorus

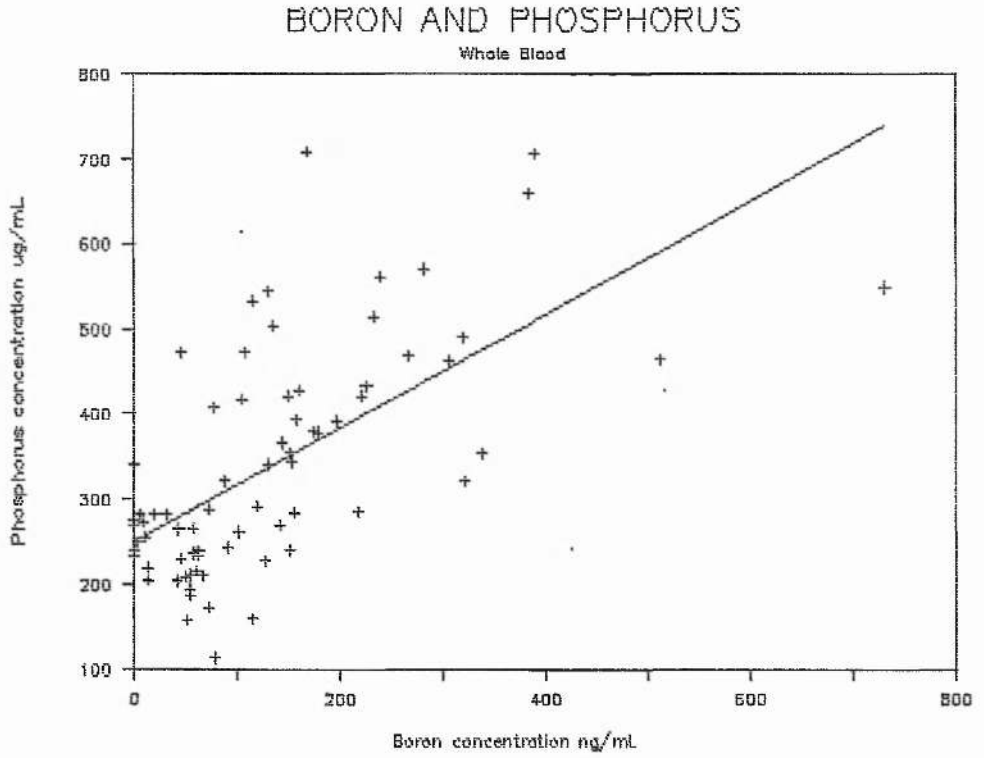
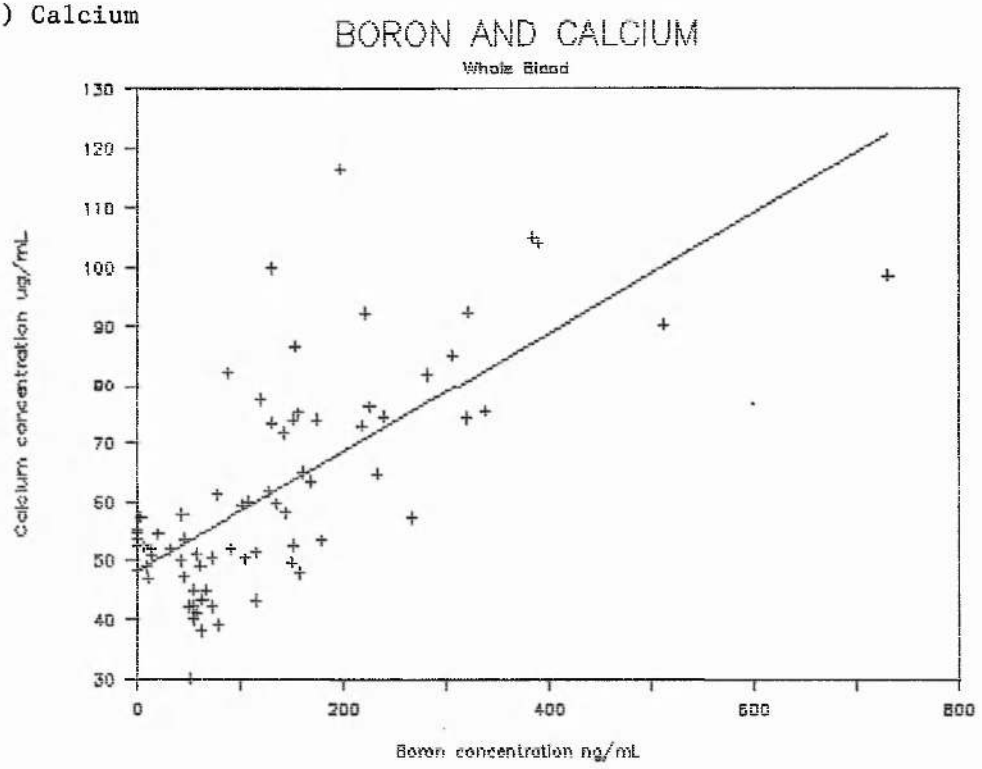
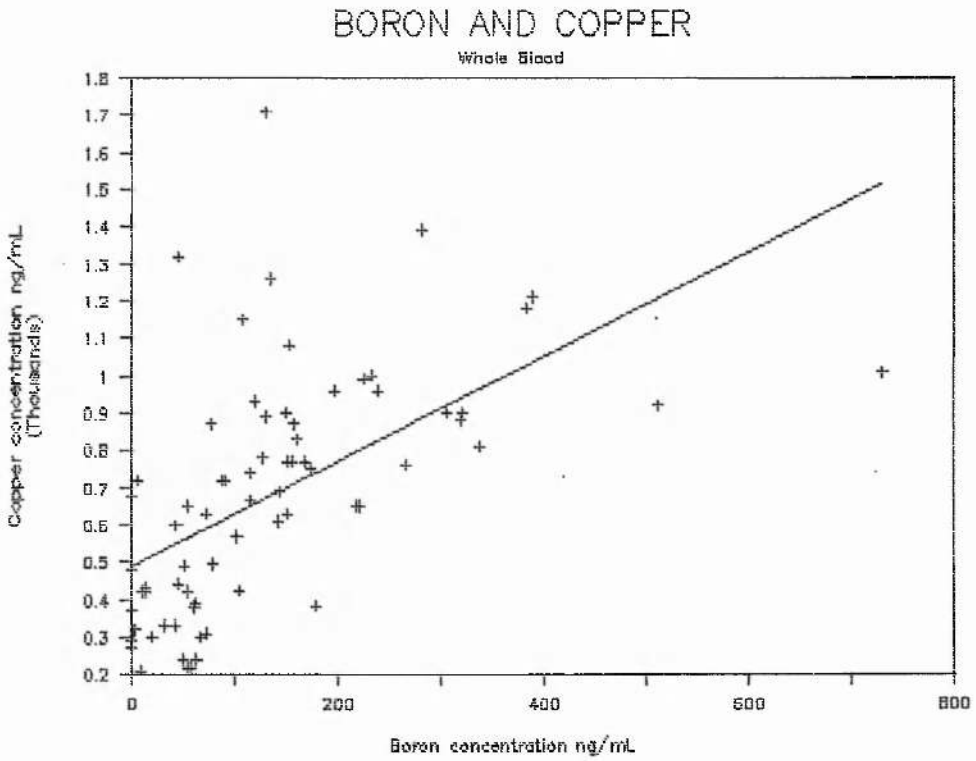


Figure 30 (con.): Boron associations in whole blood

c) Calcium



d) Copper



3.2.4.2. Hair and nails

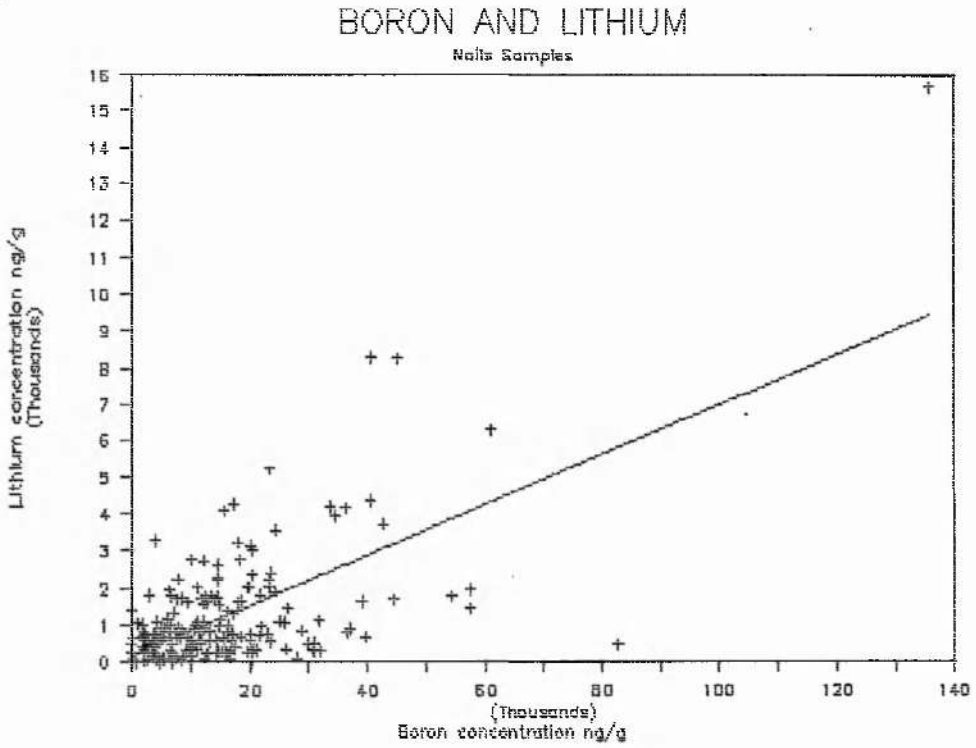
Hair and nails show a reasonable number of associations between boron and other elements, although rather less are at the very highly significant level. Those of boron with magnesium and lithium in nails are shown in Figure 31. The number of associations in these tissues is not surprising, considering their role as excretory pathways. Lithium and rubidium, both non-essential elements, are common to the two tissues. Arsenic and chromium , again in both hair and nails, are essential, but are found at very low levels within the body. By contrast, zinc is a major trace element constituent of hair and nails and its observed association with boron is therefore interesting.

3.2.4.3. Urine and saliva

Saliva shows a small number of elemental associations with boron, all of which are with non-essential elements; barium, cerium, aluminium and lead. These can probably best be explained by elevation through recently eaten food or similar substances. Urine, however, shows a large number of elemental associations; those of boron with lithium and phosphorus are shown in Figure 32. This is probably due to the fact that ingestion of a food rich in one mineral is unlikely to be rich in that mineral alone. Such a diet would probably result in the excretion of quite high levels of a wide range of minerals, if in

Figure 31: Boron associations in nails

a) Lithium



b) Magnesium

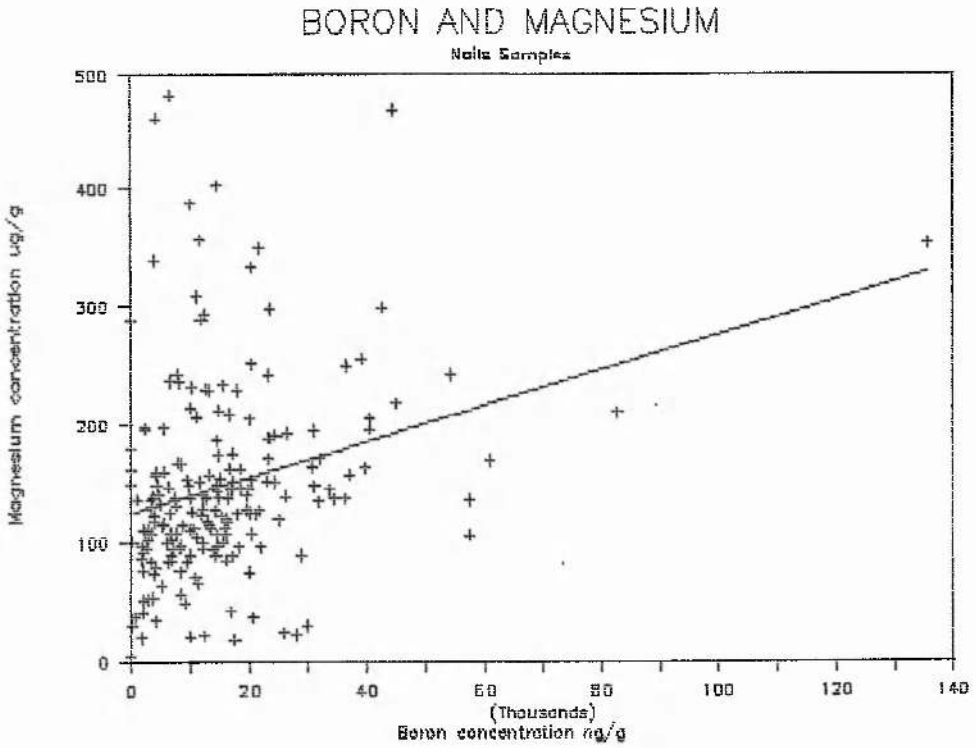
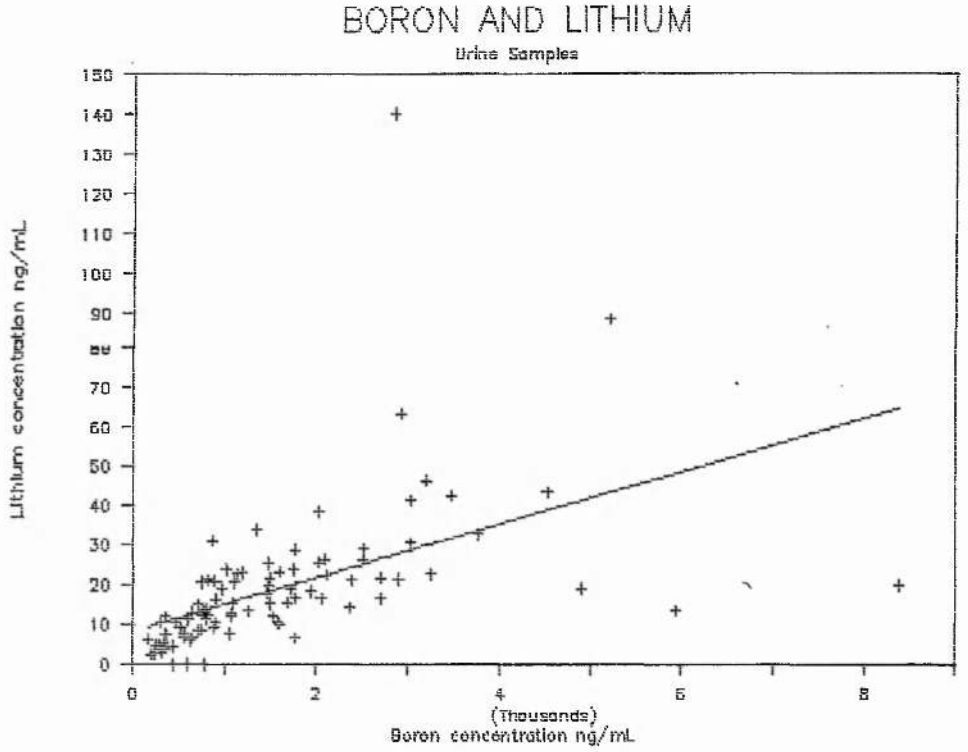
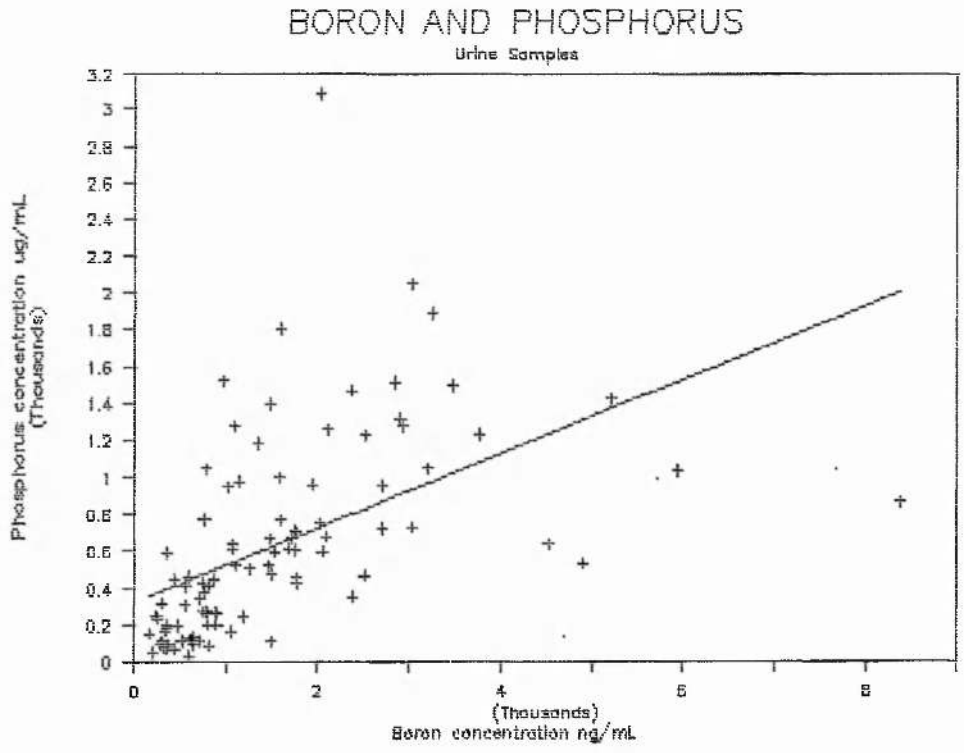


Figure 32: Boron associations in urine

a) Lithium



b) Phosphorus



excess to the body's requirements. In addition, all three elements are examples of those which are preferentially excreted by the urinary route.

3.2.4.4. Bone and brain

A large number of relationships between boron and other elements are seen in both these tissues. Most of these are non-essential; among these, lithium and scandium are again present. Strontium is known to accumulate in bone (1), and aluminium in brain (135,136), which may, in part, explain the observed statistical association with boron.

3.2.4.5. Synovial fluid

Only molybdenum and phosphorus show any degree of association with boron in this matrix. The sparcity of such relationships is similar to blood serum; all three fluids are similar in composition. The presence of the element phosphorus as one of the associated elements is possibly due to the presence of fragments of bone within the synovial fluid itself.

To summarise, many of the elements involved in associations with boron are non-essential, for example, lithium, scandium, rubidium and caesium. However, some interesting findings have been made; namely the

close relationship of boron with calcium, phosphorus, magnesium, copper and other essential elements in blood. These may be significant in the light of work carried out by Neilsen, suggesting that boron may play a role in the metabolism of several minerals within the body.

3.3. Summary

Finally, the findings of this part of the study can be summarised:-

i) Detection limits were evaluated for the comparatively new analytical technique of ICP-MS; namely 2.6ng/mL.

ii) Values are given for boron in a range of standard reference materials, for future comparison; IAEA A13 animal blood, IAEA H4 animal muscle, NIST SRM909 human serum, NIST 1577A bovine liver and SINR 0920 Chinese hair.

iii) Boron levels are, for saliva and synovial fluid, in the order of 30-40ng/mL, blood 150 ng/mL, urine 1µg/mL, hair, bone and brain 2-4 µg/g and nails 10µg/g, fresh weight or volume.

iv) Blood appears to follow an approximately normal frequency distribution, while remaining sample distributions are more skewed in shape. Synovial fluid, bone and brain samples show wide ranges of boron concentration.

v) There are slight variations in boron levels with sex in urine, and age in nails, saliva, urine and whole blood.

vi) Boron seems to have some degree of association with nonessential elements such as lithium, particularly in urine, hair and nails samples. Moreover, in whole blood, this was also found for boron with calcium, phosphorus, magnesium and copper, the elements with which Neilsen has suggested interactions, following alterations of dietary boron (114,126).

4. TRACE ELEMENTS IN HUMAN TISSUES AND FLUIDS

4.1. Introduction

4.1.1. Biological systems

4.1.1.1. Blood and blood fractions

Blood is a connective medium with a complex liquid intercellular matrix, plasma, in which various components, for example, red blood cells, leukocytes and platelets, are suspended (1). The two primary functions of blood are transport and regulation of homeostasis.

Blood can dissolve or suspend many different substances and transport them to and from cells as it circulates around the body. For example, the nutrients absorbed by the body - amino acids, lipids, vitamins and minerals - are transported from the gastrointestinal tract and carried to cells. In addition, red blood cells, utilising haemoglobin, bind oxygen in the lungs and then distribute it, while hormones are carried from various adrenal glands to target organs. Blood also transports the body's waste products, for example, carbon dioxide to the lungs for expiration, and other substances for excretion via the kidneys or intestine. Blood is involved in the maintenance of homeostasis within the body by regulating fluid volume, pH, body temperature; components

of blood play a role in protection against infection and blood loss from the body.

Blood plasma is 90% water, 1% inorganic substances, 6-8% protein, while the remaining fraction consists of substances such as glucose, oxygen, carbon dioxide, lipids, enzymes and hormones. The proteins have a number of roles, including immunological reactions, inflammatory responses, binding of specific elements, vitamins and some lipids, a buffering action, and the exertion of osmotic pressure. The main groups of plasma proteins are albumin, prealbumin and globulins. Among the last group, fibrinogen is important - this protein takes part in the clotting process and disintegrates along with the platelets. Serum is formed when whole blood is allowed to clot. Thus serum differs from plasma in that the protein fibrinogen is no longer present.

Erythrocytes, or red blood cells, are involved with the operation of active transport systems in the membrane. They contain a 33% solution of haemoglobin, and enzymes of glucose metabolism. Leukocytes, or white blood cells, are major components of the body's defence mechanisms, and contain enzymes such as alkaline phosphatase and collagenase. Platelets are tiny cells whose main role is the coagulation of blood.

4.1.1.2. Saliva

This fluid has a mainly mechanical role i.e. assisting in mastication, swallowing and in speech. It contains two types of secretions; mucous for lubrication, and serous. The latter contains ptyalin, an enzyme for digestion of starches. Saliva contains potassium and bicarbonate concentrations above those in plasma, but lower sodium and chloride levels (1).

4.1.1.3. Urine

The composition of the urine depends on the nutritional status, the state of metabolic processes, and the ability of the kidney to cope with the material presented to it (1). Blood passes continually through the kidney; various substances in the plasma are able to move through the semi-permeable membrane of the kidney glomeruli. This filtrate is modified within the kidney tubules and nephron collecting duct to form the urine which is excreted. Urine is largely made up of urea and sodium chloride, with additions of ammonia and various salts. There are also smaller amounts of sugar, and oxalic, citric and free fatty acids. Trace quantities of cholesterol and metals are also excreted in the urine, as are various hormones and vitamins. Normal urine will contain red and white blood cells, and other aged epithelial cellular material.

4.1.1.4. Hair and nails

Hair is a down growth of epidermal (outer layer) cells into the dermis, forming a hair follicle set at an angle to the skin; the hair is produced by mitosis in the follicle base (1). The main component of hair is the cortex, containing the most resistant keratin; coarse hairs contain a central medulla. The cortex is surrounded by the cuticle, on which overlapping cuticular scales are arranged, to provide a protective covering. At its lower end, hair has an expanded bulb, embedded in the skin; blood vessels flow through these folded dermal layers and supply nutrients which ensure hair growth. This growth is cyclic; the length of cycle depends on the hair location. The period of growth is known as the anagen phase, after which the follicles shrink down and the matrix cells degenerate, in the catagen phase. During the resting period, or telogen phase, part of the follicle is lost, the hair develops a club-shaped proximal swelling and is dislodged (1,74).

Nail is a hardened corneal layer and is comprised of a main "body", with a root and a free edge (1). The nail rests on the nail bed (derived from the epidermal layer) and has a cuticle above its root. The active growing area lies under the root and is shown by a white "half-moon".

4.1.2. Trace elements in the human body

4.1.2.1. Ingestion, absorption and excretion

Most nutrients and toxic substances are taken into the body via the gastrointestinal tract, although the inhalation and accumulation of dust can result in high levels of elements within the lungs (1). Information about the elemental composition of the diet, however, is only a rough guide to the nutritional value of the diet. The ultimate measurement of mineral availability from a particular dietary source must be its contribution to the maintenance of body function (137). Factors affecting mineral availability tend to fall into two main groupings, dietary or physiological.

Dietary factors include the impact of soil and geochemistry on content e.g. selenium in China (138). In addition, the chemical form of an element within the diet must be considered e.g. selenate and selenite, haem-Fe and non-haem-Fe (139). Interrelations between nutients in the diet can be critical, for instance, protein and zinc uptake, carbohydrate and copper uptake (138,139). The effect of food refining is also important: processing of food can result in considerable loss of trace elements, but the availability of some elements after extrusion cooking has shown an increase (137,138). This latter fact is probably due to the reduction of phytate; the outer layers of grains

and seeds contain the major part of many trace elements but also many substances that could influence trace element uptake, such as fibre, phytic acid and tannins. Mineral-mineral interactions may also take place. Iron and manganese have shown behaviour of this type in animal and man (140), while high levels of tin have been found to impair zinc absorption (141,142). Fortification of products, such as infant formulas, with trace elements can also precipitate imbalances. In all these cases, interactions may occur at the level of intestinal uptake and transfer, or may cause an alteration in the systemic metabolism of nutrients (138).

Several physiological factors affect the mineral solubility and rate and efficiency of uptake from the intestines of the different nutrients in thier various chemical forms. Because of the body's control mechanisms, wide variations in rates of excretion, as well as intestinal absorpton, may occur with the essential elements. The stage of development, nutritional status, state of health, pregnancy and lactation, and the ability of individuals to adapt to variations in nutrient supply can all influence the availability of elements for absorption and metabolism. For example, iron-deficiency amaemia results in an increased ability to absorb iron (142); in addition, iron absorption is also influenced by the level of iron in the diet consumed over the previous 1-3 days (143). Deviations in elemental absorption may be long or short term; reasons for prolonged variations

include disease. Tissue levels of essential elements may be altered due to metabolic diseases; concentrations of potentially toxic elements may be raised after abnormally high exposure (1).

In the gastrointestinal tract, only a part of the ingested quantity is absorbed and distributed around the body by the bloodstream. The unabsorbed part is excreted in the faeces which is added to by bile, pancreatic juice and intestinal secretions. This process is quite slow; with zinc, for example, faecal samples can contain quantities of the element from 12-30 days before collection (139). The absorbed portion is transferred to the different organs and tissues. Most is then excreted in the urine, with some endogenous intestinal losses (as described above), and minor quantities via hair, nail, sebum and sweat (1). The amount of element excreted by each route varies: for example, high percentages of boron, molybdenum and arsenic, amongst others, are absorbed by the gut, and thus their urinary excretion is high. For elements such as iron, copper and zinc, the amount eliminated in the urine is 1, 2 or 3%, respectively, of the amount ingested. For other elements, such as cadmium, manganese and tin, there is very poor absorption in the gastrointestinal tract, with the result that the waste is passed via the faeces (1,144).

4.1.2.2. Blood

Due to homeostatic control, the level of essential elements in blood is not always a good indicator of adequate or deficient supply (1). Abnormal levels of copper, iron, selenium or zinc have been seen in certain populations, such as high blood copper in some native Australian or New Zealand settlements or low zinc in sections of Middle East populations. For elements such as arsenic, chromium, mercury, nickel and lead, blood concentrations become elevated under conditions of exposure. Lead, mercury and cadmium bind very readily with red cells, indicating that serum is not a preferred specimen for analysis of these elements (145). The level of lead in blood is a good indicator of current exposure, and reflects a dynamic equilibrium between exposure, absorption, distribution and elimination of lead in situations of relatively high exposure. It has been shown that the cadmium level in the blood of smokers is noticeably greater than nonsmokers, and that blood mercury levels are increased in those that freqently eat fish (146,147).

For many diagnostic purposes, it is considered useful to analyse blood samples in conjunction with analyses of other specimens, such as urine or hair.

4.1.2.3. Urine

A specimen of urine is a good indication of the nutritional condition, and the state of metabolic processes within an individual. For a number of elements, including copper and zinc, below average levels are seen in urine under deficient input; this data should, however, always be supplemented by information from blood (and its fractions) and hair to aid in interpretation of results. If the complete range of specimens display lowered values, some kind of deficiency is likely. Some paradoxical situations occur, however, such as increased levels of zinc in the urine of alcoholics (148). Urine can also be useful as a specimen in the cases of occupational exposure, by elements such as arsenic, chromium, mercury, nickel and lead (149-152). For mercury, urine concentrations are useful if an individual is exposed to metallic mercury or to inorganic salts, but not to alkyl mercury salts. In the latter case, urinary excretion does not reflect any increases of mercury concentration in the blood (1).

4.1.2.4. Hair

Hair may be used as an indicator of the general level of exposure to potentially toxic elements, or the supply of essential elements. Since growth of hair is slow, this is a more long-term indicator than those

previously described. In addition to deposition during matrix formation or by sweat or sebum, external contamination is a trace elemental source.

For most elements, no quantitative physiological relations have been shown between hair and elemental intake, resorption, blood or tissue concentration. Mercury, particularly as methyl mercury, is an exception to this (153). Elements are transferred to the hair, at various rates; mercury and zinc, for example, have a high turnover unlike cadmium (154). Moreover, the biological meaning of levels of essential elements in hair often remains doubtful, because fluctuations of these elements, due to the homeostatic control mechanisms in the pool which supplies hair, may obscure the direct reflection of a nutritional deficiency (1). Therefore, elevated zinc concentrations have been found in hair despite acute zinc deficiency (155).

4.1.2.5. Other samples

Very little work has been carried out on either nails or saliva. One recent study used toenails in an attempt to monitor selenium status in various populations from high and low selenium status areas (156). Another group have attempted to correlate hair and nails samples from a rural U.S. population since the two matrices are chemically related

(157), and show variations in Alzheimer's patients (158). Saliva has had some use as a biological monitor of fluorine in the body and lithium in patients undergoing lithium therapy (159,160).

4.1.3. Concentration of chemical elements

4.1.3.1. Distribution in the body

Biological samples are made up of the basic structural elements carbon, hydrogen, nitrogen, oxygen, phosphorus, sulphur and calcium (in hard tissues). Calcium, chlorine, potassium, magnesium and sodium make up a group of elements which are required for electrolyte balance, with HCO_3^-, SO_4^{2-} and HPO_4^{2-}. The remaining essential elements, along with environmental contaminants, occur in minute quantities. These minor and trace groups of elements are not necessarily distributed evenly around the body; a number of tissues act as major storage depots.

Bone is a target tissue of many elements; in particular, calcium and phosphorus, but strontium, lead, barium, fluorine and uranium also accumulate here preferentially. According to the ICRP Reference Man, composed by Snyder et al. (161), over 90% of each of these elements are found in the skeleton. Other elements, including magnesium, rubidium, aluminium and manganese are at higher concentrations than in

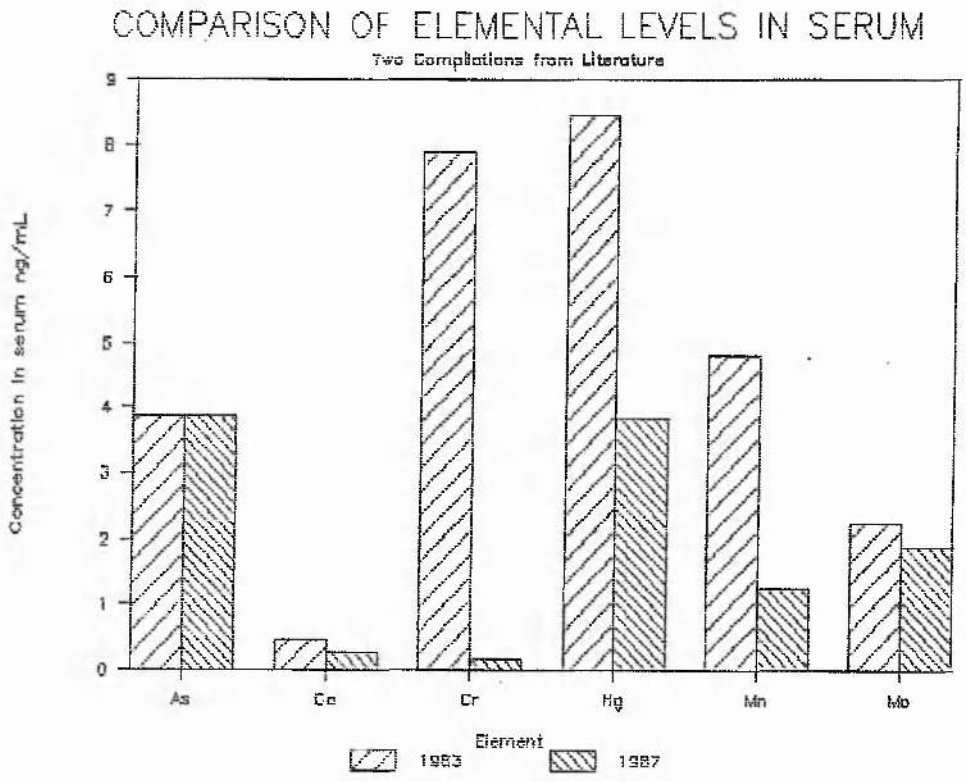
soft tissues. Teeth are also high in elements such as strontium and calcium. Levels in muscle are generally much lower; in this matrix, however, zinc is a particularly important trace metal. Liver and kidney tend to accumulate large quantities of trace elements. For example, 21% of manganese is stored in the mitochondria-rich liver (2), along with sizeable quantities of copper and iron, while 20% of the body's cadmium is sequestered in the protein metallothionein in the kidney (2). Other target body components are erythrocytes and spleen for iron, thyroid for iodine, and semen, prostrate gland, hair and nails for zinc. Lung tissues may have considerable levels of a number of elements, for example aluminium, fron inhaled dust. Solid tissue, including hair and nails, tend to concentrate trace elements to a higher degree than body fluids.

4.1.3.2. Trends in literature data

Before trace element researchers cam make clinical judgements about possible elemental roles in disease states, well-founded reference values or "base-line" data are required for comparison. As analytical techniques are improved, however, it is important that the literature data be re-evaluated, and reference values revised, where necessary.

Figure 33 shows a comparison of two compilations of literature data for serum concentrations of selected elements (145). This reflects the

Figure 33: Comparison of literature data for serum



fact that many reference values in human tissues and fluids are generally thought to be at lower levels now, than a few years ago. Particularly striking, in Figure 33, is the example of chromium. Even quite recently, values of 6-40 ng Cr/mL have been published in the literature (162-6) which are probably seriously affected by contamination and other methodological errors. Other authors, reporting values of approximately 1-6 ng/mL, and 0.56 ng/mL (166,167) are still above the range of values which is becoming a general consensus. This suggests that the "true" value of chromium in serum is in the order of 0.1-0.2 ng/mL (168-170).

Progress will be aided in the refinement of reference values by the development of new biological reference materials with elements at ultra-trace levels (145), and the improved certification of existing materials (77).

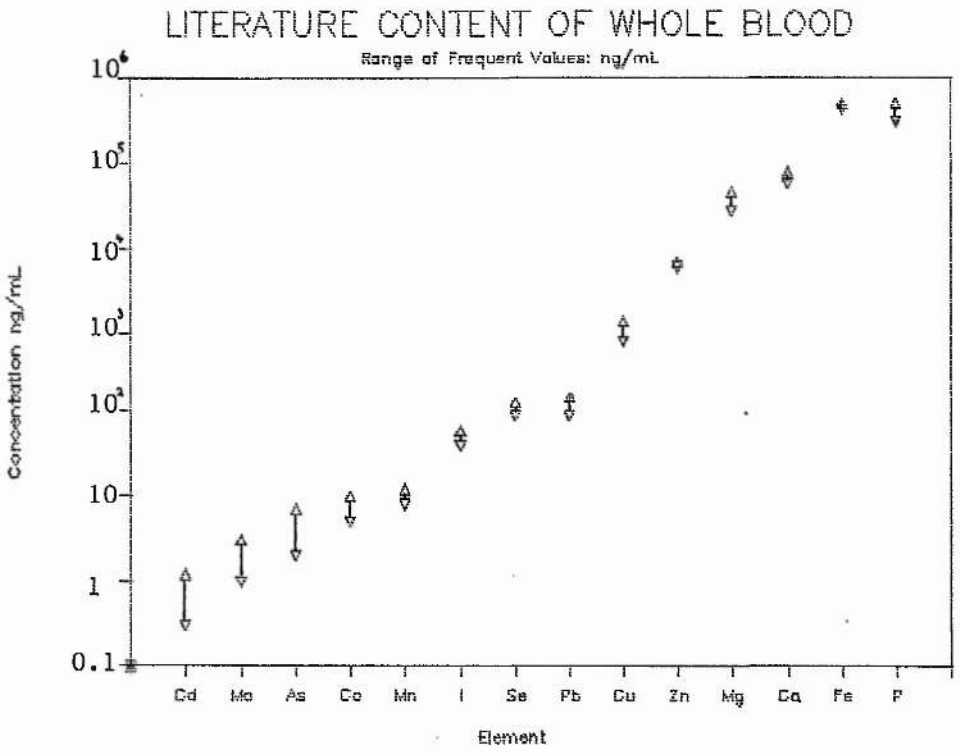
4.1.3.3. Summary of literature values

Figure 34 shows some of the most recently available literature data on the elemental content of whole blood, blood serum, hair and urine. The data displayed are values frequently obtained for the elements in question (1), that is, populations with abnormally deviating levels are not included. The reasonably small number of different elements quoted is an indication that reference values of several other elements in the tissues or fluids selected have not yet been established satisfactorily.

In general, elemental levels in hair are higher than in the fluid samples of blood and urine, as expected. For most elements, the range of frequent values is wider in both urine and hair than in serum and whole blood, since the former matrices are both excretory pathways. Several elements are at higher levels in whole blood than in serum, including zinc, lead, manganese and cobalt; many of these can be found at still higher concentrations in the red blood cells fraction of

Figure 34: Literature trace element values in human specimens

a) Whole blood



b) Blood serum

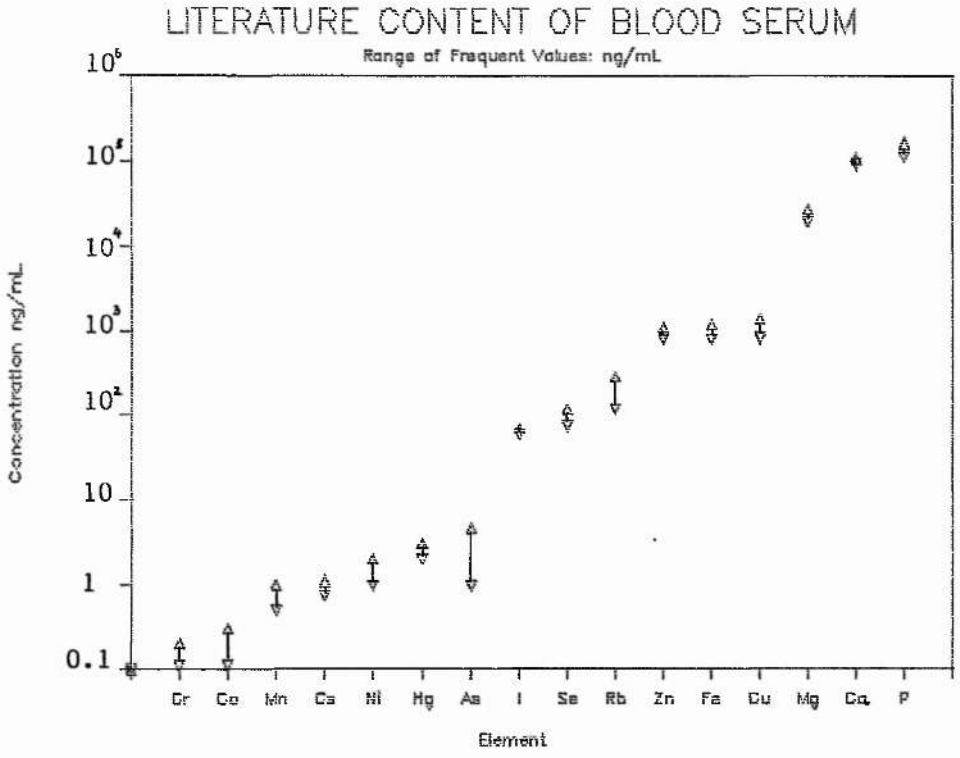
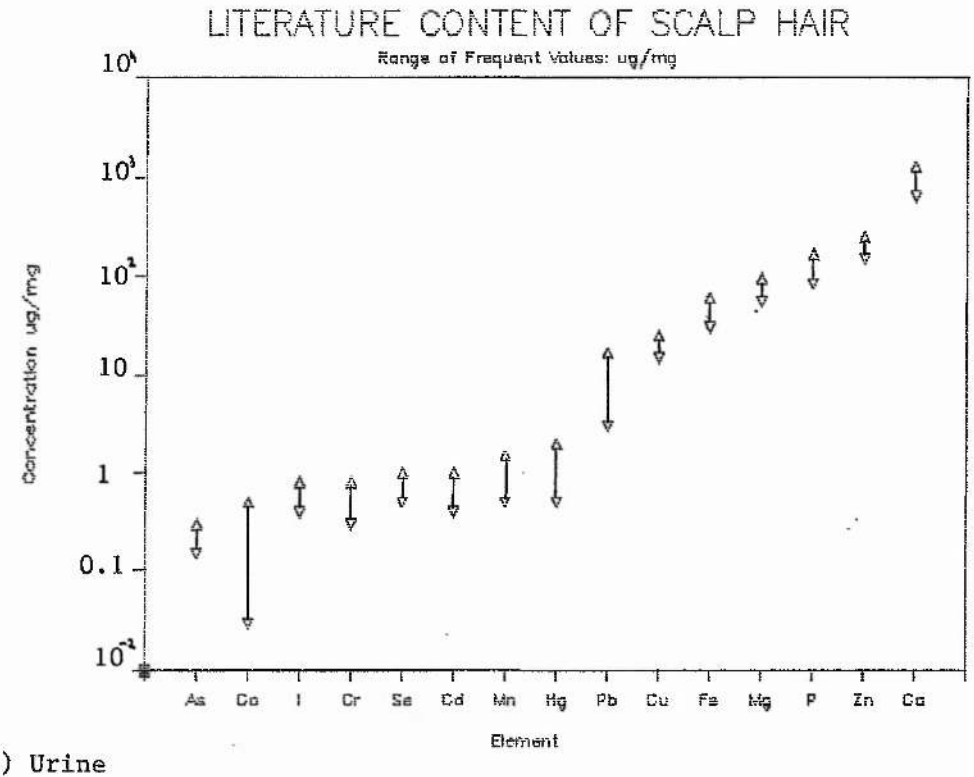
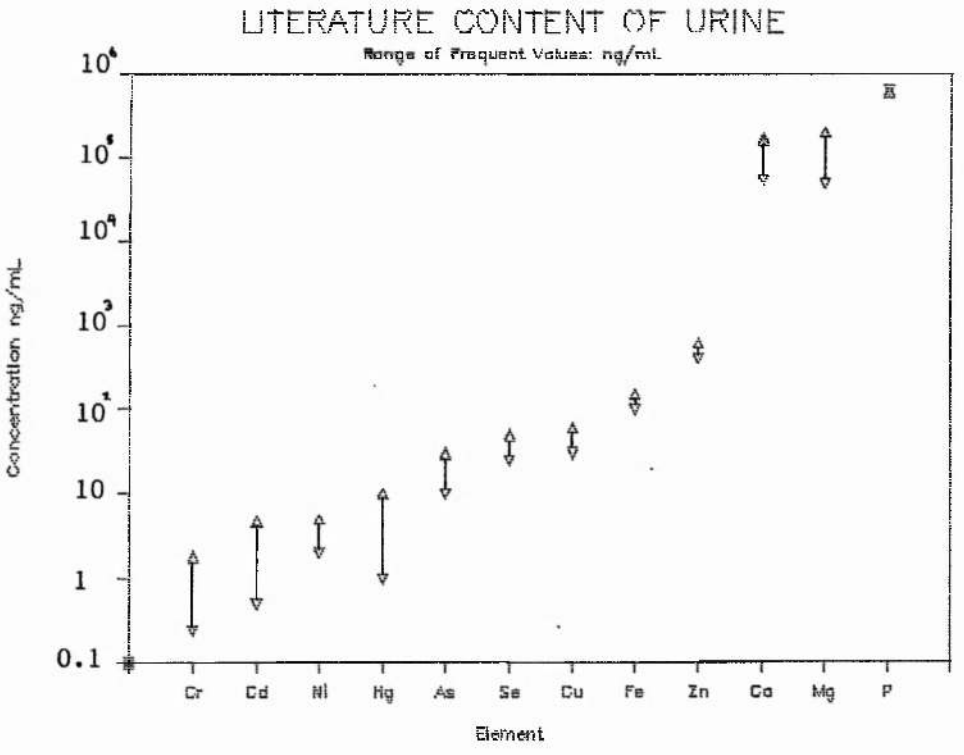


Figure 34 (con.): Literature trace element values in human specimens

c) Scalp hair



d) Urine



blood (145). Copper is one exception to this; most of this element is complexed to ceruloplasmin, a plasma protein (1).

4.2. Results and Discussion

Human tissue and fluid samples were prepared and analysed, using ICP-MS, as described in Chapter 2., parts 2 and 3. The results of this analysis are described in the following sections.

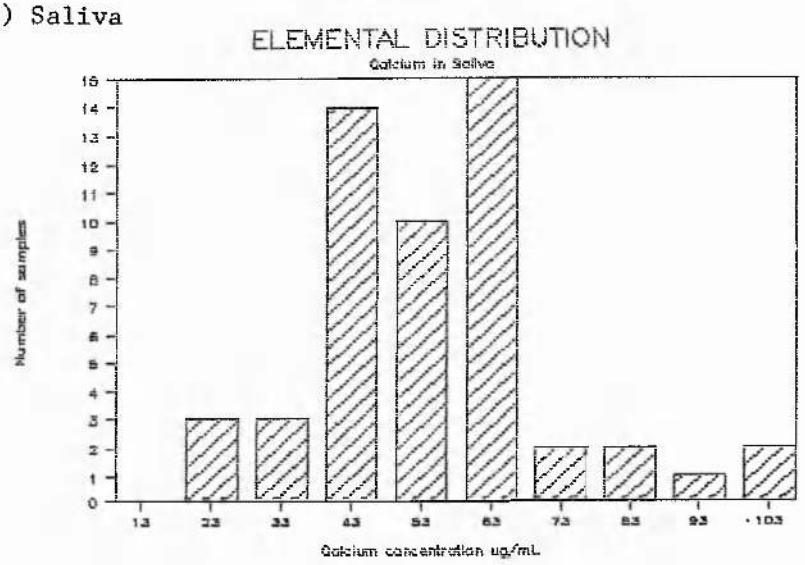
4.2.1. Distribution of data

Graphs were plotted to show the frequency distributions of the elements within the main sample groupings of saliva, urine, whole blood, hair and nails. Figures 35 to 41 show these distributions for a number of representative elements i.e. calcium and phosphorus (essential micronutrients), copper, zinc and molybdenum (essential trace and ultra-trace elements), strontium and lead (non-essential/toxic trace elements). For both essential and non-essential elements, the same basic results are seen. In whole blood, all samples seem to be approximately normally distributed, while most elements in the remaining tissues and fluids follow a log-normal distribution.

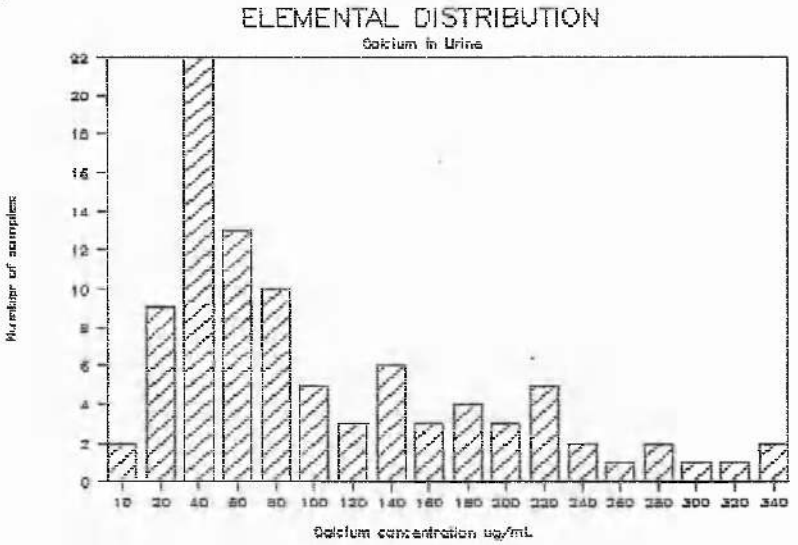
It has been suggested that the shape of the distribution curve for a trace element in tissue can be used as a method of determining whether

Figure 35: Frequency distributions of calcium data

a) Saliva



b) Urine



c) Whole blood

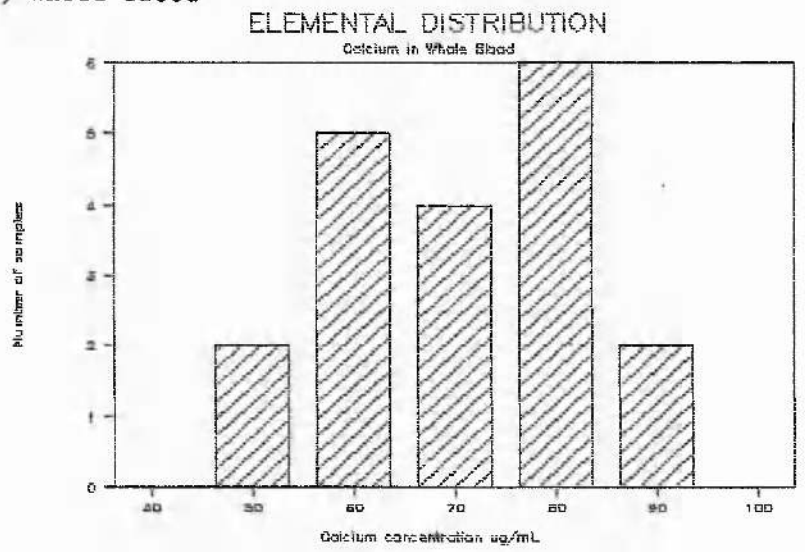
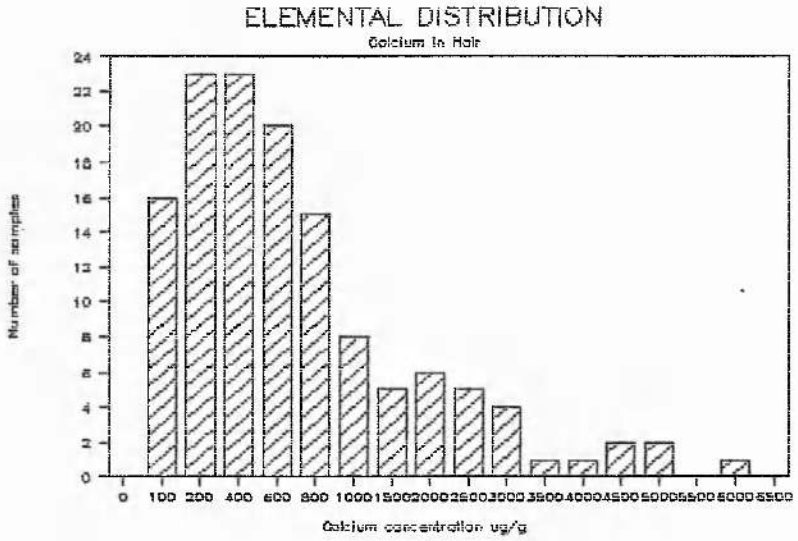


Figure 35 (con.): Frequency distributions of calcium data

d) Hair



e) Nails

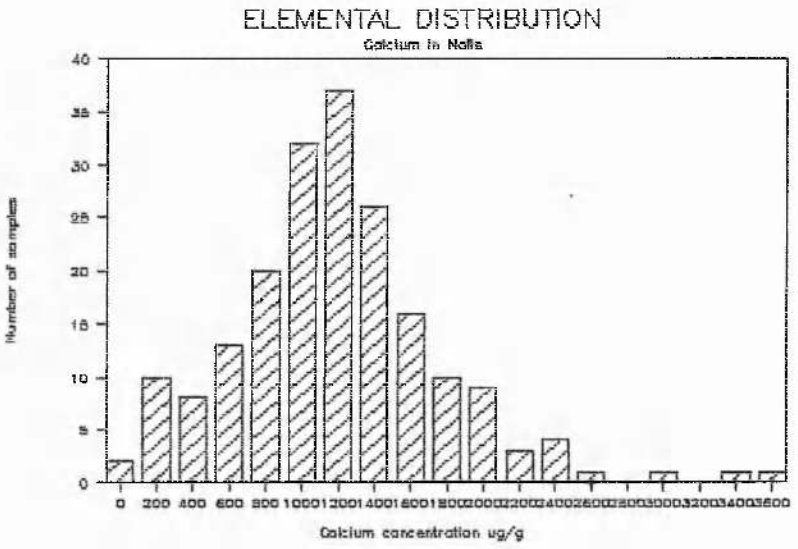
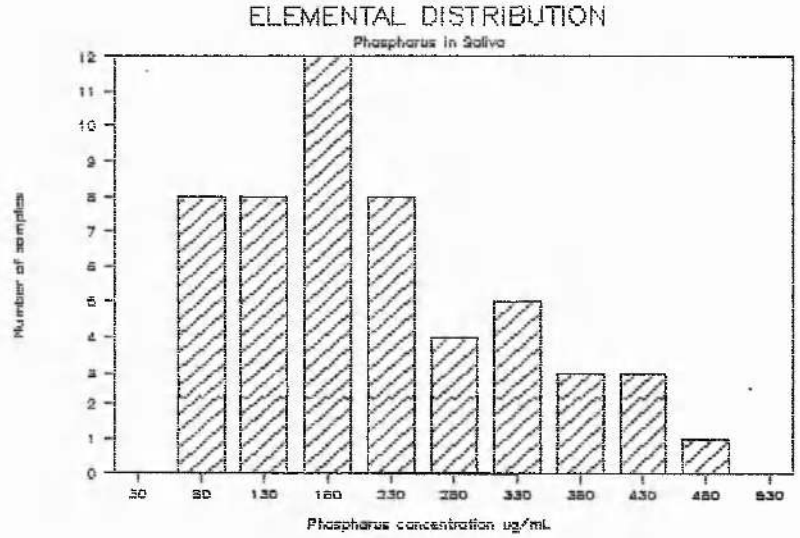
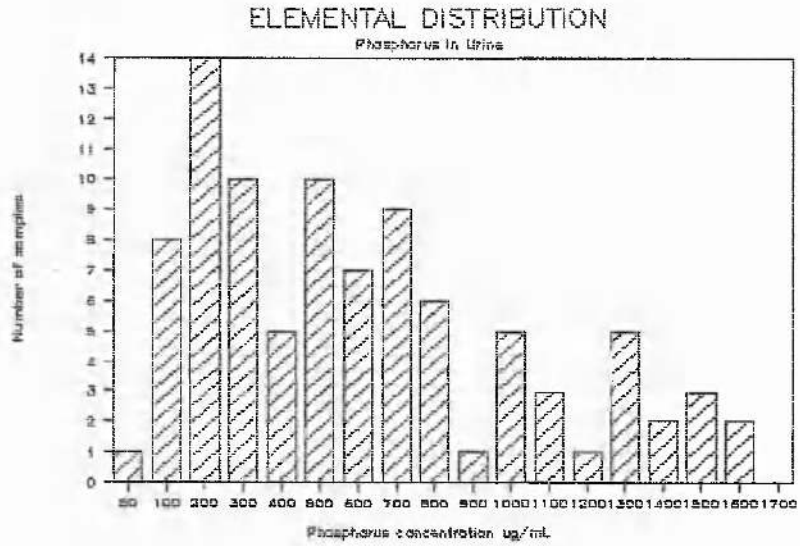


Figure 36: Frequency distributions of phosphorus data

a) Saliva



b) Urine



c) Whole blood

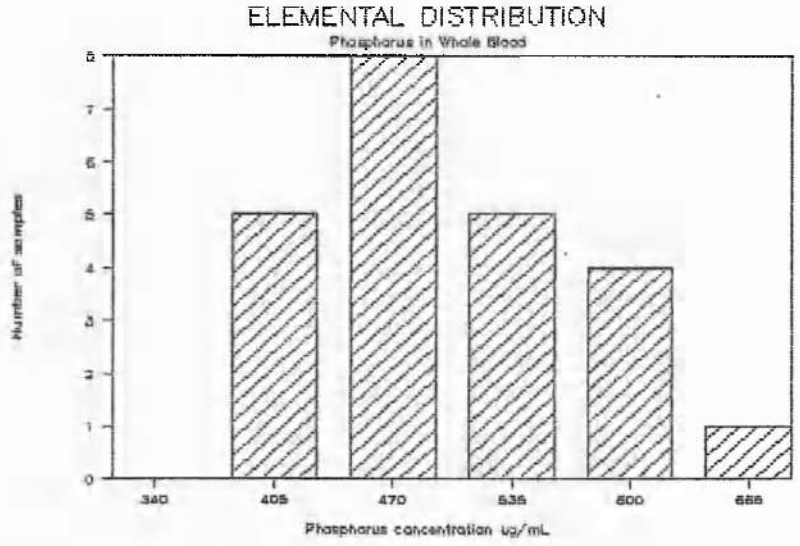
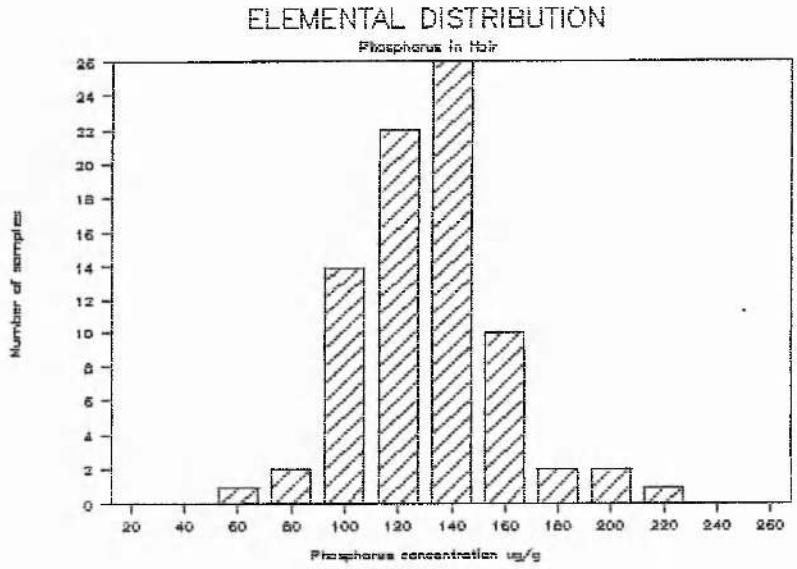


Figure 36 (con.): Frequency distributions of phosphorus data

d) Hair



e) Nails

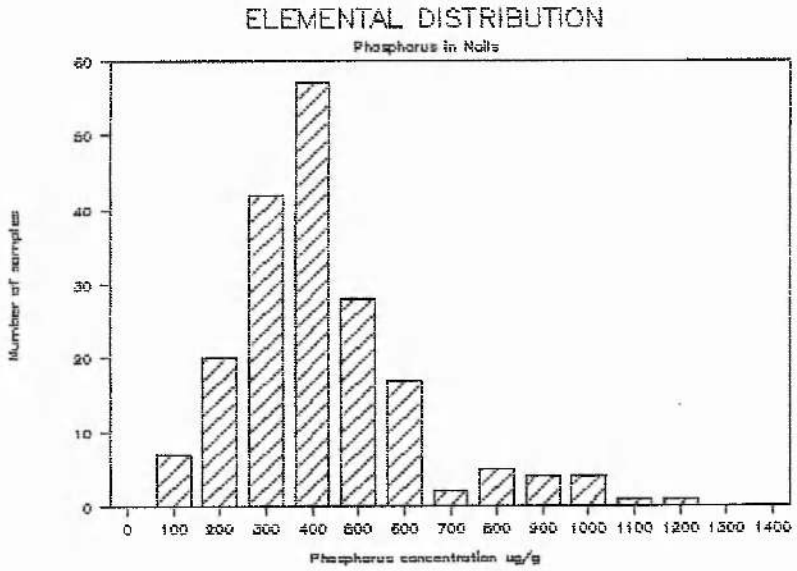
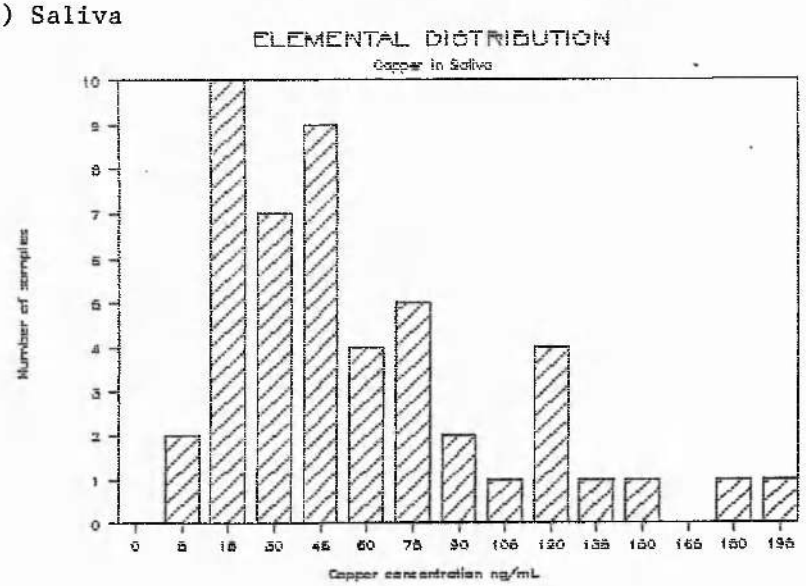
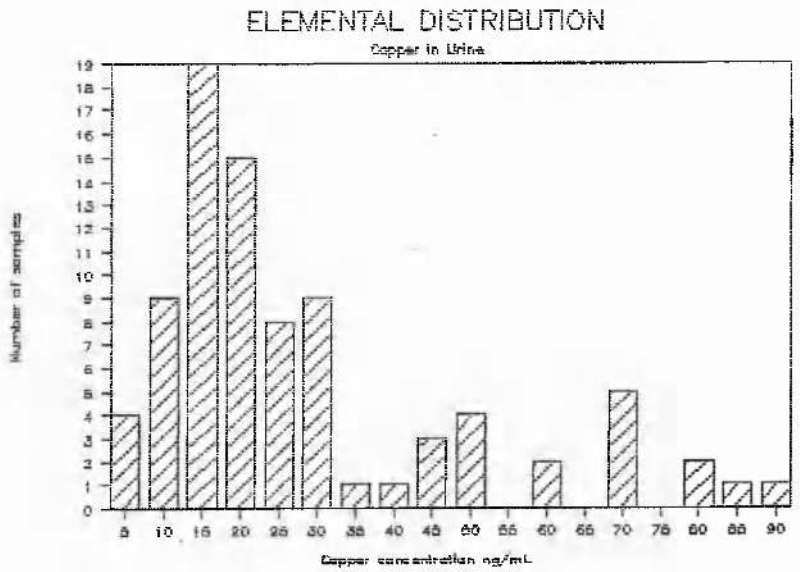


Figure 37: Frequency distributions of copper data

a) Saliva



b) Urine



c) Whole blood

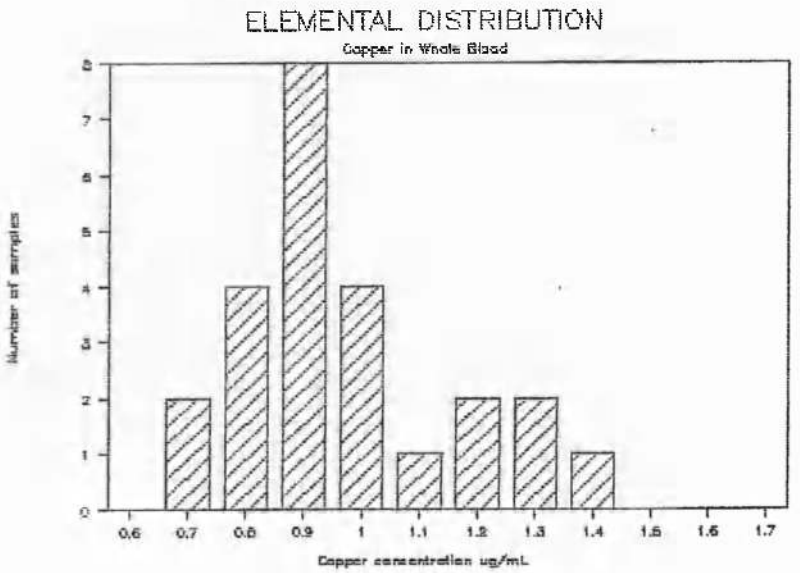
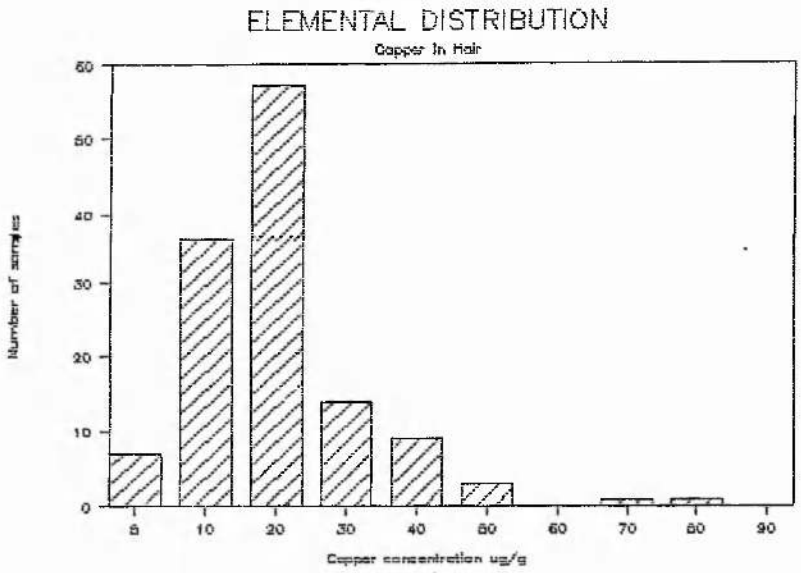


Figure 37 (con.): Frequency distributions of copper data

d) Hair



e) Nails

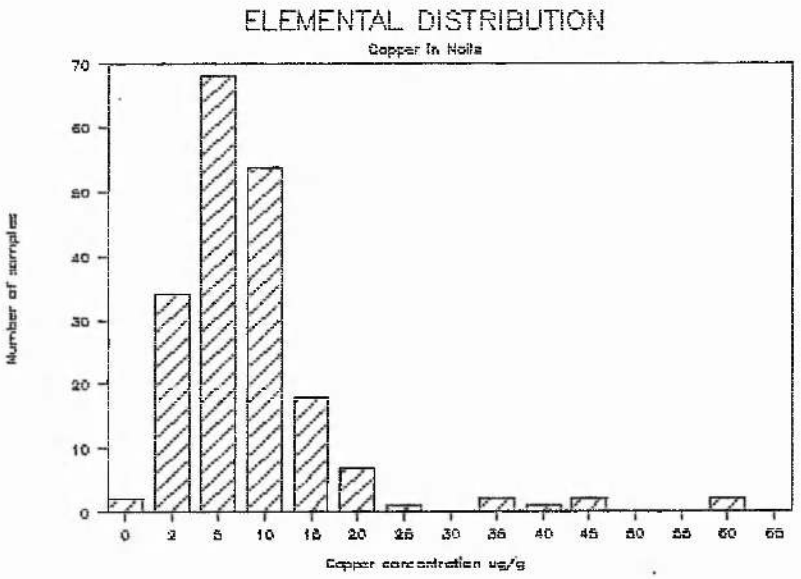
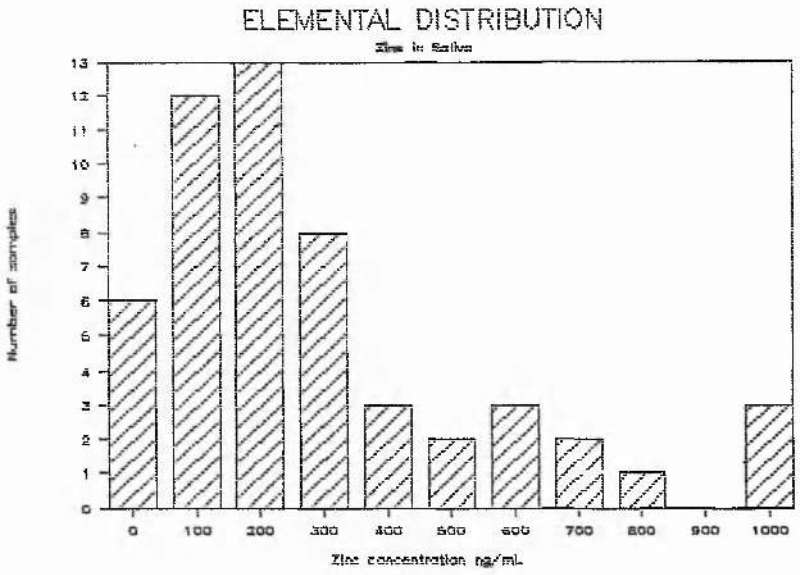
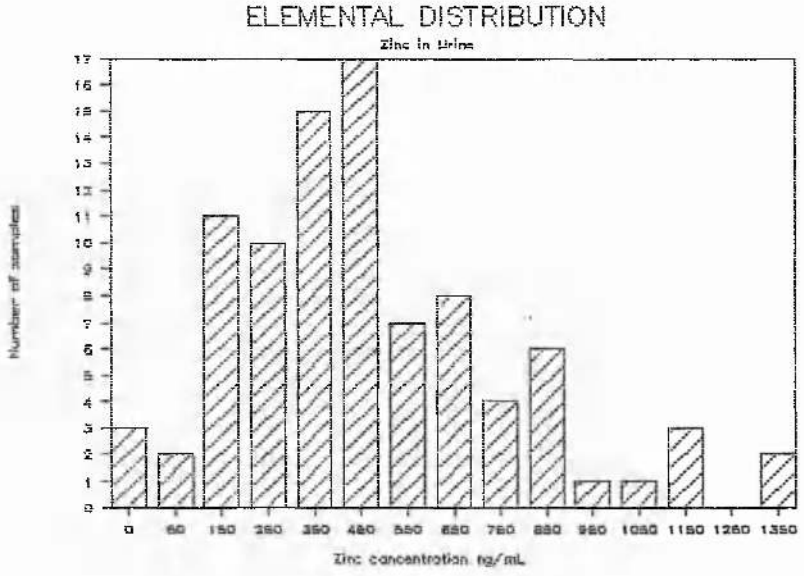


Figure 38: Frequency distributions of zinc data

a) Saliva



b) Urine



c) Whole blood

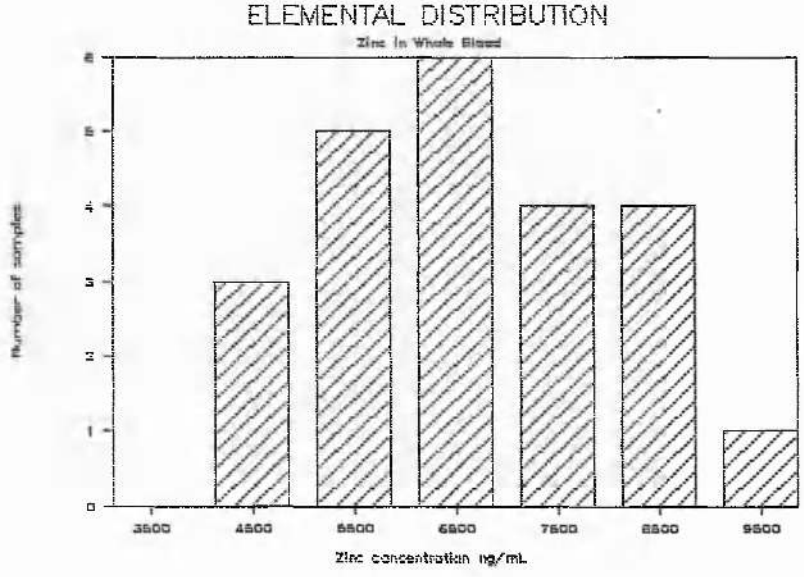
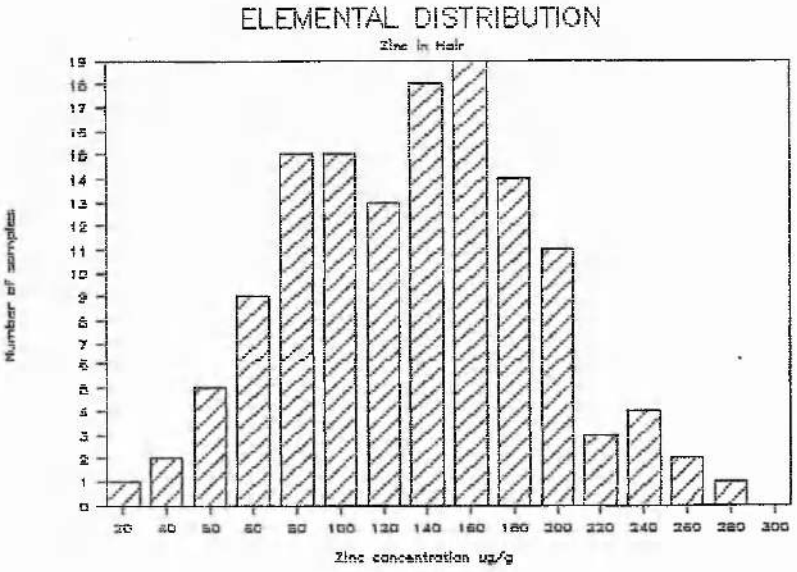


Figure 38 (con.): Frequency distributions of zinc data

d) Hair



e) Nails

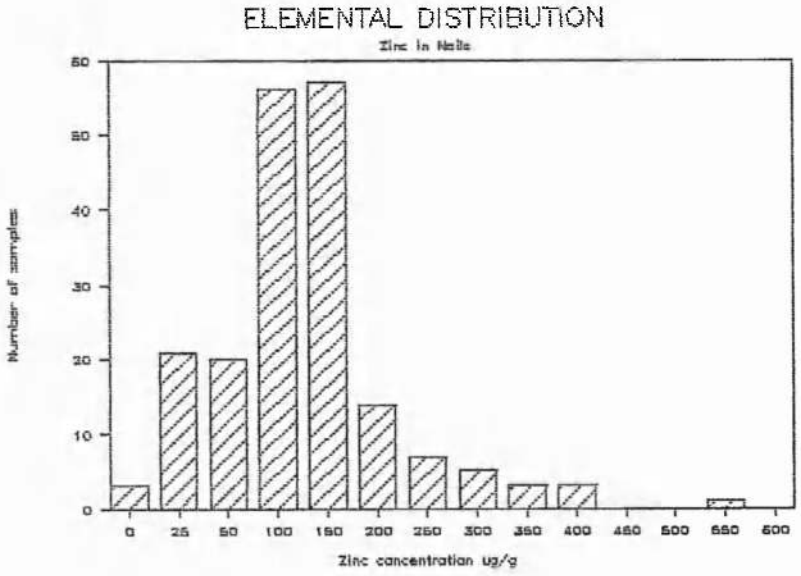
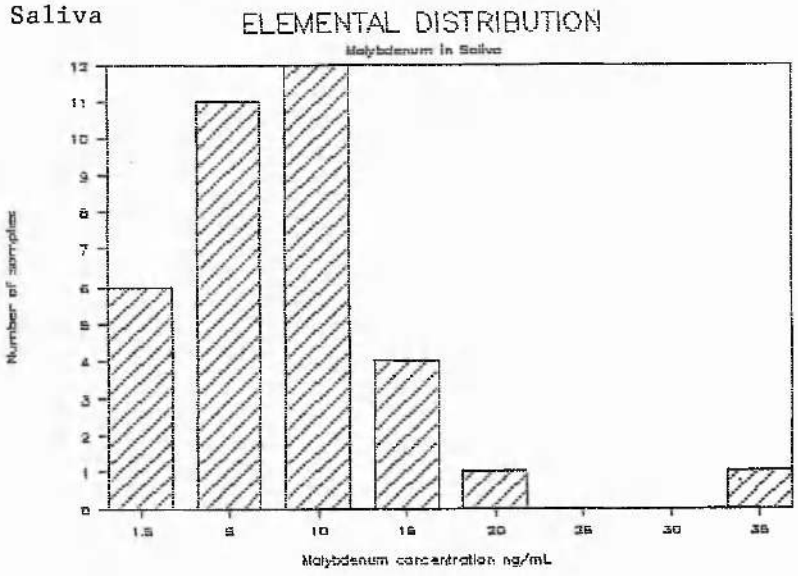
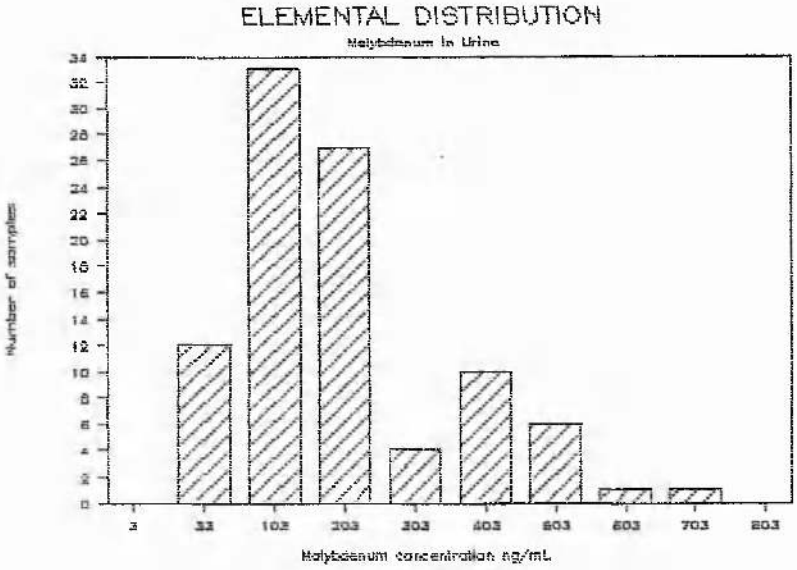


Figure 39: Frequency distributions of molybdenum data

a) Saliva



b) Urine



c) Whole blood

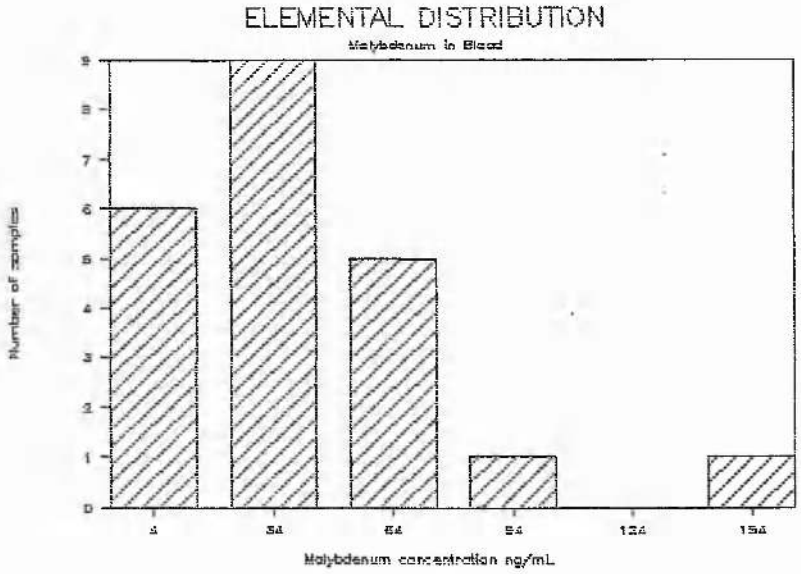
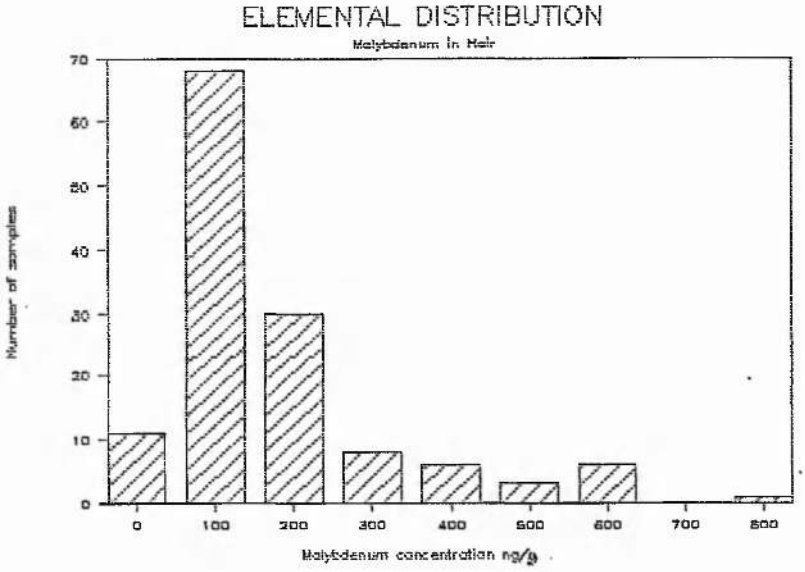


Figure 39 (con.): Frequency distributions of molybdenum data

d) Hair



e) Nails

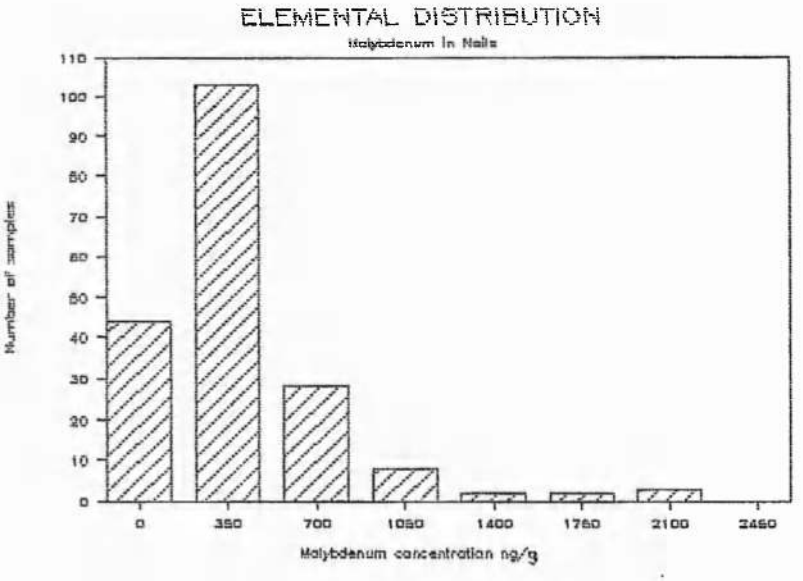
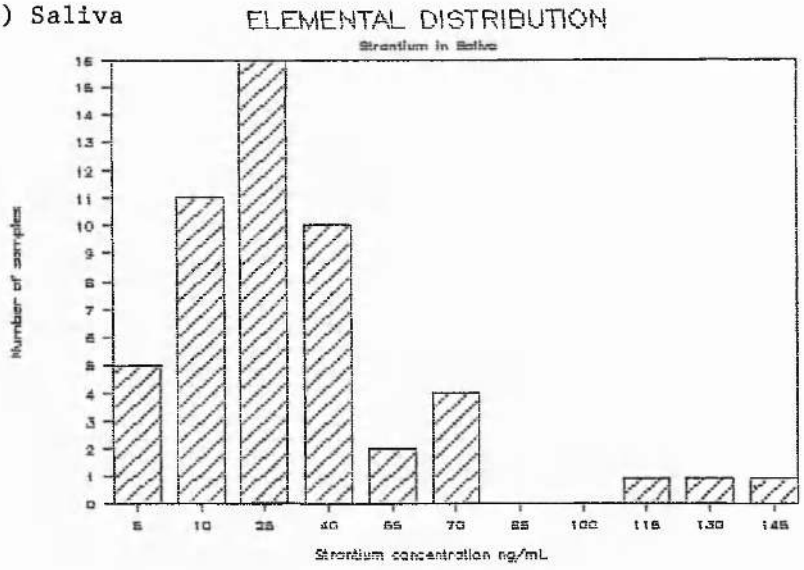
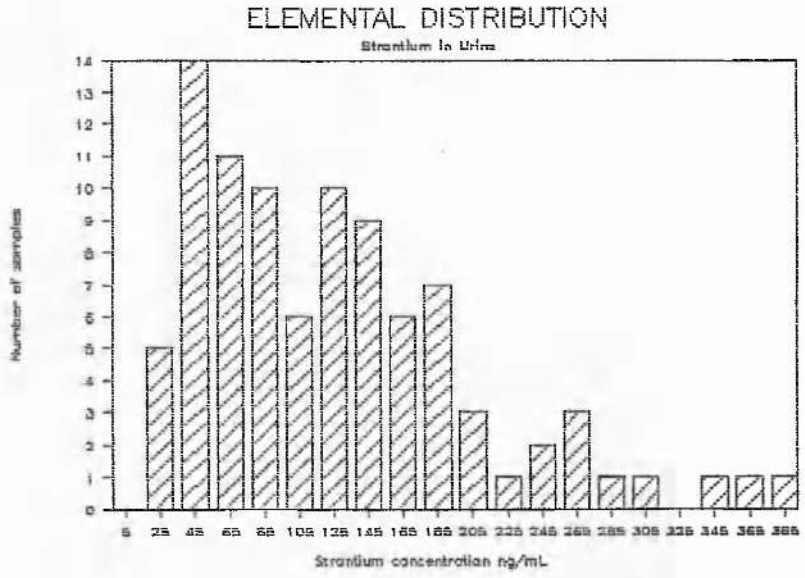


Figure 40: Frequency distributions of strontium data

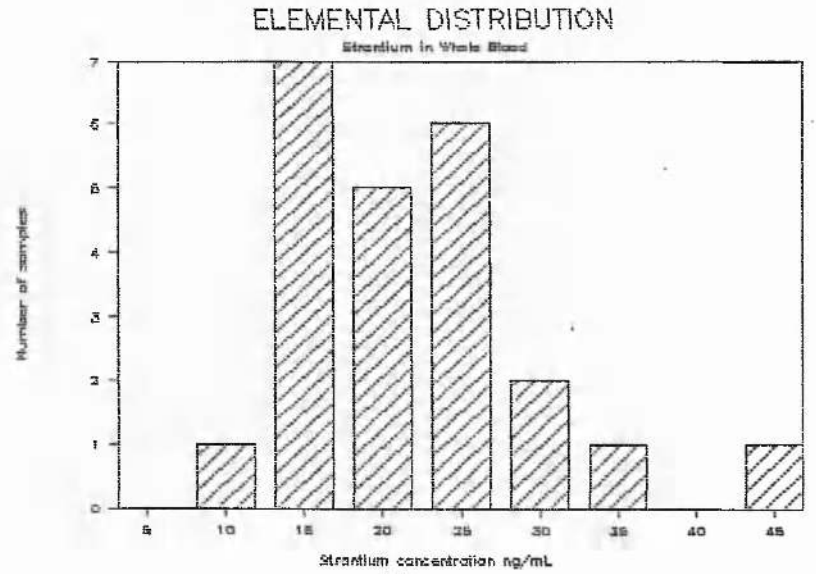
a) Saliva



b) Urine



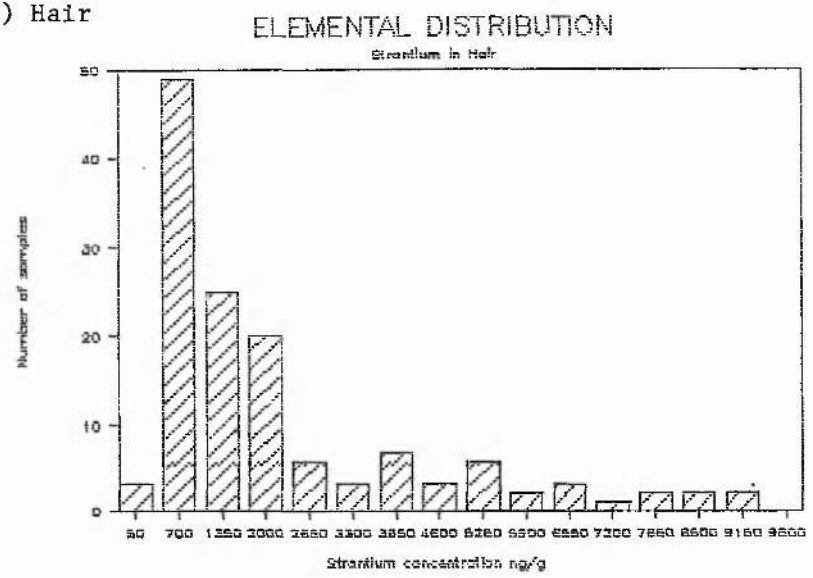
c) Whole blood



153

Figure 40 (con.): Frequency distributions of strontium data

d) Hair



e) Nails

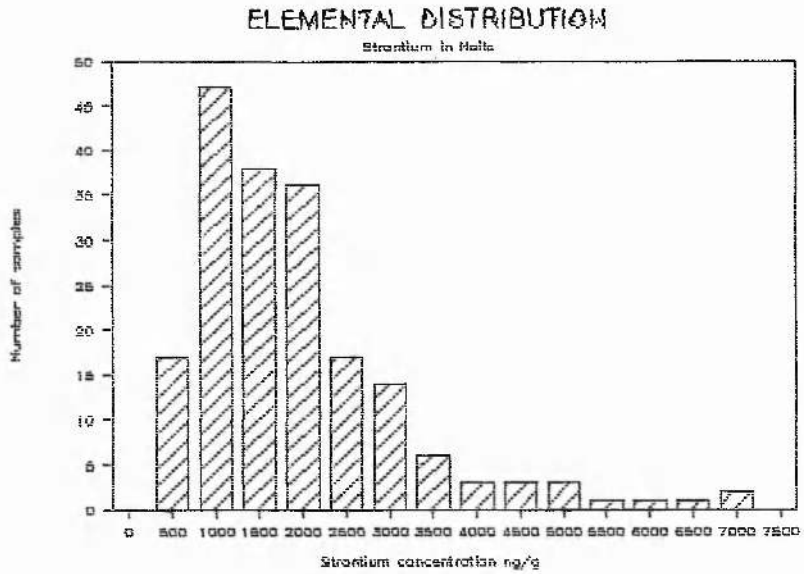
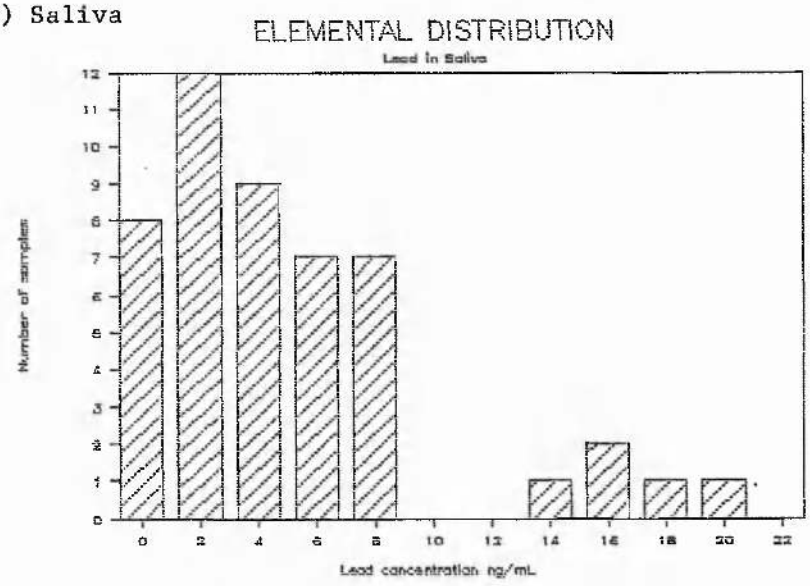
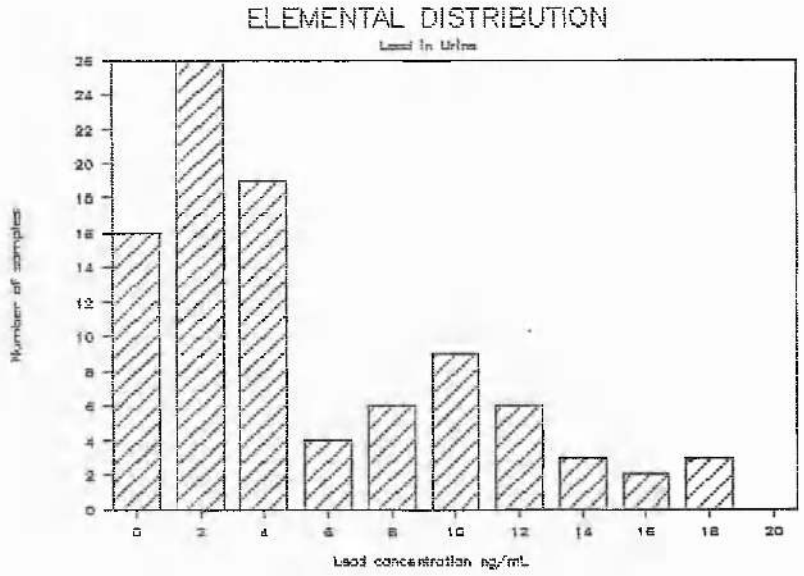


Figure 41: Frequency distributions of lead data

a) Saliva



b) Urine



c) Whole blood

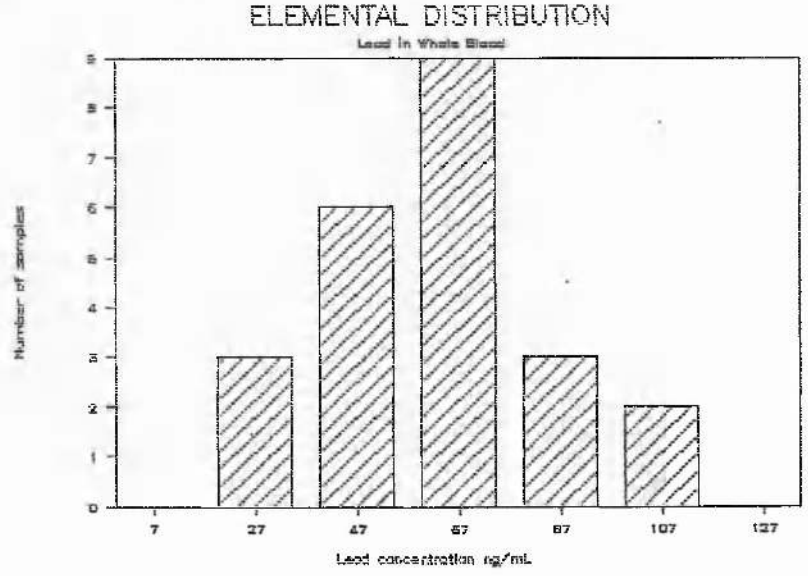
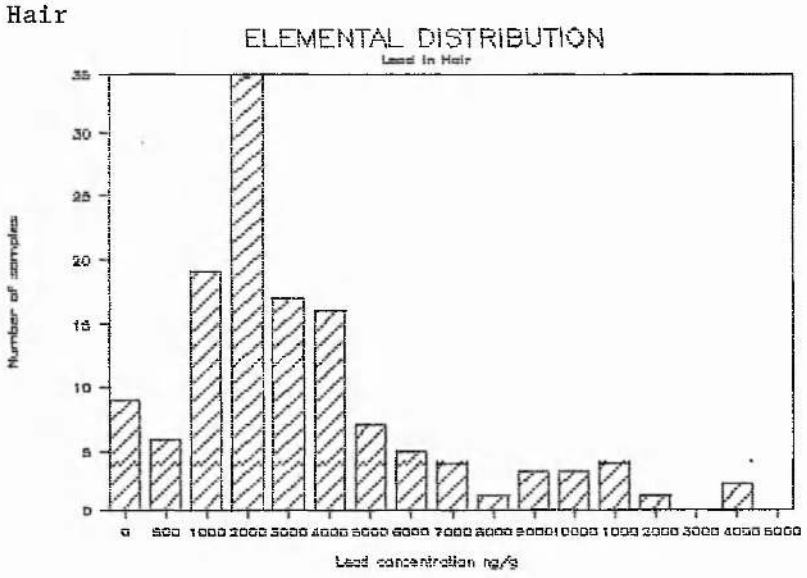
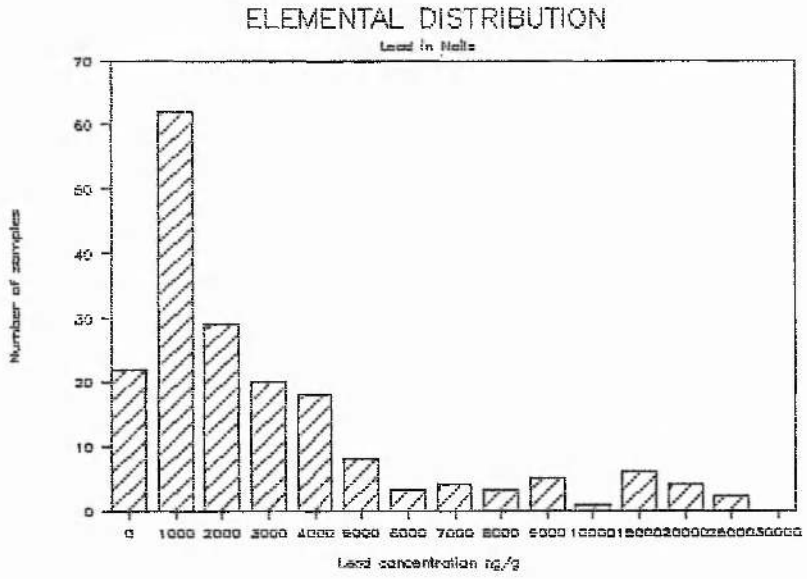


Figure 41 (con.): Frequency distributions of lead data

d) Hair



e) Nails



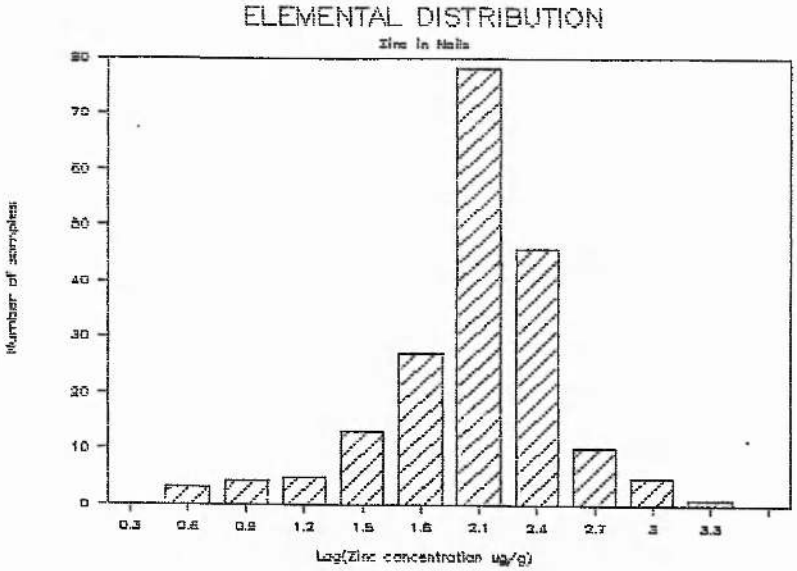
the element is essential (4). For the essential elements, an internal control mechanism has been postulated with a resulting symmetrical distribution, whereas the level of non-essential elements is completely dependant on the degree of contamination from the environment (4,5,145). Moreover, the type of tissue or fluid, and the role it plays within the body is obviously of critical importance. All elements in this study, essential, non-essential and toxic, demonstrate an approximately normal distribution in whole blood. The number of samples in this tissue was comparatively small, which gave rise to the lack of "smoothness" in some of the curves obtained. Moreover, in the case of molybdenum, a fraction of the samples were at or below the detection limit of ICP-MS for this element (under the conditions of sample volume and dilution used); thus the complete upward slope of the distribution was ill defined. As the body is thought to exert homeostatic control over essential elements, such as calcium and copper, the curves obtained followed the expected shape. The fact that similar distribution graphs should have resulted from the measured strontium and lead data is more surprising, since strontium has no known role within the body and lead is known to be toxic. This suggests that blood, in its role as a transport mechanism, carries the environmental contaminants to various organs, where they are stored. It is likely that the elemental levels in these tissues increase to produce a distribution which reflects the degree of environmental exposure (5). Moreover, a number of elements are

known to be associated with cells within the blood; for example, lead, mercury and cadmium with erythrocytes (145). These cells have a limited life; it is possible that the continuous formation and breakdown of cells would preclude any build-up within the blood of the elements attached.

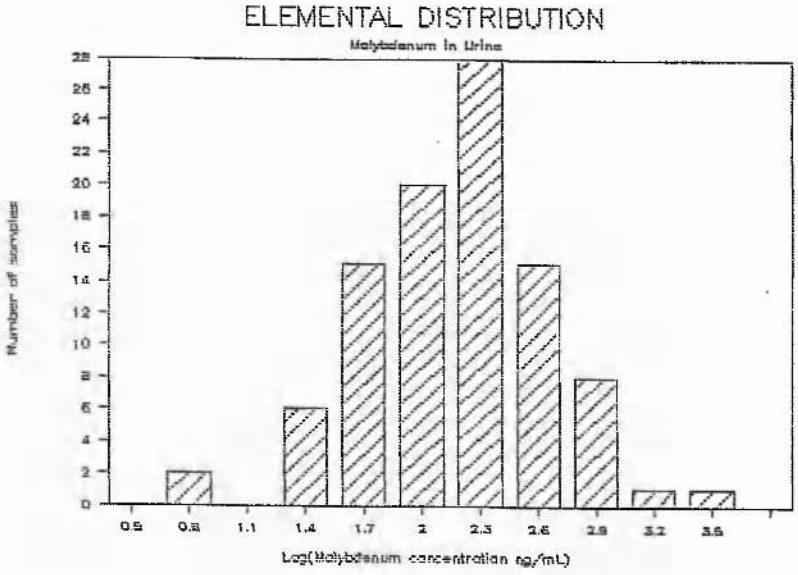
Most elements in the remaining sample matrices of saliva, urine, hair and nails demonstrate log-normal distributions. Figure 42 shows a few examples; they differ from Figures 35-41 in that the number of samples is plotted against the logarithm of the elemental concentrations (divided into ranges, as before). The exceptions to this are phosphorus and zinc in hair, and calcium in saliva. Little is known of the role of trace elements in saliva; previous published research has been confined mainly to specialised studies where fluoride or lithium levels required monitoring (159,160). Saliva is made up of mucous and enzymatic components (1,160); how elements are distributed between these two components is not known. The shape of the calcium distribution suggests that this element, unlike the others, is under homeostatic regulation in this fluid or that calcium has some specific role to play within saliva. The fact that the remaining elements display log-normal curves is not surprising; this matrix could be easily affected by a number of factors, including dental hygiene, dietary intake and environmental conditions such as dust.

Figure 42: Logarithmof frequency distributions

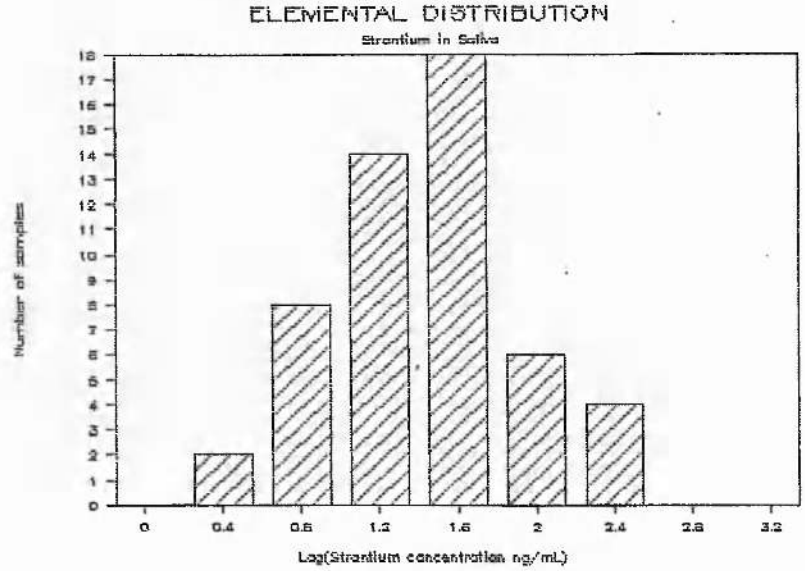
a) Zinc in nails



b) Molybdenum in urine



c) Strontium in saliva



The dietary intake of an individual also has a direct bearing on the elemental content of urine samples. The similarly skewed distributions obtained for all the elements is an indication of the variability of intake, absorption and excretion of nutrients and environmental contaminants. As the water content of urine is variable, it would be interesting to see if a 24-hour collection of urine would produce different results, particularly for the essential elements (171); unfortunately, this option was not available in this study.

Urine has a use in identifying some elemental deficiency states, for example, copper, selenium and zinc, where this depletion is reflected in a reduced urinary output (1). Similarly, high occupational exposure of elements such as arsenic, chromium, mercury, nickel, lead and thallium (149-152) can be revealed by an increased output. Urine is an excretory pathway, not a tissue where homeostatic regulation results in a well-defined, narrow concentration range.

Hair and nails also act as excretory pathways; as with urine samples, most elements display a log-normal pattern of distribution. Exceptions to this are the normal distributions of phosphorus and zinc in hair, this suggests an important part in the production and structure of hair. Interestingly, these findings were not replicated in nail tissue, despite the similarities in chemical structure. There is some conflict in the literature over the expected elemental data

distribution in hair (there is little published data on nails); some authors have reported that the homeostatic control of copper and iron within the body results in symmetrically shaped frequency distributions (145) while others have described log-normal curves for all trace elements except zinc (157). In this study, essential elements, such as copper, calcium and manganese were found to mirror the behaviour of non-essential elements in producing log-normal distributions. It can be assumed, therefore, that for most essential elements, hair and nails act only to a very small extent as storage tissues.

4.2.2. Elemental levels

In this section, the data obtained for saliva, urine, hair, nails, blood serum, whole blood, clot and packed cells are summarised in a series of tables (Tables 20 to 27). Ranges of values found in the literature are presented along with the measured concentrations, where available. In the previous section, most elements were found to follow log-normal frequency distributions in all samples except blood. Therefore, median values were quoted for elements in saliva, urine, hair and nails, while mean values with associated standard deviations were reported for whole blood and the blood fractions.

4.2.2.1. Saliva

Median elemental levels in this matrix tend to be rather low, with many values falling close to or below detection limits , as shown in Table 20. Despite the generally low levels, several samples were seen to have high elemental concentrations; this wide range of values is demonstrated by many elements, including boron, aluminium, zinc and tin, i.e. aluminium ranging from <0.6-2225 ng/mL . Saliva samples follow a log-normal frequency distribution of concentration; the exception being calcium, as described in Section 4.2.1.

It is interesting to compare saliva data with the measured values for serum. While most elements are found at considerably lower levels in saliva, phosphorus has approximately the same median concentration, 170µg/mL, in both fluids. The range of values is markedly different, however; in saliva, the range:mean ratio is seven times larger than in serum. Even in calcium, normally distributed in both fluids, the range:mean value is a factor of three greater in saliva. The levels of rubidium in saliva are five times higher than in serum (500 c.f. 100ng/mL), while caesium levels are twice as great (1.6 c.f.0.7ng/mL). This may be linked with the observation that the concentration of potassium in saliva also exceeds that in plasma (1); both rubidium and caesium have been shown to resemble potassium in its pattern of distribution and excretion (172,173). A relationship between these

Table 20: Elemental composition of saliva: ng/mL unless stated

	This Study		
	Median	Minimum —	Maximum
Li	0.7	<0.6 —	422.5
B	44.5	<13 —	4383
Mg[a]	4.48	1.23 —	18.35
Al	89.2	<0.6 —	2225
P[a]	169.8	30.46 —	719.7
Ca[a]	49.06	14.54 —	115.7
Sc	<0.2	<0.2 —	2.4
Fe	113	<1.5 —	2712
Mn	15.4	<0.2 —	160.6
Ni	10.3	<1.6 —	128.7
Co	<3.0	<3.0 —	29.7
Cu	39.78	2.44 —	659.7
Zn	189.0	<1.0 —	999.8
Ge	<1.1	<1.1 —	5.08
Rb	527.1	124.8 —	1210
Sr	17.3	1.6 —	1737
Mo	2.9	<1.5 —	37.9
Cd	0.8	<1.5 —	18.8
Sn	1.2	<0.9 —	183.9
Sb	<1.0	<1.0 —	11.4
Cs	1.6	<0.5 —	14.6
Ba	3.6	<1.5 —	788.5
La	<0.7	<0.7 —	78.6
Ce	<0.6	<0.6 —	5.1
Hg	<1.2	<1.2 —	20.6
Pb	3.2	<0.07 —	115.2

a: µg/mL

elements has been shown to exist for a variety of physiological processes (174,175). They can also act as nutritional substitutes in yeasts and bacteria (176,177) and to a lesser extent, and over a short term, in higher animals (178,179). Elements such as iron, copper and zinc display similar median values in saliva and urine; 40 and 20ng Cu/mL, 189 and 391ng Zn/mL respectively (comparing Tables 20 and 21). These elements are relatively poorly absorbed by the body, with 1,2 and 3% respectively being excreted in the urine (1).

4.2.2.2. Urine

The elemental levels in urine reflect, to a large extent, the dietary intake of various elements and the percentage of this intake which is absorbed: the data is displayed in Table 21. Reference values are given as comparisons; deviations have been described in the literature due to dietary deficiences, metabolic disorders or environmental exposure (1). Elements in urine tend to follow a log-normal frequency distribution, thus indicating a strong dependence on intake rather than stringent homeostatic control.

Median values of boron, molybdenum and rubidium are high in urine (1079, 108 and 811 ng/mL respectively) ; all these elements are well absorbed in the gut with only a small fraction of the amount ingested being excreted via the faeces. Cobalt, copper, iron, mercury,

Table 21: Elemental content of urine with literature comparison: ng/mL unless stated

	This Study			Literature[b]		
	Median	Minimum	Maximum	Minimum		Maximum
Li	15.2	<0.6	88.4	-		-
B	1079	163.8	5943	709	–	919
Mg[a]	60.43	0.16	376.1	48	–	200
Al	19.8	<0.6	1675	60		-
P[a]	488.1	30.3	2045	24	–	621
Ca[a]	65.88	2.01	422.4	55	–	164
Sc	1.5	<0.2	7.5	-		-
Fe	130	30	3840	180		-
Mn	1.7	<0.1	132.8	3	–	300
Ni	9.1	<1.6	736.9	2300	–	8.5
Co	0.4	<0.4	20.6	98		-
Cu	20.3	<2.0	144.2	17	–	300
Zn	391.0	<5.0	1938	193	-	2040
Ge	1.0	<1.1	4.9	-		-
Rb	810.7	<0.6	2375	941	-	1520
Sr	108.6	5.04	464.7	100		-
Mo	107.6	3.36	803.4	10	–	10
Cd	0.5	<1.6	17.9	0.64	–	8.9
Sn	2.0	<1.0	21.8	13		-
Sb	0.3	<0.8	6.6	-		-
Cs	5.4	0.5	16.4	80		-
Ba	1.8	<1.5	731.5	89		-
La	<0.1	<0.1	6.4	-		-
Ce	<0.1	<0.1	1.4	-		-
Hg	1.2	<1.6	15.2	4.3	–	114
Pb	2.7	<0.5	29.4	6.3	–	110

a: µg/mL
b: Iyengar 1978 (133)

strontium and zinc fall into the category of "medium" absorption. These elements are found at lower levels in the urine than those mentioned above, taking into account their relative concentrations within the body, and also compared with their levels in some other biological fluids. For example, copper has a median urine level of 20ng/mL which is two orders of magnitude lower than in serum. Other elements, such as cadmium, aluminium, manganese, nickel and tin, are very poorly absorbed by the gut (median levels of 0.5, 19.8, 1.7, 9.1, and 2ng/mL respectively) - the large unabsorbed portion appears in the faeces (1). The rare earth elements of lanthanum and cerium also have low urine concentrations(both <0.1ng/mL), suggesting that the same pattern of poor absorption and faecal excretion is followed. Wide ranges are seen, as in saliva, with a few examples of unexpectedly high values e.g. aluminium, 1.7μg/mL, and nickel, 2.3μg/mL. Such sample values probably give some indication of environmental exposure.

4.2.2.3. Hair and nails

As is usual with solid biological matrices, trace elements are found at higher levels here than in most fluids; see Tables 22 and 23. Concentrations in hair and nails may reflect the level of intestinal absorption in some situations or the accumulation within the body (145). Trace elements may leave the body via nails and hair along with

Table 22: Elemental content of scalp hair with literature comparison: ng/g unless stated

	This Study			Literature[b]		
	Median	Minimum	Maximum	Minimum		Maximum
Li	95.1	<0.6	3192	-		-
B	827	<13	23621	5000		-
Mg[a]	31.7	5.2	775	19	-	163
Al[a]	7.2	0.6	50.1	4.2	-	29.3
P[a]	121	5	512	83	-	165
Ca[a]	431	46.6	7206	146	-	3190
Sc	1.3	<0.2	129	-		-
V	33.5	<1.5	429	5	-	70
Cr	228	<1.4	3676	130	-	3650
Fe[a]	10.2	4.2	153.5	5	-	44.7
Mn	323	52.4	3066	250	-	5700
Co	16.2	<3.0	407	30	-	1050
Ni	441	<1.6	8763	600	-	6500
Cu[a]	13.4	1.4	203	11	-	34
Zn[a]	130	22.1	319	99	-	450
Ge	3.2	<1.1	108	2300		-
Rb	4.7	<0.6	236	210	-	500
Sr	1048	20.7	9079	46	-	920
Mo	88.7	<1.0	1110	64	-	205
Cd	185	<1.6	5918	240	-	2700
Sn	301	<1.0	8180	1000		-
Sb	79.1	<0.8	460	90	-	3000
Cs	1.2	<0.5	103	370	-	1100
Ba	399	<1.5	8740	550	-	4200
La	13.9	<0.1	3155	150	-	650
Ce	15.1	0.3	1823	-		-
Pb	1969	50	18780	3000	-	25400

a: µg/g
b: Iyengar 1978 (133)

Table 23: Elemental content of nails with literature comparison: ng/g unless stated

	This Study			Literature[b]	
	Median	Minimum	Maximum	Minimum	Maximum
Li	753	<0.6	8299	-	-
B[a]	12.27	<0.01	82.74	33	0
Mg[a]	137	20.8	467	16.2	125
Al[a]	36.6	<0.6	244	132	927
P[a]	340	45.8	3784	180	990
Ca[a]	1044	31.6	3459	368	3400
Sc	9.7	<0.2	969	-	-
V	86.6	<1.5	1422	<150	-
Cr	708	<1.3	7002	6200	-
Fe[a]	36.9	6.2	287	27	347
Mn	993	138	8383	40	2100
Ni	591	<2	10000	33	11900
Co	38	<3	1586	<200	-
Cu[a]	4.7	0.3	130	11.2	53
Zn[a]	97	2.5	922	73	304
Ge	6.7	<1.1	251	-	-
Rb	70.8	<0.7	1750	3100	-
Sr	1408	88.4	28060	17	650
Mo	121	<1.5	5794	<150	5600
Cd	131	<1.5	4802	80	3400
Sn	486	<1.0	7340	12000	-
Sb	146	<0.8	6133	<30	750
Cs	5.2	<0.5	14710	6600	-
Ba	1518	139	35089	7500	-
La	64.7	4.1	683	310	-
Ce	111	4.3	1319	600	-
Pb	1557	<1.0	116	13800	39000

a: µg/g
b: Iyengar 1978 (133)

other routes, such as sweat and sebum. Wide concentration ranges of elements are seen, including many detection limit values, with log-normal frequency distributions seen for most trace metals (exceptions being zinc and phosphorus).

Zinc, phosphorus, calcium and magnesium are found at substantial levels in both nails and hair. Zinc is higher in hair, whereas the latter three elements are at greater concentrations in nails. Several other elements are generally found at microgram per gram levels or above, for example copper, aluminium and iron, followed by boron and lead. Several of these are elevated in the nails compared to the hair. This is probably because nail has a greater density than hair. Nails were also more difficult to clean than hair; acetone was used to ensure that all traces of lacquer were removed, as well as residual grease, but some impurities may have been engrained. Rubidium is unusual in that it is found at lower levels (medians of 4.7 and 71ng/g for hair and nails) within these two solid tissues than in most biological fluids, such as blood and saliva (1.2 and 0.5µg/mL respectively). Despite being within the literature range quoted, manganese levels are possibly slightly elevated due to extraneous additions from the scissors or clippers used, although it is hoped that the washing regime removed such effects.

4.2.2.4. Blood and blood fractions

Whole blood and its fractions of serum, clot and packed cells were analysed; the data is presented in Tables 24 to 27. As most elements follow an approximately normal distribution, the results of analysis by ICP-MS in this study are presented as the arithmetic mean, with the standard deviation in parentheses. Most elemental concentrations are in good agreement with the literature (no values for packed cells or clots were readily available).

For manganese, however, a value of 3.8 ng/mL in serum was found. Some recent studies suggest that the "true" value for this element is in the order of 0.6 ng/mL. This variation may be due to contamination of the blood by the manganese content of the stainless steel needle. It is possible that whole blood and the remaining products show similarly elevated values, although as the reference data are poorly defined, the measurements fall within the expected range. Another possibility is the rupturing of red blood cells during sample collection with "leakage" from the red cells into the serum, since most literature sources report a higher level of manganese in red blood cells than in serum (145,180-2). The element is almost totally linked with a β_1-globulin in plasma, and forms a firmly bound porphyrin compound in red cells (183-5). Other elements which show some variation in their values are aluminium and barium. In

Table 24: Mean elemental content of blood serum with literature comparison: ng/mL unless stated

	This Study		Literature[c]
	Mean(s.d.[a])	Minimum — Maximum	Minimum — Maximum
B	201(127)	45.5 — 460	0.12 — -
Mg[b]	19.7(2.9)	14.73 — 25.80	19.9 — 27.5.
Al	37(21)	5.8 — 82.9	2.1 — 10
P[b]	168(25)	124 — 222	115 — 163
Ca[b]	119(20)	91.5 — 169	92 — 109
Sc	33(16)	7.3 — 68.6	<1 — -
Fe[b]	2.1(0.6)	0.88 — 3.1	0.8 — 1.87
Mn	3.8(1.6)	1.2 — 6.5	0.5 — 1
Ni	13.3(3.9)	6.4 — 21.1	0.4 — 2
Co	0.4(0.3)	<0.4 — 1.2	0.11 — 8
Cu[b]	1.3(0.4)	0.65 — 2.2	0.815 — 1.64
Zn[b]	0.95(0.23)	0.52 — 1.45	0.67 — 1.83
Ge	1.2(0.7)	<1.1 — 2.6	<4 — 290
Rb	113(21)	81.5 — 152	40 — 580
Sr	38(17)	14.8 — 67.6	20 — 46
Mo	5(3)	<2 — 11.5	0.6 — 27
Cd	0.3(0.3)	<0.2 — 0.8	0.1 — 12
Sn	7(8)	<1 — 19.9	1 — 30
Sb	0.1(0.1)	<0.1 — 0.5	<0.3 — 3.2
Cs	0.7(0.3)	0.2 — 1.2	0.74 — 1.3
Ba	207(60)	91.4 — 296	25 — 80
La	2.3(1.3)	0.9 — 4.0	5.5 — -
Ce	44(18)	15.4 — 66.5	-
Hg	0.3(0.5)	<0.3 — 1.5	1.3 — 3
Pb	2.3(1.2)	0.3 — 4.0	<1 — 5.4

a: standard deviation
b: µg/mL
c: Iyengar 1978 (133)

Table 25: Mean elemental content of whole blood with literature comparison: ng/mL unless stated

	This Study			Literature[c]		
	Mean(s.d.[a])	Minimum	Maximum	Minimum		Maximum
B	133(106)	<26	511	40	–	740
Mg[b]	29.5(9.7)	16.1	57.7	27.1	–	45.5
Al	348(600)	<2	3987	2	–	720
P[b]	335(127)	160	707	311	–	510
Ca[b]	61(17)	39	105	57.5	–	78
Sc	26(16)	0.5	81.2	7.6		-
Fe[b]	315(72)	200	417	301	–	530
Mn	41(39)	<0.5	198	1.6	–	75
Ni	295(254)	<3	864	4.8	–	106
Co	8(34)	<6	68	0.3	–	9.9
Cu	663(300)	220	1390	640	–	1280
Zn[b]	4.4(2.7)	0.6	10.5	4.8	–	9.3
Ge	20(22)	1.4	77	440	–	5000
Rb[b]	1.2(0.3)	0.6	1.9	1.2	–	6.0
Sr	19(10)	4.9	49	16	–	95
Mo	50(80)	<3	418	1	–	75
Cd	7(6)	<3	24	1	–	7.4
Sn	13(18)	<2	74	8.5	–	290
Sb	16(30)	<2	129	1.2	–	4.7
Cs	3(2)	0.2	9	3	–	4.7
Ba	30(32)	<3	96	41	–	95
La	2(4)	<0.5	17	-		-
Ce	7(6)	<0.4	18	<2		-
Hg	15(17)	<2	78	5	–	20
Pb	43(33)	0.6	150	50	–	400

a: standard deviation
b: µg/mL
c: Iyengar 1978 (133)

Table 26: Mean elemental content of clot: ng/g (fresh weight) unless stated

	Mean(s.d.[a])	Minimum – Maximum
B	283(71)	159 – 376
Mg[b]	48.8(4.5)	39.3 – 54.0
Al	88(46)	<1.2 – 147
P[b]	511(47)	427 – 588
Ca[b]	66(15)	39.5 – 85.8
Sc	54(32)	3.5 – 107
Fe[b]	614(42)	549 – 662
Mn	20(12)	<0.3 – 42
Ni	11(18)	<3 – 49
Co	0.9(1.3)	<0.9 – 3.4
Cu[b]	0.73(0.11)	0.55 – 0.94
Zn[b]	10.6(0.8)	8.5 – 11.7
Ge	102(18)	74 – 133
Rb[b]	2.5(0.3)	2.2 – 3.3
Sr	16.9(3.6)	11.7 – 24.5
Mo	7(8)	<3 – 18.3
Cd	1.8(2.0)	<1.6 – 5.2
Sn	7(11)	<2 – 28.2
Sb	2.6(4.3)	<1.5 – 10.5
Cs	4.5(1.4)	1.5 – 7.1
Ba	205(29)	170 – 252
La	7.4(4.3)	1.5 – 13.6
Ce	71(18)	40 – 99
Pb	167(46)	96.4 – 246

a: standard deviation
b: µg/g

Table 27: Mean elemental content of packed cells: ng/g (fresh weight) unless stated

	Mean(s.d.[a])	Minimum — Maximum
B	186(37)	122 — 238
Mg[a]	41.3(9.3)	24.1 — 54.1
P[a]	475(107)	283 — 581
Ca[a]	48.4(6.8)	36.8 — 58.1
Sc	12(8)	4 — 29
Fe[a]	458(87)	289 — 535
Mn	20(30)	<0.3 — 75
Ni	115(183)	<3 — 454
Co	1.3(2.0)	<0.9 — 5.2
Cu[a]	0.36(0.08)	0.26 — 0.48
Zn[a]	6.6(1.9)	3.4 — 8.5
Ge	11(4)	6 — 18
Rb[a]	1.3(0.3)	0.7 — 1.7
Sr	14(10)	0.6 — 31
Mo	74(104)	<3 — 296
Cd	0.7(1.7)	<0.5 — 5.0
Sn	0.7(1.3)	<0.6 — 3.8
Sb	11(10)	<0.9 — 30
Cs	3.0(0.5)	2.4 — 3.7
Ba	62(18)	35 — 95
La	1.8(0.9)	<0.5 — 3.1
Ce	28(6)	21 — 43
Hg	2(2)	<1.7 — 5
Pb	67(39)	13 — 128

a: standard deviation
b: µg/g

packed cells, the levels of aluminium could not be detected in any samples, while in whole blood, in particular, there are some extremely high values i.e. a maximum of 4µg/mL. Barium appears to be at high mean levels in serum and in the clot, compared to the other fluids (207 and 205ng/mL or g, in serum and clot, with whole blood and packed cells of 30 and 62ng/mL or g). As the clot and serum were from the same aliquot of blood, this is probably due to some sample mis-handling during separation stages i.e. the possible use of a glass pipette or container by clinical staff (see section 5.2.3.2.).

Copper is unusual among trace elements at being found at greater quantities in serum than in erythrocytes, and thus in whole blood (2,145). These results are confirmed by the data shown in Tables 24 to 27 (1.3µg/ml in serum compared to an average value of 0.6µg/mL for the remainder). A large proportion of copper is bound to the globulin protein ceruloplasmin, which is critical in the mobilisation of iron, particularly from reticuloendithelial systems and/or the liver (186). Copper is also attached to the plasma protein albumin, by which it can readily pass to erythrocytes (187). The great difference in iron concentration between serum and whole blood, clot and packed cells is due to the occurrence of iron in haemoglobin in the red blood cells and as transferrin in plasma, in a ratio of nearly 1000:1. A very small fraction of iron, as ferritin, is also found in red and white blood cells and plasma. Approximately 80% of blood zinc

is bound to erythrocytes, with about 17% in plasma, and the remaining fraction in leukocytes and platelets (188). The measured values of zinc reported in Tables 24-7 reflect this fact; the clot and packed cells have the highest measured zinc (11μg/g in the clot c.f. 0.9μg/mL in serum). In plasma, 30-40% of zinc is firmly bound to an α_2-macroglobulin, with 60-70% loosely bound to the albumin (189).

Many other elements were found to be at higher levels in whole blood, clot or packed cells than in serum. These include cadmium, mercury, lead, cobalt and rubidium; literature findings describing a greater affinity for red blood cells were therefore confirmed (146,172-3,190). For example, lead and rubidium were found to have mean values of 0.3 and 113ng/mL in serum and 43 and 2500ng/mL in whole blood, respectively. Some authors have suggested that plasma lead has a ceiling value, possibly related to the binding capacity of a low molecular weight protein or polypeptide of the serum, and that erythrocytes represent a large repository for lead, maintaining plasma lead concentrations between narrow limits (191,192). The comparatively high values of molybdenum in the whole blood and other cellular components, compared to serum, were rather unexpected. This element is thought to be evenly distributed between red blood cells and plasma (145,193). It has been suggested that it is transported in blood and urine as the molybdate ion (MoO_4^{2-}) (194). Caesium is found at approximately 6.5 times higher levels in packed cells than in serum

(77); these findings were confirmed in this study, with mean values of 3 and 0.7ng/mL respectively.

4.2.3. Distribution of elements in human clinical specimens

4.2.3.1. Variations in tissues and fluids

Figure 43 (a-g) demonstrates graphically how elemental levels differ from one biological tissue/fluid to another. Where possible, elements in the same group are listed together e.g. calcium, magnesium, barium and strontium, from Group IIA, and transition metals, for example, copper, zinc and iron.

It is possible to identify a few similarities in behaviour. For example, rubidium and caesium are quite alike in distribution among fluid samples, despite the difference of some orders of magnitude (1200ng Rb/mL c.f. 3.2ng Cs/mL in whole blood). They appear to behave differently in the nails and hair; rubidium is particularly low in hair, while caesium follows the more established pattern of moderately elevated levels in solid tissues. Strontium follows the pattern of calcium quite closely, particularly in being higher in serum than in the remaining blood samples. Strontium has demonstrated some degree of interaction with calcium; many research articles have concentrated on its retention in bone with particular reference to the abundant ^{90}Sr

Figure 43: Range of elemental levels in various human specimens

a) Rb, Cs.

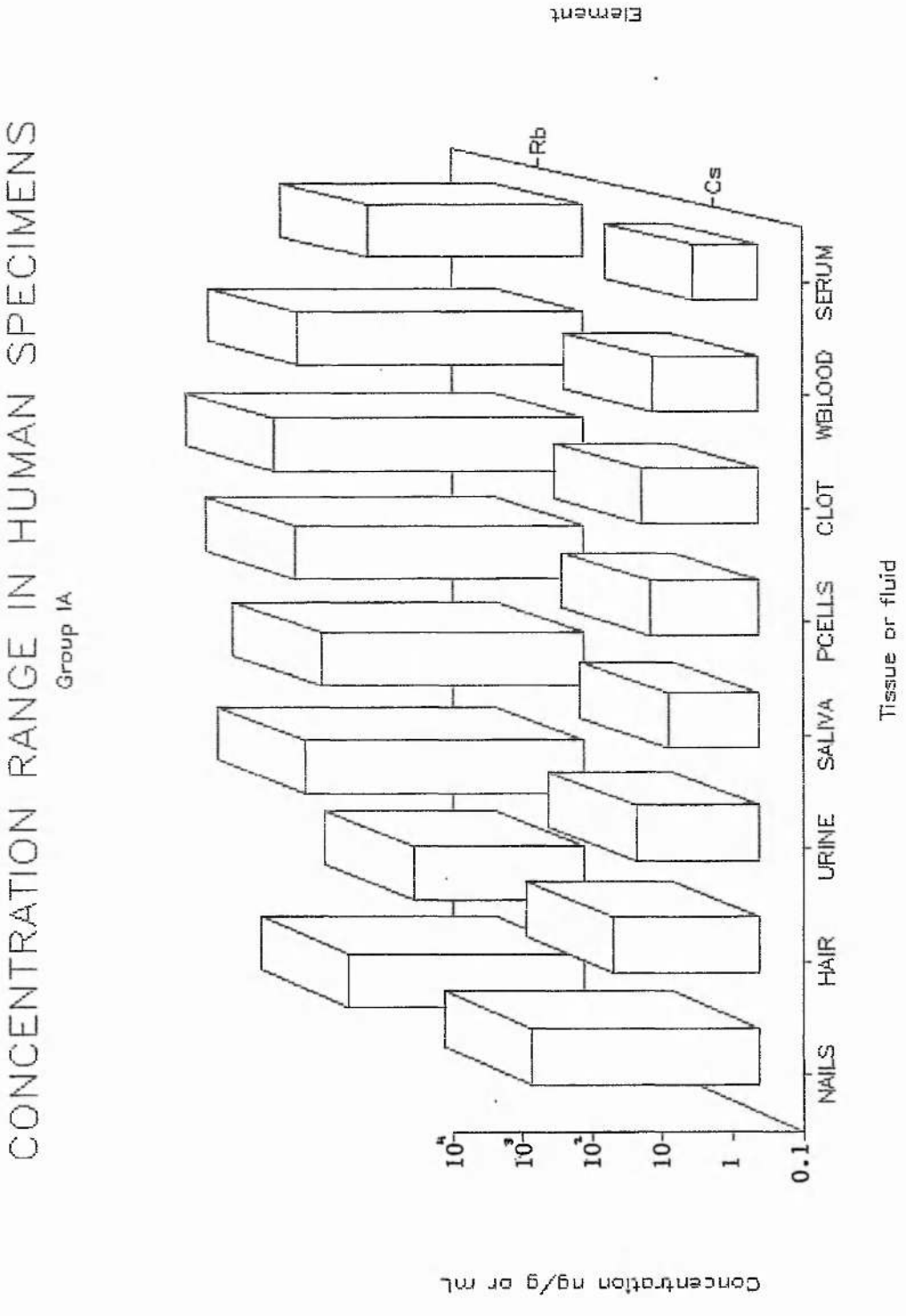


Figure 43 (con.): Range of elemental levels in various human specimens

b) Ca, Mg, Ba, Sr.

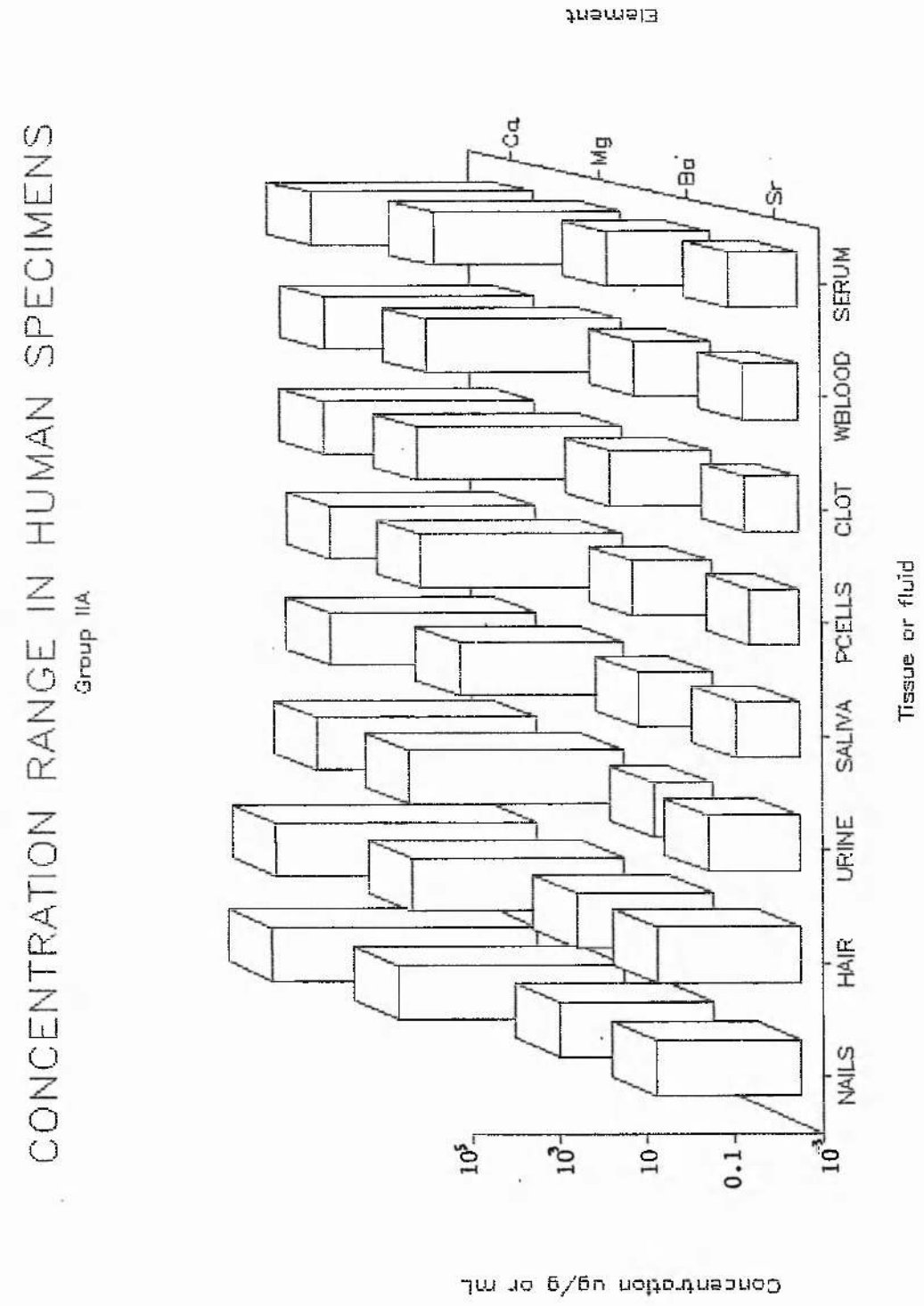


Figure 43 (con.): Range of elemental levels in various human specimens

c) P, B, Sb.

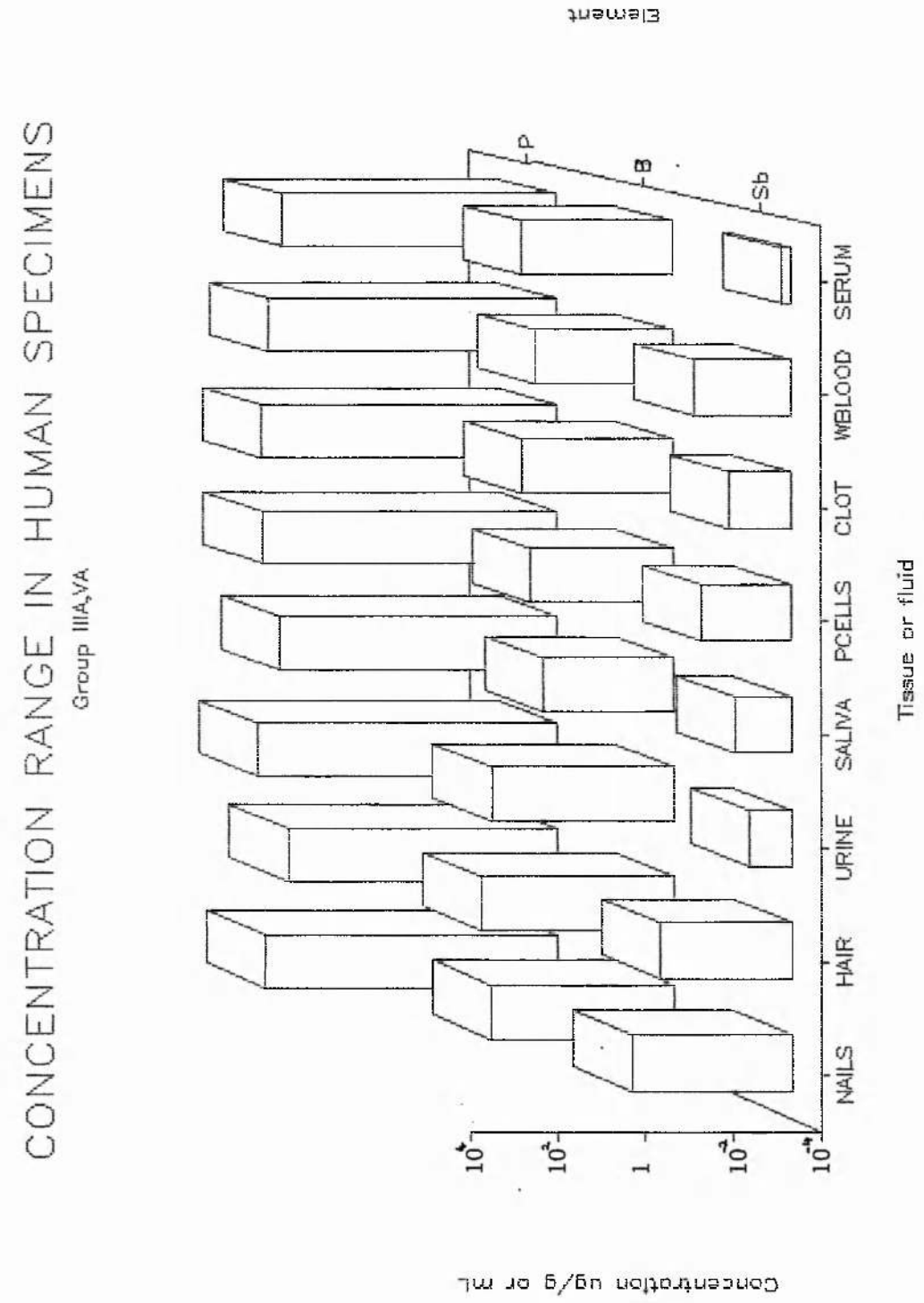


Figure 43 (con.): Range of elemental levels in various human specimens

d) Pb, Ge, Sn.

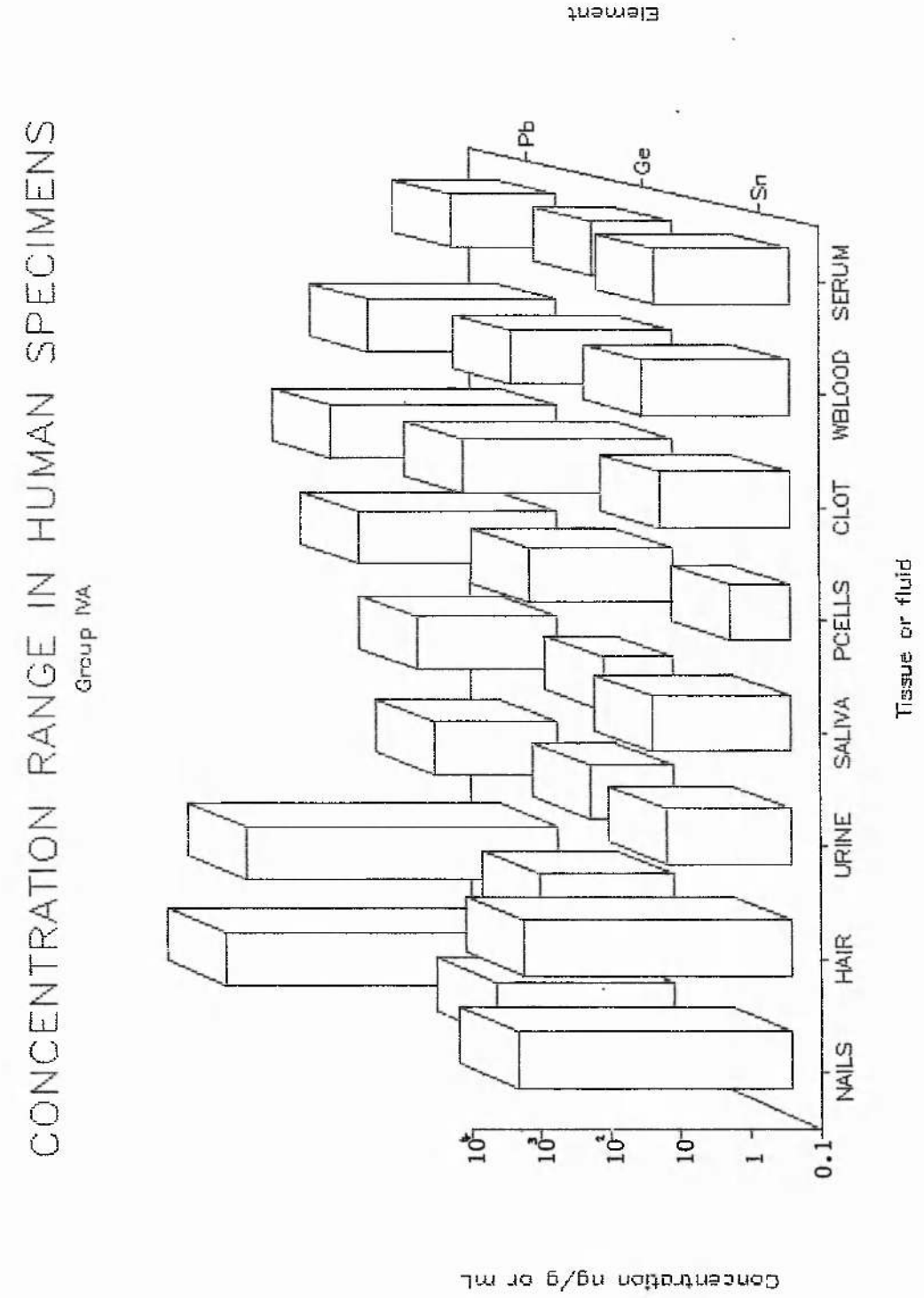


Figure 43 (con.): Range of elemental levels in various human specimens

e) Mn, Co, Ni, Sc.

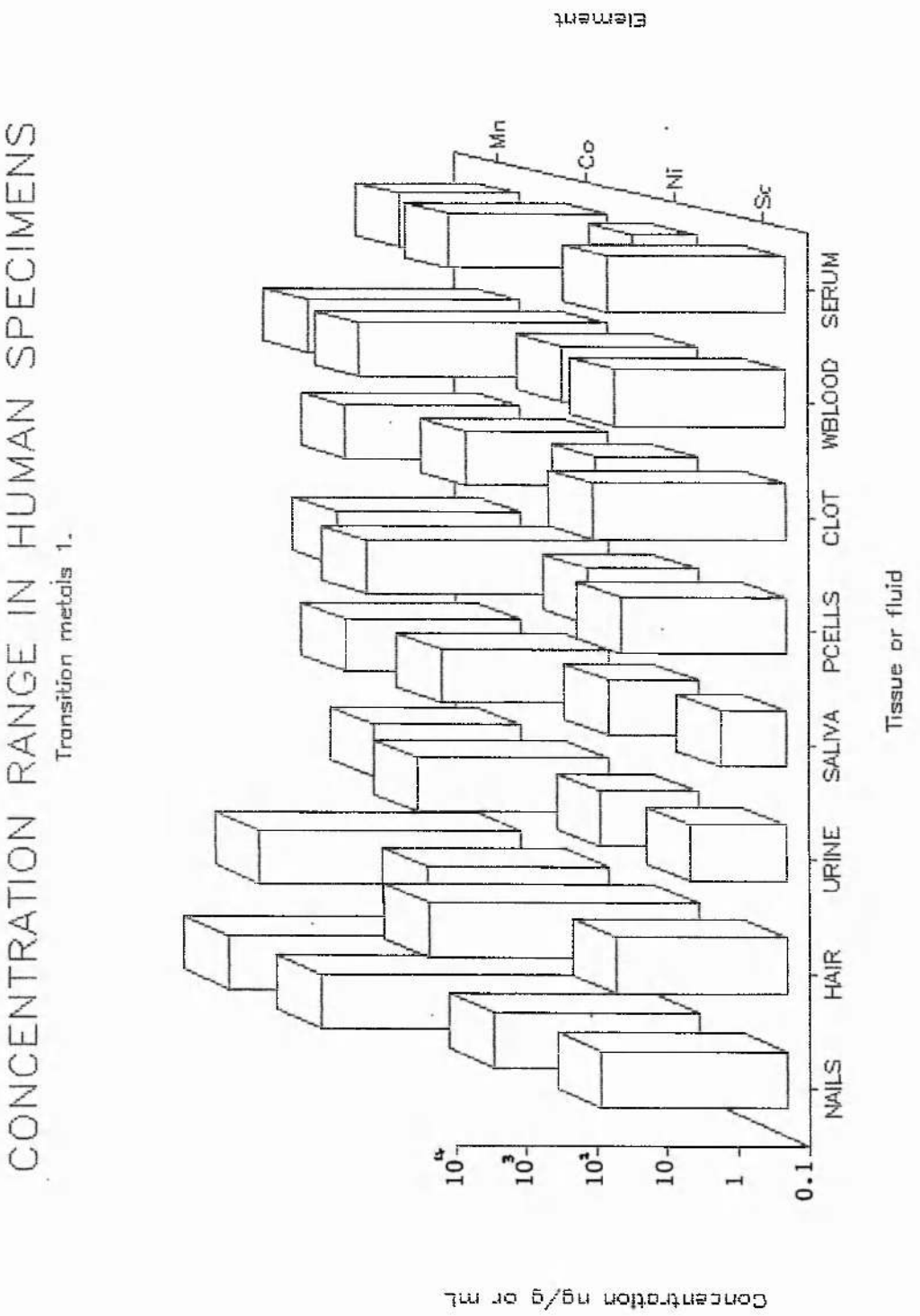


Figure 43 (con.): Range of elemental levels in various human specimens

f) Fe, Zn, Cu.

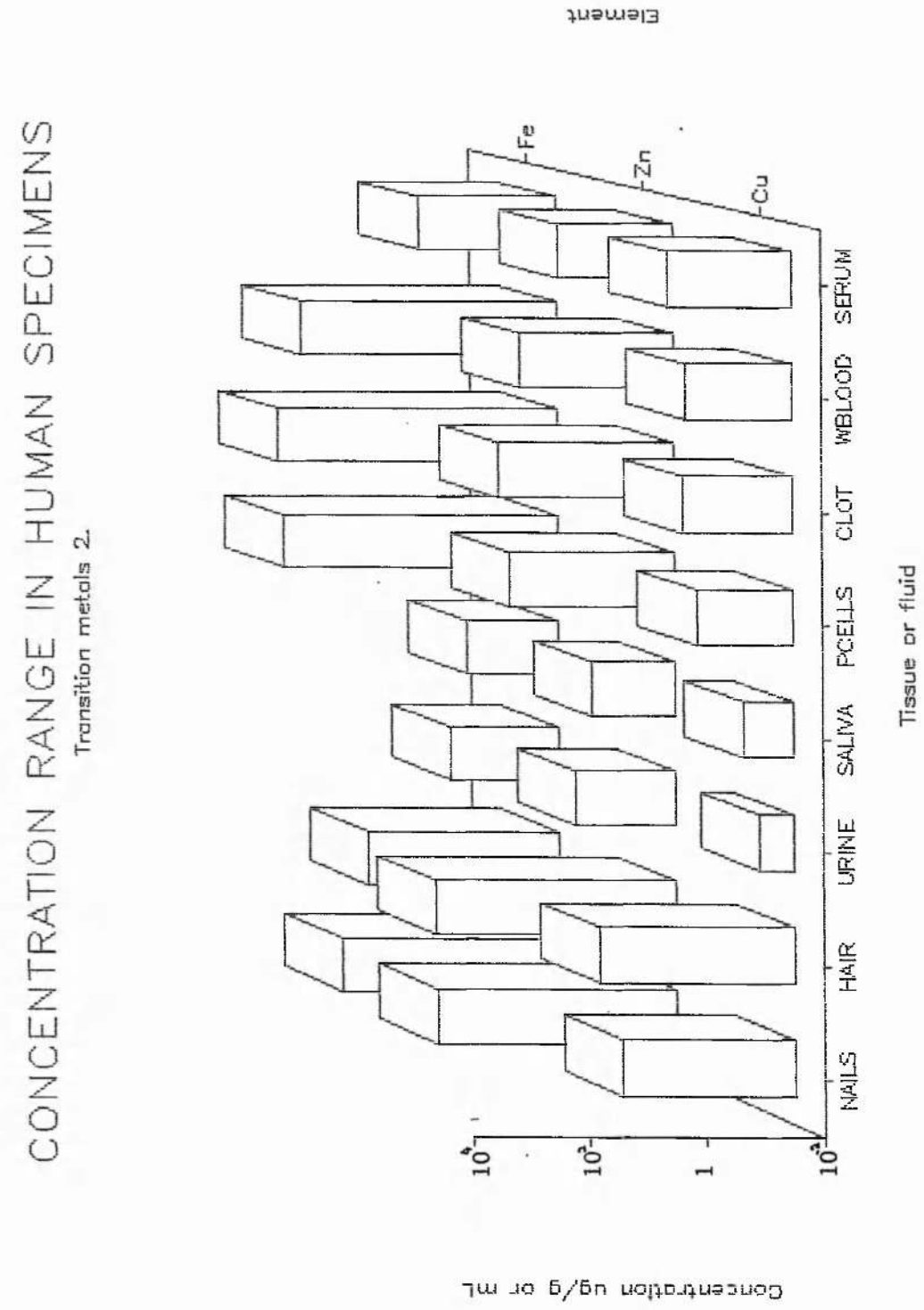
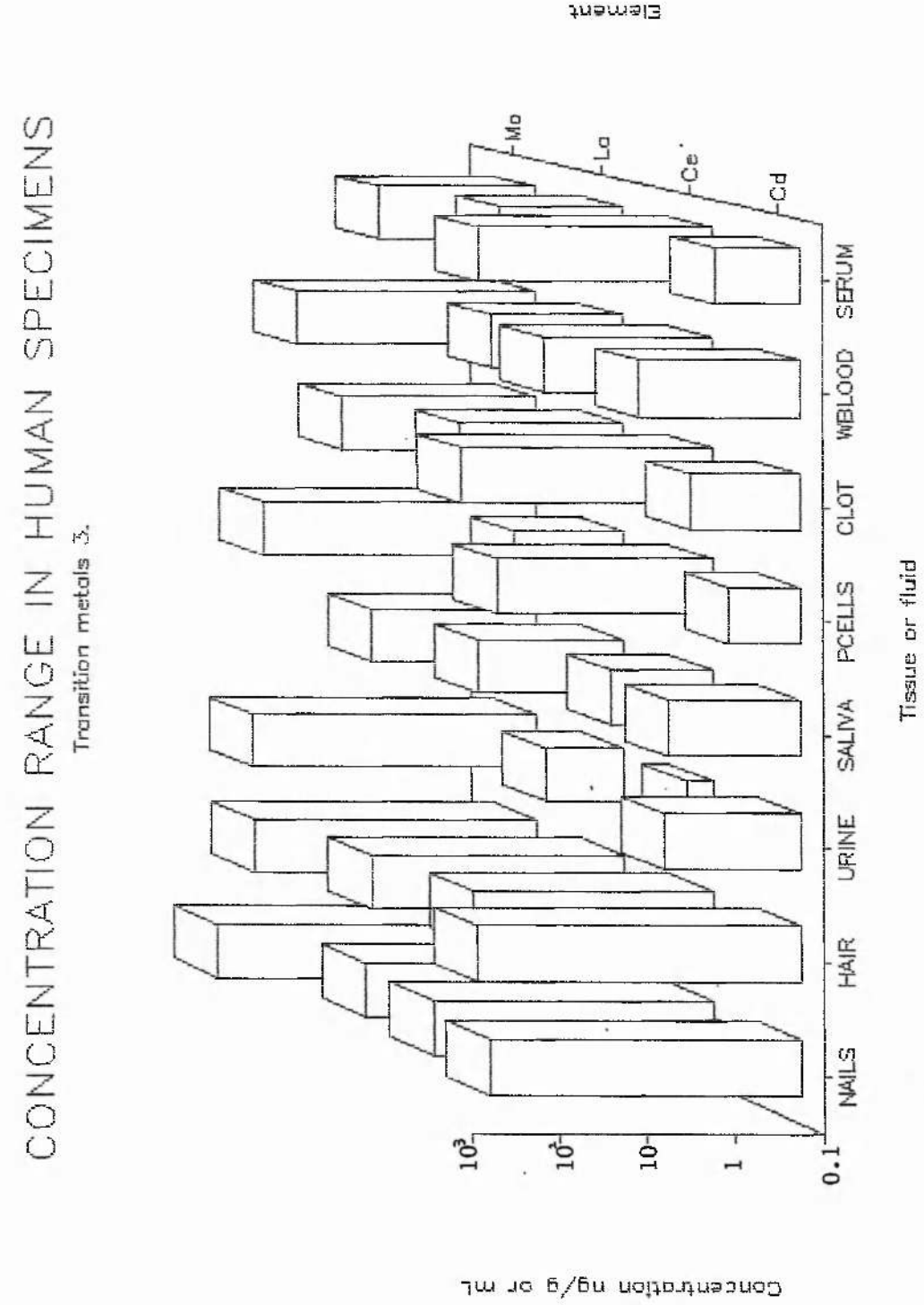


Figure 43 (con.): Range of elemental levels in various human specimens

g) Mo, La, Ce, Cd.



by-product of nuclear fission (2,195). There is, however, a biological discrimination that occurs against strontium compared with calcium i.e. Sr:Ca ratios in bones and bodies of animals are lower than Sr:Ca ratios of diets (196). This is mainly due to the fact that calcium is absorbed more readily than strontium, and strontium has a comparatively higher urinary excretion than calcium. Barium does not seem to follow a similar pattern of behaviour; this element is poorly absorbed, with little retention in tissues (except bone) or excretion by urine (a median level of 1.8ng/mL). Magnesium also appears to behave differently from the other Group IIA metals; its urinary excretion is particularly high (60µg/mL), and its levels in saliva are comparatively low (4µg/mL).

Lead and germanium seem to have approximately the same pattern of distribution; lead and tin also show a high degree of accumulation in hair and nails. The relativly low urine levels indicate that none of these elements are well absorbed by the gut. Elemental accumulation by the hair and nails is not unusual - this is shown by many other elements, including cadmium, the rare earths, molybdenum, antimony and boron. Phosphorus, as an essential micronutrient, is found at substantial levels in most tissues and fluids. Its excretion in the urine is high (488µg/mL median value), which contrasts greatly with antimony (0.2ng/mL), also a Group VA element. It is interesting to see that where absorption of dietary intake is high, such as boron and

phosphorus, the urine levels are within an order of magnitude of the levels in hair. This is also demonstrated by cobalt and molybdenum.

Higher elemental levels in erythrocytes rather than in plasma are seen in many elements, for example, manganese, lead, antimony, zinc and iron. The latter two elements are shown in Figure 43f with copper, also a transition metal. Copper is higher in serum than in red blood cells; apart from this, these three elements show approximately similar patterns of distribution i.e. they are at high levels in hair and nails and low in saliva and urine.

4.2.3.2. Inter-element associations

Regression analysis was carried out between various pairs of elements from the different sample tissues and fluids (the packed cell and clot sample groups were not included here because of their limited size), to investigate whether variations in the level of one element resulted in similar behaviour in the other. As before, the regression coefficient, r, was evaluated; from this, a t-value was calculated and used to identify significant correlations.

The essential micronutrients displayed numerous associations, both among themselves and with other trace elements, in a wide range of tissues and fluids. For example, magnesium, calcium and phosphorus

were "very highly significantly" positively associated (+S**: 0.1% level) in serum, whole blood, saliva and urine. This was seen to a lesser extent in hair or nails; however, more trace elements showed apparent relationships here, such as, calcium with phosphorus, zinc, copper or lead (+S**), phosphorus with zinc (+S**) and copper with zinc or lead (+S**). Some of these relationships are shown in Figures 44 to 47. The data displayed, particularly for hair, indicates how important it is to examine data graphically, and not to rely "blindly" on statistical techniques (197). It seems likely that zinc and phosphorus interact in some way within hair; the same cannot be said for copper with zinc or lead, even though associations, significant to the 0.1% level, were found in all these examples. In the latter cases, the data appear to be distorted by a small number of high concentration samples while most of the remaining data seem to be clustered in a completely different pattern. This is due to a small number of individuals having elevated levels of a number of elements; this could be a reflection of a high exposure to, or a metabolic imbalance of these elements at the time when the hair was actively growing, or, perhaps, of some treatment carried out to the hair by that individual.

Surprisingly few associations between essential trace and toxic elements, such as mercury and lead, were seen in blood. Several essential trace elements and micronutrients, however, showed a close

Figure 44: Associations between elements in hair

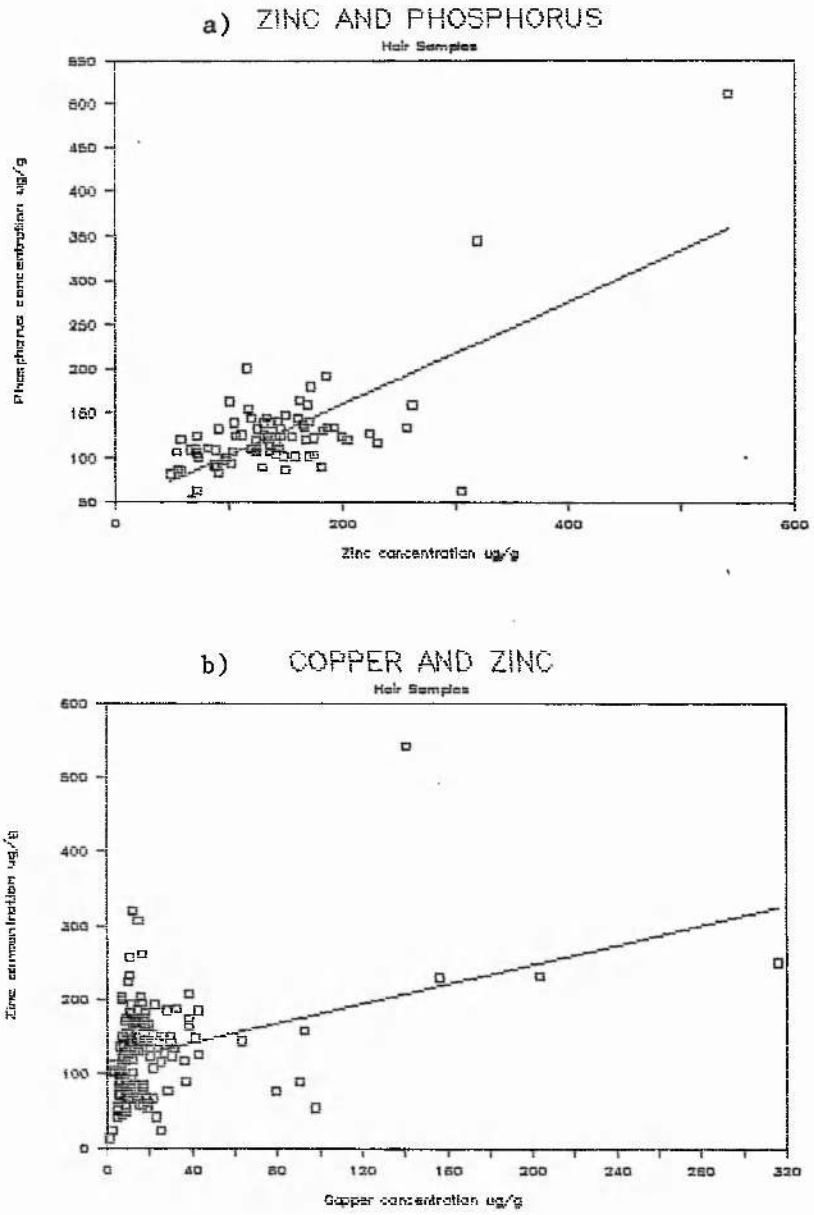


Figure 45: Associations between elements in saliva

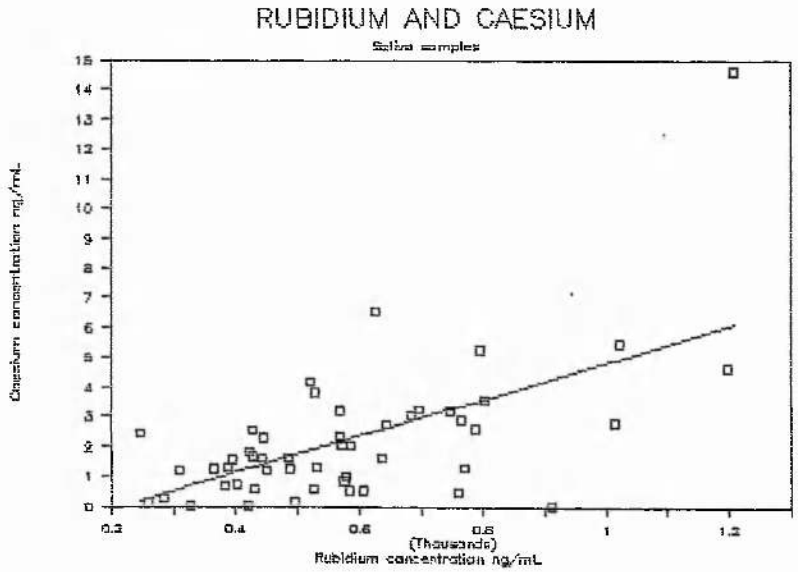
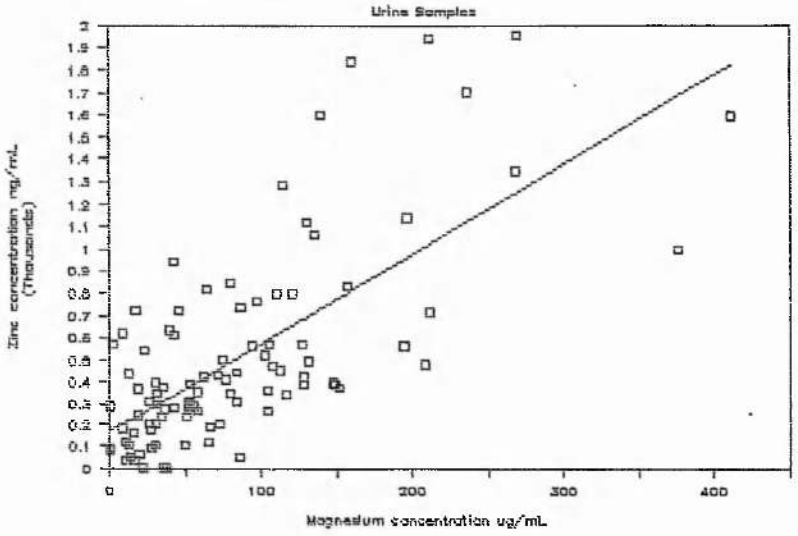
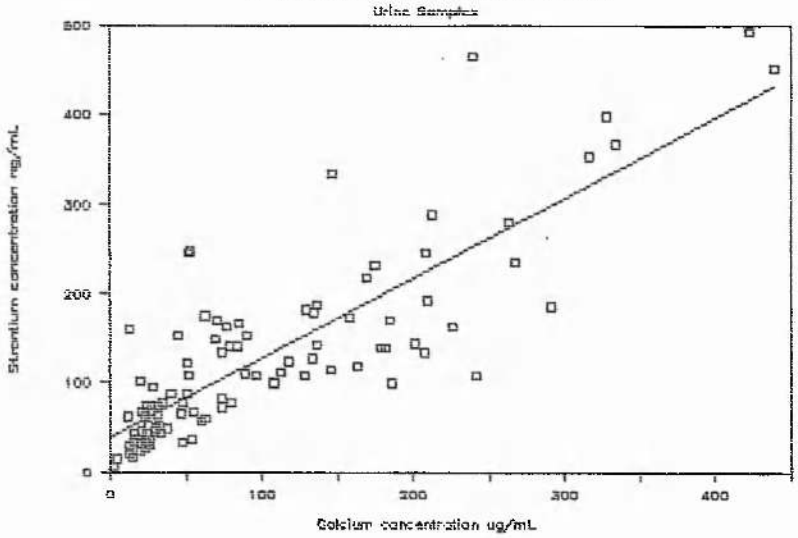


Figure 46: Associations between elements in urine

a) MAGNESIUM AND ZINC



b) CALCIUM AND STRONTIUM



c) RUBIDIUM AND CAESIUM

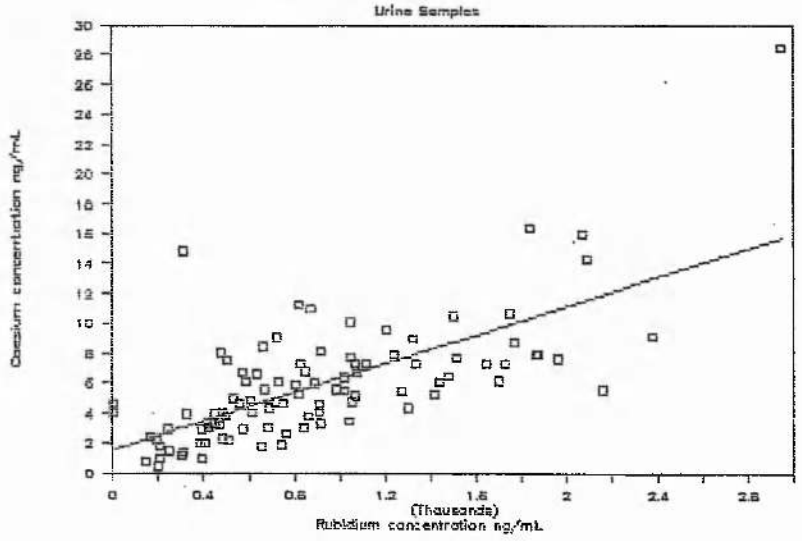
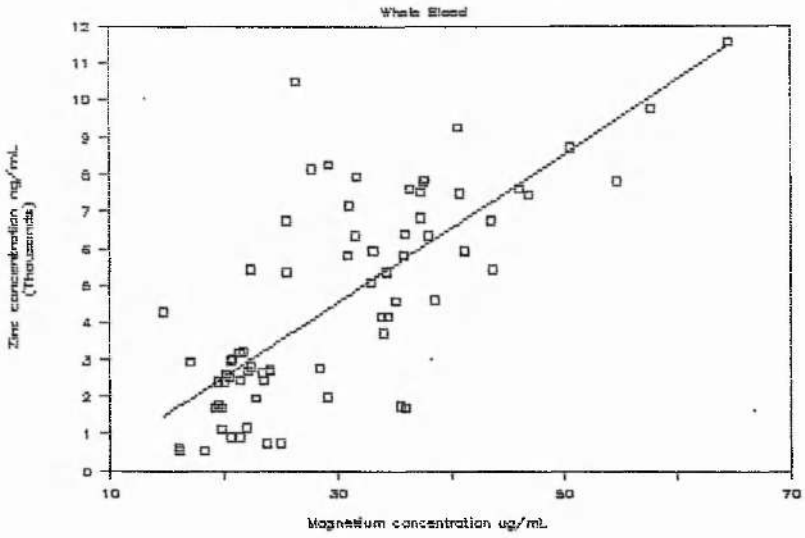
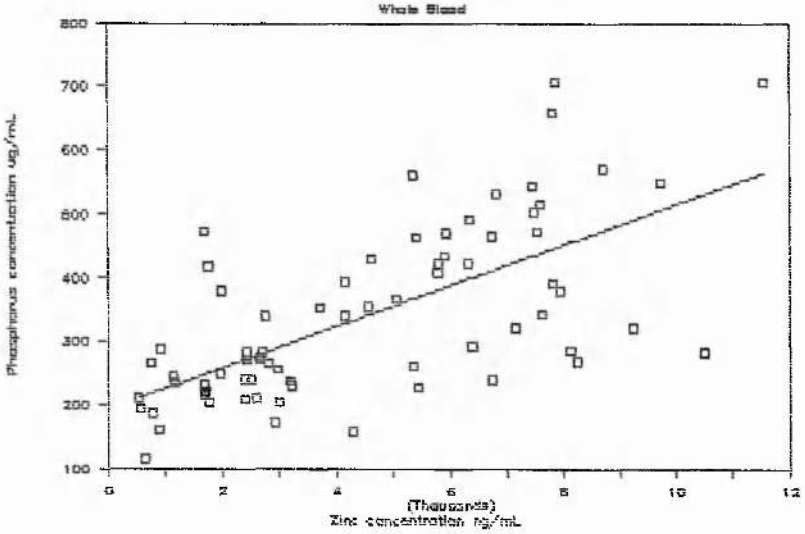


Figure 47: Associations between elements in whole blood

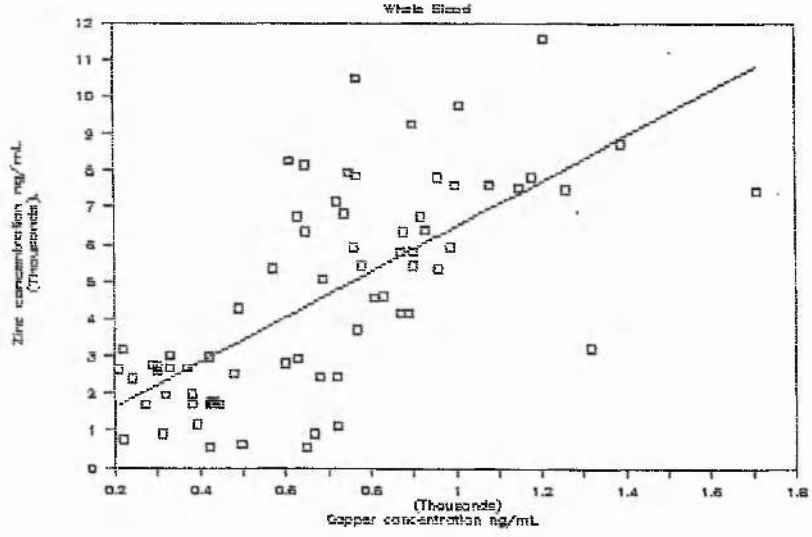
a) MAGNESIUM AND ZINC



b) ZINC AND PHOSPHORUS



c) COPPER AND ZINC



degree of association with each other in both serum and whole blood; Figure 47 displays zinc with magnesium, phosphorus and copper, all in whole blood (+S**). Calcium showed a "highly significant" relationship (+S*: 1% level) with strontium in whole blood; these elements were also linked in urine and nails (+S**). Reports have been made in the literature about interactions between calcium and strontium (195). In saliva and urine, however, more relationships were seen involving toxic elements (particularly lead; mercury was at low levels throughout). Moreover, in these two fluids, the elements rubidium and caesium showed a very high degree of positive association; see Figures 45 and 46c. The similarities in behaviour of these two elements have already been described, particularly in their reported tendency to replace potassium (172,173). The concentration of potassium is comparatively high in saliva (1); rubidium and caesium also seem to have elevated levels. The association of these two elements in saliva suggests, therefore, that they behave in a similar way within this fluid. Their relationship in urine is indicative of the patterns of absorption and excretion within the body. Rubidium and caesium do not form any associations in blood, nor in hair and nails.

It is interesting that very few significant correlations were shown between pairs of elements in nails, particularly in comparison with hair. This suggests either a different way of accumulating trace elements into the matrix (even though hair and nails are thought to be

similar in chemical structure), or perhaps that either nails or hair are affected by exogenous deposits of elements to a markedly different degree (157). External factors which might cause a change in elemental levels, and thus their interrelationships, might be dirt, dust and lacquer, for example, although washing procedures were used to try and reduce such effects.

4.2.3.3. Inter-matrix associations

Regression analysis was carried out on each element, in various types of tissue and fluid. For example, the variations of copper in hair were compared with copper in blood, and so on. This was to continue and expand on a study reported in the literature, where correlations between hair and nails samples were investigated using INAA (157).

Vance et al. found that the levels of nonessential elements were generally correlated in hair and nails, whereas those of essential trace elements were not (157). This study does not agree totally with these findings; aluminium, calcium, chromium, copper, lithium, magnesium, molybdenum and strontium showed some degree of positive correlation (at the 5% level; exceptions of molydenum and strontium at the 0.1% level). Vance at al. suggested that hair and nail may not incorporate internal trace elements in exactly the same way, despite their similarity in chemical composition. It is also realised that

different methods of preanalysis treatment and basic population differences (influenced by factors such as diet and environment) may cause some variation between results. It is interesting to note that calcium and strontium, with their suspected interactive behaviour, are among the elements listed.

The levels of calcium and strontium also displayed some correlation in saliva and urine (+S*); in addition to these were caesium, nickel and molybdenum, also at the 1% level of significance. The importance of these associations is not known; saliva may simply have been altered by ingestion of nutrients and other elements in other forms (although a standardised collection protocol was used to try and prevent this), while urine is clearly dependent on the dietary intake and its absorption by the body.

Each element's levels in blood and hair were also correlated against each other; the concentration in blood represents information about immediate, metabolic levels, while in hair, a record is provided over a time scale of weeks and months (75). Therefore, a correlation is not necessarily expected, although a chronic deficiency or exposure might be shown in both types of specimen. Here, positive correlations were seen for aluminium, molybdenum, lead and rubidium; of these elements, only molybdenum is essential - this element has been reported in the literature as having important interactions with copper and inorganic

sulphate (2,194). Moreover, increasing dietary molybdenum has been shown to raise the elemental content of hair and wool, in cattle and sheep (198).

Few correlations were seen between blood and urine; the elements of interest here were boron, magnesium and zinc, all at the 5% level of significance (+S). By contrast, a number of elements showed correlation of their levels in hair and urine. These elements were boron, aluminium, scandium, manganese, copper, barium, rubidium and caesium. Of these, only copper and manganese are essential, however; among the remaining elements were rubidium and caesium, which have demonstrated marked similarities in behaviour throughout the body, in this study and reports in the literature.

4.2.4. Variations due to age

The population was subdivided into two groups; children (below 18 years) and adults (18 years and above). A comparison of the elemental levels in saliva, urine, whole blood, hair and nails was carried out, using the Student's t-test; the results are summarised in Table 28.

No statistically significant differences were seen between the two groups in the data obtained for whole blood. In saliva, levels of boron, zinc and antimony were higher among the group of children, at

Table 28: Comparison between the elemental levels in clinical specimens collected from adults and children, male and female.

	Elemental differences[a]							
	Saliva		Urine		Hair		Nails	
Li							C<A	
B	C[b]>A[c]		M[d]>F[e]				C<A	
Mg			M>F					
Al		C<A	M<F					
P			M<F		C<A		C<A	
Ca			M>F					
Sc		C<A	M<F				C<A	
Mn		C<A	M<F					
Ni		C<A	M<F					
Co		C<A						
Cu			M<F					
Zn	C>A		M>F		C<A			
Ge		C<A						M>F
Rb								M>F
Sr						F>M		
Mo					C<A			
Cd		C<A	M<F					
Sb	C<A							
Ba						F>M		
Hg		C<A	M<F					

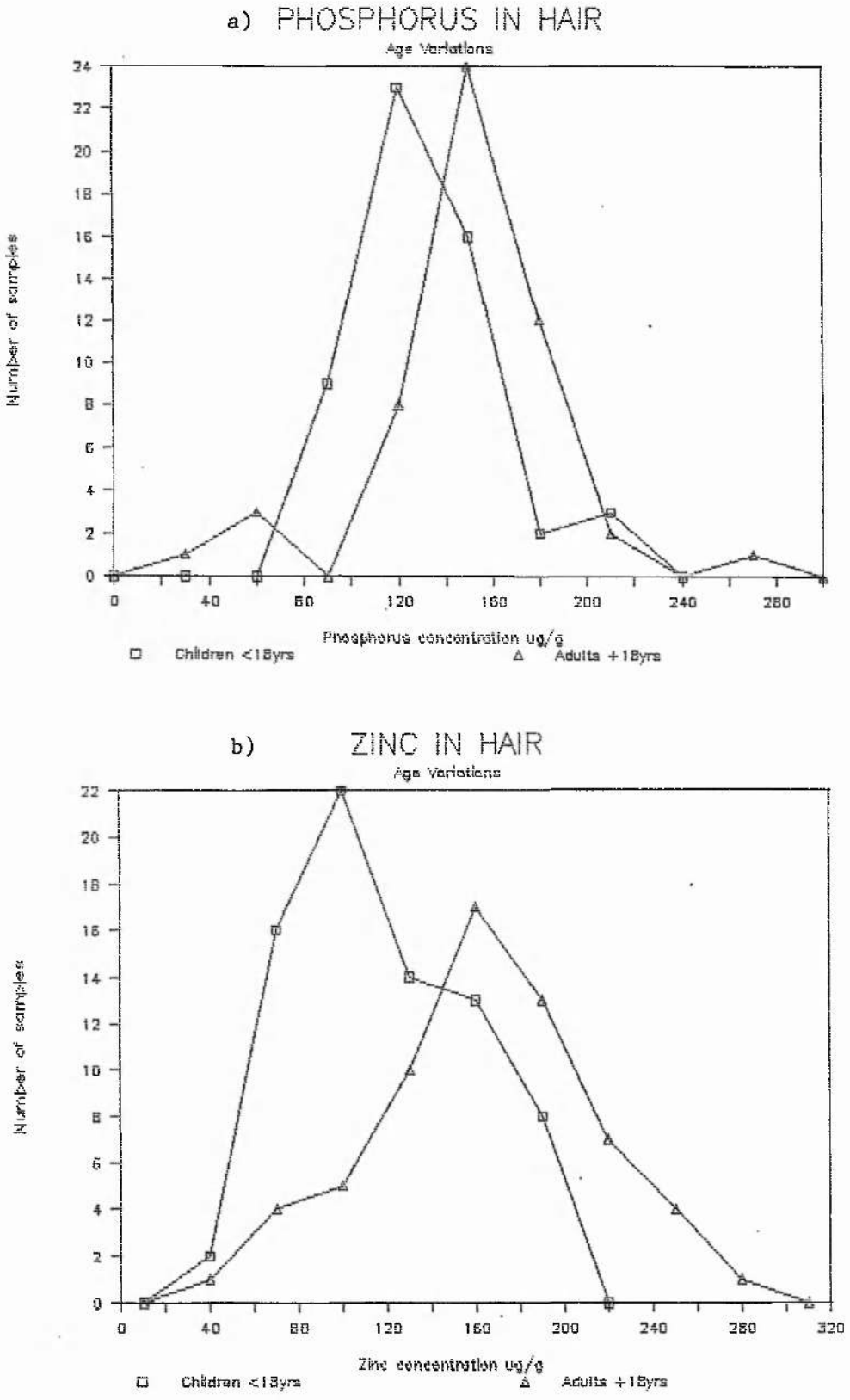
a: differences significant at the 1% level are shown
b: children (<18)
c: adults (+18)
d: male
e: female

the 1% level of significance (S*). There may be some physiological reason for this - knowledge of the roles of various elements in saliva is extremely sparse because very few studies have been carried out (160,54). It is more likely, however, that environmental and/or dietary factors are involved in this observed disparity between children and adults.

In urine, the situation was reversed; several elements were lower in children than in adults. These elements were aluminium, scandium, manganese, nickel, cobalt, germanium, cadmium and mercury - the majority of these are nonessential or toxic elements. As urine concentrations are directly related to intake, the comparatively low elemental content of urine in children may be linked to the fact that they eat less, on average, than fully grown adults. It is also interesting to note that all these elements are poorly absorbed from the gut i.e. the fraction of daily intake excreted in the urine is low. This is reported in the literature (1,144); also, the comparatively low levels of these elements in urine can be seen in Table 21 and Figure 43 (a-g).

The levels of zinc, phosphorus and molybdenum are low in the hair of children compared to adults; see Figure 48 a-b. Zinc and phosphorus are strongly bound to the S-H groups in hair; moreover, these elements were the only ones to follow a normal frequency distribution in this

Figure 48: Variations in elemental level with age



tissue. Lithium, boron, phosphorus and scandium were low in the nails of children when compared to those of adults; the reasons for these diferences is not clear; adults may be more exposed to these elements from external, environmental sources.

4.2.5. Variations due to sex

Samples of saliva, urine, blood, nails and hair were divided into male and female groups, and a comparison was made between the two groups, using the Student's t-test; see Table 28. No differences between the sexes were seen for either blood or saliva; in hair, strontium and barium were elevated in females, while in nails, germanium and rubidium were at increased levels in males. No reason is known for these findings - none of these elements are essential.

In urine, a large number of sex-related differences were shown. Boron, magnesium, phosphorus and zinc concentrations were elevated in urine from men, while the reverse was true for aluminium, scandium, manganese, nickel, copper, cadmium and mercury. A simple explanation might be that men, in general, consume larger quantities of food than females, and thus have a greater trace element (and other nutrient) intake. They would therefore excrete a larger quantity of trace elements than women. This rather simplistic argument suggests that men should excrete an elevated trace elemental content in the urine for

all elements; this was not seen. It is interesting to note, therefore, that the elements in which male values in urine exceeded those of females, are those where a sizeable proportion of dietary intake is absorbed by the body and excreted in the urine, for example, boron and phosphorus. The remaining elements, where female urine levels are higher than males, are those with poor absorption, for example, aluminium, manganese and cadmium. The larger unabsorbed portion is passed via the faecal route.

4.3. Summary

Having completed this section of the study, the work can be summarised as follows:-

i) Most elements demonstrated approximately normal frequency distribution curves for whole blood and log-normal distributions for the remaining sample groups; exceptions were calcium in saliva, phosphorus and zinc in hair. For some ultra-trace elements, the patterns of distribution became a little unclear, due to the proximity to the detection limit (the product of the "instrumental" detection limit and the dilution factor).

ii) Tables of elemental levels were presented for a range of readily accessible human tissues and fluids; the multielement capability of ICP-MS was used to provide a broad range of data. This included

information on saliva and nails, which are not well documented in the literature.

iii) Graphs were plotted to demonstrate how the elemental levels in the various tissues and fluids varied, for example, across a group of the Periodic Table. Inter-element and inter-matrix associations evaluated using linear regression analysis were discussed. Several pairs of essential elements showed relationships in blood, for example, while the largest number of inter-matrix correlations were seen between hair and urine.

iv) Finally, variations in elemental content indicated in the different age and sex groupings, for all tissues and fluids, were summarised. Of these, urine levels showed the largest number of variations between males and females, children and adults.

5. TRACE ELEMENTS AND RHEUMATOID ARTHRITIS

5.1. Introduction to Rheumatoid Arthritis

5.1.1. Epidemiology

Rheumatoid arthritis (RA), the most common arthritic disorder, is a chronic inflammatory disease which varies widely in severity and rate of onset (199). Diagnosis of the disease is based on the recognition of certain clinical and laboratory features specified by the American Rheumatism Association (ARA), which have recently been revised (132). These include morning sickness, joint swelling, symmetrical joint involvement and X-ray erosions of cartilege and bone (200). Despite the widespread use of the ARA diagnostic criteria, there is still some variation in the resulting values of disease frequency found in the literature. A consensus of opinion suggests that 1-3% of the world population suffer from RA (199-202), although one study gave a figure of 20% for disease prevalence in the UK, USA, New Zealand and similarly developed countries (203). It is well documented that women are more likely to suffer from RA than men, in a ratio of approximately 2-3:1. It appears that climate, dampness and social status are unrelated to disease prevalence (199), despite the opinion held for years that a hot climate was beneficial. For example, it is refuted by the observations that Jamaica and Mauritius have high

Table 29: % Population with definite rheumatoid arthritis (200,204)

Region	Males %	Females %	Both %
Wensleydale-Leigh (U.K.)	0.47	1.60	1.07
Rotterdam (Holland)	0.5	1.2	-
Michigan (U.S.A.)	0.3	0.7	0.5
Puerto Rico	0.16	0.4	0.34
Japan	0.4	0.7	0.55
Finland	1.4	5.4	3.0
Czechoslovakia	0.30	0.76	0.51

incidence of arthritis within their poulations (203) and Eskimos have low incidence (201). Some South African evidence indicated an increase in disease prevalence from 0.87% to 3.3% when a section of its native population moved from a rural to an urban environment (205). In the UK, however, people from rural and urban environments suffer equally, as do white and black populations in the USA (202). Table 29 shows the levels of disease found in different parts of the world.

5.1.2. Aetiology

The aetiology of RA is still uncertain; the two major hypotheses are the infective and autoimmune, although as knowledge grows in both fields, the distinction between the two becomes blurred (200). Other areas of research have been investigated; the findings can be summarised (132,199,201):-

5.1.2.1. Genetic factors

A weak influence of heredity has been shown by family and twin studies. More recent research has shown the importance of the major histocompatability complex (MHC) in immune response, although an individual MHC product may not of itself be necessary for initiation of disease (200).

5.1.2.1. Infection

No organism is consistently found in the joints or blood, although a number of agents have been investigated, such as mycoplasma, diphtheroids, and a variety of viruses (200). Work still continues, however, since several viral infections, for example, rubella and serum hepatitis, may produce a transient arthritis very similar to rheumatoid.

5.1.2.3. Autoimmunity

There is much evidence of immunological involvement: for example, high proportions of mononuclear chronic inflammatory cells, especially lymphocytes, found within an affected joint, and the presence of a variety of autoantibodies, especially rheumatoid factor (RF) (200). Antigens, usually foreign to the host, can elicit an immunological response from the body by production of specific antibodies. Demonstration of antibodies to immunoglobulin G (IgG), nucleoproteins and collagen in serum and synovial fluids of patients with RA suggests an impairment discrimination between self and non-self (host and foreign cells) in this disease.

5.1.2.4. Endocrine factors

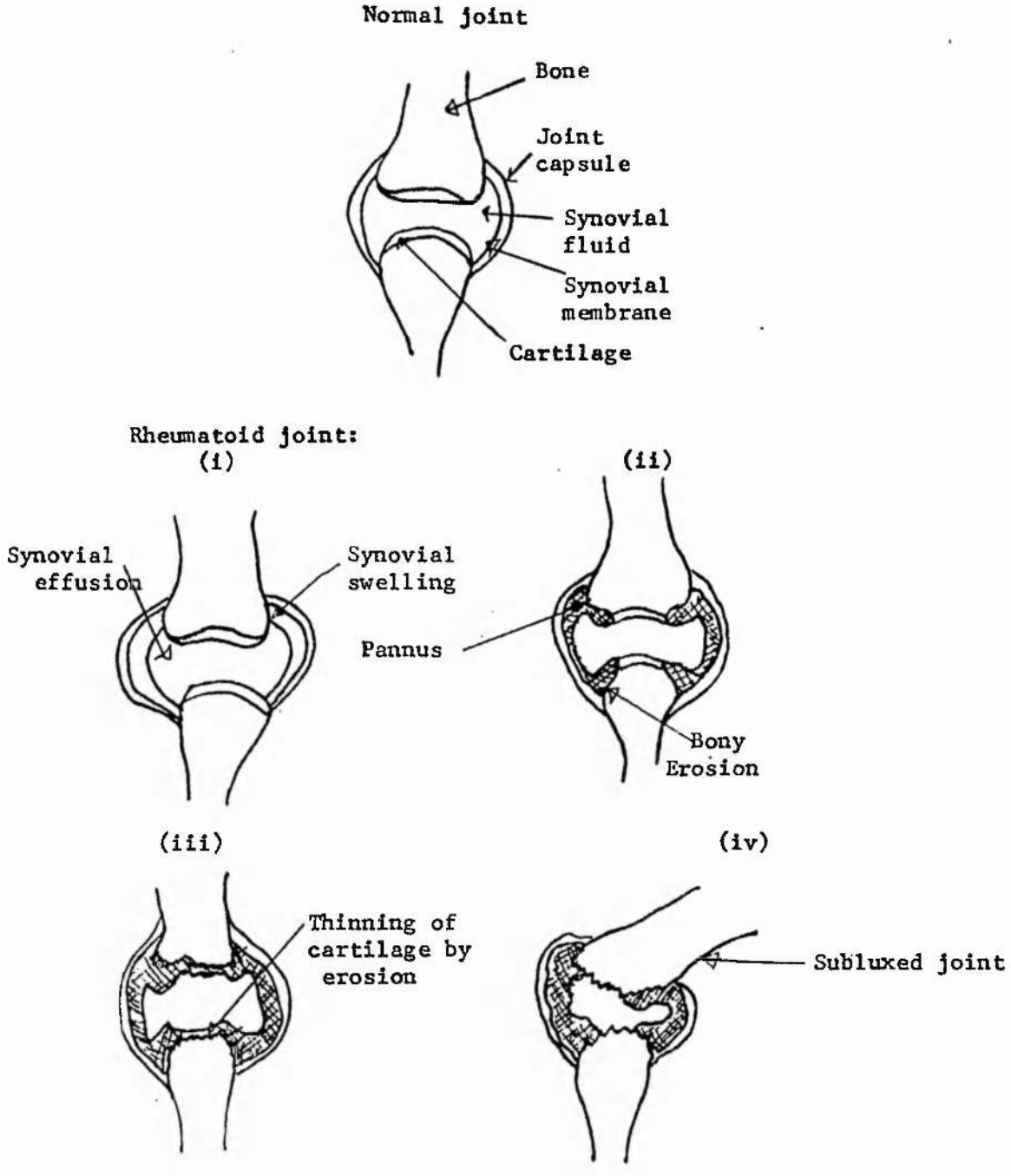
There are no known consistent factors, although some observations have been made and will be described. For example, several studies have shown that the prevalence of RA has declined while the oral contraceptive pill has been used (200). In addition, remissions in the severity of the disease are common in pregnancy and exacerbations common during menopause. Adrenocortical steroids and corticotrophin (ACTH) produce a decrease in disease activity, while RA is uncommon in association with hypothyroidism.

5.1.3. Pathogenesis and pathology

RA is a generalised disorder of connective tissue involving extra-articular structures as well as joints (199-201). In particular, it attacks synovial joints - fingers, knees, toes - and this attack is typically symmetrical in form i.e. both knees. The onset of the disease may be sudden or gradual (over months and years) and is characterised by periods of exacerbation followed by remission, rather than a constant deterioration.

Figure 49 clearly shows the pathogenesis of RA. A "normal" synovial joint is shown, and then the various stages of attack are detailed. The first stage (i) is early inflammation of the joint, leading to swelling of the synovial membrane and exudation of increased synovial fluid. This effusion adds to the swelling of the joint, with a very high protein and white blood cell content. In the second stage (ii), the swelling continues. A mass of granulation tissue or "pannus" is formed and starts to encroach on the bone and cartilage causing destruction of joint surfaces and weakened ligaments. The loss of tissue can be detected radiologically as "erosion". As the disease continues to grow in severity (iii), the granulation tissue, formed in continuity with the inflamed synovial membrane, continues to replace bone and cartilege throughout the joint. Adhesions between the joint surfaces may form. The fourth stage (iv) shows how the weakened

Figure 49: A synovial joint; normal and rheumatoid (i - iv)



ligaments and bone destruction may cause subluxation and complete dislocation. Fibrous or bony fusions may be produced with resulting stiffness, shortening or complete rigidity of the joint.

5.1.4. Synovial fluid

Almost all types of articular disease tend to increase the volume of synovial fluid. Examination of this fluid in RA can play an important part in the diagnosis of complicating factors in the disorder such as bacterial infection (200). Only a film of synovial fluid separates the moving parts in normal, healthy joints. Generally, the free fluid, clear to pale yellow in colour, is so viscous that it cannot be recovered by needle aspiration . Moreover, the protein concentration in fluid obtained from postmortem cases is sufficiently altered, compared to normal cases, to preclude its use in studies as a substitute for normal fluid (132).

Synovial fluid is a dialysate of plasma with the addition of hyaluronate, an important aspect in lubrication mechanisms, synthesised by cells in the synovial tissue. Proteins are present at lower concentrations than in plasma (approximately 1.3%) and 60-75% of that protein is albumin. The larger molecules, such as fibrinogen, large globulins, and certain complement components are excluded, due to their molecular size. The above is true for "normal" and "non-inflammatory" (for example, osteoarthritis) synovial fluids, although the latter group may have slightly raised protein levels. The absence of fibrinogen explains the absence of clot formation. By contrast, when the synovium becomes inflamed, the fluid clots after aspiration

by needle, the concentration of each protein approaches that of plasma, and the complement activity increases (132,200). All factors present in plasma are then present in the synovial fluid.

A very limited number of trace element studies have been carried out on synovial fluid samples. One study (206) used synovial fluid from 33 control subjects (post-mortem) and 13 RA patients; the findings were copper, iron, aluminium, rubidium, lithium and strontium in all fluids; barium, silver, manganese and caesium inconsistantly in both groups; nickel not detected. It found increased levels of copper, iron, and aluminium in the RA group. Another, more recent study (207) also noted that copper was elevated in rheumatoid effusions compared with "controls" (osteoarthrosis). A similar observation was made for zinc, present in both normal and rheumatoid synovial fluids (208).

5.1.5. Treatment

To date, no prevention or cure has been found for RA; treatment is carried out in order to alleviate symptoms wherever possible (199-201,212).

The first group of drugs administered to an arthritic patient is that of nonsteroidal anti-inflammatory drugs (NSAIDs), for example, aspirin. These are agents that reduce the symptoms of established

inflammation within a few days, without serious side-effects. Chronic inflammatory arthritis is often moderated, without being completely suppressed, by NSAIDs.

If this is unsuccessful, the next stage is treatment by slow-acting antirheumatic drugs (SAARDs). This large, diverse group includes the anti-malarial drugs, gold compounds and penicillamine. They are delayed in their effect, eventually producing reversal of symptoms in 70-80% of cases. Side-effects may be more serious than with NSAIDs; the anti-malarials may cause damage to eyes. Gold and penicillamine, at worst, may cause suppression of cell-production in the bone marrow. These drugs are given systemically.

The final groups of drugs, if NSAIDs and SAARDs prove unsuitable or inadequate, is the corticosteroids. These may have alarming side-effects if used at the dosage required to suppress disease completely, such as the hairy "moon-face", and perforated and bleeding gastric ulcers. The total dose can be reduced by local injections; this is a useful method of treatment if just one joint is swollen.

5.1.6. Trace elements and rheumatoid arthritis

Increasing interest has been shown in alterations of trace element levels and metabolism in chronic inflammatory diseases during recent

years. Some recent findings seem to indicate that the redistribution of cellular metals is an extremely sensitive marker of an inflammatory process not evident by clinical symptoms (209). A number of elements of particular concern to RA will be discussed in turn.

5.1.6.1. Gold

Injected gold salts have been used to treat RA since the 1920's (210); most of such drugs consist of gold and sulphur-containing-complexing agents e.g. gold thiomalate. After intramuscular injection and absorption of gold thiomalate, the gold becomes bound to plasma proteins and most of the thiomalate is liberated in the free form (211). Gold salts are microbial and anti-inflammatory and can alter immunological functions. A wide range of enzyme systems are influenced by gold; it is known to enter lysosomal enzymes which contain important compounds of RA. The phagocytic activity of synovial macrophages from RA patients is significantly reduced in the presence of gold (212). Other reported effects include a redistribution of trace elements within the body, including a reduction in serum concentration of several trace metals such as tin, molybdenum, manganese, barium and caesium but not copper, iron or zinc (213).

5.1.6.2. Boron

The element boron, as described in Chapter 3, has been linked to arthritic disorders. Work carried out in New Zealand (91,203) suggested that areas with very low availability of soil boron (<0.5 μg/g) tended to have high proportions of arthritis, for example the populations of Mauritius and Jamaica. The levels of 0.5-1.5 μg/g typical in the USA, UK, RSA, New Zealand and Australia were regarded as inadequate, particularly compared to Israel. In that country, and limited areas elsewhere, with >1.5 μg/g boron, there were very few cases of arthritis. The author also reported that a remarkable improvement in the severity of arthritic cases was seen when boron supplementation was given; however, no double-blind trials were carried out. In a more recent study, bone and tooth samples of sixteen rheumatoid arthritics and fourteen controls were analysed for boron (128). The results indicated that arthritic individuals had significantly lower levels of boron in their bone.

5.1.6.3. Copper

Serum and synovial fluid copper levels rise in patients with rheumatoid arthritis . Since almost all of the copper in both serum and synovial fluid is complexed to ceruloplasmin, an acute phase reaction protein, these levels rise as well in the course of the

disease (207,214). Direct correlations with disease activity, articular index, erythrocyte sedimentation rate, and antioxidant activity of the serum were found with serum copper and ceruloplasmin. Inverse correlations were found with serum iron and zinc (215-7). Anti-rheumatic drugs associated with a lowering of copper level include glucocorticosteroids, gold and penicillamine. The latter is also used in treatment of Wilson's diseases (copper toxicity) because of its potent copper-chelating properties (132).

5.1.6.4. Zinc

Serum zinc levels have been shown to be low in patients with RA (211). They have also been shown to correlate inversely with osteoporosis associated with the disease (218). An original trial (219) was based on the hypothesis that zinc is essential for the normal synovium and is depleted in rheumatoid synovitis; zinc supplementation was attempted with promising results. Further trials were carried out based on whole-body amounts of zinc. Those with low levels were treated by supplementation while others were given penicillamine. Despite large differences in whole body zinc levels, no difference in disease severity was found. Since then, more negative evidence has been supplied (220,221). This suggests that variations in plasma zinc are due to the inflammation of RA, and no convincing aetiologic nor therapeutic roles should be attributed to zinc. Reports have also been

made of reduced levels of zinc in the granulocytes of patients with inflammatory disorders (209), and increased zinc concentrations in synovial fluid and urine of RA patients (208).

5.1.6.5. Selenium

Selenium is known to act as a component of the membrane protecting enzyme, glutathione peroxidase, which utilises glutathione for the breakdown of peroxides (222). Elevated levels of peroxides have been found in RA serum and synovial fluid; the activity of enzymes protecting against free radical damage have been suggested to be insufficient (223,224). Low selenium levels have been found in RA serum (222,225) and supplementation with this element has been attempted. Although serum selenium increased, there was no significant improvement in the condition of the RA patients, measured by a number of laboratory and clinical parameters.

5.1.6.6. Other elements

Anaemia is a frequent finding in patients with RA and its severity roughly parallels the activity of the disease (226). Iron supply to the marrow may be limited either by a reduction in whole body iron or by defective release of iron from the reticuloendothelial system into

the plasma pool. In the latter case, simple iron therapy would not be useful.

Increased nickel concentrations in sweat, serum and urine have been found for RA subjects compared to controls. It was suggested that sweating is an important route of elimination for nickel; the high nickel levels in RA could be explained by reduced sweating due to impaired physical activity (227).

Other recent reports include abnormal cellular stores of calcium, magnesium and strontium in patients with rhuematoid arthritis; erythrocytes and granulocytes were measured (228,229). Granulocyte calcium is an important metal for the phagocytic and secretory activities of inflammatory cells (230), while strontium stimulates degranulation of granulocytes (231).

5.2. Results and Discussion

Samples of brain and bone tissues, and synovial fluid (as listed in section 2.2.1.) were digested and analysed as described on Chapter 2, sections 2 and 3. The results of these analyses are presented in the following sections, along with a discussion of the findings.

5.2.1. Analysis of brain tissue

5.2.1.1. Elemental levels

A group of brain samples was analysed by ICP-MS; the results are presented in Table 30, in ng/g or µg/g fresh ("thawed") weight. The median value, together with the range of values (minimum to maximum) is given for each element. The data were not necessarily expected to follow Gaussian curves; in addition, the use of the median minimised the effect of outlying values on the distribution as a whole. Some samples were found to have elemental levels below the limit of detection of ICP-MS (taking into account the mass and dilution factors involved), and are presented as such. The trace elemental content of brain is also shown graphically, in Figure 50, indicating clearly how elements vary in concentration within a tissue matrix. For example, magnesium is found at levels approximately three orders of magnitude greater than manganese.

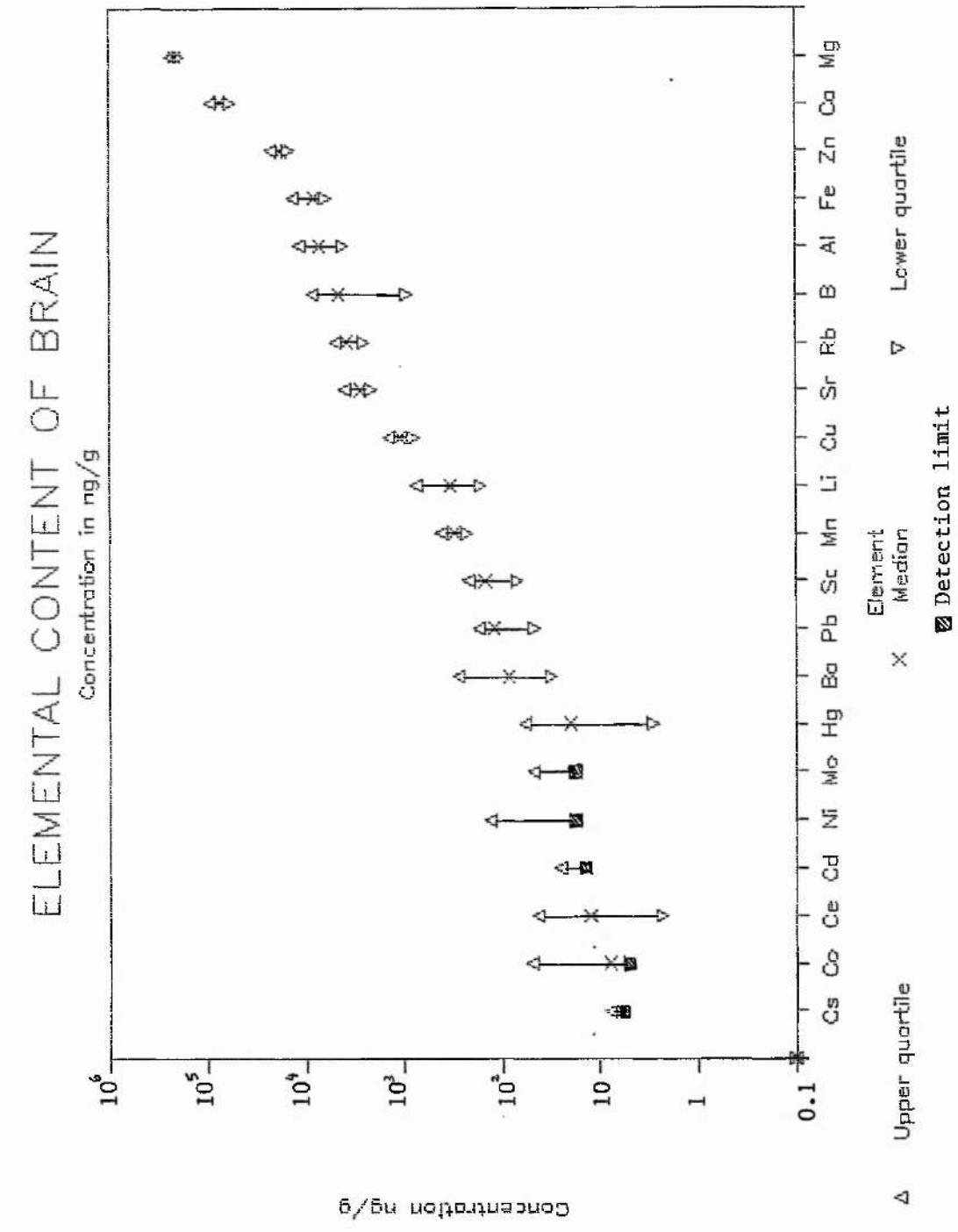
Table 30 also shows how the data obtained in this study for brain tissue compare with the information available in the literature about this sample matrix. A number of elements, such as manganese, zinc, rubidium and strontium are at comparatively high levels within brain tissue, as compared to other matrices, such as blood. For example, manganese has an average value in brain of approximately 300ng/g,

Table 30 : Elemental content of brain with literature comparison : ng/g fresh weight unless stated.

	This study		Literature[c]
	Median	Range	Range
Li	342	4 — 1888	- -
B[a]	4.65	0.13 — 22.0	0.06[b]
Mg[a]	214	66.1 — 598	114 — 690
Al[a]	7.18	0.47 — 28.4	0.5 — 3.6
Ca[a]	73.1	28.7 — 169	130[b] -
Sc	150	7 — 948	210[b] -
Mn	312	58 — 7963	200 — 1930
Fe[a]	8.31	0.12 — 57.5	45.4 — 73.5
Co	8	<5 — 610	55 — 210
Ni	<14	<14 — 3620	140 — 400
Cu[a]	1.09	0.04 — 126	2 — 10.6
Zn[a]	17.8	5.81 — 111	12 — 84
Rb[a]	3.82	0.53 — 14.1	1.5 — 12
Sr[a]	2.75	1.09 — 8.13	0.08[b] -
Mo	<16	<16 — 267	16.3 — 210
Cd	<14	<14 — 558	500[b] -
Sn	<9	<9 — 3611	60 - 550
Cs	<6	<6 — 481	0.7[b] -
Ba	86	<13 — 1812	6[b] -
Ce	13	<2 — 251	- -
Hg	20	<3 — 430	5 — 280
Pb	123	<1 — 7224	130 — 500

a: concentration in µg/g fresh weight
b: only one value available
c: Iyengar 1978 (133)

Figure 50: Elemental content of brain; ng/g (fresh weight)

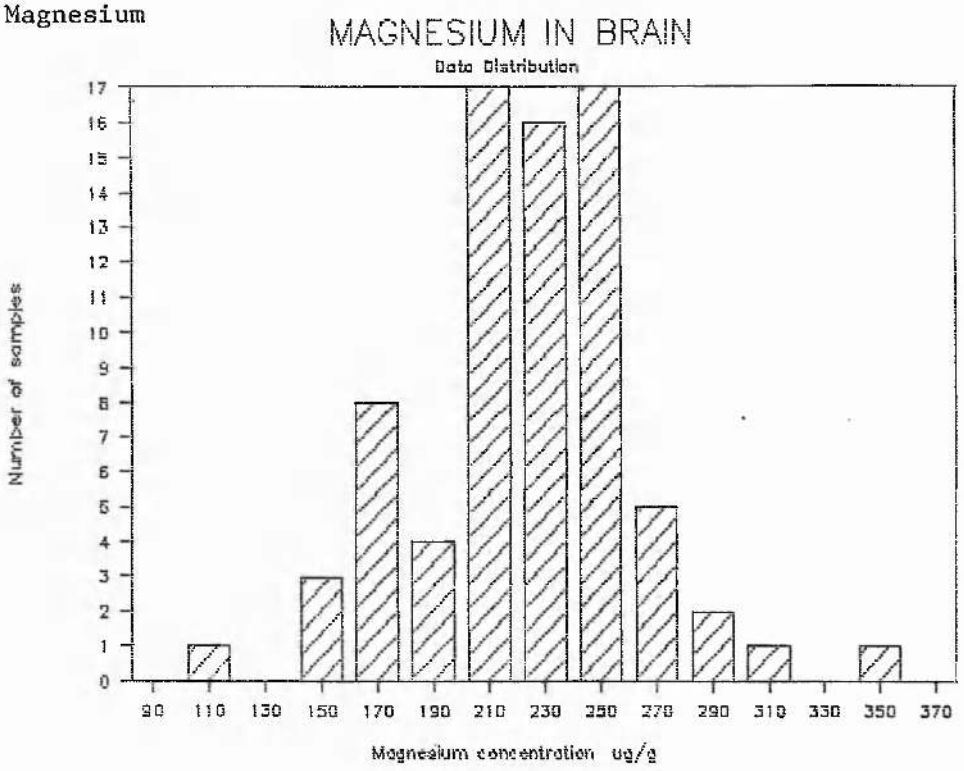


compared to measured levels of approximately 4 and 40ng/mL in serum and whole blood, respectively, while strontium is found at approximately 2μg/g in brain, and 40 and 20ng/ml in serum and whole blood. Within the group analysed in this study, several samples seemed to contain unexpectedly high elemental levels. This included metals such as copper, barium and tin. In most cases of these high levels, both hippocampus and basal ganglia samples (from a single individual) showed a similarly elevated content i.e. copper values of 119 and 126μg/g respectively, compared to a median of 1.1μg/g. Therefore, the altered trace metal composition is probably a naturally occuring abnormally high level in the individual, from environmental exposure or a metabolic disorder. An example of the latter is Wilson's disease, where the brain is known to demonstrate high concentrations of copper (2,139).

The distribution of several elements within the brain tissue was examined; Figure 51 shows the curves obtained for magnesium, aluminium, copper and lead. For aluminium and lead, in particular, there is considerable spread in the data shown on the graphs. Neither element is essential, so the variation is probably an indication of environmental exposure. Elevated levels of aluminium have been linked with Alzheimer's disease; these are thought to be localised in areas of neurofibrillary degeneration (232), and would probably not be seen in bulk tissue samples such as those used in this study. A gradual

Figure 51: Elemental frequency distribution in brain

a) Magnesium



b) Aluminium

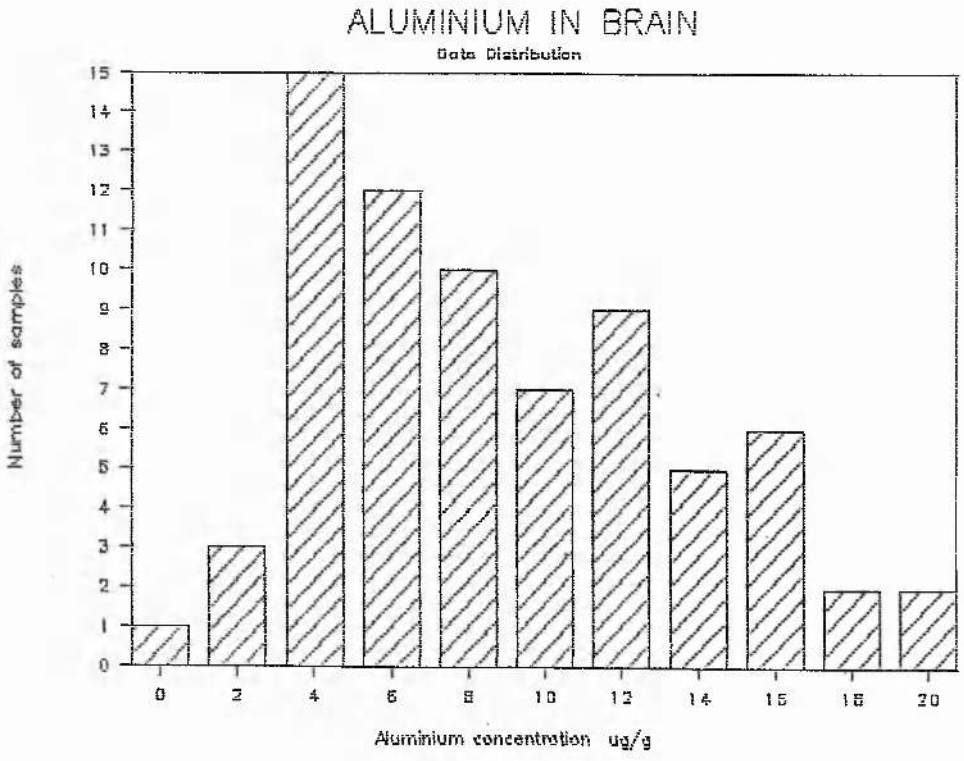
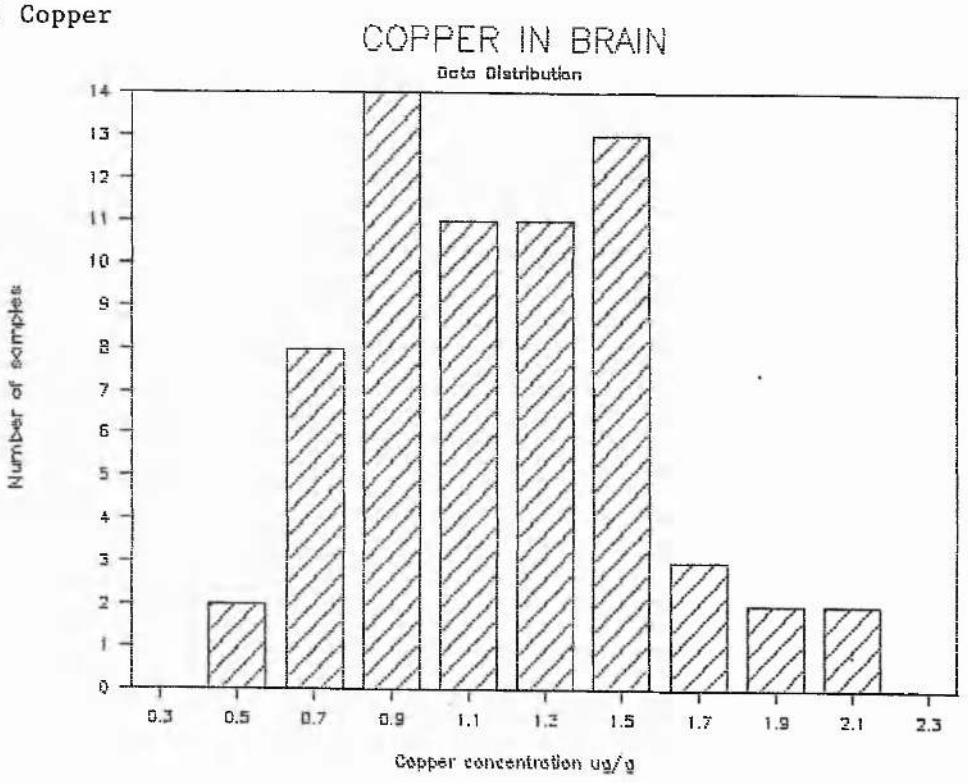
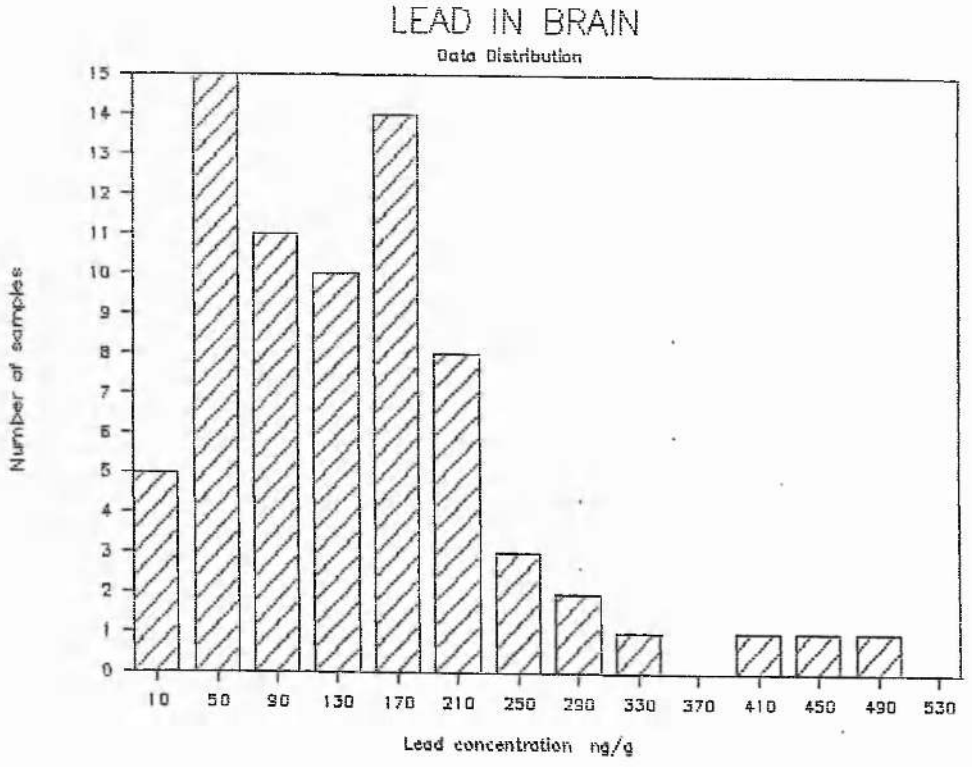


Figure 51 (con.): Elemental frequency distribution in brain

c) Copper



d) Lead



accumulation of aluminium from the environment is thought to occur in the brain and other tissues (such as lung) (233). This also applies to a number of other non-essential elements and would account for some scatter in the distribution curve. For example, more than half of the brain samples analysed contained levels of cadmium which could not be detected using solution ICP-MS (bearing in mind the dilution factors involved) i.e. <14ng/g; despite this, a maximum value of 558ng/g was also seen.

5.2.1.2. Regional variation

In this study, samples were taken from the hippocampus and basal ganglia regions of the brain. The basal ganglia appear to be involved in the coordination of movement, while the hippocampus is thought to be linked with memory. A number of authors have reported variations in elemental composition in different regions of the brain (1). For this reason, the values for brain tissue obtained in this study were examined to see if there were any such significant differences for these two particular regions.

Statistical analysis was carried out using the "matched pair" t-test. This involved evaluating the elemental difference between each pair of samples, and calculating a t-value from the mean and standard deviation of these differences:-

Equation 5 $t_{n-1} = \frac{x_d \sqrt{n}}{s_d}$ where n is number of sample pairs
x_d is the mean difference
s_d is the standard deviation

This method was used because the variation among samples is sufficiently large that smaller inter-regional differences might otherwise be obscured.

When the t-test was carried out, however, no variations in elemental content between basal ganglia and hippocampus regions were found to be significant (at the 5% level or above), for any of the elements measured in this study.

5.2.1.3. Variations due to age.

As the brain samples were taken from a relatively narrow agegroup of middle-aged and elderly people (matched in numbers of male and females), regression analysis was not carried out on the data. Instead, the samples were divided into two groups, of 51-69 years, and ≥70 years. The Student's t-test was used to differentiate between the two groups; there were, however, no significant variations (at the 5% level or above) in elemental content for any of the minor or trace elements measured in this study. This result was confirmed by

calculating and comparing median and quartile values; again, no significant differences were seen.

There have been some reported literature findings concerning variation of elemental content with age. For example, aluminium and caesium have been shown to increase, and phosphorus and rubidium to decrease, with increasing age (232,233). However, subjects or patients were generally of a wider range of ages i.e. an adult population of 20-99 years (233).

5.2.1.4. Variations due to sex

The brain samples were taken from an equal number of age-matched males and females (67 ± 8 and 68 ± 8 years respectively). The Student's t-test was used to investigate if any gender-based variations in trace element composition might be observed. Again, the median and quartiles were employed to examine the data graphically. This statistical analysis revealed no sex-based distinctions in elemental content of brain samples taken from basal ganglia and hippocampus regions of the brain.

5.2.1.5. Comparisons between rheumatoid athritics and controls

In this study, forty brain samples were from RA subjects, and thirty-two from controls. The elemental data obtained by ICP-MS for the two subdivisions were compared using the Student's t-test. At the 5% level of significance (S), calcium and strontium both had depressed levels in RA subjects. At the 1% level (S*), RA individuals had significantly lower lithium levels.

These variations, calculated by t-test, can be seen clearly in Figure 52. For a number of elements, in addition to those mentioned above, the content of RA samples appeared to be slightly lower than in the control group. This is true, notably, for iron, copper and zinc. The reverse is true for manganese, barium and lead, amongst others. It is interesting that, graphically, for both copper and manganese, there appears to be an easily distinguishable separation between the data obtained from the two sample groups. This illustrates how a t-value (obtained from mean and standard deviation) can be distorted by outlying data points, and that it is important to study data by observation in addition to simple statistical manipulation.

The reasons for the elemental variations in brain tissue taken from RA and control subjects are unclear. Iron and copper are known to interact, with deficiencies leading to anaemia (1); a high percentage

Figure 52: Comparison between rheumatoid arthritic and control brain (I)

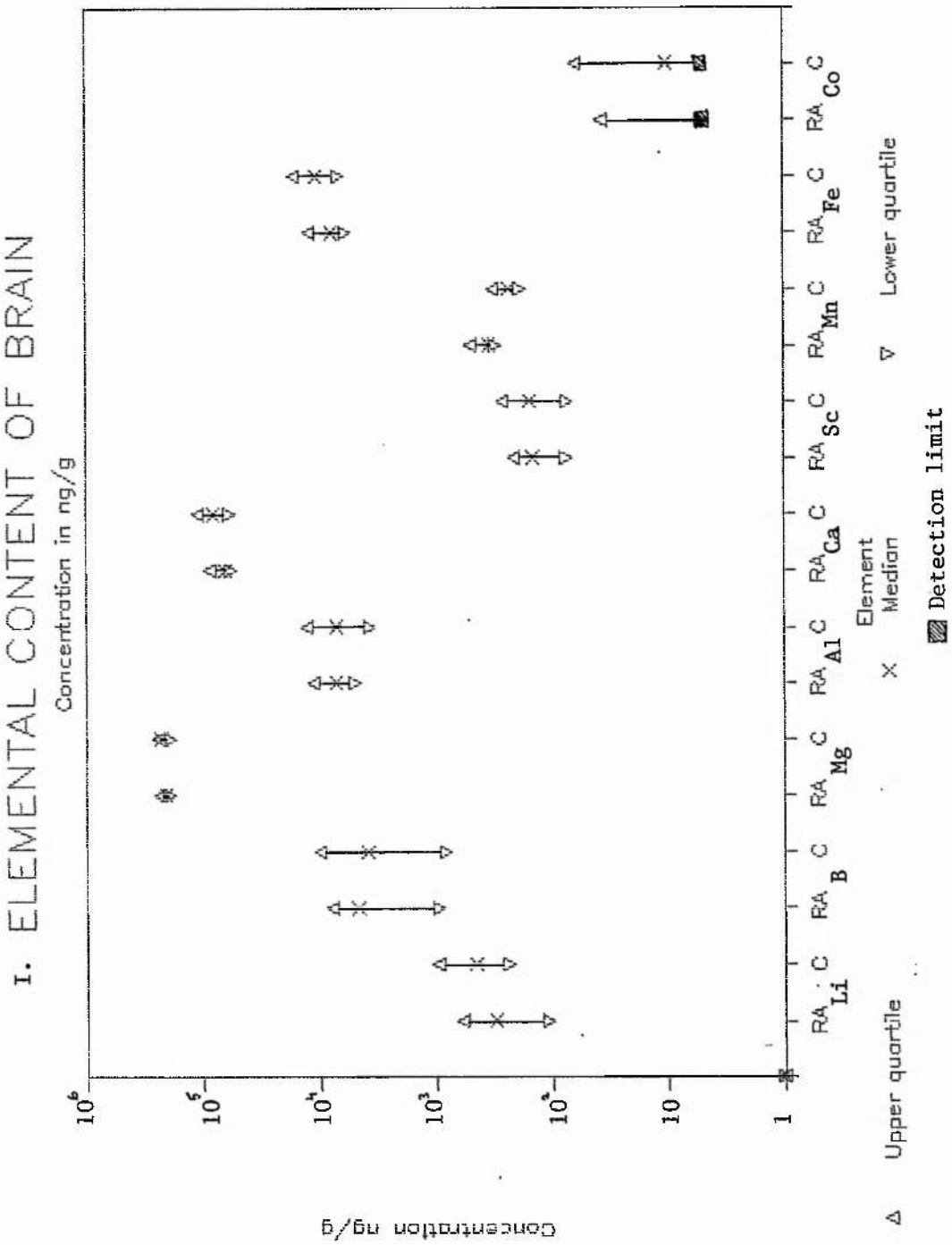
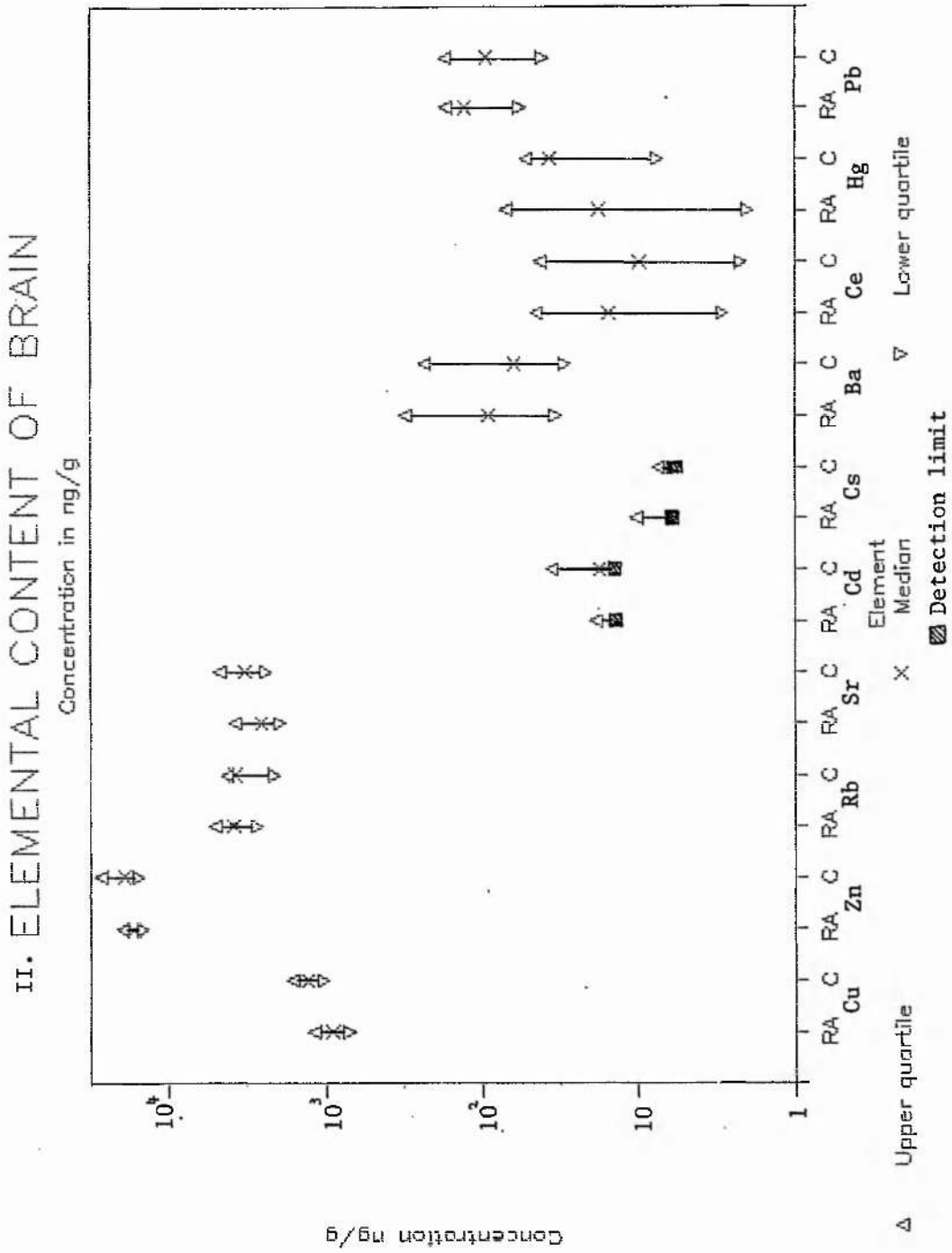


Figure 52 (con.): Comparison between rheumatoid arthritic and control brain (II)



of RA subjects are found to be anaemic. Another observed finding concerning copper and RA is that serum copper levels are elevated in the disorder; this is thought to be concerned with the inflammation that characterises RA (214). Abnormal cellular distributions have been reported for calcium and strontium in blood taken from patients suffering from inflammatory disorders, such as RA (209,228-9). Calcium is, moreover, a major component of the bone and cartilage which undergoes erosion. It also forms crystals, with phosphorus, which are found in the joint tissues and fluids of other arthritis disorders.

Elevated manganese concentrations have been found in the red blood cells of RA patients (234); in addition, studies have shown that excess of this element leads to neurological disorders (1). Lithium is found in human systems at low levels i.e. 0.7 and 15ng/mL in saliva and urine, respectively. It does not have any specific function within the body (1), but is used as a drug in the treatment of manic depressives (2).

5.2.2. Analysis of bone tissue

5.2.2.1. Elemental levels

Table 31 shows the values obtained from analysis of bone material by ICP-MS. Results are presented in ng/g or µg/g fresh ("thawed") weight

Table 31. Elemental content of bone with literature comparison: ng/g fresh weight unless stated.

	This study		Literature[c]
	Median	Range	Range
Li	310	240 — 400	- -
B[a]	2.13	0.15 — 9.38	0.74 — 0.9
Mg[a]	1272	467 — 3627	700 — 1800
Al[a]	5.74	2.52 — 31.2	3 — 5
Sc	375	130 — 920	1 — 4600
Fe[a]	104	40.8 — 445	3 — 2040
Mn	775	180 — 3840	190 — 3000
Ni[a]	4.89	3.15 — 6.33	110 -
Co	275	60 — 890	10 — 4600
Cu[a]	4.04	0.46 — 96.9	1 — 25.70
Zn[a]	49.9	30.8 — 212	50 — 170
Rb[a]	2.84	1.63 — 15.1	0.1 — 5.11
Sr[a]	37.1	14.9 — 90.7	42.6 — 237
Cd	<14	<14 — 80	1280[b] -
Sn	<9	<9 — 40	800 — 3000
Sb	40	<10 — 330	10 — 300
Cs	<6	<6 — 750	9 — 98
Ba[a]	2.51	9.38 — 60.2	4.12 — 19.8
La	<8	<8 — 3120	<200 — 6600
Hg	<2	<3 — 420	450[b] -
Pb[a]	1.57	0.76 — 5.72	10 — 42.5

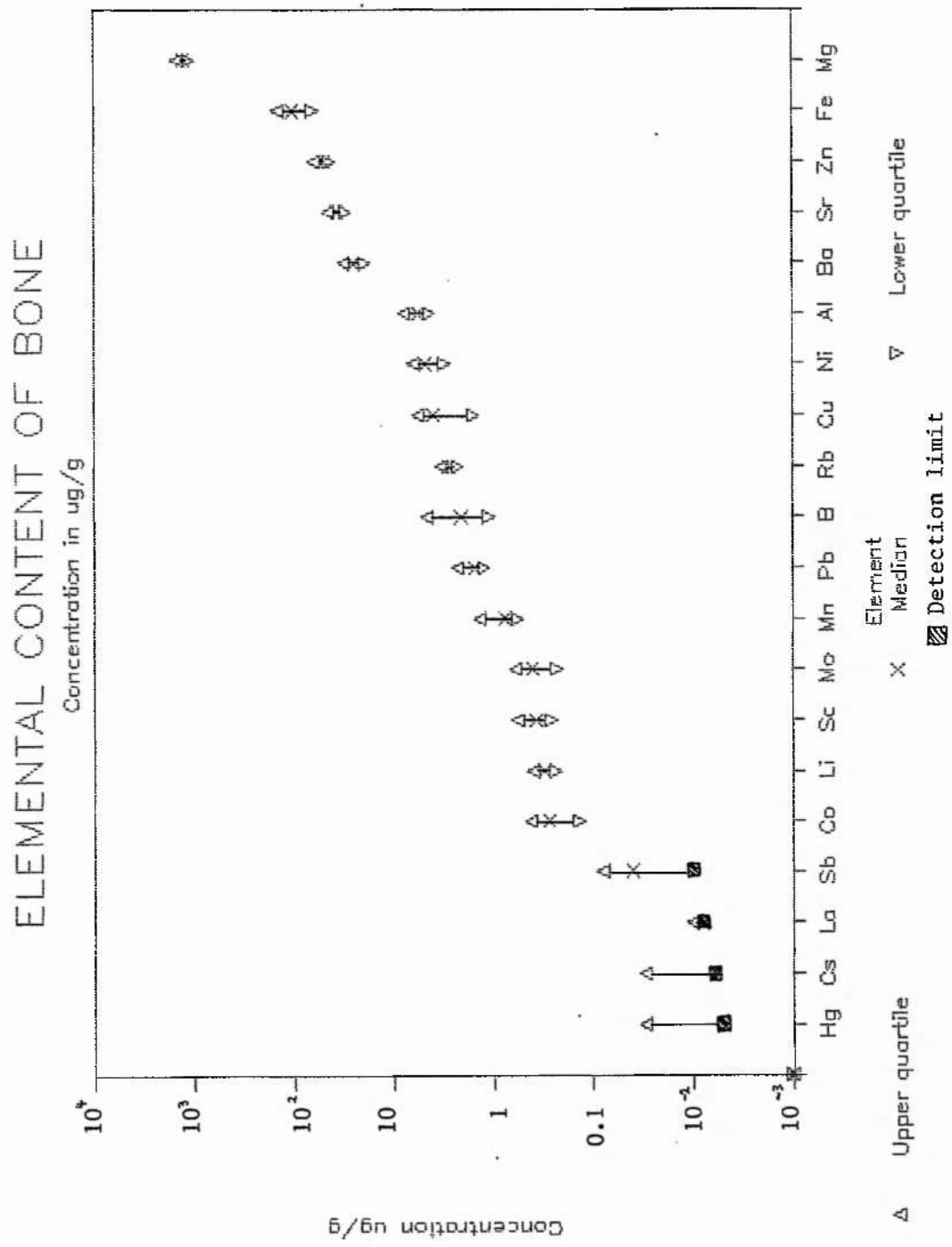
a: concentration in µg/g fresh weight
b: only one value avilable
c: Iyengar 1978 (133)

as medians and ranges. Where any given element within a sample was at too low a concentration to be measured by ICP-MS (taking dilution factors into account), this is shown by stating the detection limit and quoting the result as "less than" (<) this value. In this table, literature data are also given for comparison, while Figure 53 shows the measured information graphically.

For many elements, only a limited number of studies have been carried out to characterise their content in bone tissue. Some of the values quoted as references in Table 31 are taken from just one or two studies, some of which contained a small number of samples (<5). Some references quote bone composition in "ashed" weight; these have been omitted from the table. For most elements, agreement between this study and the published data is good, although a wide range of concentrations is visible in both groups.

Bone is composed of osseous tissue, a tissue made hard by the deposition of inorganic substances, or calcification. The inorganic fraction is made up of crystals deposited on collagen fibres. These crystals are mainly hydroxy-compounds of calcium phosphate, but also contain smaller quantities of magnesium and zinc, for example. This agrees well with the high levels of magnesium and zinc, in the order of 1000 and 50 µg/g respectively, which were found in this study. The crystals are surrounded by a hydration shell which allows free ion-

Figure 53: Elemental content of bone; µg/g (fresh weight)

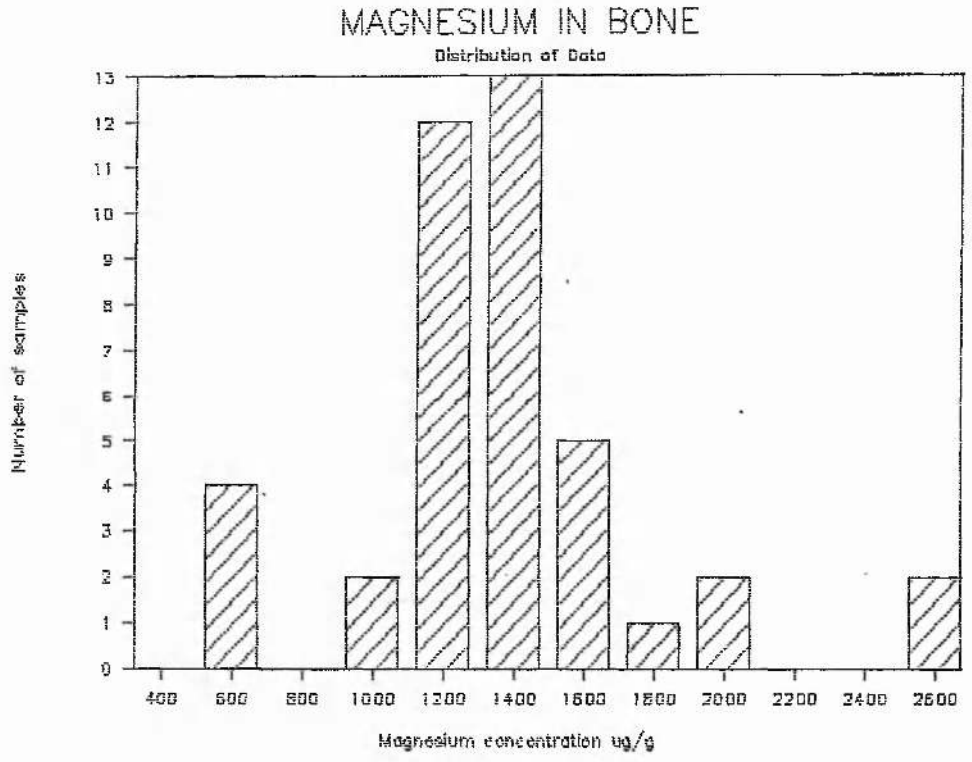


exchange between the extracellular fluid and the crystal interior; inside the crystals, there is a slow turnover rate of ions. Some elements, such as strontium, barium and lead are known as "bone seekers" because they can be readily absorbed into the crystals (1). Thus a high concentration of these elements was expected, and was shown: median values in the order of 30, 3, and 2 μg/g respectively. In addition, many other elements, such as manganese, copper and rubidium, are found to be at comparatively high levels of concentration; sizeable percentages of the total body content of these elements are stored in the skeleton (1), for example, manganese was at levels of approximately 800ng/g, 200 times the level measured in serum. Iron is found in appreciable quantities in bone, but this can vary greatly; ranges of 41-444μg/g were found in this study, with 3-2040μg/g described in the literature (see Table 31). This depends on the bone marrow content of the sample under analysis.

Figure 54 shows the frequency distribution of magnesium, copper, zinc and strontium levels in the group of bone samples analysed in this study. Despite a few outliers at both ends of the concentration range, magnesium (Figure 54a) shows a very narrow, symmetrical curve. By contrast, copper (Figure 54b) displays a very wide scatter of data. This is either one broad, ill-defined peak, or two distinct maxima, with several outlying high values. Boron in bone also showed a tendency to "cluster" (section 3.2.2.5). A larger number of bone

Figure 54: Elemental frequency distribution in bone

a) Magnesium



b) Copper

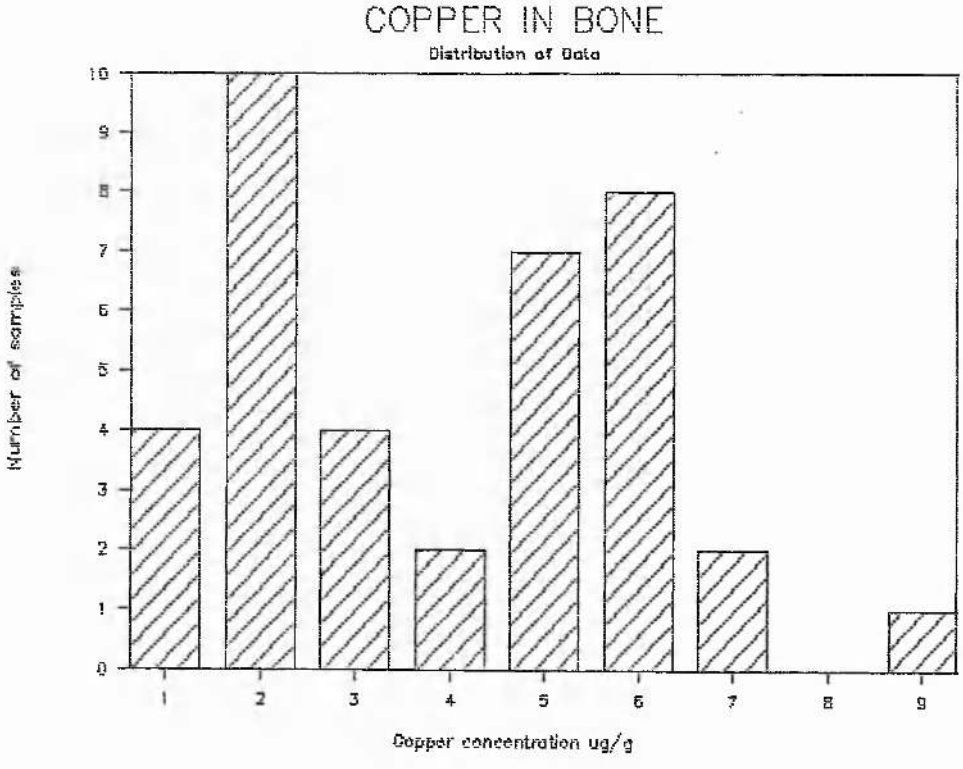
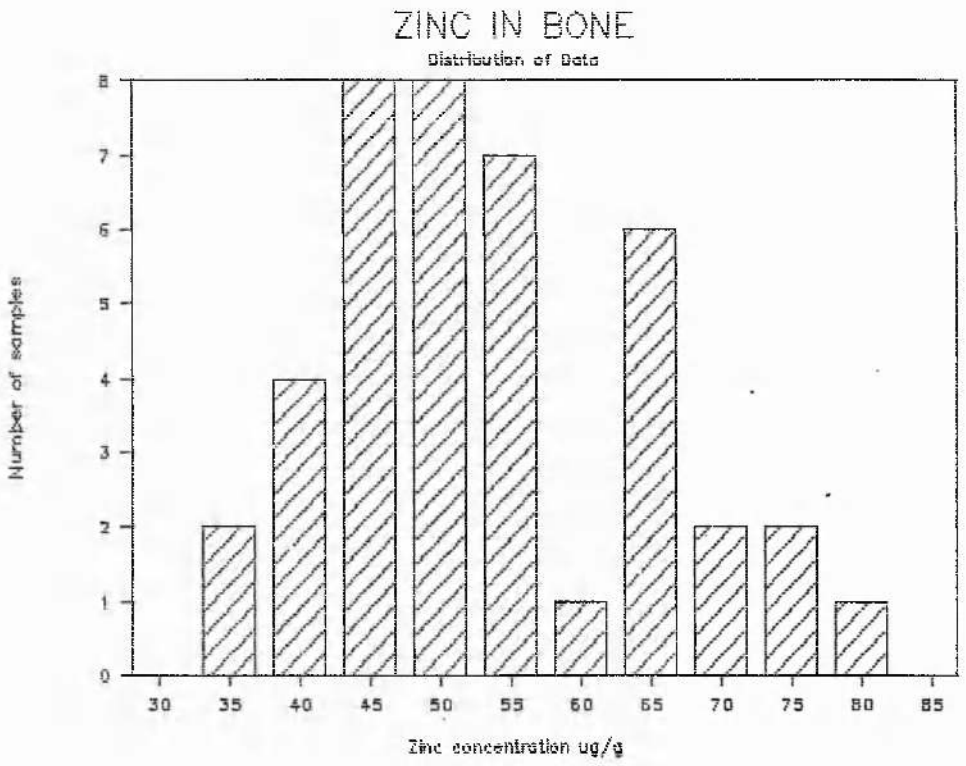
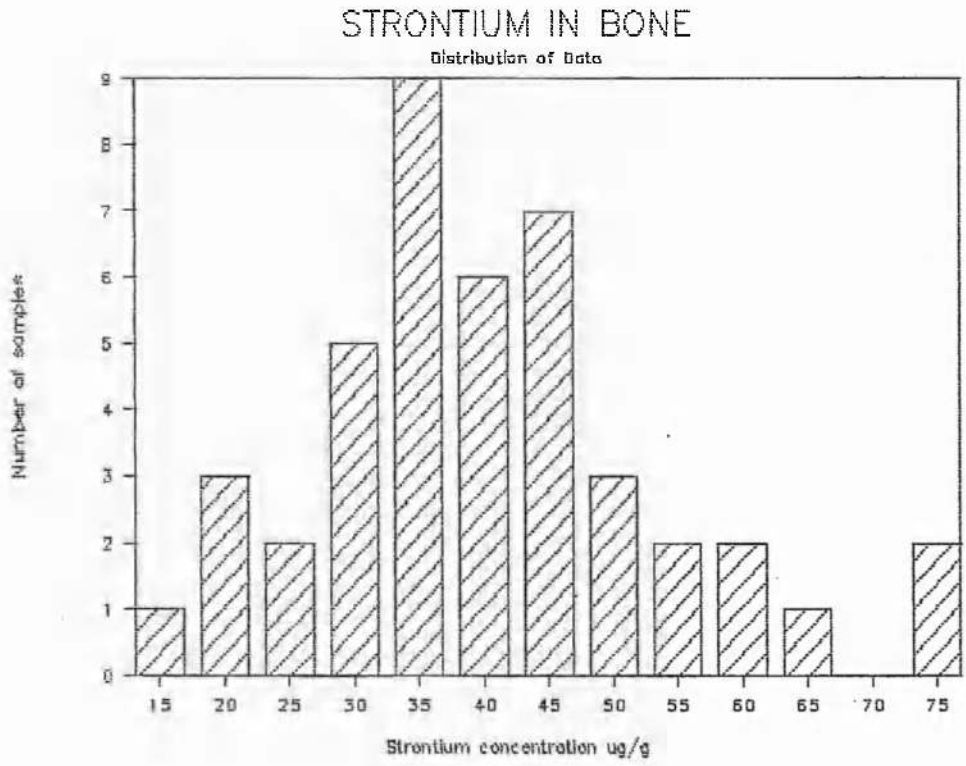


Figure 54 (con.): Elemental frequency distribution in bone

c) Zinc



d) Strontium



samples would possibly aid in reducing these effects. Moreover, it must be remembered that bone is a inhomogenous sample matrix, and that samples were taken, from various sites, from patients of RA (a bone disorder) as well as controls. Therfore, a scattering of data points is not unexpected. Zinc (Figure 54c) also shows a broad peak, although much better defined and with fewer outlying values than copper. Strontium (Figure 54d) in bone follows a relatively smooth curve, although there is the same tendency, as with zinc and particularly with copper, for the main peak to divide.

5.2.2.2. Regional variation

The sample group was too small to permit investigation into possible effects of age or sex on the elemental content of bone.

The bone samples used in this study were mainly cross-sections taken from the rib or from the knee joint. A comparison was made between elemental data from these two subdivisions. First of all, the Student's t-test was used; from this, magnesium was shown to be at sigificantly lower levels (S: 5%) in the rib, compared to the knee. Further investigation was carried out by superimposing one data distribution on the other. This showed that the peaks of both curves were in approximately the same place; the deviation was mainly due to one or two outlying data points. Because of this, and because many of

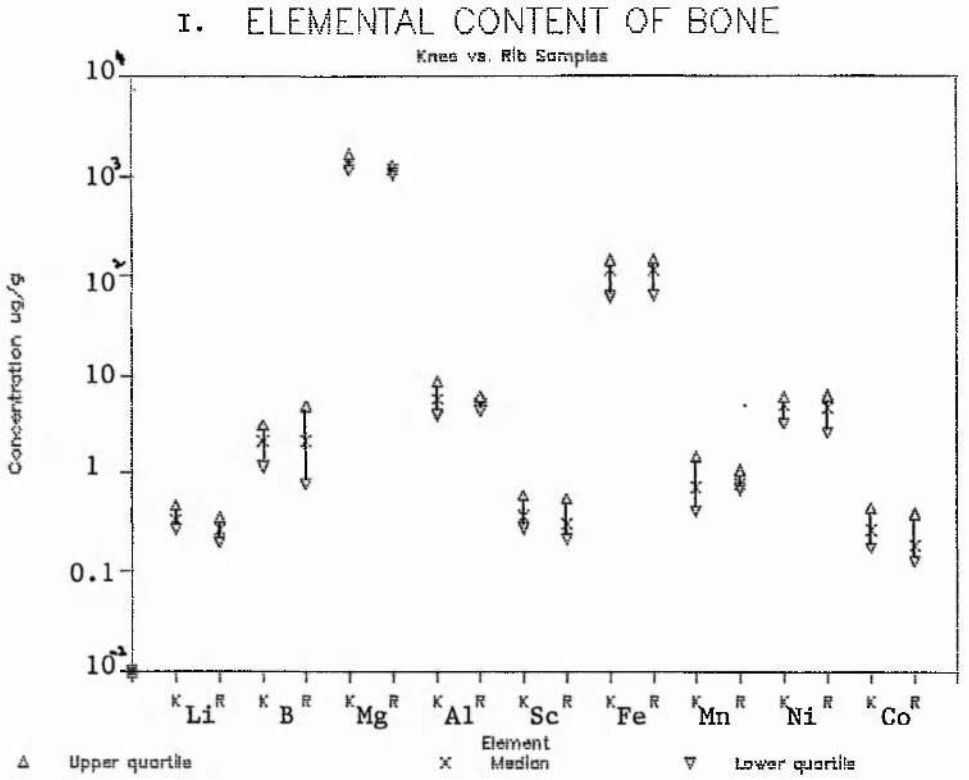
the elements did not follow Gaussian statistics, a graph was plotted of the median and interquartile range; see Figure 55.

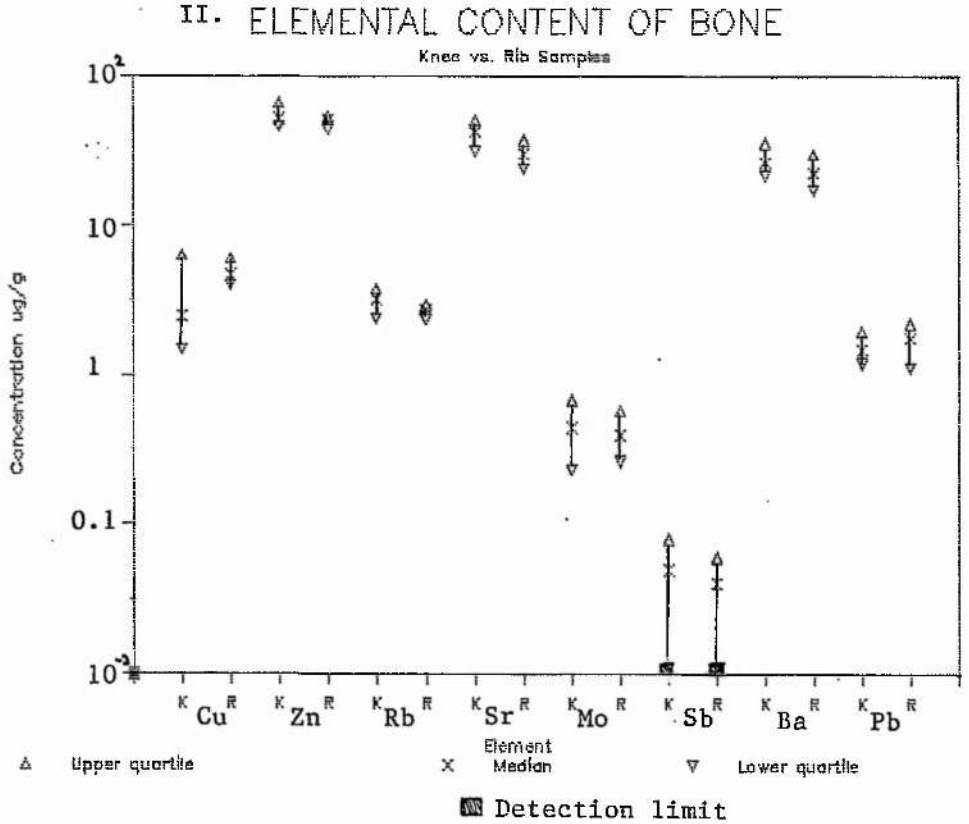
There seems to be a general trend that cross-sections of rib are marginally lower in trace element content than the cross-sections of the knee joint. The one exception to this is copper, with a considerably higher median concentration in the rib. Such a large variation probably explains the large degree of scatter (and the apparent presence of two maxima) in the copper distribution graph (Figure 54b). That there should be a difference in elemental composition of bone samples is not surprising, since bone can be either spongy or compact in form, depending on the section of bone and its location within the body. Another factor is that rates of bone formation and resorption vary in different parts of the skeleton; this may also have some effect.

5.2.2.3.Comparison between rheumatoid arthritics and controls

Twelve bone samples were taken from RA subjects, and ten from controls. The groups were compared by using the Student's t-test and by plotting median and interquartile ranges. By the t-test, two elements show differences at the 5% level (S); these are iron, where RA bone samples have an elevated elemental content, and lithium, where the reverse is true. Antimony levels in RA bone are "highly

Figure 55: Regional variations in bone elemental content





significantly" higher than in control samples (S*:1% level). At the 0.1% level (S**), RA bone samples are depleted in boron and scandium, compared to the control group.

These results are shown graphically in Figure 56; in addition to those elements described above, many of the remaining show a slight, though perhaps not significantly, lower elemental content in RA bone. Of these, nickel seems to be severely depleted in bone from RA patients.

The finding for lithium in bone mirrors its behaviour in brain samples, while for iron, the situation is reversed i.e. decreased in brain and increased in bone. Increased nickel levels have been reported in the serum, urine and sweat of RA sufferers (227). Antimony and scandium are both inessential elements which have not, to date, shown any essential role within the body. Recent studies for boron, however, have attempted to demonstrate that this element has a role to play in animals and humans; interestingly, with regard to this study, it may be involved in calcium metabolism and thus in the maintenance of healthy bones (93). As previously described, some reports have suggested that boron supplementation can play an important part in the treatment of RA (91,203). Finally, these boron results confirm findings by Ward (128), where prompt gamma ray neutron activation analysis was used to measure bone and teeth from RA and control subjects.

Figure 56: Comparison between rheumatoid arthritic and control bone (I)

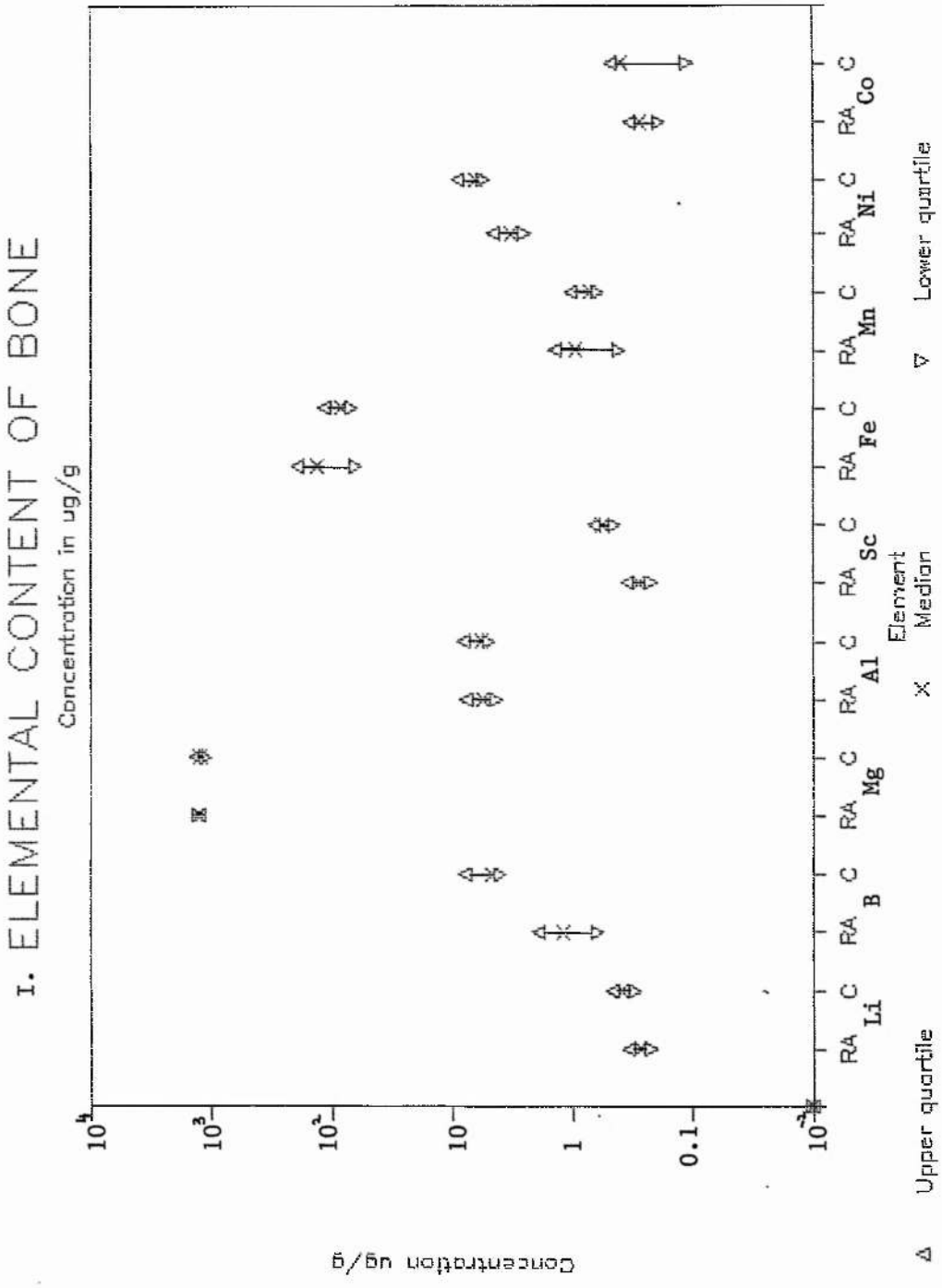
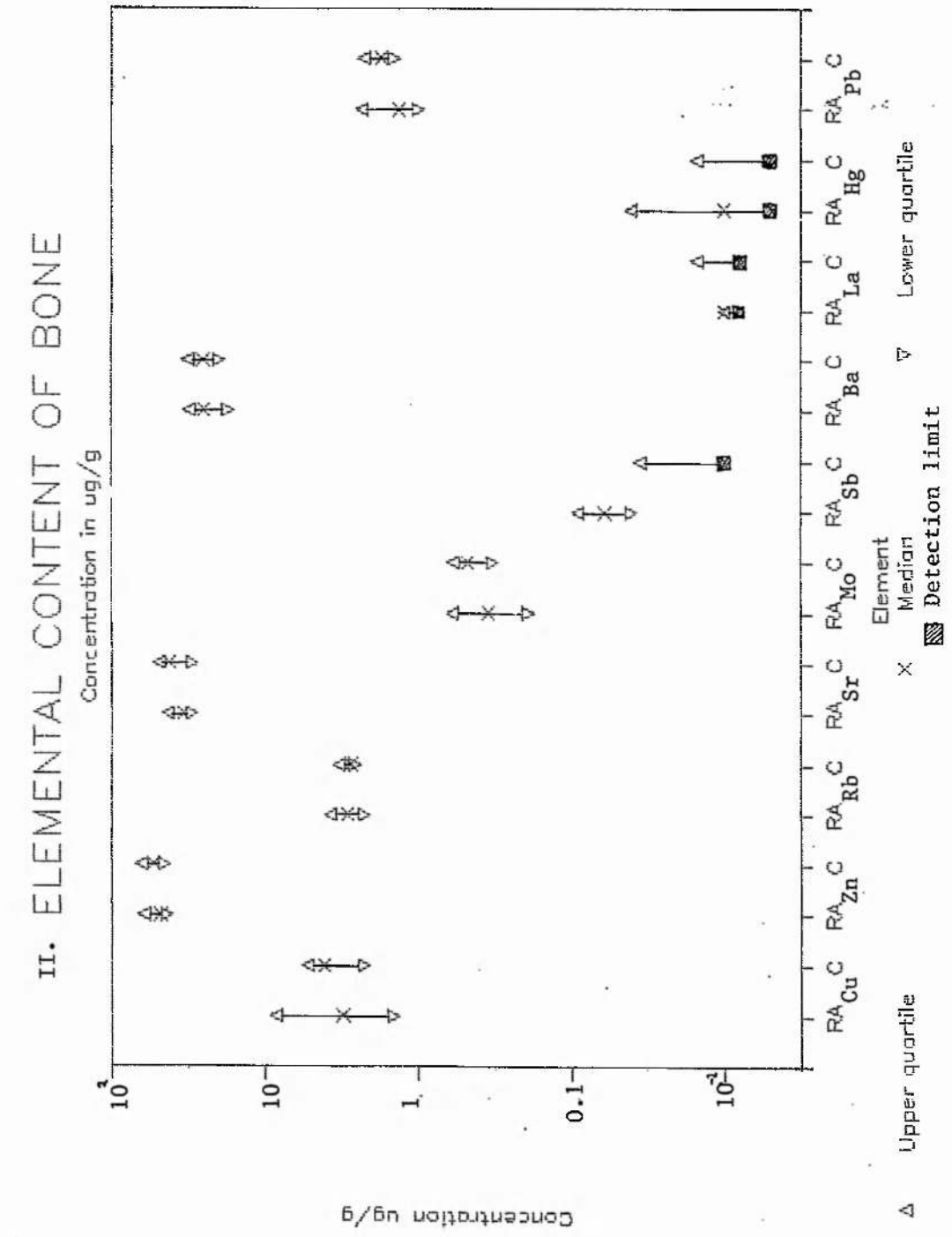


Figure 56 (con.): Comparison between rheumatoid arthritic and control bone (II)



5.2.3. Synovial fluid

5.2.3.1. Elemental Levels

The trace elemental levels obtained from analysis of synovial fluid by ICP-MS is shown in Table 32. The data are presented as median and range (minimum to maximum). Where sample levels fell below the detection limit of this fluid by ICP-MS, the sample value was described as being "less than" the calculated limit of detection e.g. <0.36 ng/mL in the case of lithium ("instrumental" detection limit multiplied by the dilution factor). All results were quoted in ng/mL or µg/mL with volumes based on fresh samples i.e. no drying of samples was carried out. In 1962, a multielement study was carried out on synovial fluid, taken from RA subjects and post-mortem "controls", using emission spectography (206). These results, in ng/g fresh weight, are presented in comparison.

Figure 57 shows these experimental data graphically, in order of increasing median value. Some elements show a wide interquartile range, for example, lithium, barium, aluminium and lead, while other elements have a more narrow spread of values, such as calcium, magnesium, copper, rubidium, and caesium. This could be for a number of reasons. For example, the essentiality of a particular element may result in it being controlled within a range of values in the body.

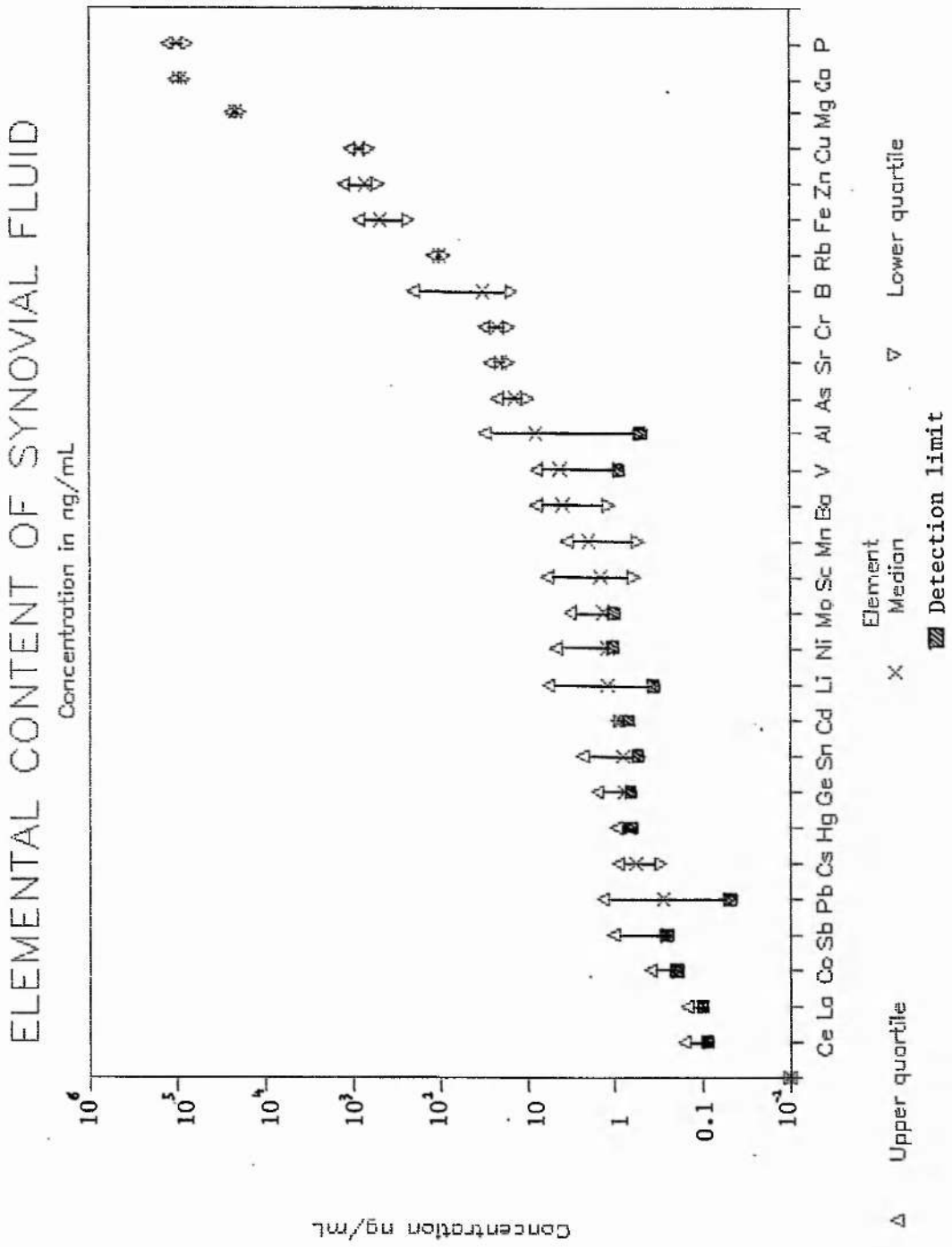
Table 32: Elemental content of synovial fluid with literature comparison : ng/mL or g (fresh weight) unless stated.

	This study ng/mL			Literature[b] ng/g		
	Median	Minimum	Maximum	Minimum		Maximum
Li	1.2	<0.4	46.6	-		-
B	32.3	<7.8	2410	-		-
Mg[a]	21.57	14.16	35.86	-		-
Al	7.9	<0.4	618	40	—	1570
P[a]	99.86	28.08	295.4	-		-
Ca[a]	93.84	36.46	147.9	-		-
Sc	1.5	<0.1	61.1	-		-
V	4.2	<0.9	20.6	6	—	700
Cr	22.0	5.2	85.5	-		-
Fe	477.0	22.4	7049	20	—	320
Mn	2.0	<0.1	53.4	7	—	400
Ni	1.2	<1.0	52.2	-		-
Co	<0.2	<0.2	10.1	-		-
Cu	814.3	155.6	3485	40	—	1570
Zn	714.2	100.1	3160	247	—	1273
Ge	0.8	<0.7	4.8	-		-
As	13.9	3.0	121.9	-		-
Rb	100.9	50.5	252.2	110	—	1300
Sr	19.9	8.9	70.2	-		-
Mo	1.3	<1.0	10.4	-		-
Cd	<0.9	<0.9	12.0	-		-
Sn	0.8	<0.5	11.8	-		-
Sb	<0.3	<0.3	235.3	-		-
Cs	0.6	<0.3	3.8	40	—	560
Ba	3.9	<0.9	47.5	90	—	300
La	<0.1	<0.1	1.7	-		-
Ce	<0.1	<0.1	11.3	-		-
Hg	<0.7	<0.7	29.7	-		-
Pb	0.3	<0.1	65.4	-		-

a: µg/mL fresh

b: Niedermeier 1962 (206)

Figure 57: Elemental content of synovial fluid; ng/mL



Moreover, these samples were taken from a group of subjects with various arthritic disorders, which may alter the magnitude and spread of an element within the body in general, and the synovial fluid in particular. A number of elements were not detected in more than half of the synovial fluid samples i.e. their median levels were below their respective detection limits. These elements included cerium, lanthamum, cobalt and mercury.

Table 33 shows a comparison of elemental levels between the synovial fluid and serum samples measured in this study. This is because synovial fluid and plasma or serum are very similar in composition; synovial fluid is a dialysate of plasma. Most of the synovial fluid data obtained here appears to be in the same order as the literature values for serum. Copper and zinc data are close to, but tend to be rather lower than expected in serum. The iron values in synovial fluid also seem to be considerably lower than the typical range in serum. These findings agree with some reports in the literature (206-8). In addition, one factor which may be involved here is the fact that the synovial fluid samples were not taken from a "control" or "healthy" population, but from patients suffering from arthritic disorders. Many individuals with RA are anaemic (226), so a lowered concentration of iron is perhaps not unexpected. Other values which deviate considerably from their serum equivalents are vanadium and chromium. That these elements are susceptible to polyatomic interferences when

Table 33: Comparison between concentrations of synovial fluid and serum measured in this study: ng/mL (fresh) unless stated.

	Synovial fluid		Blood serum	
	Minimum	Maximum	Minimum	Maximum
Li	<0.4	46.6		
B	<7.8	2410	45.5	460
Mg[a]	14.16	35.86	14.73	25.80
Al	<0.4	618	5.8	82.9
P[a]	28.08	295.4	124	222
Ca[a]	36.46	147.9	91.50	169
Sc	<0.1	61.1	7.3	68.6
V	<0.9	20.6	<0.1[b]	
Cr	5.2	85.5	0.4[b]	
Fe	22.4	7049	88	3100
Mn	<0.1	53.4	1.2	6.5
Ni	<1.0	52.2	6.4	21.1
Co	<0.2	10.1	<0.4	1.2
Cu	155.6	3485	650	2200
Zn	100.1	3160	520	1450
Ge	<0.7	4.8	<1.1	2.6
Rb	50.5	252.2	81.5	152
Sr	8.9	70.2	14.8	67.6
Mo	<1.0	10.4	<2	11.5
Cd	<0.9	12.0	<0.2	0.8
Sn	<0.5	11.8	<1	19.9
Sb	<0.3	235.3	<0.1	0.5
Cs	<0.3	3.8	0.2	1.2
Ba	<0.9	47.5	91.4	296
La	<0.1	1.7	0.9	4.0
Ce	<0.1	11.3	15.4	66.5
Hg	<0.7	29.7	<0.3	1.5
Pb	<0.1	65.4	0.3	4.0

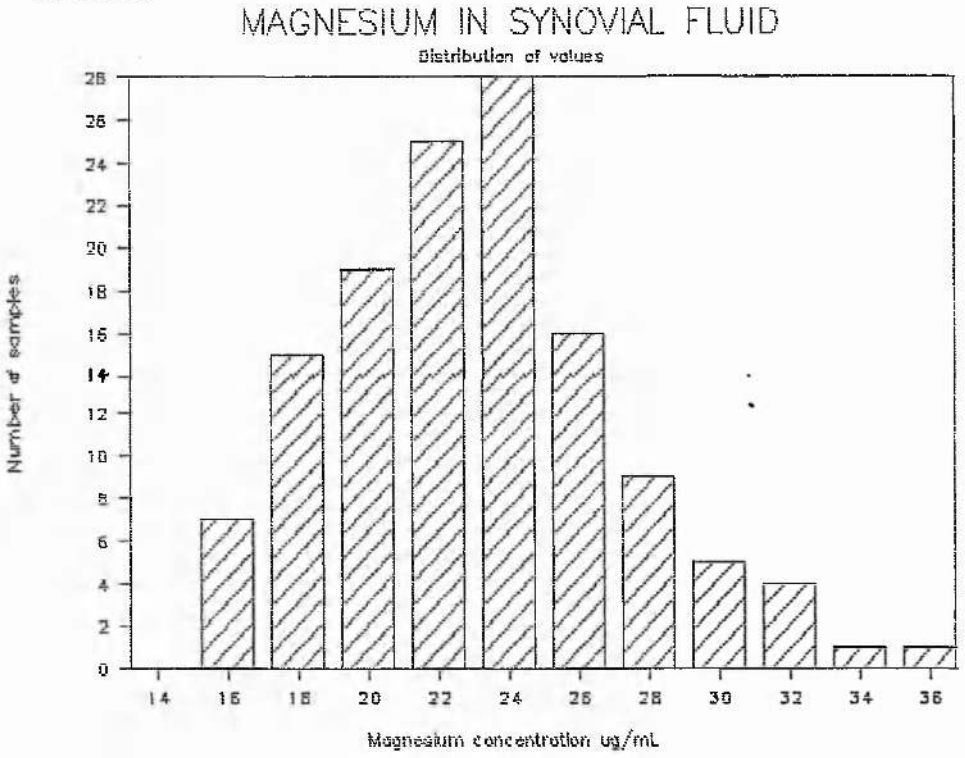
a: µg/mL fresh
b: literature values (77)

using ICP-MS has been described earlier (section 2.1.5.); this is the probable explanation for the disparity in serum and synovial fluid values. A polyatomic interference between argon and chlorine is also likely to be the cause of the high values obtained in this study for the element arsenic, even though the amount of comparative data is small. These values are presented as reference data, only, for future comparison using an alternative technique. There was little comparative data available for a number of other elements in addition to arsenic, particularly those at nanogram or subnanogram levels, for instance, lithium, germanium, barium, tin and lanthanum. Most of these elements have not yet demonstrated a useful role within the body.

As it has been postulated that essential elements follow normal or Gaussian statistics within the body (4,5), the distribution of values obtained for several elements in synovial fluid have been plotted. Those of magnesium, calcium, iron, copper, zinc, rubidium, strontium and caesium are shown in Figure 58. True normal distributions were not necessarily expected because the samples were taken from arthritic patients and not from healthy individuals. In fact, magnesium and calcium data, Figures 58a and b, are quite symmetrical in form, while the other elements show a greater or lesser degree of positive skew. As previously described, RA patients tend to suffer from anaemia, which may partly explain why the peak of the iron-distribution curve, Figure 58c, is further to the left than expected. Literature suggests

Figure 58: Elemental frequency distribution in synovial fluid

a) Magnesium



b) Calcium

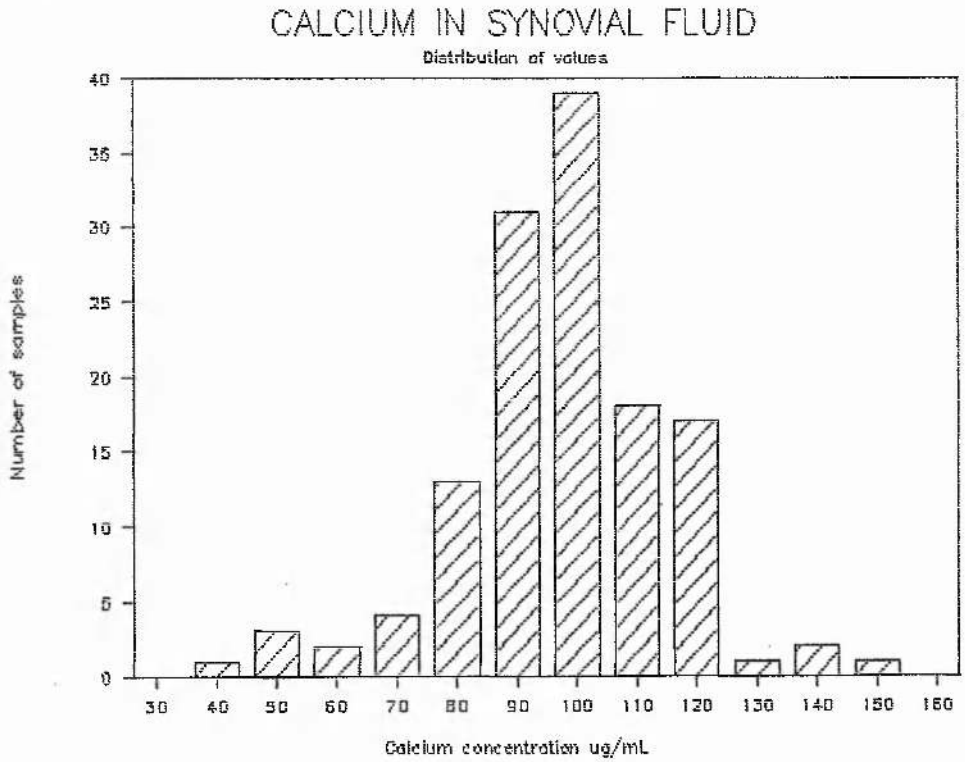
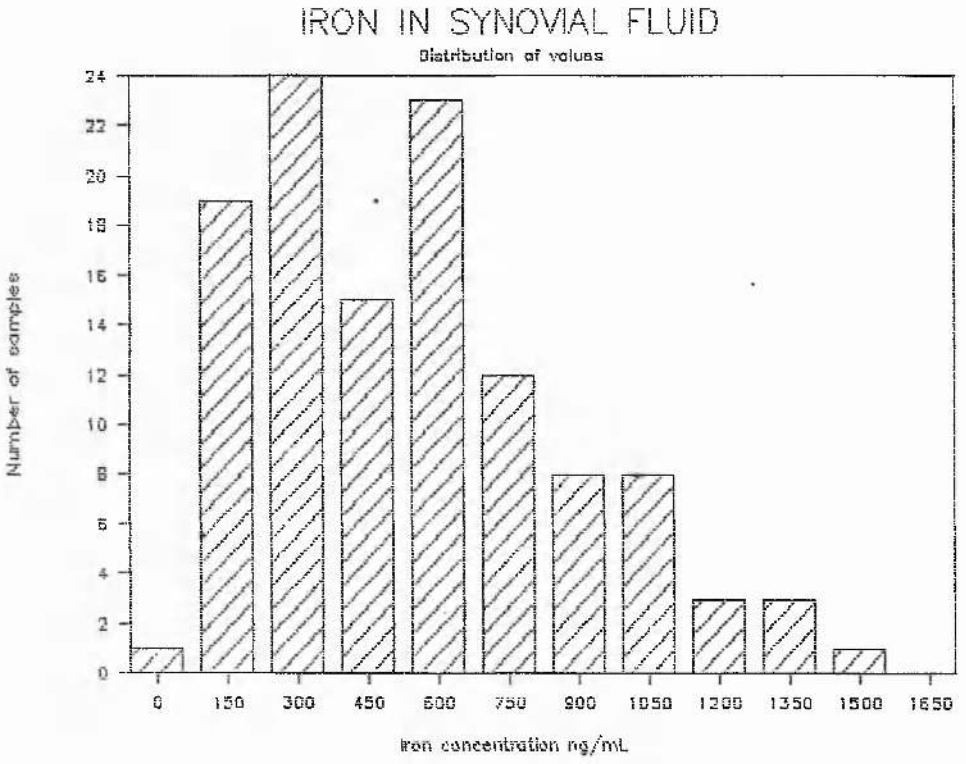


Figure 58 (con.): Elemental frequency distribution in synovial fluid

c) Iron



d) Copper

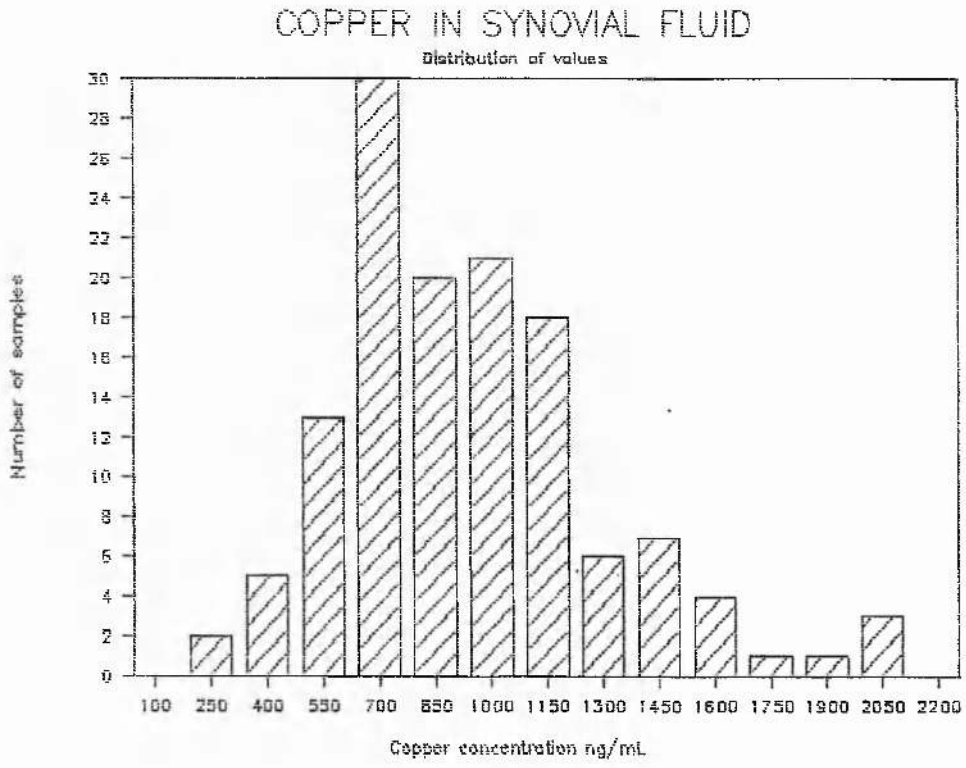
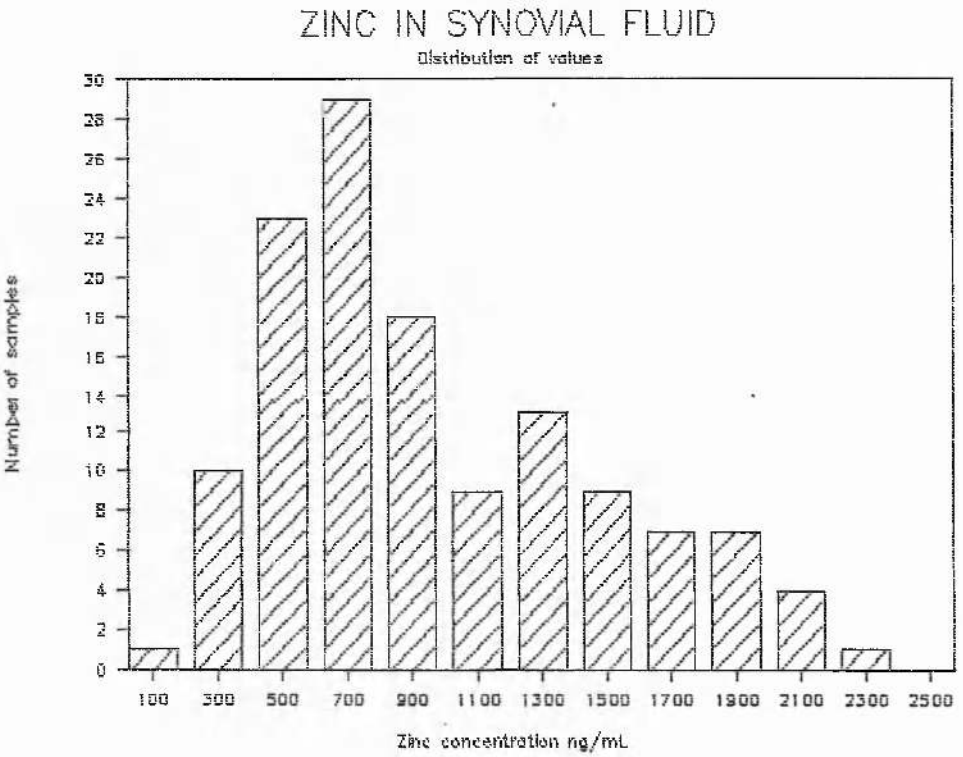


Figure 58 (con.): Elemental frequency distribution in synovial fluid

e) Zinc



f) Rubidium

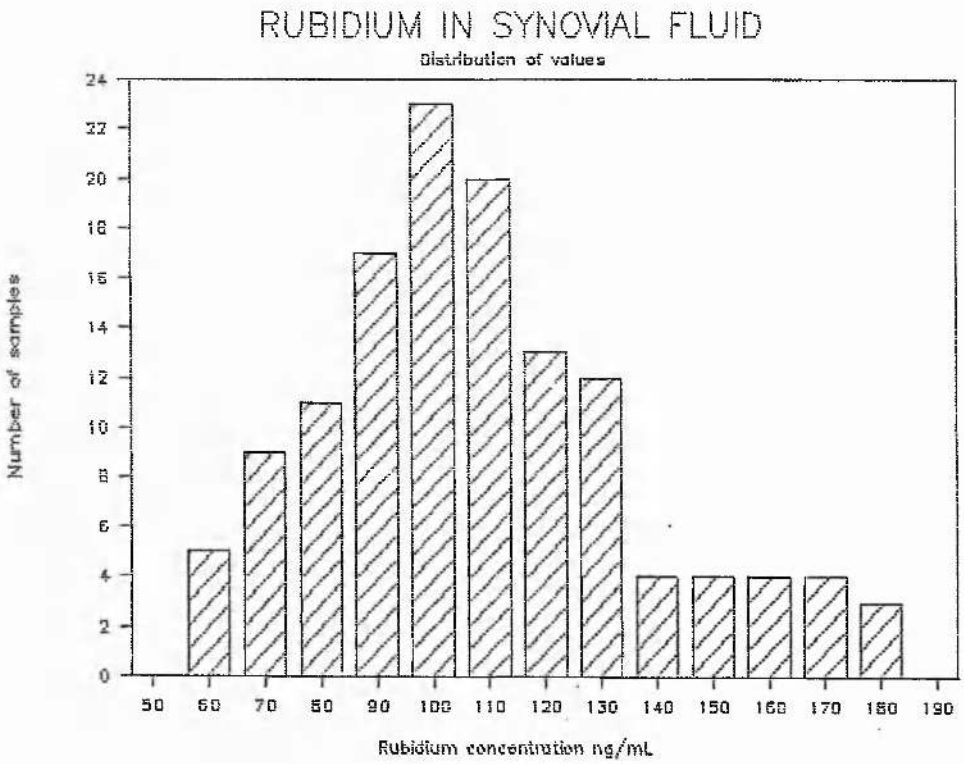
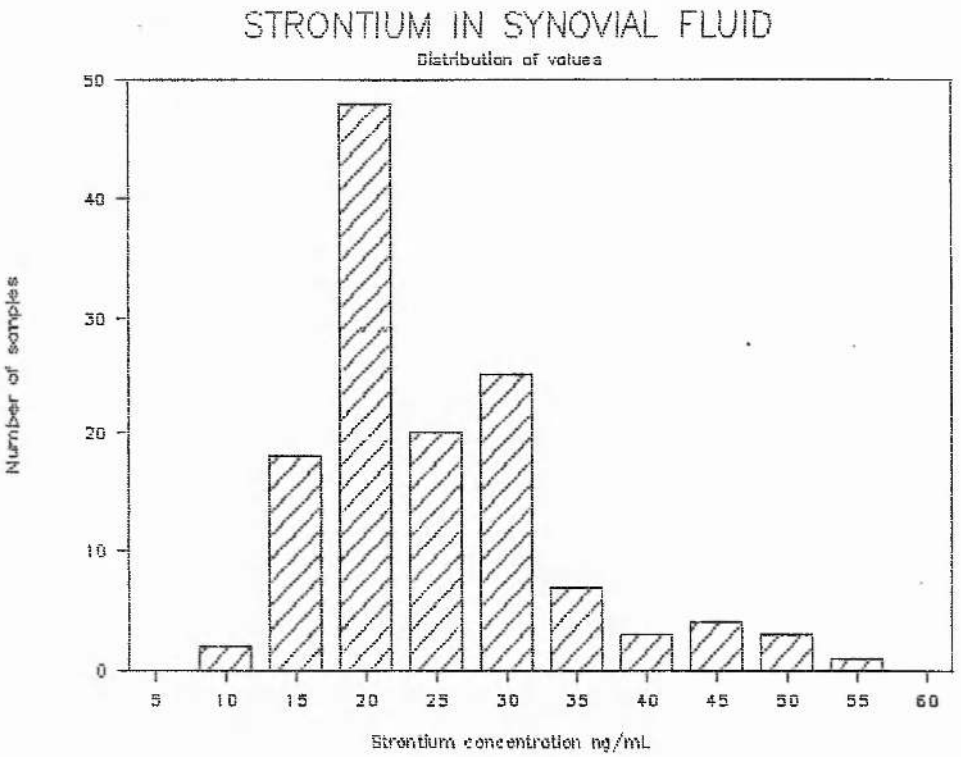
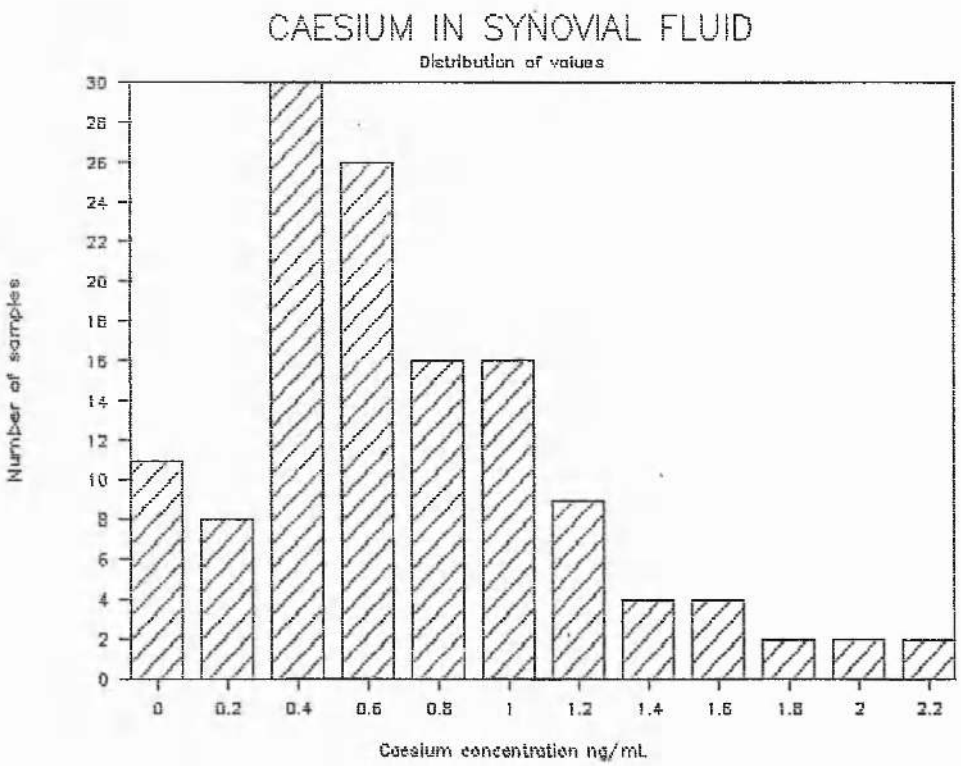


Figure 58 (con.): Elemental frequency distribution in synovial fluid

g) Strontium



h) Caesium



that copper and zinc may be increased in the synovial fluid of RA sufferers (207,208); this factor may result in the scatter of data around and gentle gradient of the downward slope of both copper and zinc curves (Figure 58d and e). Little is known about strontium and rubidium in humans, neither of which are essential elements; in synovial fluid, both elements show smooth and steep upward curves, with a more gradual and scattered decrease (Figure 58f and g). Elevated strontium has been noted in the granulocytes of sufferers from inflammatory disorders (209). Caesium is an example of an element whose levels in some samples were too low to be determined; see Figure 58h. This means that the upward slope of the graph is rather unclear; the data, however, still appears to have a positive skew.

5.2.3.2. Cellular and acellular synovial fluid

Following aspiration of synovial fluid from the knee joint, samples were divided into two. One aliquot was stored directly; the other was spun down, and the supernatant removed and stored prior to sample preparation. These samples were then analysed by ICP-MS, to compare synovial fluid with and without cells.

Statistical analysis was carried out in two ways. Firstly, the "matched pair" t-test was used (Equation 5, section 5.2.1.2). Using this method of comparison, significant differences in elemental

composition were seen for three elements. For phosphorus, the acellular fluid had a "highly significantly" (S*: at the 1% level) lower elemental content than the "whole", cellular synovial fluid. There were very highly significant differences (S**: at the 0.1% level) for rubidium and barium. Compared to cellular synovial fluid, the fluid without cells was lower in rubidium but higher in barium.

To study the data graphically, the median, upper and lower quartiles of cellular and non-cellular synovial fluid were plotted, and are shown in Figure 59. The inter-group distinction is still clear for barium, but less obvious for phosphorus and rubidium. For most of the other elements, the median value of spun fluid samples tends to be lower than the whole fluid, although the differences are not statistically important because of the size of the interquartile range. This is not surprising, since it is likely that some fraction of the trace element content of synovial fluid would be bound within the fluid cells.

Data distribution curves for cellular and acellular fluid were drawn for the three elements mentioned specifically in this section. Those for barium and rubidium are shown in Figure 60. The difference between the sample groups for barium is very clear, whereas it is considerably less so for for rubidium (and was worse still for phosphorus). The result for barium is rather strange since the cellular sample is

Figure 59: Variation between cellular and acellular synovial fluid (I)

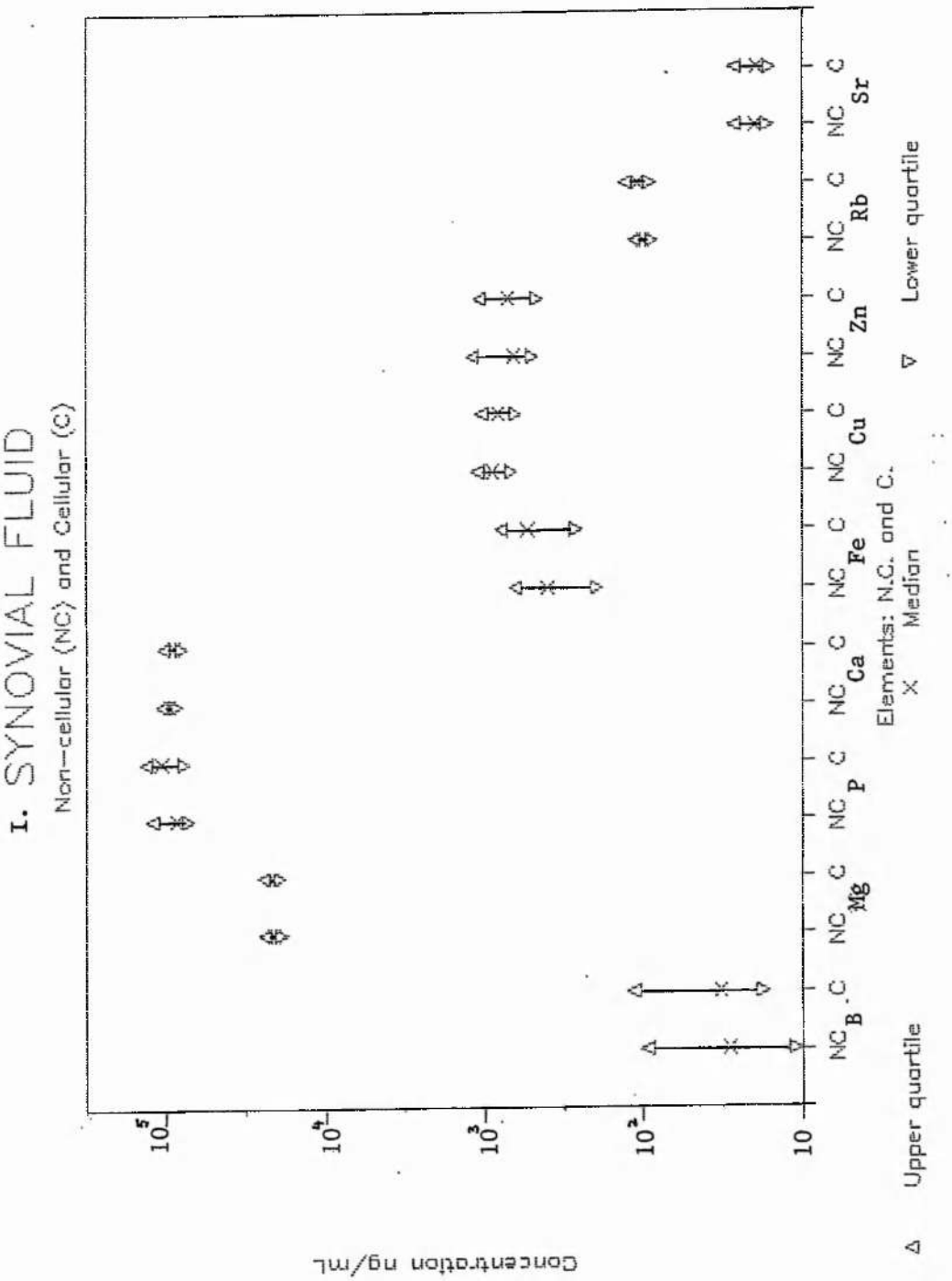


Figure 59 (con.): Variation between cellular and acellular synovial fluid (II)

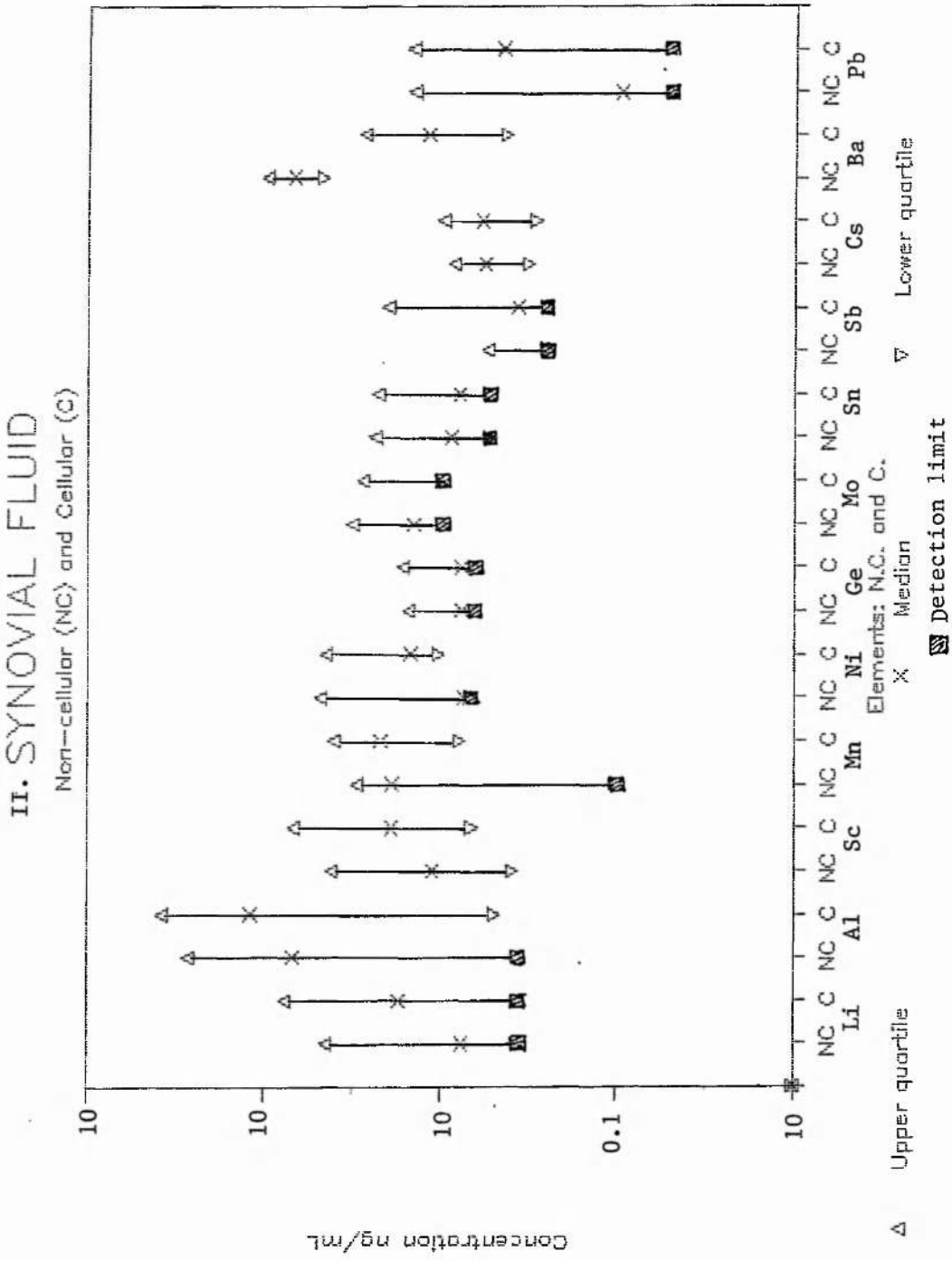
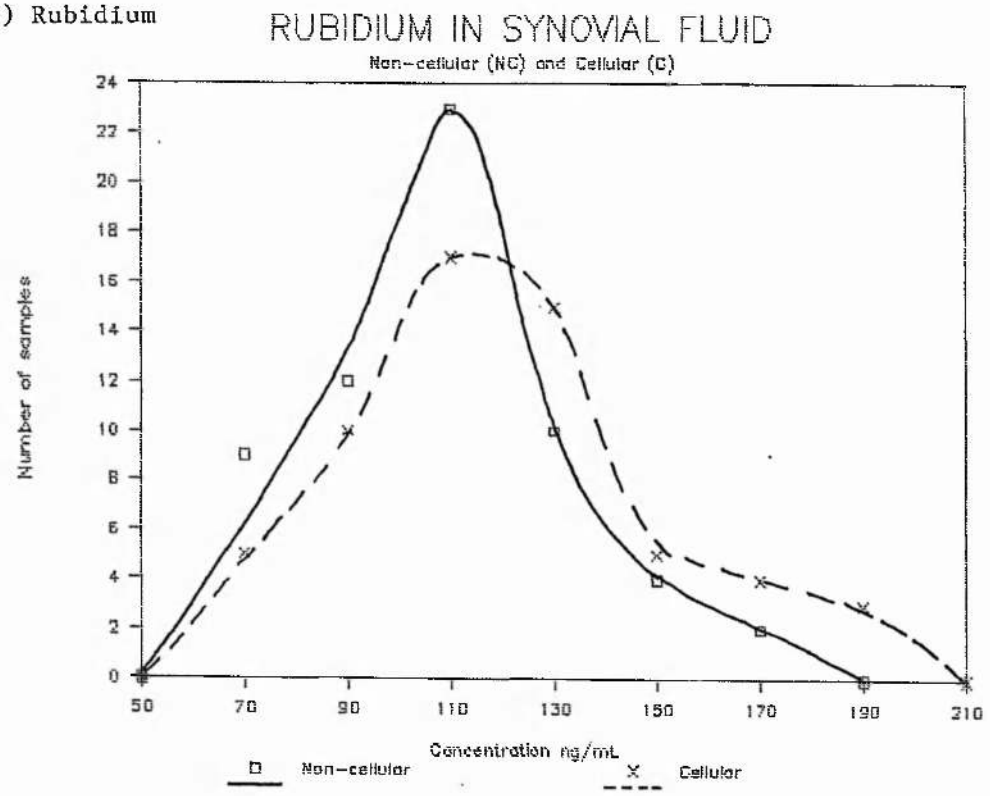
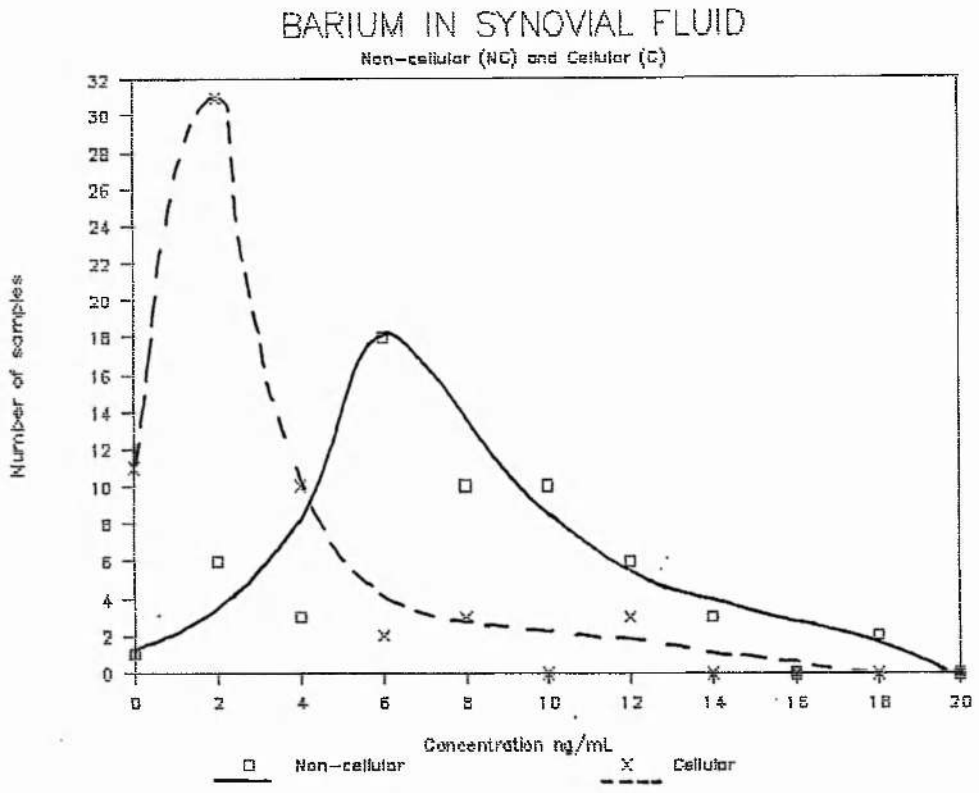


Figure 60: Frequency distributions of cellular and acellular synovial fluid

a) Rubidium



b) Barium



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"whole", untreated synovial fluid, which is then spun to give the acellular fluid by removal of the cell fraction. This suggests that there is a source of barium contamination somewhere in the spinning-down or in the separation of the acellular supernatant, for example, from the glass pipette used in separation. Errors might also be introduced from the sample tubes used (although plastic or polypropylene vessels were always utilised to try and prevent this), or perhaps, by contact with the sides of the tubes during centrifugation.

5.2.3.3. Variations due to age and disease duration

The subjects, from which synovial fluid was taken, ranged in age from 14 to 86 years. Regression analysis was carried out on the data to establish if there was any correlation between age and elemental concentration. Values of regression coefficient, r, were again substituted into Equation 4 (section 3.2.3.2) to give a t-value and thus establish significance. The only element to show any association with the age of the individual was molybdenum, significant at the 5% level. Figure 61 shows the measured molybdenum values plotted against age in years, with a calculated regression line drawn through the data points. The data appear to be widely scattered; the trend, as shown by the line, is very hard to see.

Elemental concentrations were also correlated against disease duration, which varied fron a few days to 20 years. No significant associations were found. Disease duration was also plotted against the white blood cell count of the fluid (clinical parameters measured in the Department of Rheumatology, University of Manchester), which is a measure of the degree of inflammation. Again, no correlation was found, indicating that disease duration is not necessarily an indicator of disease severity.

5.2.3.4. Variations due to sex

In the study population used, there were 56 samples taken from females and 67 from males. The average white blood cell count of the two groups was similar (11.4 and 10.7 x $10^3/mm^3$ respectively), enabling a reasonable comparison to be made between the two groups on the grounds of sex. The Student's t-test was used to quantify any distinctions between the two sets of data by using the arithmetic mean; no significant differences were found. To check these results, the medians and interquartile ranges were evaluated and compared. Again, no obvious distinction between groups was noted - the elements showing the greatest variation are shown in Figure 62. Not unexpectedly, iron is lower in females than in males; female individuals, however, show an interesting increased level of zinc, although this is accompanied by an extremely large interquartile range. Despite the fact that women

Figure 61: Variation in synovial fluid due to age

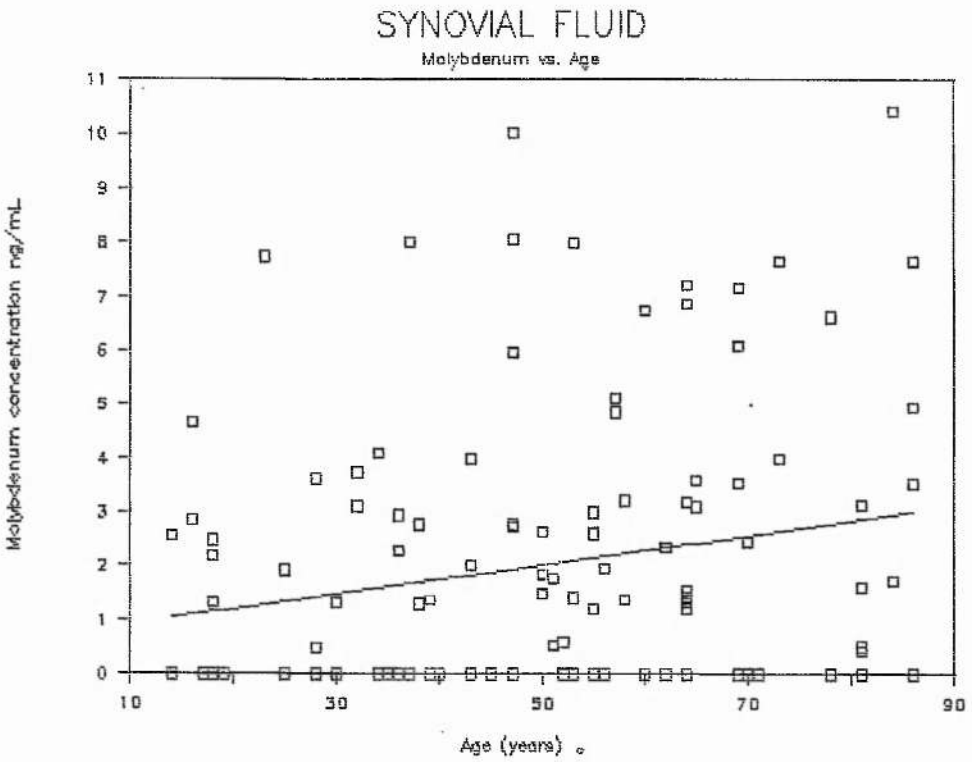
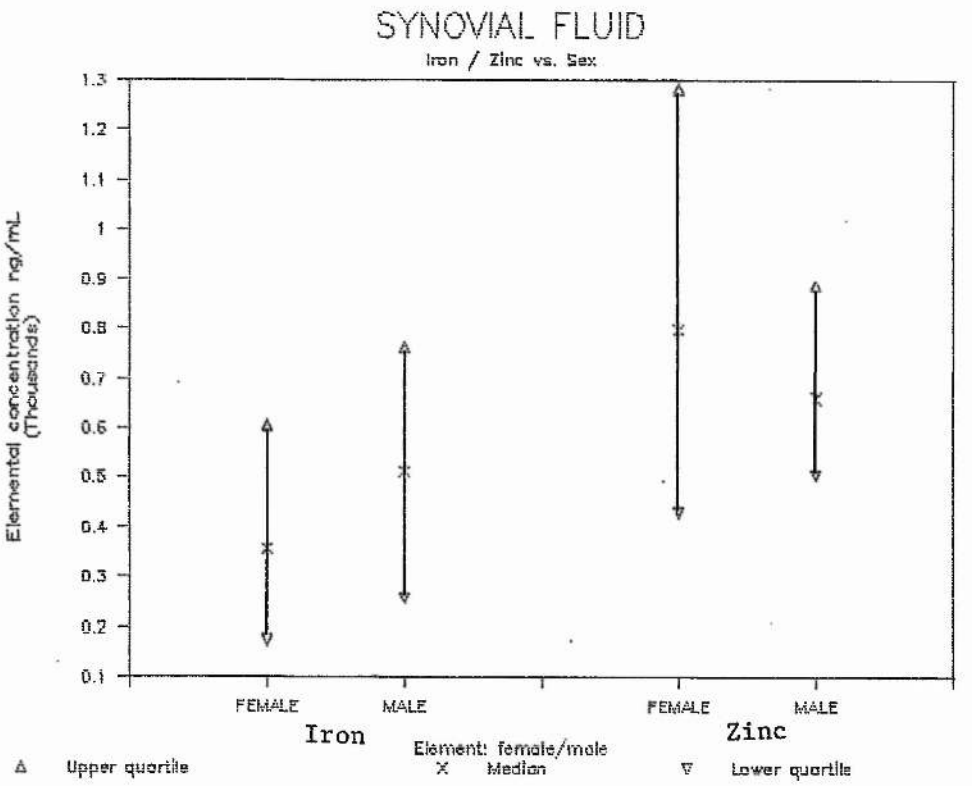


Figure 62: Variation in synovial fluid due to sex



have been found to display consistently higher serum copper levels than men (1), this is not echoed by the synovial fluid data.

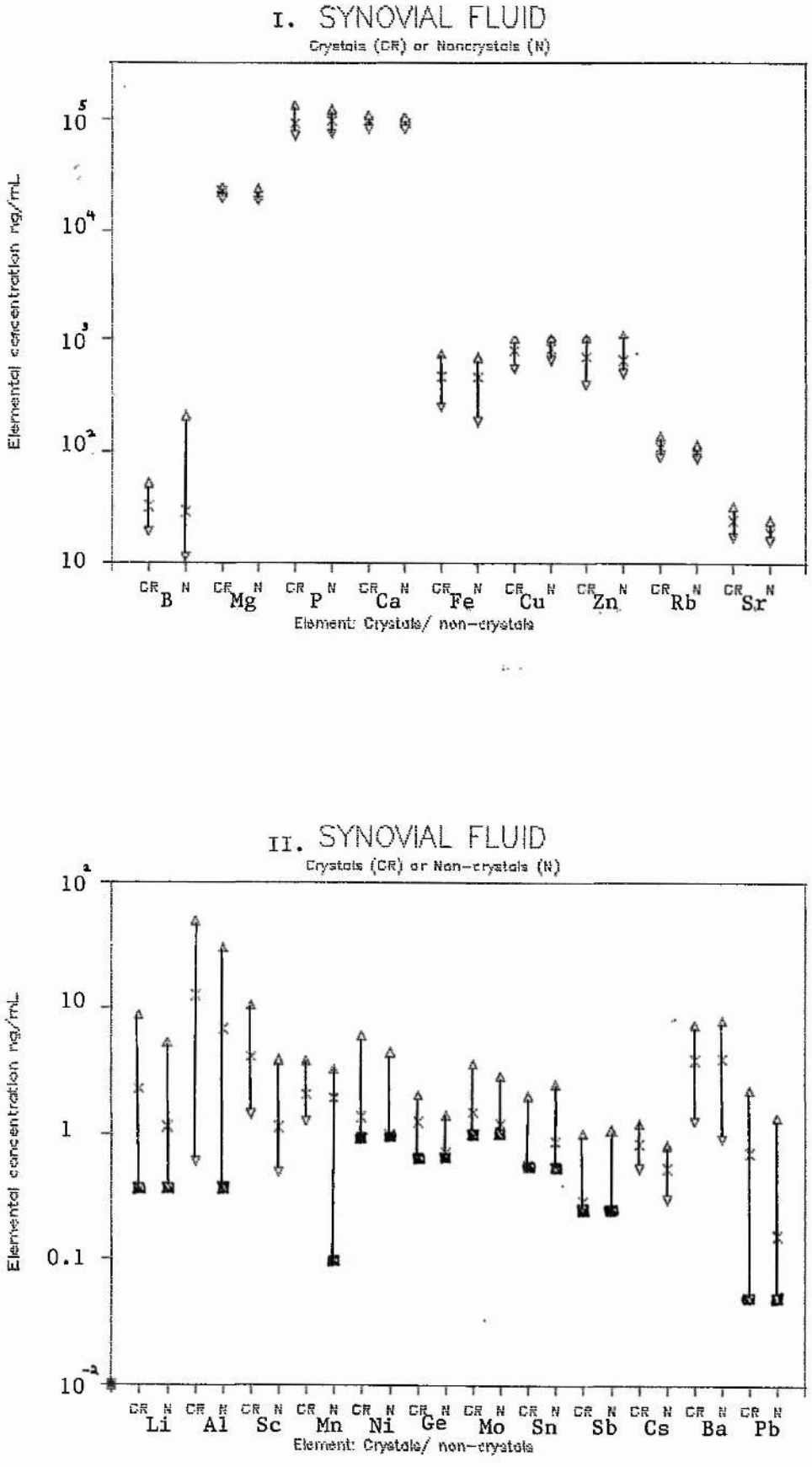
5.2.3.5. Variations due to crystal content

The crystal content of the synovial fluids were measured (Department of Rheumatology, University of Manchester), and were classified according to whether they were rare or numerous in number, intra- or extra-cellular in location, and bone-derived, apatite, calcium pyrophophate or monosodium urate in type. Samples containing crystals were compared with those absent of crystal formation. The student's t-test was used; in addition, the elemental variation between the two groups was shown graphically, in Figure 63.

Significant differences are displayed for scandium, strontium and caesium; in all these cases, the synovial fluids containing crystals show higher elemental levels than the non-crystalline. This is the basic trend for most of the elements, although in the majority of cases, the differences between the two groups are quite small.

A number of causes have been suggested for formation for calcium pyrophosphate crystals, including elevated levels of either calcium or inorganic pyrophosphate, changes in the matrix that promote crystal formation, or a combination of these. An influx of magnesium, thus

Figure 63: Variations between crystal- and non-crystal-containing synovial fluid



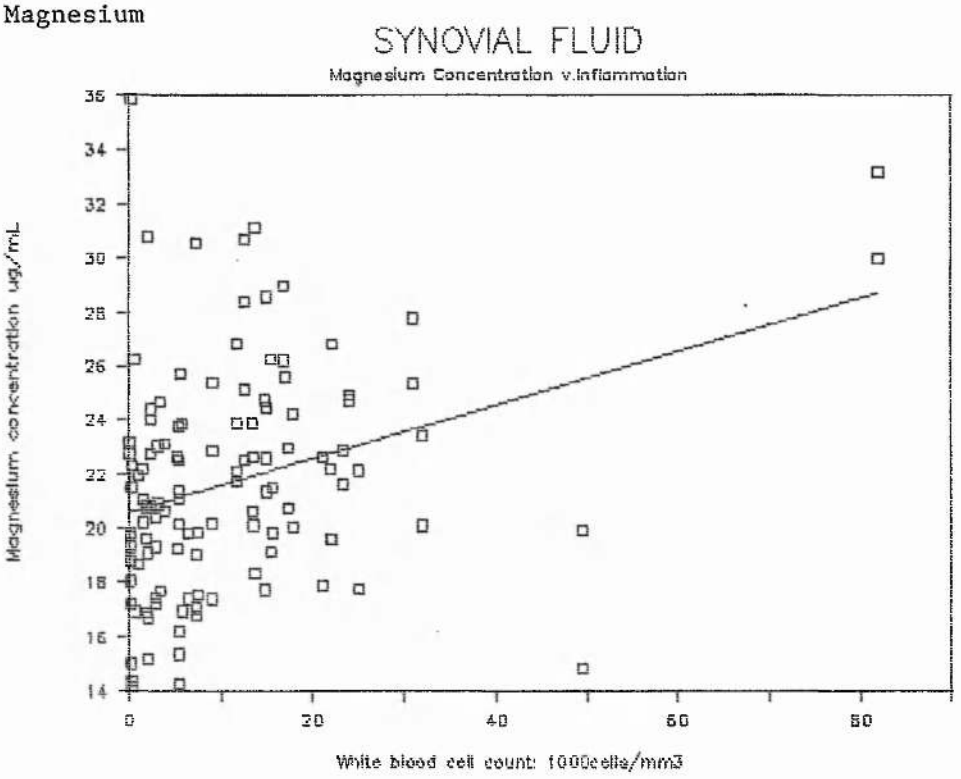
altering the matrix composition, has been shown to induce the formation of crystals, while iron can slow this process (132). Despite this, there were no significant changes in synovial fluid levels of magnesium, phosphorus, calcium or iron demonstrated in this study. Of course, numerous other factors may be involved, such as inorganic matrix components acting as nucleating agents (132).

5.2.3.6. Variations due to white blood cell count

The white blood cell count in synovial fluid increases as a joint becomes inflamed; in some ways, therefore, the increase in white blood cell count can be seen as a sign of disease severity (199-201). Elemental concentrations obtained in this study were correlated against the white blood cell count (measured in the Department of Rheumatology, University of Manchester) of the synovial fluids using regression analysis. As before, the regression coefficient, r, was used to calculate a t-value, and the significance of the data obtained. There was a highly significant association (1%) between caesium and white blood cells, while magnesium, phosphorus, copper, zinc and rubidium all showed very highly significant correlations (0.1%). These data are shown, with calculated regression lines, in Figure 64. In the case of copper and zinc, these results agree with findings that sufferers of RA (an inflammatory disorder) have elevated levels of these elements compared with osteoarthritic (OA) subjects

Figure 64: Variations in elemental content of synovial fluid with white blood cell levels

a) Magnesium



b) Phosphorus

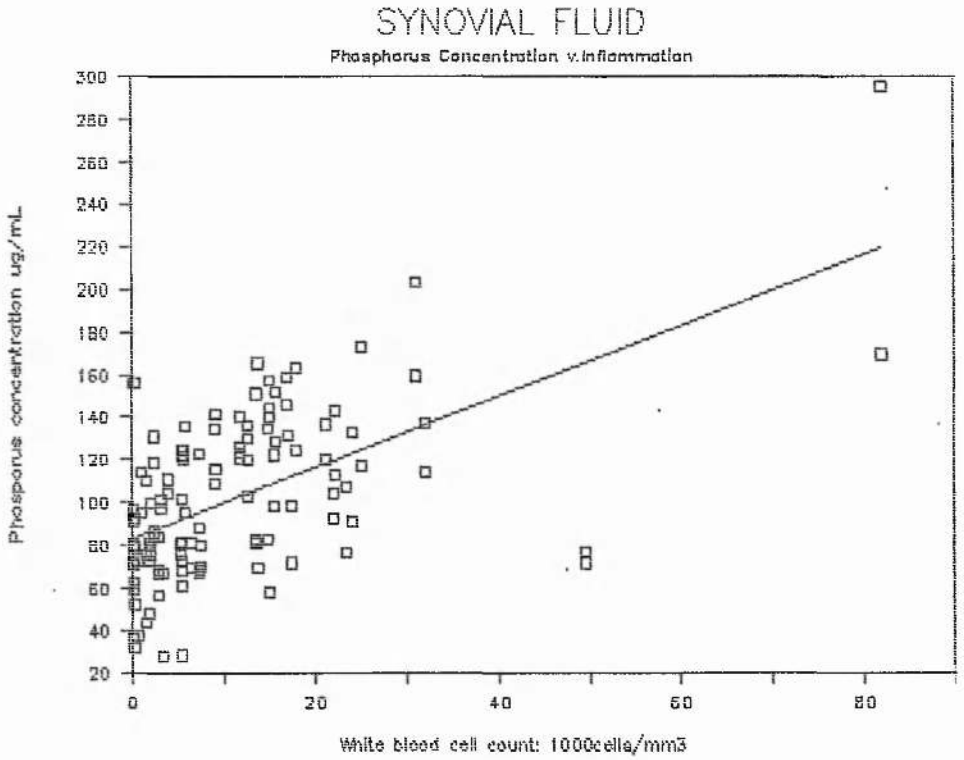
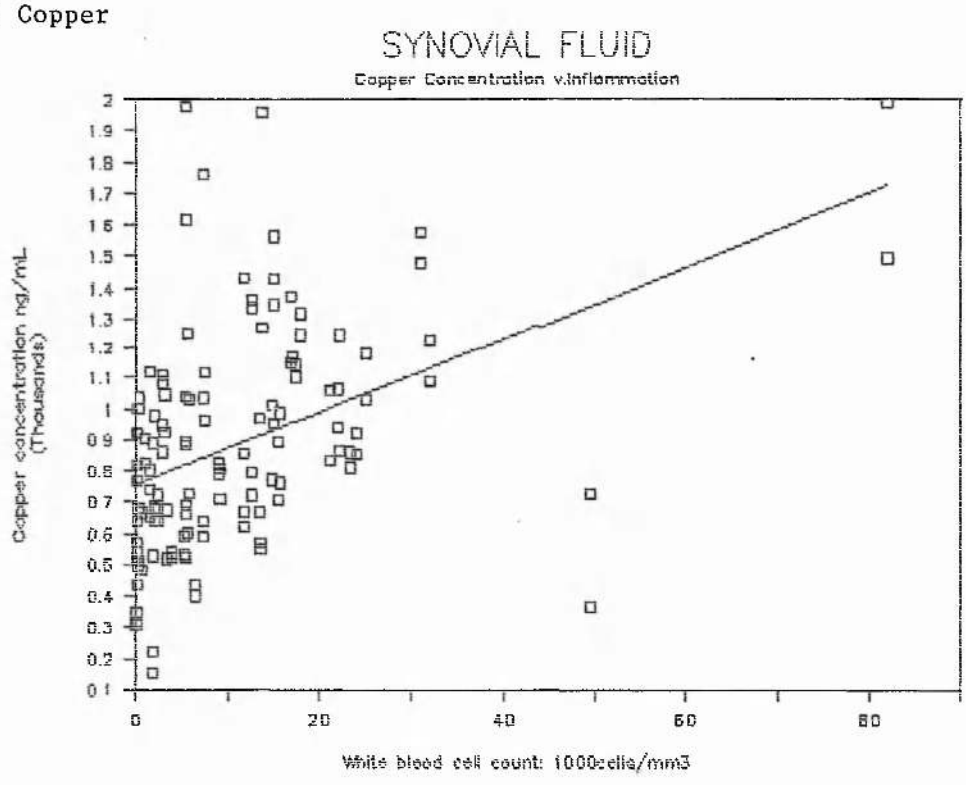


Figure 64 (con.): Variations in elemental content of synovial fluid with white blood cell levels

c) Copper



d) Zinc

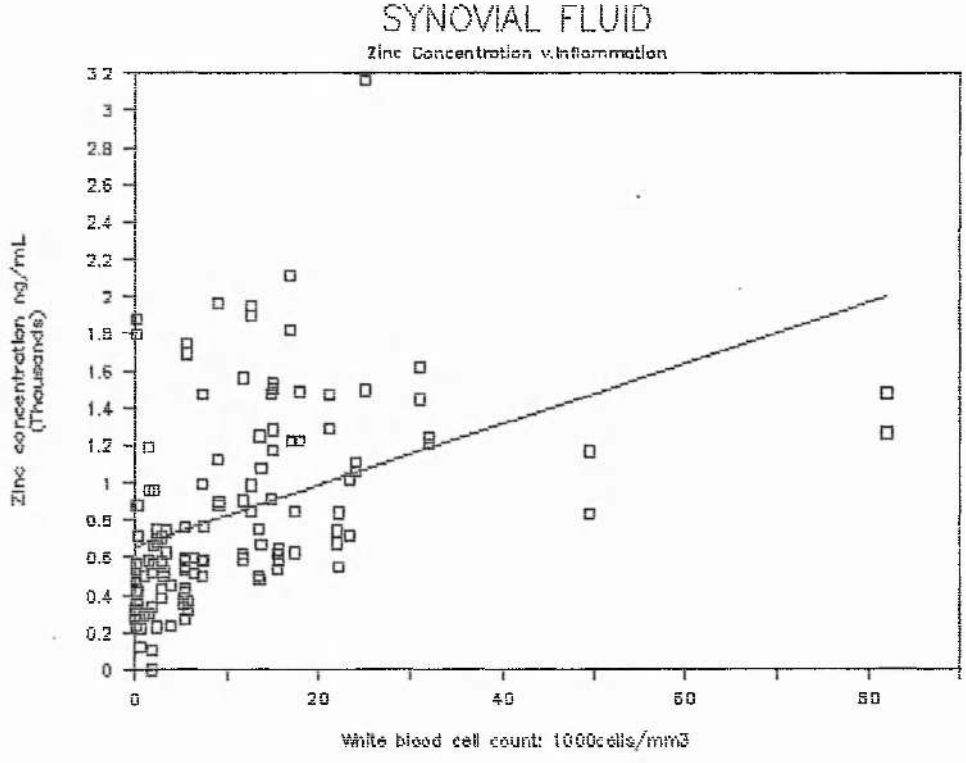
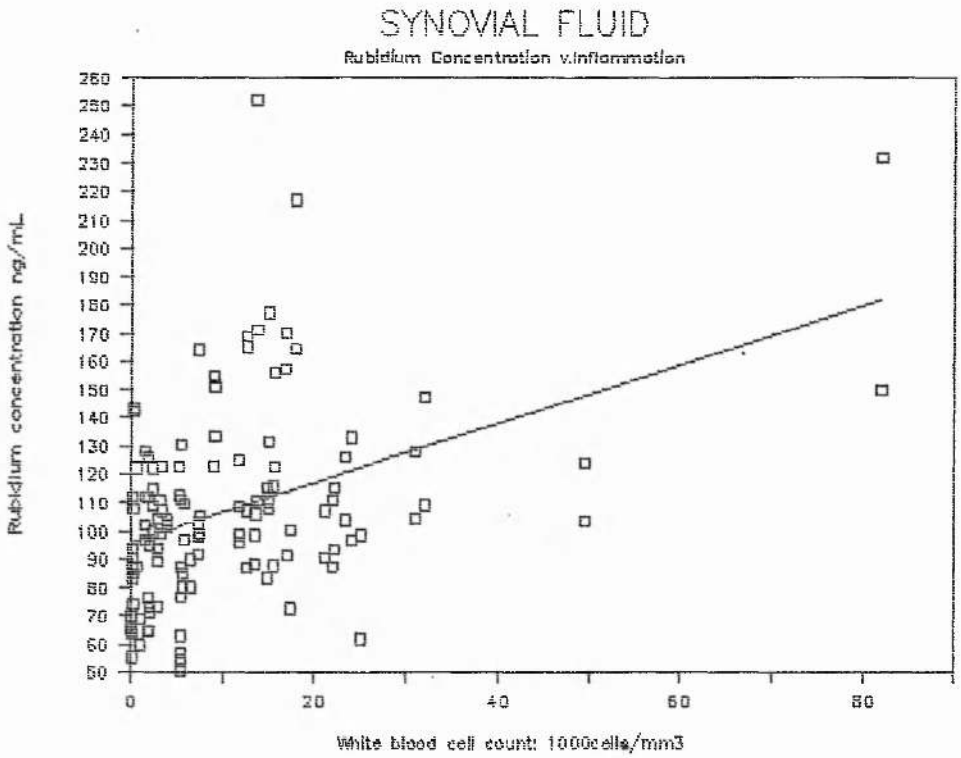
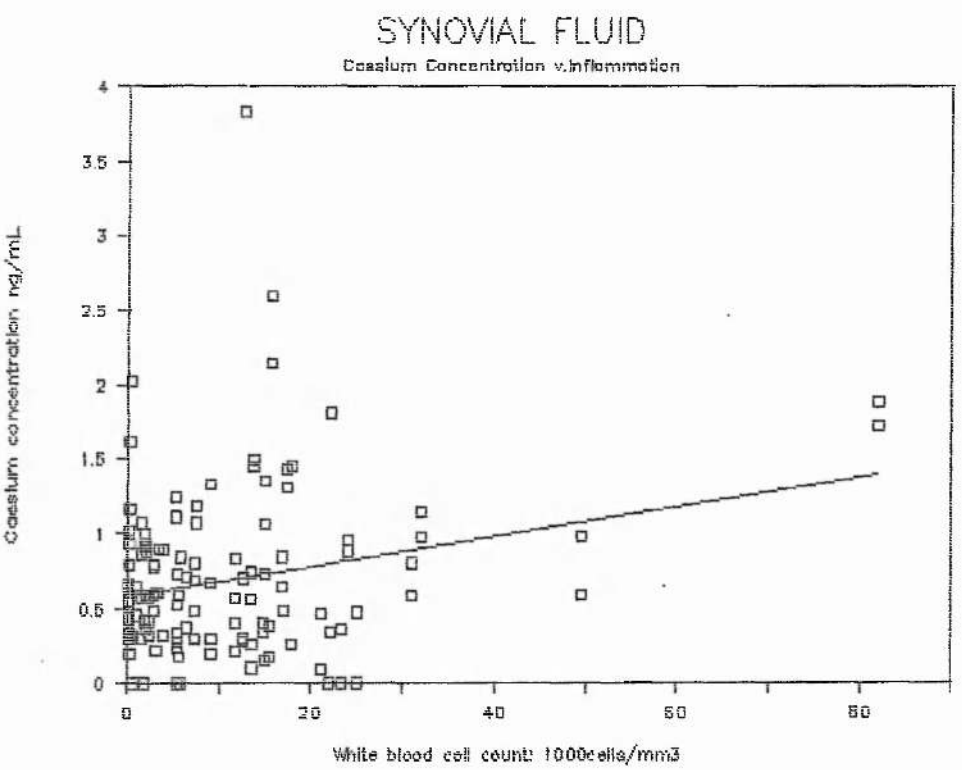


Figure 64 (con.): Variations in elemental content of synovial fluid with white blood cell levels

e) Rubidium



f) Caesium



(207,208). It is known that "inflammatory" synovial fluid contains more proteins than "non-inflammatory" i.e. it becomes closer to serum or plasma in composition. It is possible that the increase in trace element levels in synovial fluid with a high white blood cell count is due to the binding of some of these elements to the plasma proteins.

5.2.3.7. Comparison of different arthritic disorders

In this section, the trace element content of synovial fluid from OA, RA and sero-positive RA (S+RA) subjects are compared. As has been already explained, it proved impossible to obtain synovial fluid from healthy individuals to act as "controls", due to the small quantity of available fluid, its viscosity, and the discomfort which would be suffered by healthy people unnecessarily (132). Osteoarthritic, noninflammatory fluid was used as a comparison with the groups of RA fluid, instead. The samples in each sub-group are chosen according to the clinical diagnosis for each subject. Even so, the results are likely to be similar to those in section 5.2.3.6., where the degree of inflammation, assessed by the white blood cell count, was utilised.

Comparison between sets of data were made using the Student's t-test, and this yielded the elemental variations shown in Table 34. These results were checked by plotting median, and interquartile ranges; this ensured that any effect of outliers on the t-value (calculated

Table 34: Comparison of osteoarthritic, rheumatoid arthritic, and seropositive rheumatoid arthritic subjects

Disorder	Level of significance S:5% level	S*:1% level	S**:0.1%level
OA / RA			-Mg[a],-P,-Cu,-Rb
OA / S+RA	-Rb	-Mg,-P	-Cu
RA / S+RA	+B[b]		

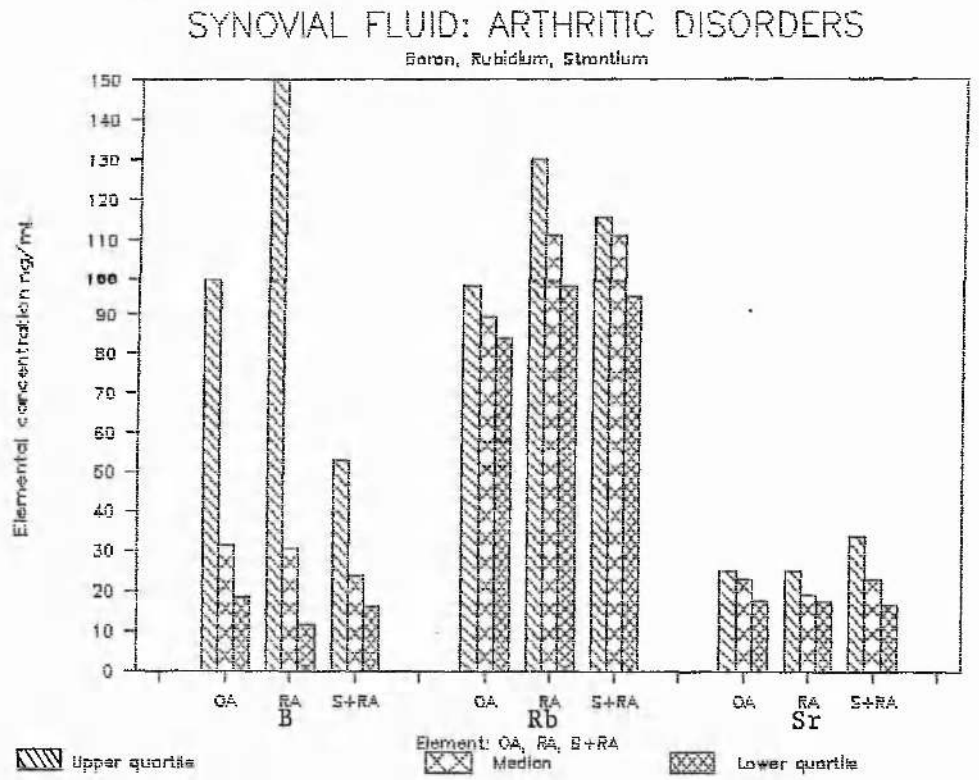
a: Mg(OA) < Mg(RA)
b: B(RA) > B(S+RA)

from arithmetic means and standard deviations) would be noticed. These graphs (see Figure 65) compare the elemental levels for the three arthritic subject groups, for the elements that were shown to be significant by the t-test i.e. boron, magnesium, phosphorus, copper and rubidium. Iron, zinc, molybdenum, tin and caesium are also shown; the variations in their median values were noted even though the t-test showed no significant variations. Finally, calcium and strontium are included as comparisons; these elements show very little variation between the different disorders investigated here.

By reference to Figures 65a and b, it can be seen that magnesium, phosphorus, copper, zinc and rubidium confirm the findings of the last

Figure 65: Elemental variations in synovial fluid from different arthritic disorders

a) B, Rb, Sr.



b) Mg, P, Ca.

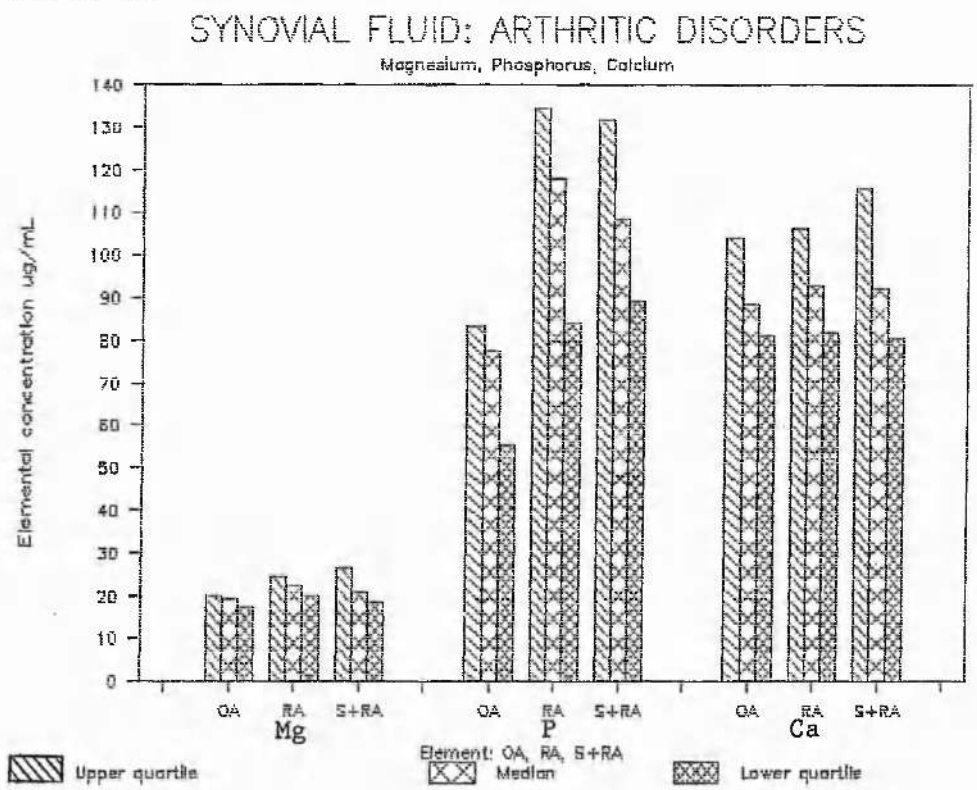
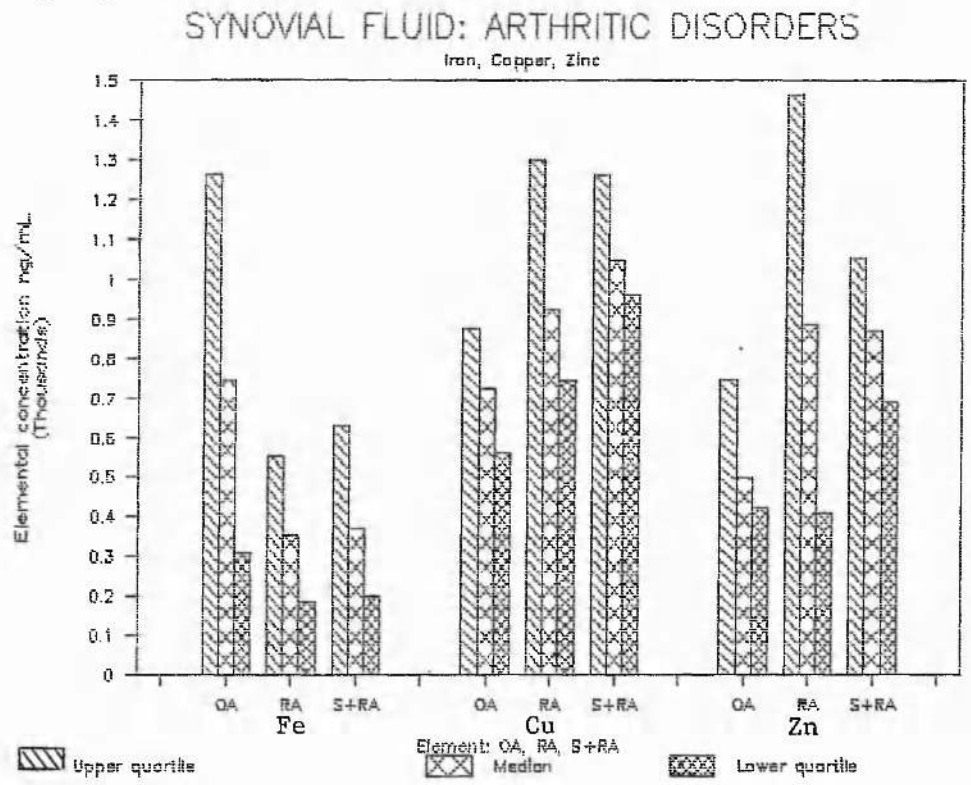
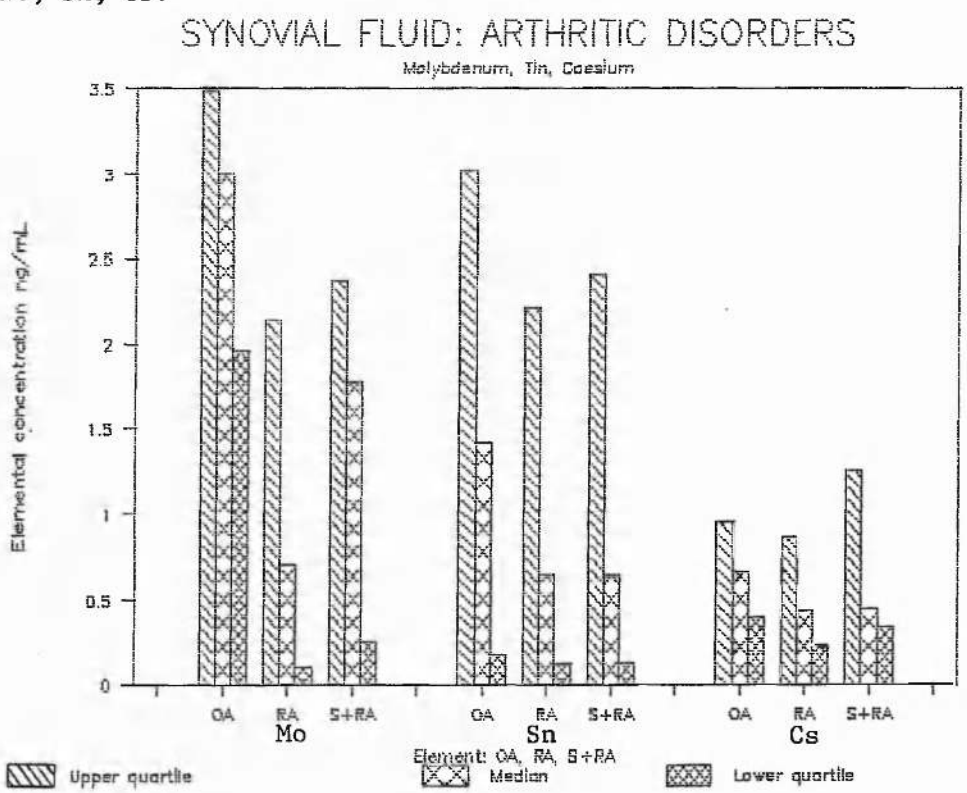


Figure 65 (con.): Elemental variations in synovial fluid from different arthritic disorders

c) Fe, Cu, Zn.



d) Mo, Sn, Cs.



section i.e. that their levels are elevated in an inflammatory disorder, such as RA or S+RA compared to OA. Of these five elements, copper is the only one to show any degree of variation between RA and S+RA subjects. The average value of white blood cells may be significant here; S+RA has a value slightly above RA (16.0 compared to 12.7 x $10^3/mm^3$) while both these groups are considerably above OA (0.6 x $10^3/mm^3$).

An interesting fact to note is that caesium shows decreased levels in the RA and S+RA compared with OA, whereas it displayed a highly significant positive correlation with white blood cells (section 5.2.3.6.). The reason for this is that RA is not the only inflammatory disease; gout is an example of another in which the white blood cell count is raised. In the latter disorder, the inflammation is due to the presence of crystals within the joint tissues and fluid. Caesium levels were raised in all crystal-containing synovial fluids (section 5.2.3.5.), and particularly so in the sub-division of individuals suffering from gout.

One element which shows a depletion in its levels in RA subjects, compared to OA, is iron. This tallies with the reports that a high proportion of RA sufferers are anaemic (226), although it disagrees with an earlier finding (206). Variations in iron concentrations were also noticed in brain and bone samples between RA and control subjects

(sections 5.2.1.5. and 5.2.2.3.). Decreased levels, for individuals with RA, were observed in other elements including caesium and tin, as shown in Figure 65d. Boron shows a depleted level in S+RA compared to the other groups, but with an extremely wide interquartile range for OA and RA groups. Boron was also found to be at lower levels in the bone of RA patients (section 5.2.2.3.). Molybdenum also displays strange behaviour. A sharp drop in its levels in the RA group is followed by an equally abrupt rise in the S+RA sub-division.

5.2.4. Summary

Having analysed brain and bone from rheumatoid arthritics and controls, and synovial fluid from a group of arthritic disorders, the following findings can be summarised:-

i) The multielemental capabilities of ICP-MS allowed a full description of the elemental content of brain, bone and synovial fluid to be made. In the 27 years since the last study of synovial fluid was made, the developments in analytical technology have provided the opportunity to measure, and obtain data for many elements which were previously unreported, such as, lithium, boron, scandium, germanium, molybdenum and lead et al..

ii) It was interesting to be able to report reduced levels of boron in the bone of arthritic subjects compared to controls (0.84±0.77μg/g c.f. 1.57±0.86μg/g), thus confirming clinical observations of the

improved condition of bone material after boron supplementation (91,203), and experimental observations (128).

iii) The alteration of trace element levels in synovial fluid taken from inflamed joints was quite evident. Previously reported findings concerning elevated copper and zinc were confirmed (206,207); other elements, such as phosphorus, rubidium and caesium were also shown to demonstrate altered behaviour. Caesium, in particular, was interesting since it displayed differences in behaviour between two inflammatory disorders i.e. increased in gout, but decreased in RA, both with respect to OA.

6. CONCLUSIONS

This study has illustrated that ICP-MS provides an accurate and precise technique for the measurement of elements in biological tissues and fluids, and biological standard reference materials. The concentrations of these elements range from subnanogram/gram (or mL) to percentage levels (%w/w), demonstrating the wide dynamic range of the technique.

One of the main aims of this study was to carry out analysis of the element boron within clinical specimens. The detection limit of 2.6 ng/mL ("instrumental" detection limit) was less good than for many other elements using ICP-MS (boron has a comparatively low degree of ionisation as calculated from the Saha equation: section 2.1.1.2.). It was, however, still suitable for most samples measured in this study, even taking into account the dilution factors involved. From measurement of NIST 1577A bovine liver, the only available standard reference material used which had accompanying information values, it appears that the precision in analysis of boron by ICP-MS is slightly less impressive. One factor here is probably the relatively high boron blank value, as measured by ICP-MS. It would appear that there were some sources of contamination in the instrumental system itself, due to elemental adhesion to the plastic tubing of the nebuliser and in the spray chamber. This also led to memory effects if great care was

not taken to wash out the system between samples. In addition, despite the use of ultra-pure, doubly distilled deionised water (DDW), there appeared to be a residual boron content of 7.7ng/mL (242). In 1% HNO_3, there was a higher level, possibly due to the glass bottle in which the concentrated acid is stored; this was 13.8ng/mL (242). Because of the high levels of boron known to be in glass or plastic, polypropylene was used in sample or standard preparation or storage whenever possible.

Despite these problems associated with measurement of boron, successful analysis of this element was carried out in a wide range of tissues and fluids. This contributed valuable data to the continuing investigation into the possibility of this element having an essential role in animals and humans. Of particular interest here was the way in which boron was positively correlated with magnesium, copper, calcium and phosphorus in whole blood. It has been suggested that changes in dietary boron may have an effect on the role in the metabolism of all these elements (93,127).

One important factor which has been noticed during the course of this study is that samples collected, however carefully, in a clinical environment, may show some slight alterations due to contamination. How this affects boron has been impossible to quantify; however, this study showed how barium levels were altered in acellular synovial

fluid (probably due to the use of a glass pipette in the separation stage) and manganese in serum (partly due to a stainless steel needle; perhaps some rupturing of red cells had also taken place). These difficulties will probably continue until research analysts are provided with an opportunity to work in a clinical setting, and are better able to monitor collection procedures.

One aspect concerning the essentiality of elements, which has been under scrutiny in this study, is the frequently quoted statement that essential elements are characterised by normal frequency distributions in human tissues. On the other hand, non-essential elements follow the environmental pattern, producing skewed curves (4,5,145). It appears from this study that this is not universally true for all biological tissues and fluids, but is dependent on the nature of the sample and its role in the body. In brain tissue, for example,essential elements such as magnesium and copper were considerably more symmetrical in their distribution than the non-essential aluminium and lead. In whole blood, however, all elements seemed to be undergoing some degree of regulation or homeostasis. This is probably due to the fact that many of its components, for example erythrocytes to which elements like mercury and lead are strongly bound, have a limited life span. Blood also acts as a means of transporting numerous substances, including chemical elements, to storage depots or functional areas within the body. In contrast, urine and saliva showed log-normal distributions

for most elements, indicating a dependence on external factors, not internal regulation. Thus, it would seem that for the elements and readily accessible (not autopsy) clinical samples analysed, the shape of the frequency distribution curve is a measure of homeostasis, not essentiality.

The investigation of elemental levels in various types of samples from arthritic and control individuals was also carried out in this study. Of particular interest, since a large portion of the work was concentrated on boron, was to find reduced levels of boron in the bone of RA patients, confirming earlier findings (128). This was not, however, accompanied by any significant variations in the boron content of synovial fluid. Other elements showing differences between RA and control tissues were lithium in bone and brain, and iron in bone, brain and synovial fluid, amongst others. A number of elements were at increased levels in "inflammatory" synovial fluid; these included copper, zinc and phosphorus, while caesium was unique in that it was elevated in fluid from sufferers of gout and not RA. These alterations in elemental levels are more likely to be symptoms of the inflammation, rather than causes of it. Further study of arthritic and control (or OA) specimens, perhaps on the smaller scale of separated cell fractions instead of the bulk material, should be able to suggest if trace elements might have a therapeutic as well as a pathophysiological role. For example, some findings in analysis of

blood cells have suggested that redistribution of cellular metals is a marker of hidden inflammatory disease (209).

In summary, ICP-MS was shown to be a very useful analytical tool for the measurement of biological specimens, enabling analysis of boron and other elements to be carried out, and in investigating certain aspects of human health and disease.

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