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CRYSTALLURIA IN VARIOUS GROUPS OF SPORTSMEN

A thesis submitted to the University of Cape Town in fulfilment of the requirements for the degree of Master of Science.

by
Tracy-Ann Cox BSc. (Hons)

DEPARTMENT OF PHYSICAL CHEMISTRY
University of Cape Town
Rondebosch, Cape
South Africa

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ABSTRACT

This thesis examines various groups of athletes to assess whether they are at risk with regard to kidney stone formation. Particle size distribution analysis (Coulter counter), ultra-structural analyses (SEM) and urine analysis were conducted.

The background to various factors relating to stone formation is discussed as well as the general theory behind the techniques employed. The methods utilized and data obtained are described.

Particle size distribution analysis and scanning electron micrographs suggest that marathon runners and cyclists may be at risk with respect to stone formation. Dehydration and urinary tract trauma are thought to occur in the former whereas dehydration only is operative in the latter. Results obtained from Na/Ca ratio analyses are found, more or less, to correspond with the particle size analyses thereby suggesting that this ratio may have potential as a useful index of stone-forming risk. The enormous spread of values amongst each class of athlete shows, however, that physical exertion is not the sole factor affecting the Na/Ca ratio .

CHAPTER 1

INTRODUCTION

CHAPTER 1

1.1 UROLITHIASIS - GENERAL

Renal stone disease has been recognised throughout medical history and is among the oldest documented medical disorders of mankind [1]. Since the days of Hippocrates it has been suggested that there may be a relationship between crystalluria and urinary stone formation. Hippocrates observed that the passage of sand in the urine was often indicative of the presence of stones in the kidneys or bladder. As early as the 3rd century BC [2], the Greek physician Ammonios used the technique of lithotrity to break up bladder stones mechanically so that they could be passed in the urine. Two hundred years later Celsus described the procedure of lithotomy but the terror of surgery encouraged the search for a potion that would dissolve stones. However, today due to medical and technological advancements [2], surgical removal of stones is routinely accomplished and better knowledge of their composition and aetiology has led to their likelihood of occurrence being reduced in susceptible subjects.

Urolithiasis is a world-wide, multi-factorial disease, with varying age, sex, and geographic correlations [3]. Other important contributary factors in the aetiology of urolithiasis include diet, profession, climate, drugs and race. South African blacks, for example, are immune. This issue will be discussed later.

In technologically developed countries [2], the disease manifests itself in adults who suffer from upper urinary tract (kidney) stones, usually composed of calcium oxalate, while stones in children are rare. The high incidence of upper tract stones, is strongly correlated with the increasingly sedentary life style in these countries [4].

In Czechoslovakia, for example, administrative employees have twenty times more stone incidents that Czechoslavakian farmers. Similarly, British naval officers have a stone incident rate twice as high as that of British seamen, presumably because their daily routine requires less physical effort. In technologically under-developed countries, it is children, mainly young boys, from the lowest economic class who are affected. The stones are from the lower urinary tract (bladder) and are composed of ammonium acid urate or uric acid.

As previously mentioned, geographic prevalence plays a role in urinary stone disease [2]. An example is seen in the south-east of the USA. The high incidence of the disease in North and South Carolina makes this region one of the stone capitals of the world [2]. Some studies have noted a correlation between this belt and the geographical distribution of soft water. Magnesium has been recognised as an inhibitor of calcium oxalate formation, and magnesium therapy for recurrent oxalate stone formers is widely practised. Deficiency of this element in the water, soil and vegetables of the south-eastern states, coupled with the hot dry climate are reported as key factors in the generation of the stone belt. The high daily intake of oxalate rich iced tea in these areas is a further contributory factor [2]. However, other reports deny that the nature of drinking water and urolithiasis are related [5,6,7].

Diet can be a contributory factor in the aetiology of urolithiasis [8,9,10]. Dietary oxalate has a significant affect on urine oxalate concentration and oxalate-rich foods are excluded from the diet of recurrent stone-formers. Dietary restrictions of dairy products are frequently prescribed in patients with calcium kidney stones due to the suspected lithogenic role of calcium. However, these restrictions have revealed a loss of bone mineral mass in some patients and therefore calcium restrictions are prescribed with caution [2].

Urinary calculi occur in a variety of shapes, colours, textures and sizes [2]. The smallest ones are crystals or crystalline aggregates about the size of a pinhead. The largest can grow to the size of a coconut [2]. The number of stones produced by any patient during a particular episode can range from one to very many, but single stones are more common than multiple ones [4]. Multiple stones can be faceted, often like rounded tetrahedra, as a result of packing and friction; they can be small and round, or even irregularly shaped, sometimes with tiny spikes. Single stones may be round, ellipsoidal, highly irregular or like a hempseed, mulberry or jackstone [4]. Stone surfaces may be smooth, uneven, or covered with large crystals. Stones differ in hardness; some can be sectioned easily, some crumble, others cannot be ground at all. Many have a well-defined nucleus surrounded by concentric bands of material; others are conglomerates with no obvious nucleus. The structure and appearance of a stone depends to a considerable degree on the texture and composition of the material present which is usually almost completely crystalline [4].

The most common constituents found in urinary stones are calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) with various compounds of calcium phosphate, magnesium ammonium phosphate and uric acid representing the majority of other substances present [11]. These constituents [4] frequently crystallize in stones as single crystals often oriented with respect to the nucleus, but they may also occur as a coherent fine powder sometimes with preferred orientation. The stones themselves may be conglomerates with or without preferred orientation, aggregates of single crystals, or mixtures. Constituents may also be amorphous. This type of material has no regular, repeating, basic structure since the component atoms or molecules are disordered [4]. Urinary stones generally consist of about 97.5% polycrystalline aggregate and 2.5% glycoprotein or mucoprotein matrix [12]. This proteinaceous material has recently received a lot of attention and many studies now suggest that it plays a primary role in stone formation [13,14,15,16].

The analysis of stones is vital for the study of urinary calculus aetiologies [13]. Knowledge of the composition, structure and ultrastructure of calculi enables investigators to accurately characterise the chemical conditions prevailing at the time of nucleation and growth. The analysis is very important, since often major components are depositied in secondary processes while deposition of minor components occurs in a primary event [13]. Analysis of a stone yields information which may enable the clinician to ascertain which clinical conditions are operative and so prescribe appropriate treatment [13]. In order to achieve this, all minor components must be identified. In addition to compositional and structural studies, analysis at the ultrastructural level can provide additional data for interpreting deposition mechanisms [13].

The formation of stones within the urinary tract in man represents a potential complication of many varied metabolic disorders [9]. In general [12] it seems that most chemical species excreted in the urine in sufficent concentration can become incorporated into a stone. The precipitation of these compounds from urine is related to some abnormality in its compostion or in its usual diurnal fluctuation in pH.

1.2 STONE - FORMING MECHANISMS

When discussing mechanisms of stone formation [17] it is very important to clearly distinguish between mechanisms of stone formation and mechanisms of stone disease. According to Finlayson et al [17], urinary stone is defined as any object resulting from a liquid-solid phase transition in urine. This definition encompasses all "ordinary" stones as well as matrix stones and crystals in crystalluria. Urinary stone disease occurs when a urinary stone does not pass with approximately the same linear velocity as the urine that surrounds it normally. Randall's plaque, which will be discussed later, is considered an example of stone disease.

Stones [9] form in a complex environment where there is constant formation of urine at variable flow rates, pH and composition. Thus, important factors such as urinary solute load, ionic strength, complexation, and pH may be constantly changing throughout a 24 hour period [9]. Even within the individual nephrons of the kidneys urine composition changes from a simple ultrafiltrate of plasma at the level of the glomerulus to a refined solution of variable concentration, composition, and pH based on the body's needs at the level of the collecting ducts [9].

Urine is an extremely complicated solution with variable composition [9]. Throughout the history of medical research [18], many hypotheses have been advanced to account for the formation of stones in the urinary tract, but a detailed mechanism of urinary stone formation is unknown [12]. The essential feature of a complete hypothesis of stone formation is that it should account for the formation, retention and subsequent growth of some critical nucleus within the urinary tract [18]. Three theories appear to be of importance in the formation of stones [19]:

1. the precipitation-crystallization theory (Vermeulen et al, 1964; Vermeulen & Lyon, 1968), regards stone formation as a physico-chemical process of precipitation of stone salts from a supersaturated urinary environment;
2. the matrix theory (Boyce & King, 1963; Boyce, 1968) considers the stone as forming in an organic matrix, analogous to the formation of bone;
3. the inhibitor theory (Howard et al, 1967; Thomas & Howard, 1959, Fleisch & Bisaz, 1962) assumes that the lack or absence of inhibitors in urine leads to stone formation .

The current scheme for stone formation, based on physico-chemical principles indicates that the initial precipitation of a component from any solution will occur if the solution is supersaturated with respect to that component [12,20]. The normal sequence likely to occur in stone formation is as follows [12]:-

initiation of precipitation→crystal growth→aggregation

Whenever phase transitions, crystal growth, and particle aggregation occur, free energy is consumed [12]. Therefore, stones can not grow unless free energy is available to drive this process [12]. This energy is derived directly from the urine supersaturation, which is a limiting factor in urolithiasis [12].

An important recent advancement in our understanding of urolithiasis concerns the evaluation of urine supersaturation [12]. By measuring urine pH, Ca, Mg, Na, K, citrate, phosphate, sulfate and oxalate, the activity of ionized Ca in urine at 25°C can be calculated. In calculating Ca ion activity the concentrations of all the other ions in the system are also calculated and therefore it is possible to see the contributions of the different ingredients in urine to supersaturation. In this way the stone-forming potential of urine can be determined [12]. A very high degree of supersaturation is necessary before spontaneous precipitation takes place [18]. The concept of the existence of a zone of "metastable" supersaturation has arisen. In this case the salt is in equilibrium with its bathing medium. In other words the salt's ion product is above its solubility product and below its formation product - at which point it can precipitate spontaneously from solution by homogeneous nucleation. Within the metastable zone a solution may exist for long periods without precipitation taking place spontaneously. However, the addition of nucleating material which may be either small amounts of the salt itself or some other material can cause homogeneous or heterogeneous nucleation respectively. Once spontaneous precipitation has occurred, crystal growth and aggregation may proceed at lower levels of supersaturation within the metastable zone [18].

Although supersaturation is necessary for the formation and growth of crystals, it alone does not predict stone formation [9]. Other factors such as inhibitors of crystal growth and aggregation, matrix and site of crystal formation and retention as well as epitaxy may be very important in stone formation in many patients [9].

Nucleation [19] describes the process by which a crystal nidus is formed, followed by the growth of the nidus into a stone through the processes of crystal growth, epitaxial growth and crystal aggregation. As noted above, nucleation [12] can be divided into homogeneous nucleation in which the nucleus is pure- and heterogeneous nucleation, which occurs on some foreign material that has narrowed the metastable region.

Crystalluria are minute crystals present in the urines of all persons, stone formers and non stone formers alike. It has been suggested by Finlayson [12] that any crystal type from the common stone crystals could act as a "nucleus" for any other crystal type. These very small "seeds" or "nuclei" are likely to be washed out with the body fluids. One or more may become attached to a cell wall thereby becoming temporarily or permanently "fixed". All the theories of the etiology of stone disease can be classified, according to the state in which the particles grow, as either fixed-particle disease, such as Randall's plaques and foreign-body encrustation, or free-particle disease, such as crystalluric particles unattached but prevented by their size from passing unhindered through the urinary tract. Carr's theory, as cited by Finlayson [12], of the etiology of stone disease requires the trapping of "large free crystalluria particles" in pericalcyceal lymphatics followed by fixed-particle growth. In the free-particle theory it is important to know, in detail, the mechanisms of particle growth and the dynamics of particle passage. In fixed-particle theory, it is necessary to also identify the pathology that gives rise to particle fixation. It has been suggested by Finlayson [12] that crystalluria particles are truely microstones, but it is not clear that free crystalluria particles could ever become large enough to cause stone disease. Studies conducted by Finlayson [12] show that nucleation and growth rates are too low to produce "free-particle" stone disease and tend therefore to support the fixed particle theory.

Other theories of stone formation mechanisms exist. A stone generally has two or more components [12]. As many as nine distinct components have been found on a small region of the surface of a single stone. These mixtures are thought to arise as a result of epitaxial growth [21]. This occurs when the geometric atomic array of a substrate surface matches the material growing on it, so that the crystal lattice of the substrate and that of the overgrowth are aligned with respect to each other . Lonsdale has presented convincing unit cell data in support of epitaxy in human calculi [21]. Although there exists much experimental evidence in support of this theory, it is nevertheless very difficult to prove. Moreover certain highly respected workers, do not believe that epitaxy is important [17]. Finlayson has pointed out that stone materials have a pronounced tendency to grow on many different substrates, which suggests that epitaxy is not necessary for urolithiasis. Werness et al [9] have also noted that the lack of a clear pattern of overgrowth makes it likely that the mechanism is a general example of heterogeneous nucleation, rather than epitaxy [9]. Whatever the actual mechanism of stone formation, research involving composition, structural, ultrastructural and micro-chemical studies might provide possible answers.

1.3 RISK FACTORS

Within the last few years a new concept has evolved to account for calcium stone-formation in the urinary tract [22]. This attributes the disorder to a combination of "risks" derived from the slightly or sometimes grossly abnormal excretion of one or more urinary constituents. According to Robertson et al [22], the six main risk factors are a high urinary pH, an increased urinary excretion of calcium, oxalate or uric acid, a low urinary volume or a reduced excretion of crystallization inhibitors (mainly acidic glycosaminoglycans (GAGS)). The relative potency of these risk factors as reported by Roberston et al [22] is in the order: ↓volume ≈ ↑oxalate > ↓GAGS > ↑uric acid > ↑ pH > ↑ calcium [22].

Figure 1.1 summarizes the overall risk factor model of stone-formation [22]. According to this scheme the first prerequisite of stones is a period of abnormal crystalluria during which a particle is formed which is large enough to become trapped in the urinary tract. The formation of abnormal crystals in turn, results from a combination of possible abnormalities in the above mentioned urinary risk factors giving rise to a high overall relative probability of forming stones. Ultimately, there is a set of epidemiological, pathophysiological and enviromental risk factors which between them account for the abnormalities in the urinary risk factors.

Figure 1.1:- A RISK FACTOR MODEL OF CALCIUM STONE-FORMATION (REPRODUCED FROM ROBERTSON ET AL [22].)

EPIDEMIOLOGICAL RISK FACTORS	URINARY RISK FACTORS	COMBINED RISK FACTORS	PHYSICAL RISK FACTORS
Age Sex Occupation Social Class Affluence → Diet Fluid intake Climate Metabolic & Genetic disorders	↑ Calcium ↑ Oxalate ↑ pH → ↓ Volume ↑ Uric acid ↓ GAG Inhibitors	Relative Probability of Forming → Ca Stones	Abnormal CaOx/CaP Crystalluria

Many enviromental and metabolic factors influence the rate of calcium stone-formation. The effects of these on the six urinary risk factors and on the overall relative probability of stones has been assessed in various studies and reports [22].

Various research groups have suggested different risk factor indices [22,23,24,25]. According to Robertson et al [26], one of the main risk factors for CaOx stone disease could be either hypercalciuria or mild hyperoxaluria. Over the past forty years, many workers have considered hypercalciuria as the main cause of idiopathic calcium oxalate stone disease. However, other investigators have emphasized that many recurrent calcium oxalate stone formers are not hypercalciuric and have begun to consider that an increase in urinary oxalate excretion is more important [26]. There are two reasons for a strong relationship between the risk of stones and urinary oxalate excretion:-

a) the marked effect which an increase in the latter has on supersaturation of urine with respect to calcium oxalate compared with an increase in calcium excretion;

b) the effect, at a constant level of supersaturation of an increase in oxalate on the Ox/Ca ratio in the urine.

Both of these effects serve to increase the volume of calcium oxalate crystals excreted in urine and therefore the percentage of large particles of crystal aggregates produced. It is the increase in the latter which determines the increase in the risk of stones. Mild hyperoxaluria thus seems to be important and more significant than hypercalciuria as a risk factor for the disorder.

The saturation-inhibition index provides another measure of the risk of calcium oxalate stone-formation in given individuals [23]. Patients with recurrent calcium oxalate stone disease excrete more crystals of calcium oxalate in their urine than normal subjects, and the crystals are generally larger and more aggregated than those passed by normal subjects [20,27]. The two main factors that have been shown to control the size and degree of aggregation of calcium oxalate crystals in urine are the degree of supersaturation of urine with respect to calcium oxalate and the concentration of inhibitors of crystallization. High levels of supersaturation promote the formation of large crystals and aggregates whereas high concentrations of inhibitors act to block the growth and aggregation of crystals. The urine of recurrent calcium oxalate stone formers has been shown to be abnormally supersaturated with calcium oxalate and also to have low levels of protective inhibitors [20]. The converse is true for the urine of normal subjects. Thus the risk of stone-formation is related to a disturbance in the relation between urine saturation and the concentration of protective inhibitors, and it may be expected that other variables such as the size of crystals excreted and the degree of severity of the disease might be related to the saturation-inhibition index [23].

It has been suggested by various workers that sodium acts as an inhibitor of stone disease by increasing the solubility of Ca in the urine by competitive binding to apatite crystals [28]. The sodium is thought to displace Ca ions in solution as well as in solid states, thus increasing the solubility of hydroxyapatite and other similar crystals [28]. Modlin [29] reported that urinary Na was significantly higher in the stone free South African black population when compared to the whites and suggested that Na acted as an inhibitor of stones. He also showed that the Na/Ca ratio was significantly higher in the blacks. However, other workers have shown that the risk of hypercalciuria is always greater in the presence of high urinary Na [24]. They have accordingly suggested that salt restrictions should be advised in subjects who have high urinary sodium to avoid the risk of hypercalciuria which in turn increases the risk of stone formation. On the other hand Singh et al [28], have shown that neither low nor high urinary sodium in the presence of moderately high calcium affects the crystallization rates of calcium, oxalate and phosphate. Neither hypernatriuria nor hyponatriuria, induced by high or low dietary sodium respectively, affects urinary calcium crystallization. These workers have concluded that sodium has neither an inhibitory nor a promotionary role in the calcium crystallization process in the urine. These observations thus highlight the controversial question of the role of Na in urolithiasis.

1.4 CRYSTALLURIA AS AN INDICATOR OF STONE RISK

Although crystals in urine have been recognised for a very long time [30], it is only recently that it has been recognised that the study of crystalluria may be valuable in the context of stone formation. All persons, whether stone formers or not, have tiny urinary crystals, invisible to the naked eye. Many scientists believe that these minute crystals and the large particles called stones are part of the same continuum. Many workers have therefore directed their research efforts at trying to characterize the relationship between crystalluria and stone disease. Robertson et al [27] have pointed out that since the days of Hippocrates, the passage of "sand" in the urine implied the presence of stone in the urinary tract, and it is a frequent symptom of certain patients with renal-stone disease. Crystalluric material contains a variety of crystalline substances including urates, phosphates and oxalates.

When considering the significance of crystalluria [31], the mere presence of crystals indicates that the urine has been supersaturated with respect to the observed species and that stone formation could occur if the crystals were to become attached to the urothelium and grow. However the absence of crystals in the urine does not necessarily imply under-saturation since the urine may be supersaturated without crystal production.

Robertson [1] has described a method for measuring calcium crystalluria in fresh urine samples maintained at 37°C using a Coulter Counter. This method proved reproducible and capable of counting all calcium crystals present in a urine sample. The technique thus indicated a possibility of studying calcium crystalluria patterns in stone formers and normal subjects in order to test the hypothesis that there is a relationship between crystalluria and stone formation. In further studies light microscopy was used to detect differences in the crystal sizes. Results from these various experimental studies showed that there are qualitative and quantitative differences between the crystalluria of recurrent calcium oxalate stone formers and controls. The urinary particle size distribution in normals was shown to be unimodal with one peak in the small diameter range (5µm) while that for the stone formers was bimodal with peaks in the 5µm and 25µm diameter range [1,20,27,30]. There were also significant differences in the average size and type of calcium oxalate crystals produced. The controls voided their precipitated calcium oxalate in the form of small, non-aggregated, monohydrate particles, [27] while the stone formers voided large dihydrate crystals, in addition to the monohydrate crystals. Moreover polycrystalline aggregates of COD up to 200µm in diameter [27] were uniquely observed in the stone formers urines. It is not known whether the difference in crystal size between these two groups is due to higher calcium and oxalate concentrations or to a deficiency of crystallisation inhibitors in the urine of stone formers [27].

Crystalluric differences between the two groups have also been reported by other workers, such as Crassweller et al [32], Finlayson [33] and Werness [9] et al. Crassweller and co-workers confirmed that stone forming individuals have a greater number of larger crystals and a greater total volume of crystals in their urine than normal subjects. They have suggested that the large crystal size in stone formers, whether oxalate or phosphate, could possibly be a determining factor in nucleation and epitaxial stone formation in association with alterations in levels of saturation and urinary inhibitors. Finlayson [33] has reported that no crystals appeared in the urine of normal subjects, but that stone formers showed a raised incidence of both calcium oxalate and calcium phosphate crystalluria.

It may thus be concluded that there exist certain qualitative and quantitative differences in the crystalluria of stone-forming and normal subjects. Moreover these differences can be used for assessing the potential to form stones.

1.5 UROLITHIASIS IN MARATHON RUNNERS

Running is becoming an ever increasingly popular pastime [34]. Aside from the obvious benefits of helping to control weight and a general sense of accomplishment, running has been linked to clinical changes that may lead to improved health. On the other hand, risks attributed to running [34] include cardiac arrhythmias and myocardial infarctions, musculoskeletal injury, heat or cold injury, motor vehicle collisions and a variety of other injuries.

Strenuous exercise also leads to specific changes in the concentration and distribution of normal body metabolites [34]. It may also lead to increased losses of essential trace elements. Since dietary intake of these trace elements may be marginal for most individuals, increased losses over extended periods would need to be replenished or compensated for in some other manner [34].

The recent rapid growth of running has provided an opportunity to study the prevalence of various disorders among runners [35]. Of particular interest is a survey [35] among entrants in the 1977 New York City Marathon which found that the incidence of urinary stone formation in these runners is greater than in the matched population [35].

This observation prompted Irving et al [36] to study this apparent phenomenon. They determined urinary particle volume distribution profiles for marathon runners and found them to be bimodal, with one peak in the 2-5μm diameter range and a second peak in the 15-32μm range thereby bearing a remarkable resemblance to the bimodal distribution pattern reported by Robertson et al [30] for recurrent stone formers. Scanning electron microscopy (SEM) and X-ray powder diffraction (XRD), revealed other features in the runners' urine which are regarded as typical of stone formers' crystalluria [36]. For example, the runners' crystalluria was predominantly COD with trace amounts of COM while another feature which resembled that of the stone former, was the presence of large COD aggregates.

Milvy and co-workers suggested that the increased prevalence of urolithiasis [35] in runners may be due to substantial dehydration to which they are continually subjected. It has been recognised that the ingestion of fluids [35] cannot keep pace with dehydration during long distance running, although a high fluid intake could help mitigate the deficit. However there appears to be several mechanisms involved in the formation of stones in marathon runners [35]. Some mechanisms may enhance while others may impede stone formation. Among the latter are the stimulating effects of muscle in preventing bone breakdown and renal calcium loss and the mechanical effect of mobility in ensuring the passage of small calculi as soon as they are formed. Among the former, dehydration is clearly very important [35].

Irving et al [36] and Rodgers et al [37] agree that dehydration may play a key role in the genesis of calculi in marathon runners. However, these workers also draw attention to an additional factor which might be of importance - the physical trauma inflicted on the urinary tract during long distance road-running. They suggest that epithelial sloughing might provide sites within the urinary tract for crystal entrapment leading to increased residence times and hence increased crystal growth. Moreover, they propose that the cellular debris itself might act as nuclei for crystal deposition processes.

1.6 OBJECTIVES

The resemblance in the crystalluric properties of stone formers and marathon runners is not only of some interest with respect to the study of urolithiasis itself, but is also somewhat alarming for athletes in general. The present study was thus undertaken to further investigate this phenomenon and to determine whether there might be a similar urinary stone risk associated with other sporting activites. With these general objectives in mind, it was decided to re-assess the marathon runners by applying a risk factor index other than particle size distribution profiles. As far as such an alternative risk factor index is concerned, the work of Modlin in his study of South African blacks was considered [29]. As mentioned in section 1.1, the occurrence of renal stones in this population group is extremely rare. Modlin reported that South African blacks have a significantly higher daily output of urinary Na when compared to the white population and also found significantly lower urinary Ca values in the former. He argued that Na can increase the solubility of hydroxyapatite by a competitive substitution mechanism of normal crystal lattice ions and drew attention to the fact that experimental evidence has shown that Na can inhibit calcification. Modlin concluded that the low incidence of urinary stones in South African blacks was due to their high daily urinary Na/Ca ratios.

In the context of the present study, it was decided that values of the Na/Ca ratio would be compared with particle size distribution results and hence the potential of the former as a stone-forming risk factor would be assessed.

In order to test the hypotheses that the stone forming mechanism involves dehydration and/or urinary tract trauma, it was also decided to investigate the risk of stone formation in other athletes participating in sports in which dehydration and trauma effects might be expected to be operative to different extents relative to runners. Two such sports are cycling and swimming.

The objectives of this research project were thus defined as follows:

(i) to determine 24 hour urinary Na and Ca (as well as Mg and K) concentrations in white and black male marathon runners;

(ii) to determine urinary particle-volume size distribution curves for black male marathon runners;

(iii) to determine urinary particle volume size distribution curves for white male cyclists and white male swimmers;

(iv) to determine 24 hour urinary Na, Ca, Mg and K concentrations in white male cyclists and white male swimmers;

(v) to characterize all urinary particulate matter using scanning electron microscopy and X-ray energy dispersive analysis.

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CHAPTER 2

GENERAL THEORY OF TECHNIQUES

CHAPTER 2

Many techniques may be applied to the study of crystalline material and thus to the study of urinary calculi. This chapter discusses some of the basic principles of the techniques employed in the present study, viz. scanning electron microscopy, Coulter counter procedures and atomic absorption spectroscopy.

2.1 SCANNING ELECTRON MICROSCOPY AND X-RAY ENERGY DISPERSIVE ANALYSIS [1,2,3,4,5]

SEM has recently gained wide acceptance as an analytical tool in urinary stone analysis [6,7,8,9,10,11,12,13,14]. When coupled with an energy dispersive X-ray analyser (EDX), the technique provides the investigator with a unique opportunity to simultaneously investigate the morphology and chemical micro-structure of stones [6].

The scanning electron microscope can be simply characterized as a closed circuit television system in which the object observed is illuminated by a constantly moving spot of electrons. (Schematic diagram, Figure 2.1)

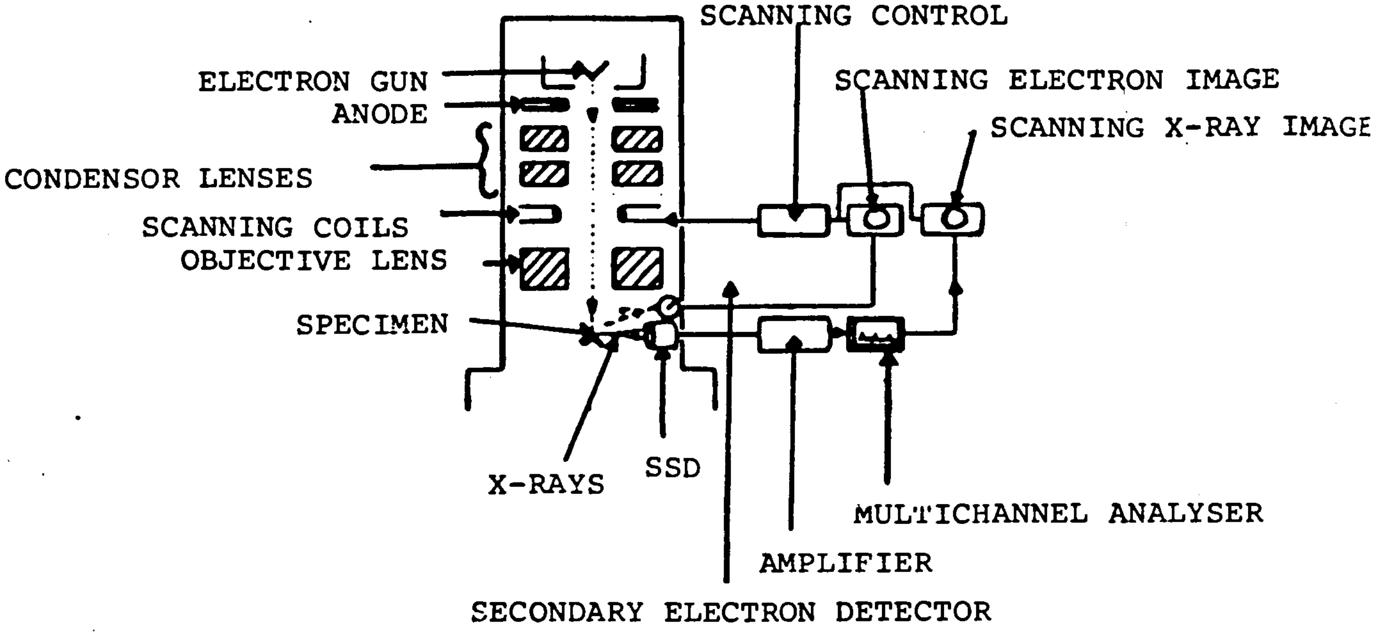


Figure 2.1: SCHEMATIC REPRESENTATION OF THE SCANNING ELECTRON MICROSCOPE
(As reproduced from [1])

Electrons are emitted by a tungsten filament located within the gun in the upper portion of the microscope column. The cathode is generally held at 20 000V below ground potential of the anode. The electron gun thus produces the electron source with energies from 1 to 50KeV [2]. Below the gun are three pre-aligned electromagnetic condenser lenses which serve to focus the electrons [2]. These lenses also serve to demagnify the beam into a small-diameter probe, which is then scanned over the specimen. In order to scan the specimen with the probe, deflection coils are placed between the last two lenses to deflect the beam in a rectangular pattern (square raster) over the samples' surface which is similar to that which occurs in a television screen. (Figure 2.2) The scan generator, which produces sweep signals to the column deflection coils, at the same time operates deflection coils in the SEM's cathode ray tubes (CRT). Due to this synchronism, there is a one-to-one correspondence between the position of the electron beam on the specimen and that of the spot on the CRT. Both the column and the specimen chamber must be under adequate vacuum (less than 10^{-4} torr), for the operation of the instrument [1]. Movements of the specimen in the x-, y-, z- directions, as well as tilting and rotation are possible by means of controls outside the specimen chamber.

The working distance (WD) in the SEM is defined as the distance from the bottom of the final lens to the mid plane of the field scanned on the specimen. The working distance can be varied in most SEMs by translating the specimen in the z-axis of the microscope. When the WD is increased, the beam convergence angle decreases, the beam becomes collimated and gives a greater depth of focus. Decreasing aperture size will have a similar effect. An increase in the z distance also decreases resolution.

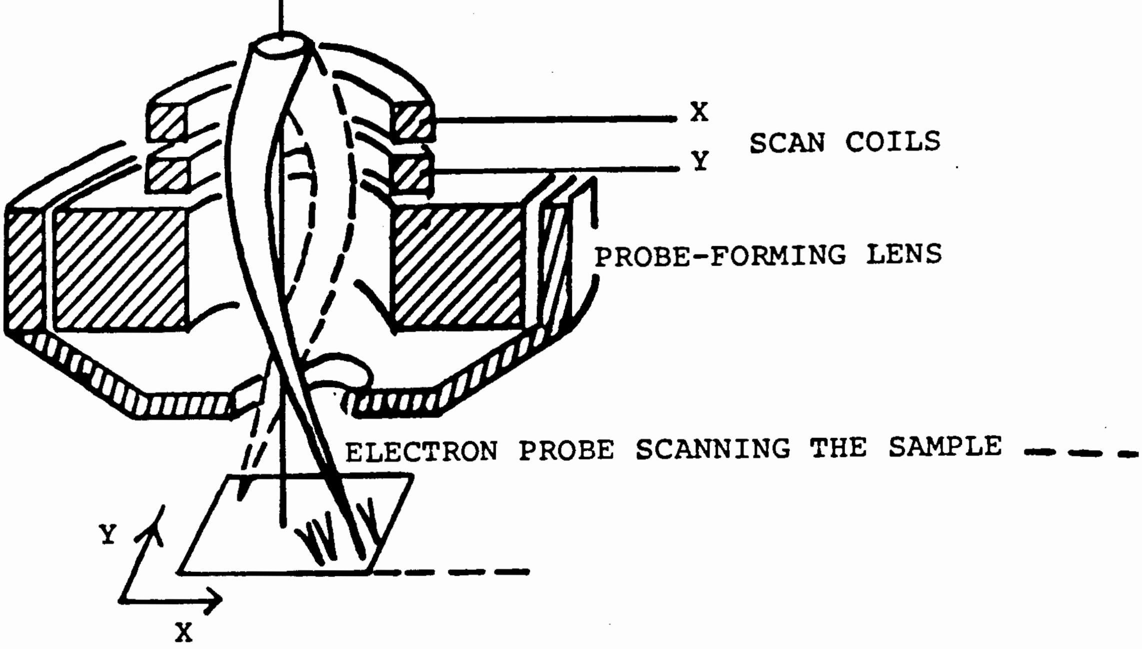
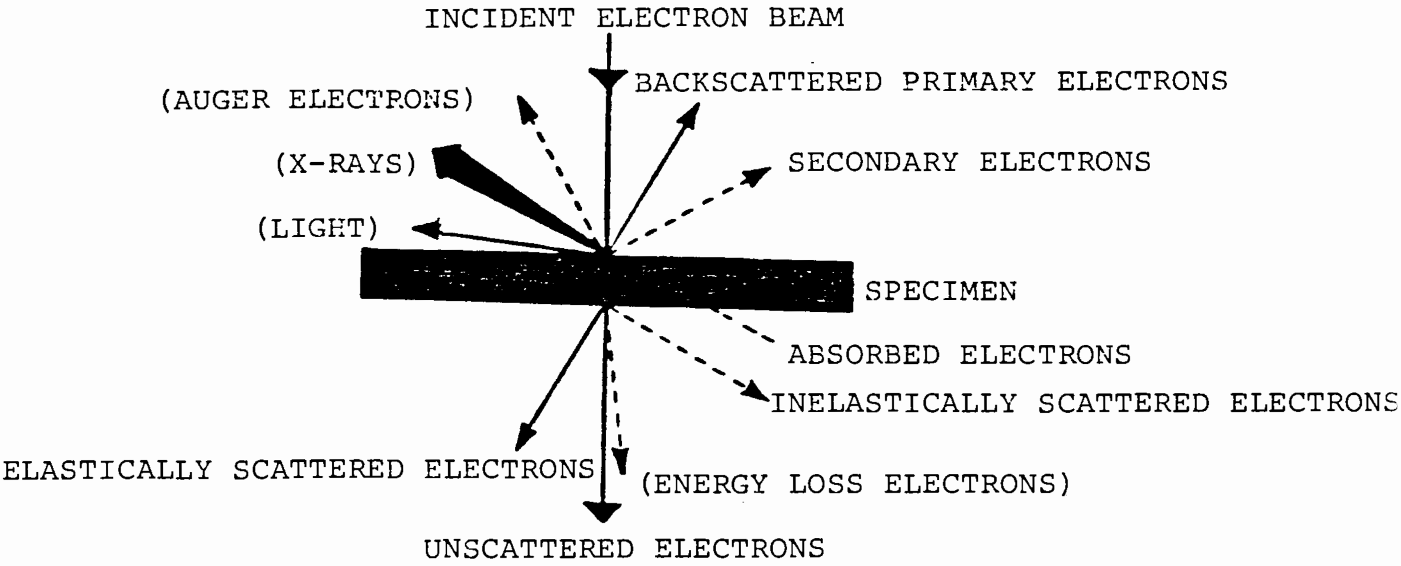


Figure 2.2: RASTER MOVEMENT OF ELECTON PROBE (Reproduced from [1])

Most modern SEMs are designed to be operated at accelerating voltages of 1 to 30 kV. If the accelerating voltage is lowered to below 5kV, less damage to the specimen due to beam heating occurs. As the electron beam is passed over or scanned across the specimen surface, the interaction of electrons with matter (the specimen) results in the production of a variety of interactions, (Figure 2.3).



<u>Figure 2.3</u>: SOME OF THE POSSIBLE RADIATIONS WHICH CAN RESULT FROM THE INTERACTION OF 5-30 kV ELECTRONS WITH A SOLID
(Reproduced from [1,3])

The excitation of secondary electrons can provide three kinds of specimen information. One of these is topographical detail. The number of secondary electrons produced depends on the surface topography as well as the composition of the specimen. The electron beam will diffuse several micrometers into the sample before all the electrons are stopped. As these electrons travel through the specimen, they ionize atoms, provided that the electron energy is greater than the critical ionization potential, E_c. As soon as this energy falls below E_c no further ionizations occur but the electrons continue decelerating until they have zero energy and still produce a certain amount of white radiation. The various interactions arise from a pear-shaped volume of the specimen below the surface and the depth of this volume depends on the penetrating power of the electron beam, that is, on the primary electron energy, and on the specimen composition, (Figure 2.4).

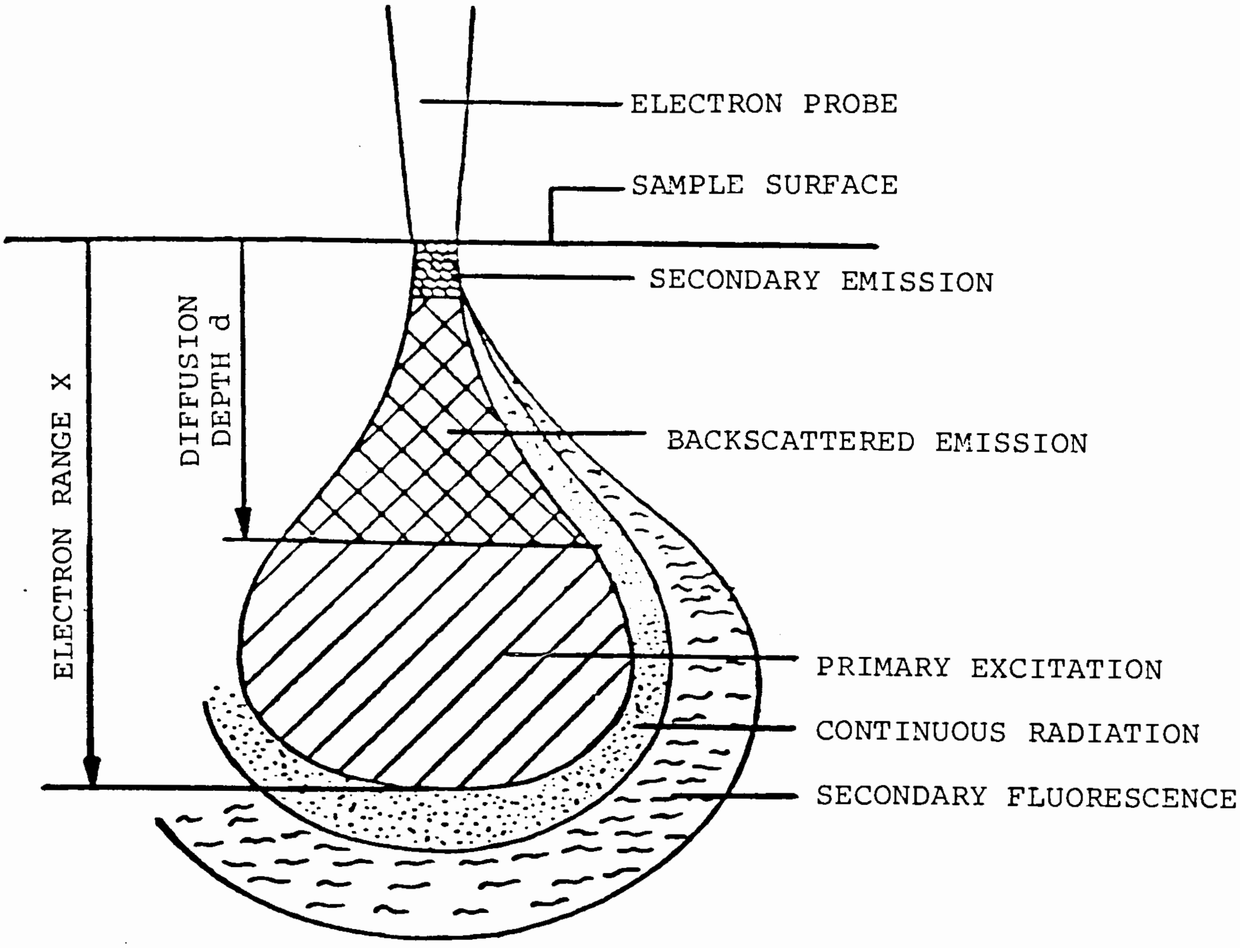


Figure 2.4: SCHEMATIC ILLUSTRATION OF DEPTH AND LATERAL DISTRIBUTION OF GENERATED SIGNALS FROM ELECTRON BEAM PENETRATION OF THE SAMPLE (Reproduced from [2,4])

It is generally necessary to evaporate a metal of high atomic number over the specimen, particularly those that do not conduct well or those that are poor emitters of secondary electrons. Specimens may be subjected to charging, which is a common serious defect in SEM studies. Coating of the specimens is therefore of primary importance. A double coating, first of carbon followed by a metal is preferred. The metal layer normally is gold. However, an alloy mixture of 60% Au and 40% Palladium has been recommended. This Au-Pd mixture provides a continuous layer with a much thinner deposit than does pure gold and is less likely to crack under the beam or on storage. It should be noted that urinary calculi and urinary crystals should preferably be coated with carbon because Au gives an emission peak which overlaps that from phosphorus. Coatings should be deposited slowly on the revolving specimens to ensure thorough and even coating. It has been shown that fast scanning at low voltage is an effective manner of examining charging or uncoated non-conductive materials. Charging can also be combated by using backscatter imaging, as the higher energy electrons ignore the stray fields on the surface.

Secondary electrons emitted from the specimen pass to the collector or detector which accelerates the electrons to strike the scintillator [2]. The electrons striking the scintillator produce photons which enter and pass through the photomultiplier, which serves to produce large numbers of additional electrons. The electrons leaving the photomultiplier produce a photocurrent that is amplified and passed to the display and record cathode ray tubes. Each of the cathode ray tubes displays is 100mm square. The SEM can provide a magnification that can be varied over a wide range while preserving focus and specimen location and depends on the nature and form of the material examined.

Using the SEM, morphology of the surface can be revealed by every kind of signal except X-rays and Auger electrons [2]. Element analysis or composition determinations can be accomplished by utilizing X-rays [3], cathodoluminescence, Auger electrons and back-scattered electrons [2]. The X-ray spectrum is unique to each element [3]. In the X-ray mode the X-rays generated when the electron beam reacts with the specimen are collected, analyzed and used to form a signal. The ability to perform X-ray microanalysis of elements in the SEM is a valuable asset. It is accomplished by coupling crystal diffraction spectrometers, energy dispersion spectrometers or a combination of both to the scanning electron microscope. The crystal spectrometer is limited in that it is unable to detect and display, simultaneously, all X-ray energies leaving the specimen whereas this is achieved with the energy dispersive spectrometer. In the latter case a multi-channel analyser is set to collect X-rays over a certain energy range [2]. Because the lower end of the range is 1keV, elements lighter than Na cannot be detected. The $K\alpha$ lines from species with atomic number 11-32 (Na-Ge) fall in the range 0-10 keV although other ranges may be selected.

A very useful application [3] of the X-ray mode involves switching off the scan and adjusting the electron beam to a particular point of interest, such as a foreign body, which remains visible in an emissive mode picture on the long delay tube. The whole range of X-rays is collected and the energy discrimination causes a spectrum to be displayed on an auxillary graphic display screen. The elements present at the particular point are thus identified [3].

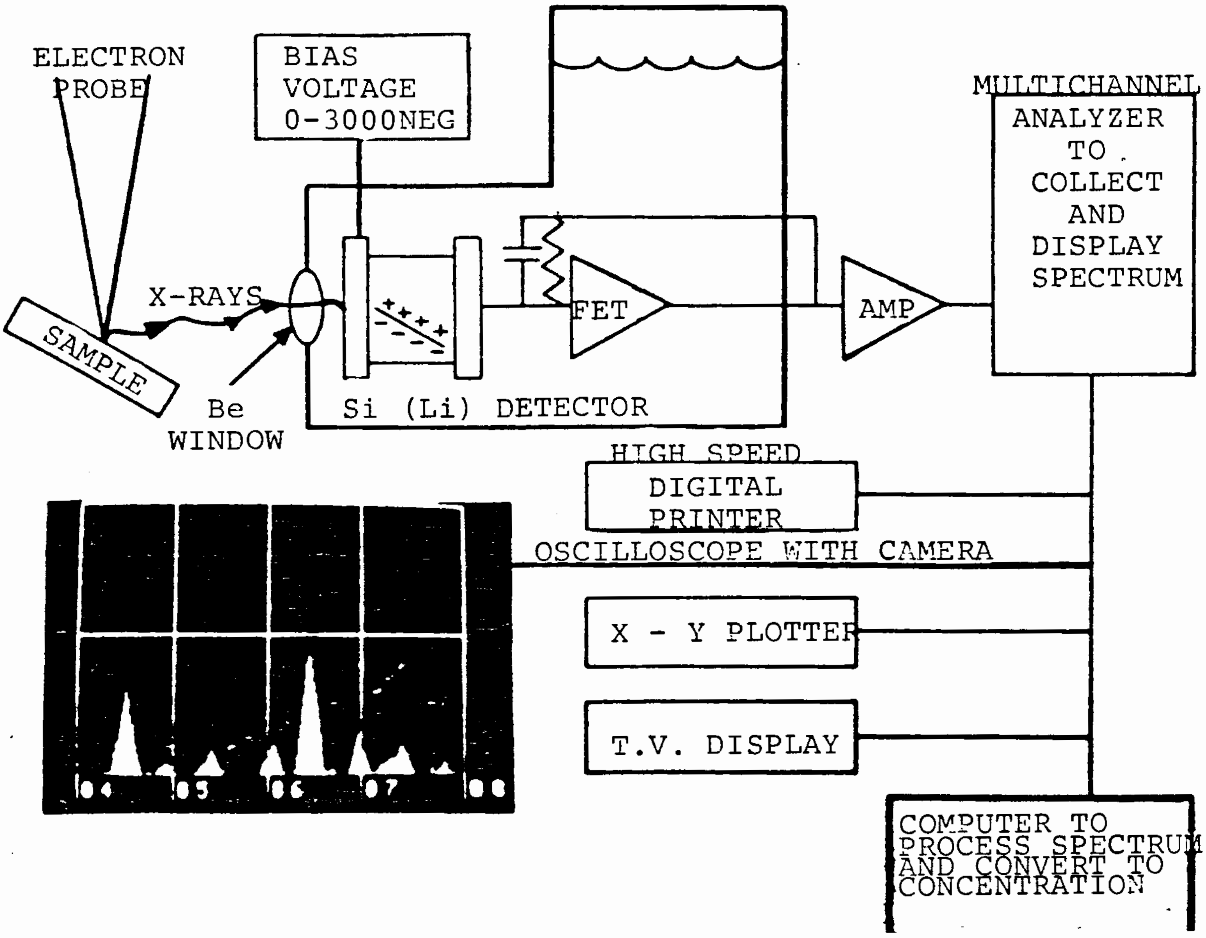


Figure 2.5: SCHEMATIC ARRANGEMENT OF ENERGY DISPERSIVE X-RAY ANALYSIS SYSTEM FOR THE SEM OR ELECTRON PROBE ANALYSER

(Reproduced from [2])

2.2 COULTER COUNTER ANALYSIS [15]

Robertson [16] has indicated that Coulter counter procedures are reproducible and capable of counting all crystals present in a sample of urine. The technique has been used by several researchers [16,17,18,19,20] for determining particle volume-size distribution profiles.

The Coulter counter was developed by Coulter in 1956. It is an electronic method for detecting, counting and measuring the size of individual particles by changes in electrical resistance that occur when the particles pass through a small orifice, one at a time. The method is quick - it can count 4000 particles a second - and it is accurate. The instrument measures particle volume, which is expressed as dv. This represents the diameter of a sphere that has the same volume as the particle. The results are expressed as the number of particles larger than a given size.

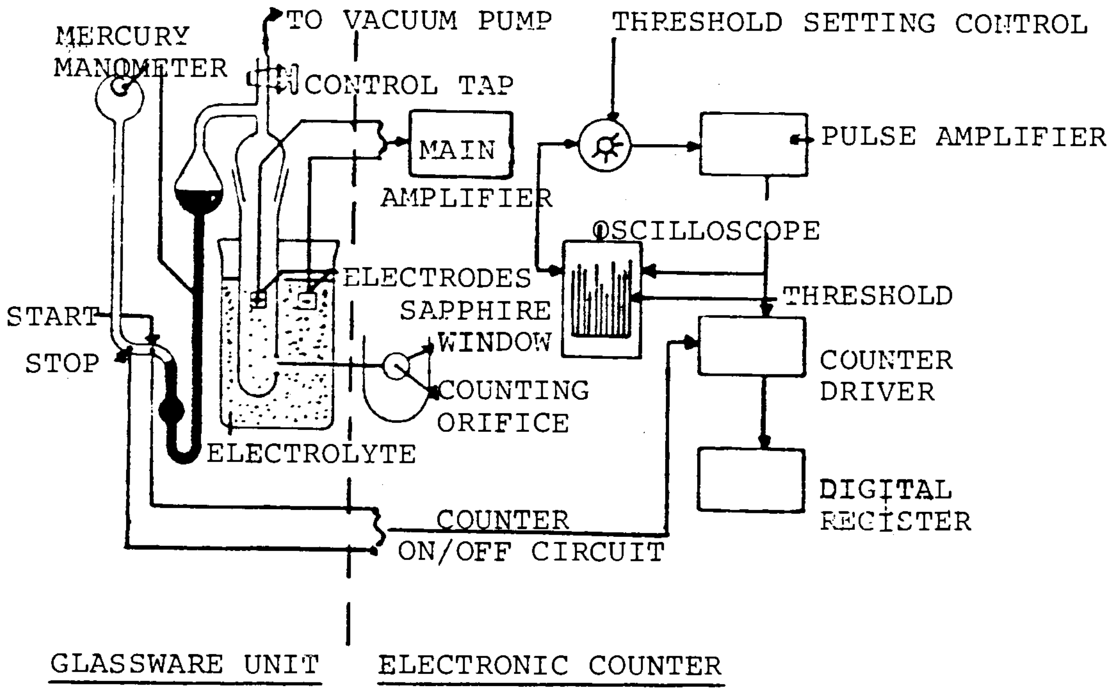


Figure 2.6: DIAGRAM (SCHEMATIC) OF COULTER COUNTER

The sample to be analyzed is suspended in a suitable electrolyte (Isoton II), in a beaker. Electrolytes provide the necessary media for counting and sizing particles with a

Coulter counter. The electrolytes require the following characteristics:-

1) correct conductivity to meet the requirements of the equipment with chosen aperture;
2) little or no solvent action on the particles to be analyzed;
3) no reactivity with the particle system to be analyzed;
4) low toxicity, fire or explosion hazard;
5) free of contaminants (filtered) prior to dispersing the particle system to be sized.

The particles are kept in suspension by a propeller stirrer. A glass tube, attached to the outside vacuum via a stopcock, has an accurately made sapphire-orifice plate fused into its lower end, and dips into the beaker and is filled with the same electrolyte. The tube is also attached to a mercury manometer with stop-start contacts controlling the counting device. There are two electrodes, one in the orifice tube and one in the beaker. A constant voltage is applied across the electrodes so that a current flows.

THE PRINCIPLE OF OPERATION

In the basic particle sensing procedure, a vacuum of approximately 165mm Hg is applied to the manometer and to the electrolyte inside the control piece and aperture tube. This vacuum establishes an imbalance in the mercury levels in the two sides of the manometer, and draws electrolyte from the sample beaker through the aperture into the aperture tube. The sample suspension then flows up through the aperture tube, through the control piece and is collected in the trap flask. There are two electrodes, one in the orifice tube and one in the beaker. A constant voltage is applied across the electrodes so that a current flows. Current polarity may be positive or negative.

A constant voltage is applied across the electrodes and this produces a current. The major resistance to the flow of current is the electrolyte in the orifice. As a particle passes through the orifice, the orifice resistance momentarily increases by an amount proportional to the volume of electrolyte displaced by the particle. This change in resistance causes a voltage pulse which is proportional to the volume of the particle.

The resultant series of pulses are electronically amplified, scaled and counted. The voltage pulses are seen on the oscilloscope screen as a pattern of vertical "spikes". The voltage pulses are also fed to a threshold circuit which eliminates those pulses below a certain voltage while those that reach or exceed a certain voltage are counted. In this way particles over a certain size are counted. The electrolyte in the aperture forms the principle resistance to the flow of current. Thus, for a given aperture size and electrolyte, response is proportional to particle volume.

Before the counter can be used it must be calibrated. This is achieved by using different sized spherical particles of known median diameter and calibrating the instrument's threshold control.

2.3 ATOMIC ABSORPTION SPECTROSCOPY [21,22,23,24,25,26]

Atomic absorption spectroscopy (AAS) has been successfully utilized in the analysis of urinary calculi [27,28,29,30,31]. Its use as an analytical tool was first proposed by Alan Walsh in Australia in 1955 [26]. The technique is simple and is used for analysing elements at trace and ultra-trace levels.

Flames are the most widely used means to produce the atom cloud in AAS. The sample is nebulized to form an aerosol which is mixed with flame support gases (oxidant and fuel). e.g. the air-C_2H_2 flame. In the hot flame, the aerosol is desolvated to form "clotlets" which absorb more thermal energy, vapourize and finally an atomic vapour is formed. The distribution of atoms in the flame is critical for some elements and the burner position must be optimized with care. The burner component has two principal functions:

1. to introduce the sample into the flame, and
2. to reduce the metal to the atomic state.

Radiation of appropriate wavelength is then passed through the atom cloud and absorption occurs. The absorption is measured and is related to the number of atoms present, viz. the atom concentration. This is the basic principle of atomic absorption analysis.

Irradiation of ground state atoms is brought about using a very narrow line source, such as the hollow cathode lamp (HCL). This lamp has a cathode comprising the element to be determined. The irradiating line from the lamp has a finite width and is operated at low currents to minimize self-absorption and other broadening processes. When the hollow cathode lamp current is increased, it broadens the irradiating line. An increase in the concentration of atoms in the cloud broadens the absorbing line. Both of these effects cause the analytical calibration curve to deviate from Walsh's theoretical linear relationship.

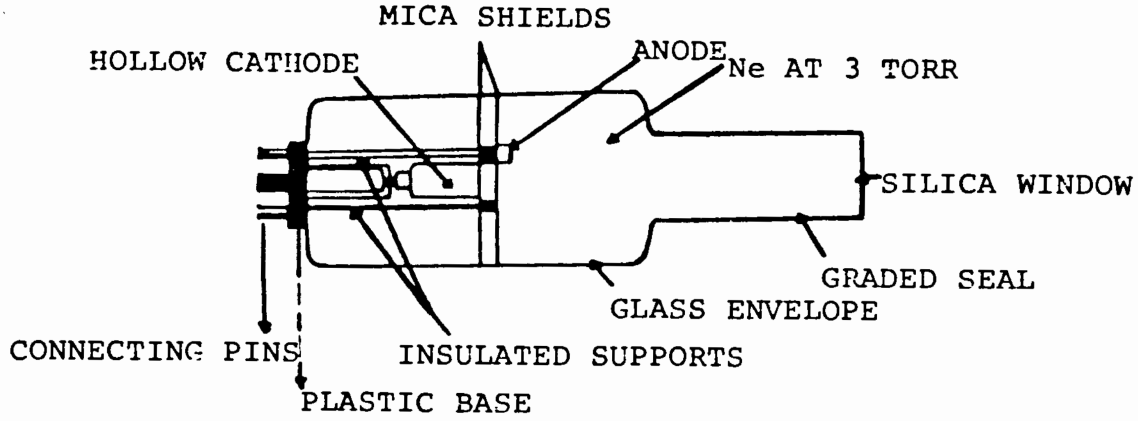


Figure 2.7: THE HOLLOW CATHODE LAMP

The absorption is measured using a standard spectrophotometer with a suitable readout system. The source, (HCL) is operated under conditions which minimize the emitted line width. The monochromator-detector system cannot distinguish between light from the hollow cathode lamp and light of exactly the same wavelength emitted by those atoms in the cloud which have absorbed energy and then have relaxed to the ground state. This difficulty is countered by modulating the source radiation mechanically ("chopper") or electronically, and tuning the detector to the modulation frequency. Emitted light from the atom cloud gives a steady signal which is electronically rejected and only the true absorption of light from the source is measured.

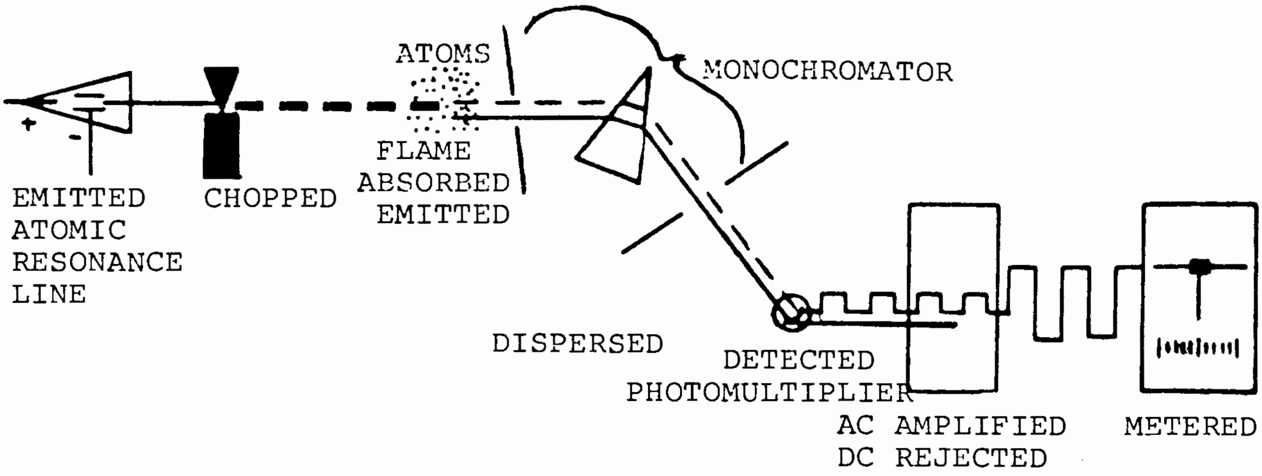


Figure 2.8: SCHEMATIC REPRESENTATION OF AN ATOMIC ABSORPTION SPECTROMETER

Operation of the AAS requires the preparation of standard solutions for the construction of calibration curves ("Calibration Method" will be presented in the next chapter). Once the instrument has been set up, the absorbances can be measured, the unknown and blank solutions can be determined and the concentrations computed. However when setting up the instrument, various conditions need to be optimized (Table 2.1).

Table 2.1: OPTIMUM INSTRUMENT CONDITIONS AND DETECTION LIMITS

CONSTRUCTED FROM THE OPTIMUM CONDITIONS PROVIDED BY PERKIN-ELMER [32].

ELEMENT	SPECTRAL BAND PASS	LINE (nm)	OPTIMUM WORKING RANGE (µg/ml)	TYPICAL SENSITIVITY	LAMP CURRENT (mA)	SENSITIVITY 1% ABS (µg/ml)
Ca	0.2	422.7	1-4	0.021	3	0.08
Na	0.2	330.2	100-400	1.6	5	0.015
Mg	0.5	285.2	0.1-0.4	0.003	3	0.007
K	0.5	769.9	1.5-6.0	0.03	5	0.04
FLAME CONDITION: Oxidizing air/C_2H_2.						

AAS is a precise technique and relative standard deviations of 1% or better can be achieved routinely with care. Even when interferences are taken in account, the technique is satisfactorily accurate. Sensitivity is good and less than 1ppm is detected for most elements. AAS is relatively free from interferences. Spectral overlap is rare while non-atomic absorption ("background") is an important interference. However it can be compensated for by using a continuum source background corrector. Ionization depletes the population of ground state atoms and is particularly marked in hot flames, or when determining the alkali metals. It is compensated for by the addition of excess easily ionized elements to samples and standards. Chemical interferences are caused by chemical reactions in the atom cloud which deplete the population of free atoms. The use of a releasing agent or a hotter flame is common, and a simple chemical separation of the analyte from the interferers is possible. Chemical interference can be a serious problem in AAS since the effects are not predictable and may seriously bias the results. Other effects which cause flame AAS interference (enhancement) are heating of the sample and the use of organic solvents.

A search for interferences is essential and can be conducted using artifical solutions in order to determine the influence of other elements on the particular element under analysis. However most of these analytical errors can be eliminated by suitable calibration. In chapter 3 it is shown that various optimal conditions have been chosen to minimize these possible interferences.

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CHAPTER 3

METHODS AND MATERIALS

CHAPTER 3

3.1 SUBJECTS AND URINE SAMPLES

Data concerning controls and athletes are given in table 3.1. White and black healthy sedentary male controls were randomly chosen from members of the University staff and from neighbouring industry while athletes were recruited at the completion of a major sporting event (e.g. marathon, cycle tour or swimming gala).

Urine specimens were collected during the 24 hours immediately after the event while a follow-up collection was effected three weeks later.

Table 3.1: CONTROLS AND ATHLETES

SUBJECTS	ABBREV	NUMBER OF SUBJECTS	AGE (YEARS)	SOURCE
WHITE MALE NORMALS	WMN	16	21-56	CHEM DEPT U.C.T
BLACK MALE NORMALS	BMN	13	18-67	LOCAL INDUSTRY
WHITE MALE MARATHON RUNNERS 1	WMMR 1	18	21-39	KELLEPRINZ MARATHON (42km)
WHITE MALE MARATHON RUNNERS 2	WMMR 2	14	21-39	FOLLOW-UP STUDY
BLACK MALE MARATHON RUNNERS 1	BMMR 1	14	19-40	PENINSULA MARATHON (42km)
BLACK MALE MARATHON RUNNERS 2	BMMR 2	8	25-32	FOLLOW-UP STUDY
WHITE MALE CYCLISTS 1	WMC 1	21	16-41	ARGUS CYCLE TOUR (104km)
WHITE MALE CYCLISTS 2	WMC 2	10	18-34	FOLLOW-UP STUDY
WHITE MALE SWIMMERS 1	SWIM 1	8	13-21	CONSTANTIA GALA
WHITE MALE SWIMMERS 2	SWIM 2	10	13-20	FOLLOW-UP STUDY

3.2 SCANNING ELECTRON MICROSCOPY AND ENERGY DISPERSIVE X-RAY ANALYSER

Urine samples at 37°C were centrifuged for ten minutes using a Piccolo table top low speed centrifuge operating at 2000 revs per minute. The deposited crystals were removed by repeated aspiration using a Pasteur pipette. Drop amounts were then filtered through a 0.22μm Millipore filter (13mm diameter) supported in a Sartorrius membrane filter clamp (GMBH Gottingen). The filter papers, with deposited crystals were then pasted onto aluminium stubs for SEM analysis. The stubs from groups WMC 1 and WMC 2 were coated with approximately 100nm of carbon at a pressure of about 1.3mPa in a Balzer's vaccuum coater equipped with a planetary sample rotator. However, all other specimens were coated with approximately 30-50nm of Au-Pd as better imaging was achieved with this coating.

Specimens were examined using both a Cambridge S180 and Cambridge S200 scanning electron microscope. The former was equipped with an energy dispersive X-ray analyser. However, as a result of a major technical fault in the S180, it was possible to subject only a few specimens to elemental analysis. Most of the samples were thus investigated using the S200 which was not equipped with an EDX facility.

3.3 PARTICLE SIZE DISTRIBUTION ANALYSIS

Urine samples for this analysis were provided by several of the subjects listed in table 3.1. Nocturnal urine specimens (i.e. the first voided urine after waking) were collected in pre-heated thermos flasks two days after the particular sporting event. These were analysed at 37°C within a few hours of voiding. A follow-up collection was also made three weeks later. A model ZM Coulter counter fitted with a 100µm diameter orifice was used for particle distribution analysis. The instrument was calibrated using Latex calibration beads (coulter Electronics, Hertfordshire) of diameter 19.00µm suspended in azide-free ISOTON II solution. Thereafter a 2.5 ml aliquot of urine was pipetted into 250ml of thermostatted ISOTON II electrolyte and mixed thoroughly and subjected to a trial count. The manometer volume used was 2000µl. However, if the concentration of particles was too high to yield a statistically reliable particle size distribution, a manometer volume of 500µl was used. All samples were continuously stirred. The instrument was set to allow 2ml of the ISOTON/urine solution to be drawn through the aperture for each counting procedure. Each sample was counted three times. The calibration settings for the Coulter counter are listed in Appendix 1.1. Appendix 1.2 lists the size-distribution data acquired for all subjects.)

In some cases, particle counting was undertaken in small steps to an upper diameter limit of 25µm while particles in the range 25-40µm were determined in one count. In other cases the counting was continued in small steps throughout the large diameter range.

3.4 ATOMIC ABSORPTION SPECTROSCOPY ANALYSIS

TREATMENT OF URINE SAMPLES

All urines were collected in polythene containers which had been washed with distilled water. The total volume of each specimen was measured using a polythene measuring cylinder. (Appendix 2.1) (Glass containers were not used as a precautionary step to avoid contamination by Na and Ca.) In order to stabilize the urine, 200µl 1M HCl was added to 2ml of each specimen in a polythene test tube. The pipetman P200 and P1000 pipettes were used for this purpose respectively .

Standard solutions were prepared for each element of interest and calibration curves were constructed. Linear plots were obtained for Ca and Na but slight curvature of the Mg and K calibration graphs occurred. In the case of the former, possible explanations could be self-absorption or non linear electronic response at high concentration and absorbance levels. A possible explanation for the non linearity of the K calibration curve could be ionization. Thus in order to avoid curvature of these graphs, Ca required a releasing agent and Mg and K an ionizing suppressant, for which the Lanthanum 5000µgml^{-1} solution was used. Na required an ionization suppressant for which KCL 2000µgml^{-1} was used (Appendix 2.1). These releasing and ionization agents were recommended by the manufacturer [1].

ANALYTICAL DETERMINATIONS

The Gibson diluter was used to prepare the samples for flame atomic absorption spectroscopy and respective releasing and ionization agents were added.

Table 3.2: DILUTION SPECIFICATIONS FOR EACH ELEMENT

ELEMENT	VOLUME OF SAMPLE (µl)	DILUENT RELEASING/ IONIZING AGENT (ml)
Ca	125	4.875 of La 5000
Na	100	5.000 of KCl 2000
Mg	DOUBLE DILUTION a) 100µl sample in 1ml water b) 100µl of a) in diluent	4.900 of La 5000
K	DOUBLE DILUTION a) 100µl sample in 1ml water b) 100µl of a) in diluent	5.000 of La 5000

As noted from table 3.2 a double dilution was preferred for Mg and K, due to the fact that the raw urine readings occurred in the non linear part of the curve. All samples were thoroughly mixed.

The Varian-1275 model flame atomic absorption spectroscope was used and was set up and optimized according to the manufacturer's instructions (Table 3.3). The air/C_2H_2 burner and Ca, Mg, K, Na hollow cathode lamps were used for all the determinations (Appendix 2.2 lists all AAS data acquired for all subjects).

Table 3.3: PARAMETERS USED FOR THE DETERMINED ELEMENT

ELEMENT	λ (nm)	SLIT WIDTH (nm)	LAMP CURRENT (mA)
Ca	422.7	0.5	3
Na	330.2	0.2	5
Mg	285.2	0.5	3
K	769.9	1.0	5

3.5 STATISTICAL METHODS

Statistical analysis was performed using the Students' t-test for independent samples. Significance was determined at the $p<0.05$ level.

REFERENCES

1. VARIAN TECTRON MANUAL. (1973). Analytical methods for flame spectroscopy.

CHAPTER 4

RESULTS

CHAPTER 4

4.1: SCANNING ELECTRON MICROSCOPY AND ENERGY DISPERSIVE X-RAY ANALYSIS

As was mentioned in chapter 3, it was not possible to conduct X-ray energy dispersive analysis on the majority of the samples. Application of this technique was in fact limited to the two groups of cyclists (viz. WMC 1 and 2). Therefore all assignments of crystal type were based on morphologic appearance alone. This shortcoming in the analysis will be discussed more fully in chapter 5.

When scanning the WMN urine specimens, no recognisable crystals were observed. However, small pieces of epithelial debris were interspersed with small urinary salt deposits. The urine of the white male marathon runners (WMMR 1 and 2) revealed COD crystals of diameter 10μm but these were sparsely distributed (Figure 4.1). COD aggregates such as that shown in Figure 4.2 were rare.

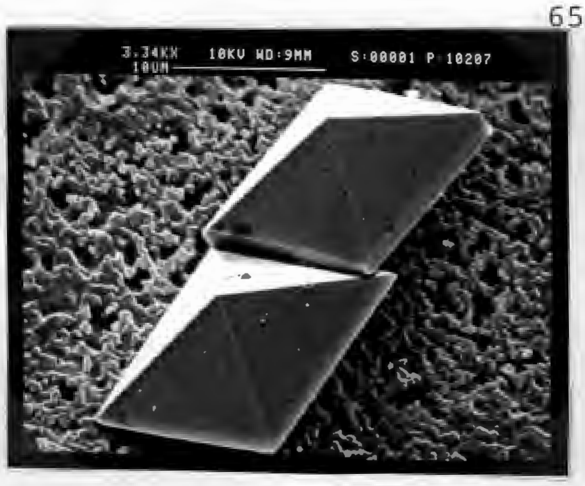


Figure 4.1

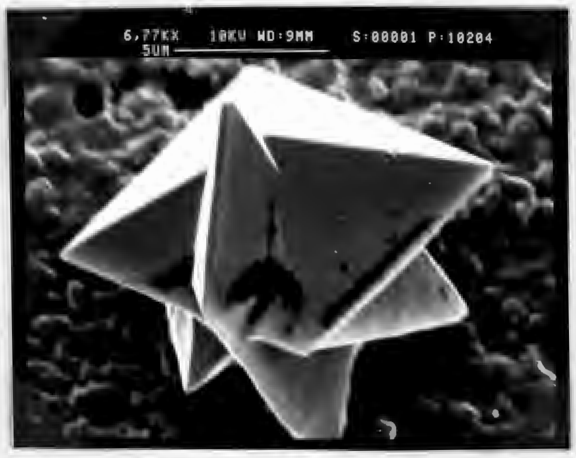


Figure 4.2

In addition urinary salt deposits were often observed as well as other crystal types as shown in Figures 4.3 and 4.4.

Figure 4.3

Figure 4.4

Of these, the former appears to have spiky crystals protruding from apatite while the latter is a deposit of unknown composition and might be epithelial debri and/or crystalline material. In both cases EDX was not performed and hence definite identification was not possible.

Four black male normals were studied. Two showed the presence of COD crystals, one such sample having profuse deposits (Figure 4.5) with an average size of about 10µm while the other was less populated and had much smaller crystals of average size 3-5µm (Figure 4.6). All four samples revealed the presence of various urinary salt deposits.

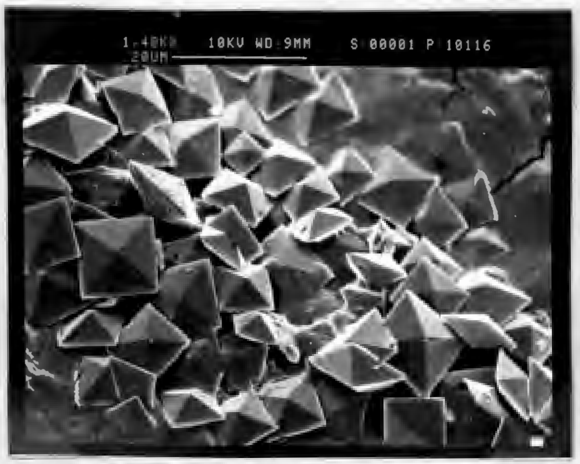


Figure 4.5

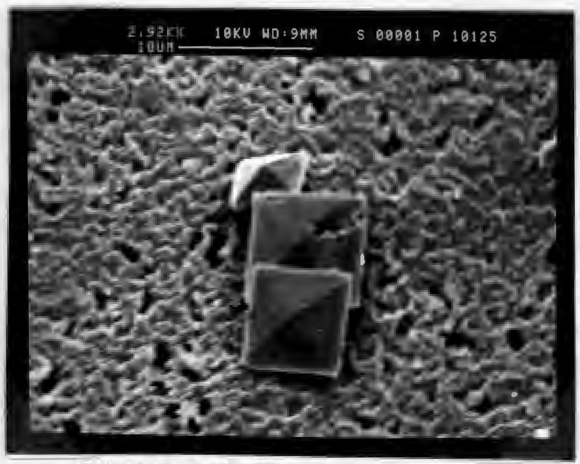


Figure 4.6

Urine samples from black male marathon runners, groups 1 and 2 displayed varying amounts of COD deposits. In group 1, the typical size of single COD crystals was about 10µm but aggregates in the 20-25µm range were observed (Figure 4.7). In one sample from this group, COD crystals displaying prismatic morphology were detected (Figure 4.8). This is not as common as the bipyramidal or octahedral morphology usually observed for COD crystals and will be discussed more fully in Chapter 5.

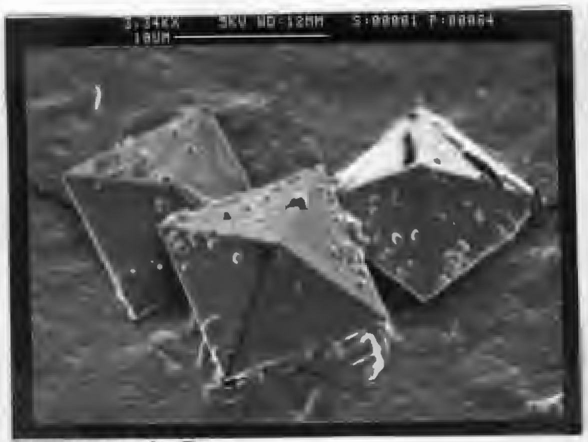


Figure 4.7

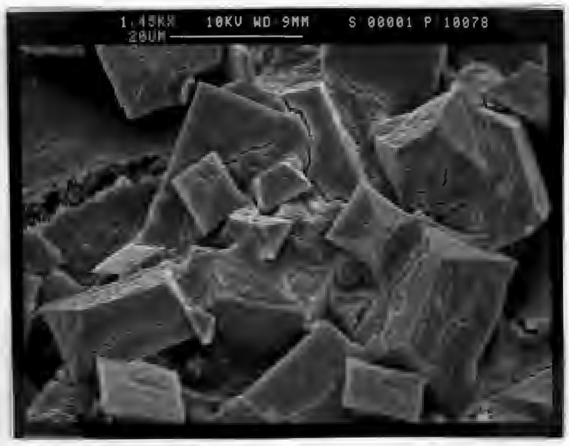


Figure 4.8

Of the six BMMR 2 specimens, two showed dense deposits of CODs. The single crystals were about 10µm in diameter

(Figure 4.9). However much larger crystal agglomerates were also observed (Figure 4.10).

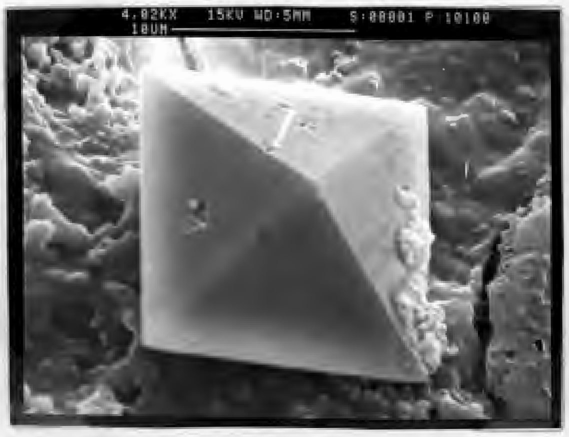


Figure 4.9

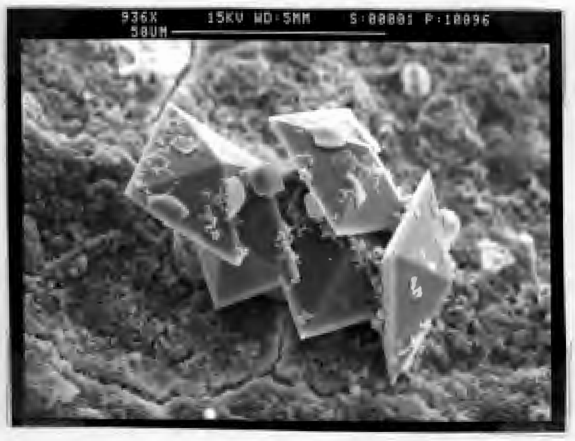


Figure 4.10

The urine samples obtained from the WMC 1 group, revealed extensive COD deposits. These occurred as large single crystals (25-40μm) (Figure 4.11) and as groups of single crystals and as clusters (Figure 4.12). In addition COM crystals were occasionally observed (Figure 4.13). These were about 10μm in cross-section.

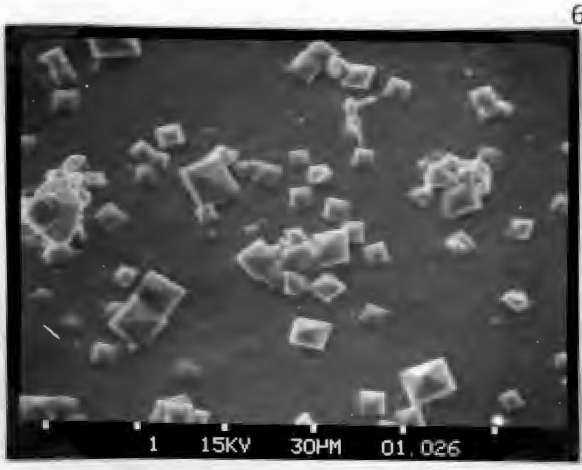


Figure 4.11

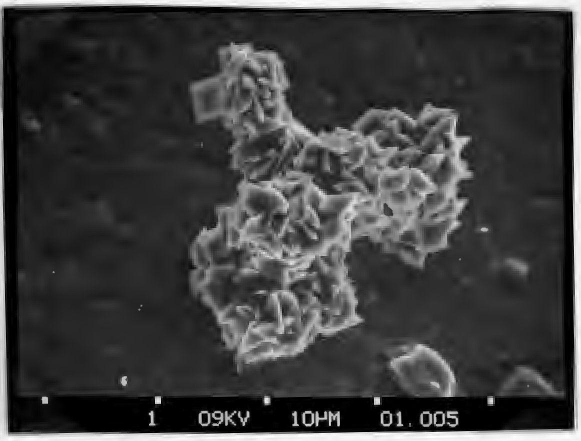


Figure 4.12

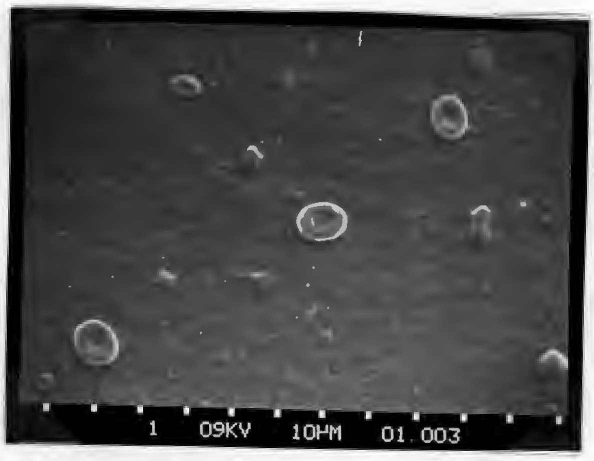


Figure 4.13

CODs were observed to varying degrees in the WMC 2 (white male cyclists). These occurred in the size range 10-30μm (Figure 4.14). Urinary salt deposits occurred in both groups of cyclists. A typical example is shown in Figure 4.15.

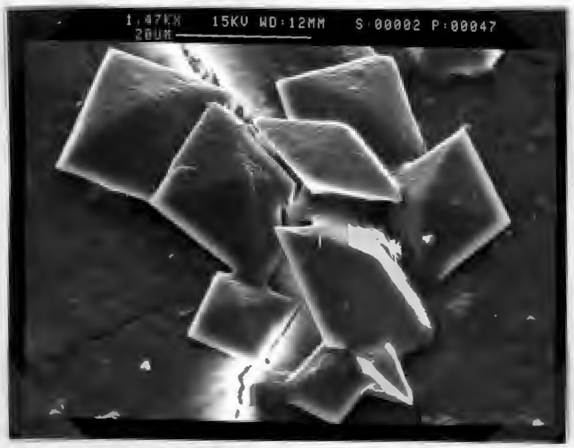


Figure 4.14

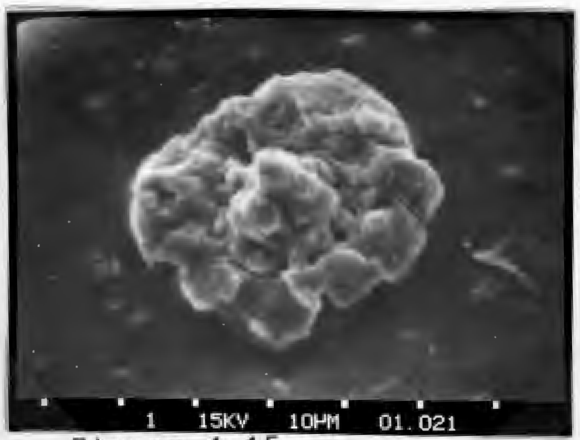


Figure 4.15

EDX showed that the composition of the latter deposits contained the elements Na, P, S, Cl and K (Figure 4.16). Occassionally the COD aggregates were coated with urinary salts as revealed in the EDX spectrum, Figure 4.17.

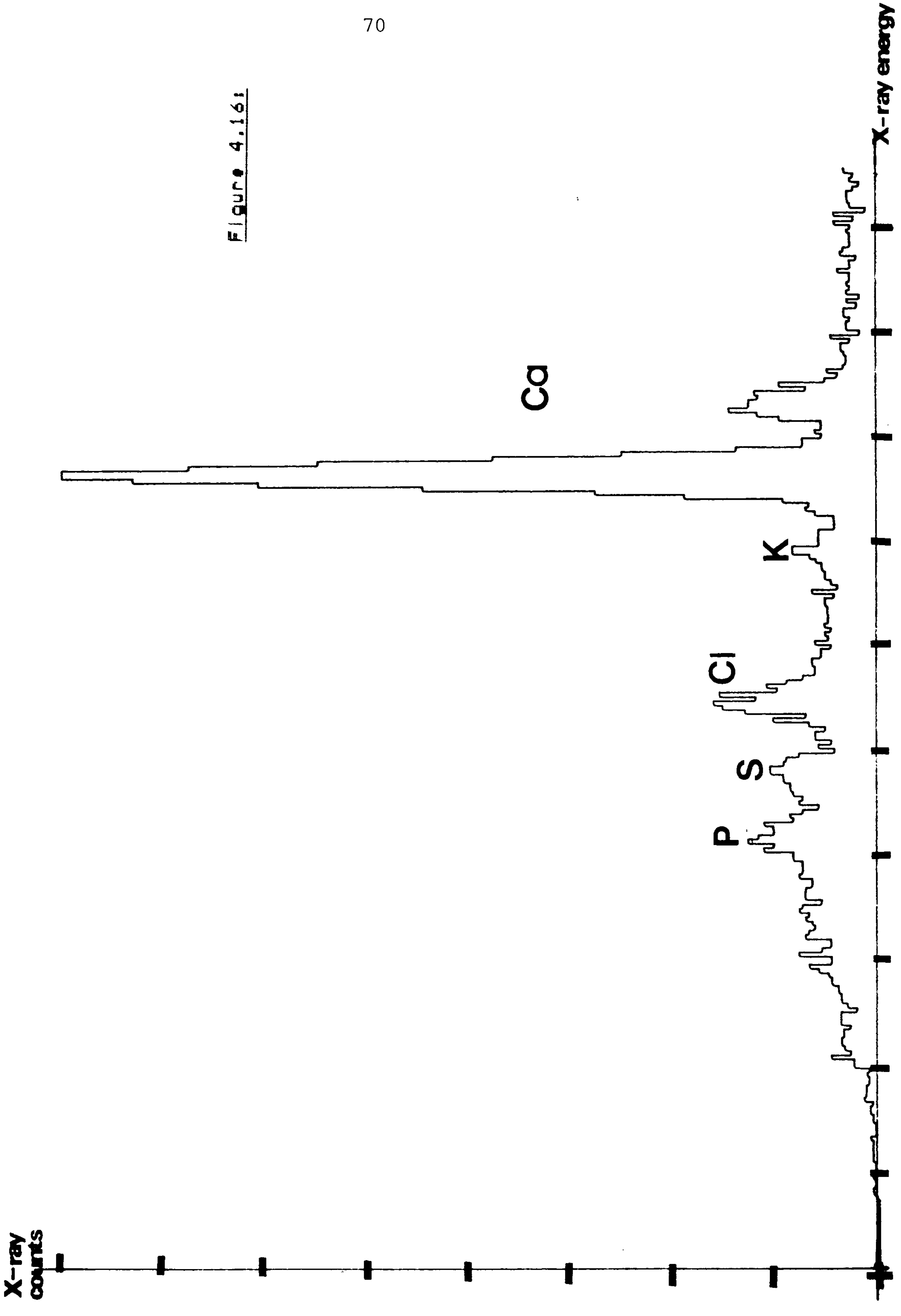
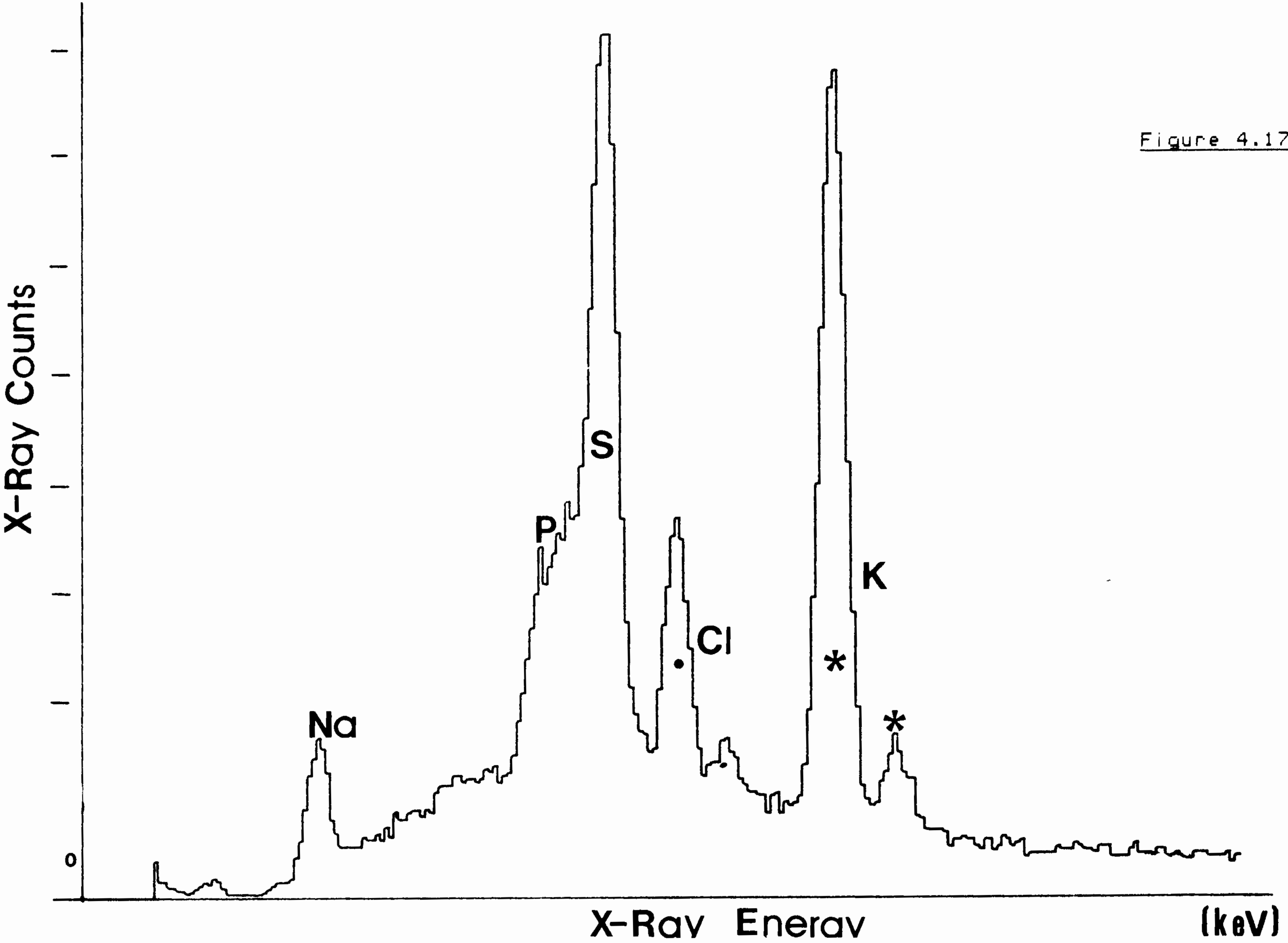


Figure 4.16:

Figure 4.17:



Extensive COD crystals both singular and aggregates, were observed in about 50 percent of specimens in the WMS 1 group. These were sometimes very large having cross-sections of up to 50μm (Figure 4.18; 4.19; 4.20; 4.21). Attention is drawn to the different morphologies of COD crystals depicted in these micrographs. A similar feature was mentioned earlier with respect to COD crystals observed in the BMMR 1 group (Figure 4.8).

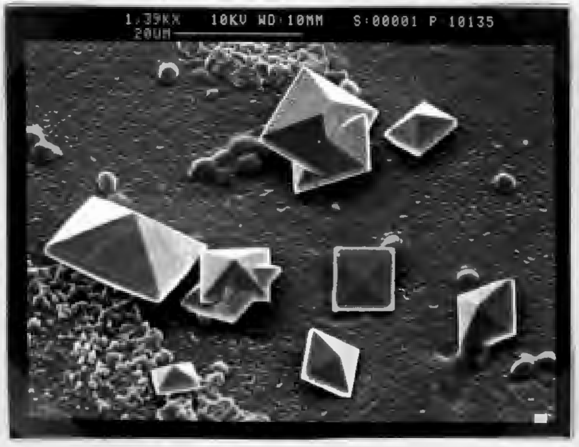


Figure 4.18

Figure 4.19

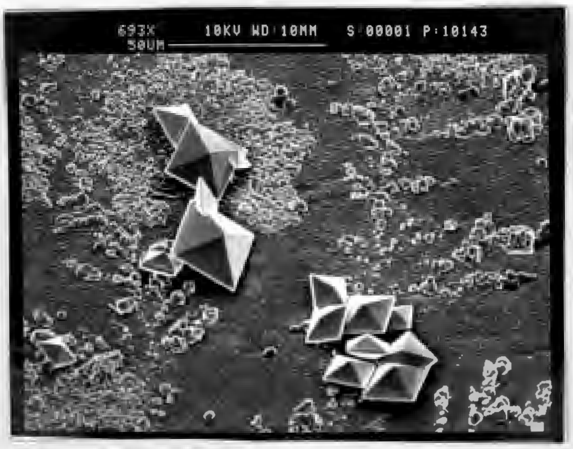


Figure 4.20

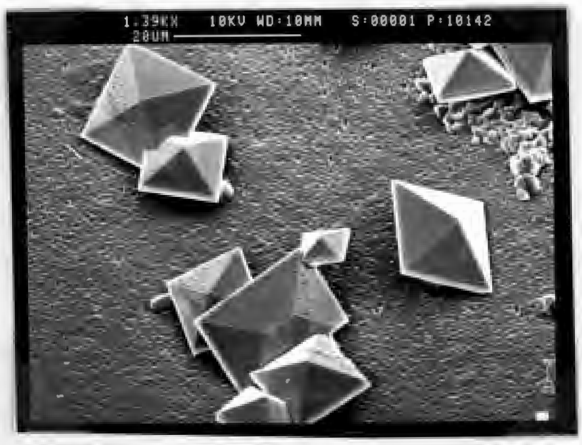


Figure 4.21

Profuse deposits of unknown composition and morphology were observed in all the urinary specimens for this group (WMS 1). An example is shown in Figure 4.22. These spherular bodies are similar in both morphology and size to that commonly reported for apatite [1,2,3]. However because of the absence of EDX facilities on the S200 Scanning Electron Microscope, it was impossible to identify these deposits with any degree of certainty.

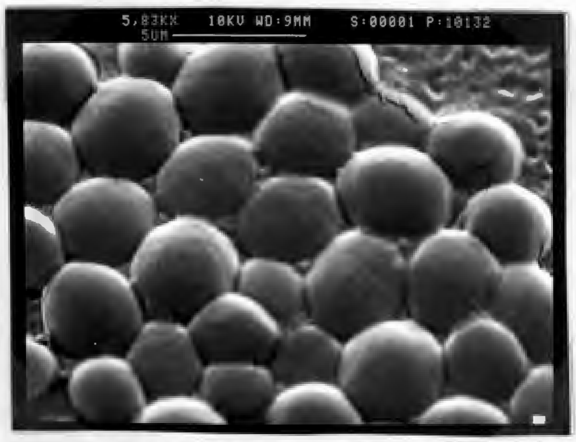


Figure 4.22

In the second group of swimmers (WMS 2), a fair amount of COD clusters was observed (Figure 4.23). In addition, individual specimens from this group revealed their own peculiar crystals. One such sample had extensive deposits of very large trapezoidal and pinacoidal crystals (Figure 4.24), similar to struvite crystals described by other workers [1,4].

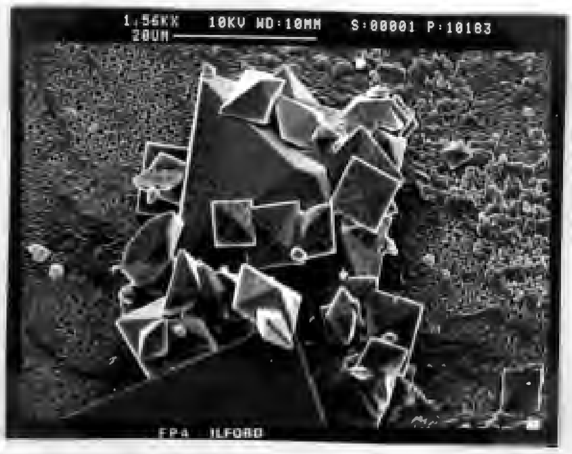


Figure 4.23

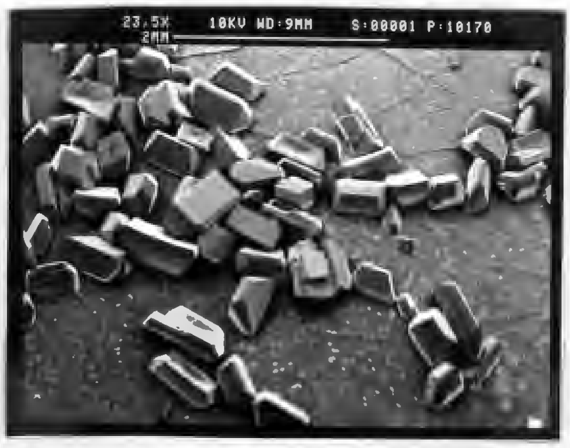


Figure 4.24

Figure 4.25 shows the size of one of these crystals while Figure 4.26 is a high magnification micrograph showing the afore-mentioned crystal in close proximity to profuse COD deposits.

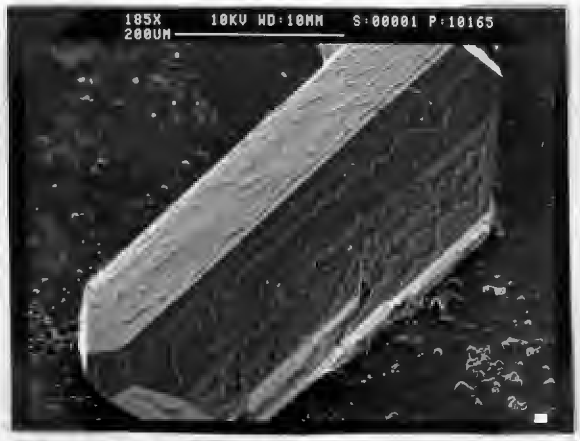


Figure 4.25

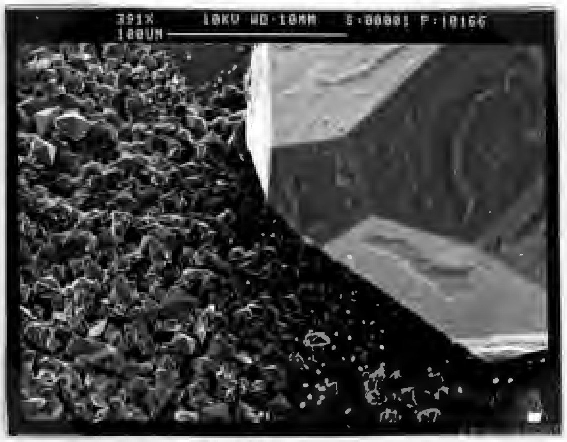


Figure 4.26

The relative sizes of the struvite and COD crystals are clearly apparent. Figure 4.27 shows a cluster of COD crystals embedded in mucoid material. The significance of this observation will be discussed in the next chapter.

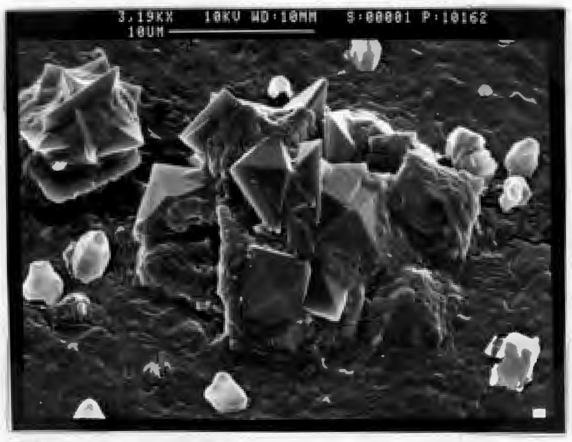


Figure 4.27

Yet another micrograph recorded for the same specimen showed COD crystals in an admixture with spherular bodies (Figure 4.28). The latter are likely to be apatite crystals.

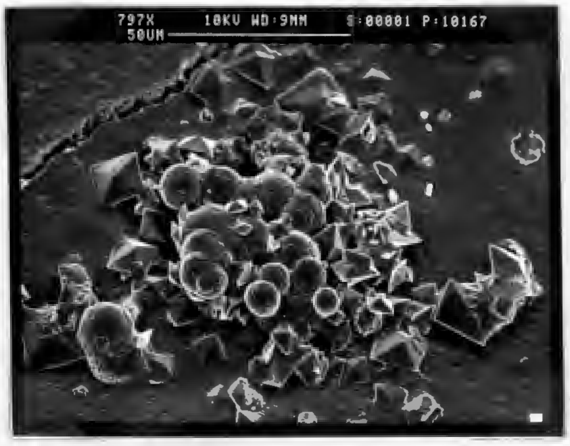


Figure 4.28

In another specimen from the second group of swimmers, rod-like and columnar crystals were observed (Figure 4.29 and 4.30). These are morphologically similar to brushite deposits reported by other workers [1,4,5].

Figure 4.29

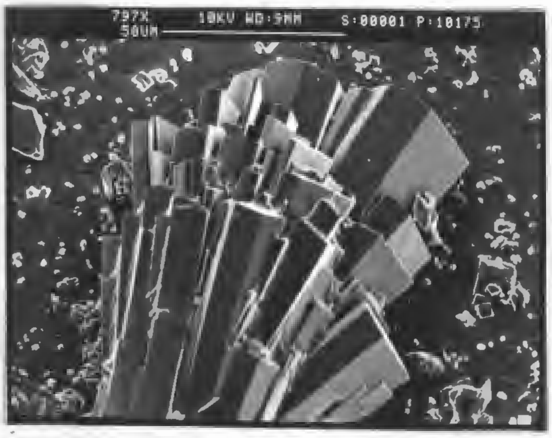


Figure 4.30

Examination of another specimen from the WMS 2 group, revealed the presence of a third type of crystalline material. These appeared as extensive deposits of needles, rods and plates (Figures 4.31; 4.32; 4.33) respectively and are likely to be uric acid deposits as they resemble similar such crystals described by Kim [1,6].

Figure 4.31

Figure 4.32

Figure 4.33

4.2: PARTICLE SIZE DISTRIBUTION ANALYSIS

The particle size distribution curves and corresponding histograms for each of the groups investigated are presented in Figures 4.34 - 4.44. In each case the curve is denoted by (a) while the matching histogram is denoted by (b).

Features of these curves that are worthy of mention are as follows:-
The WMN distribution is characterized by a high incidence of particles in the small diameter range (2,5-8,0μm; Figures 4.34(a) and (b)).
The WMMR 1 group has a different distribution in that there appears to be a second peak in the larger diameter range (8-20μm) as shown in Figures 4.35 (a) and (b). This is even more pronounced in the particle size distributions determined for the group WMMR 2 (20-40μm, Figs 4.36(a) and (b)).

The distribution for black male normals (Figures 4.37 (a) and (b)) is similar to that for white male normals although there is a large number of particles in the 8-20μm range.

The BMMR 1 group displayed distributions similar to those of their controls (Figures 4.38 (a) and (b)) while the distribution for BMMR 2 (Figure 4.39 (a) and (b)) was somewhat different to the normal group in that particles larger than 20μm were detected.

The distribution of particles in the first group of white male cyclists (WMC 1) is similar to that for WMN. However, differences in the large diameter range (20-40μm) are present in the WMC 2 distribution. (Figures 4.40 (a) and (b); 4.41 (a) and (b))

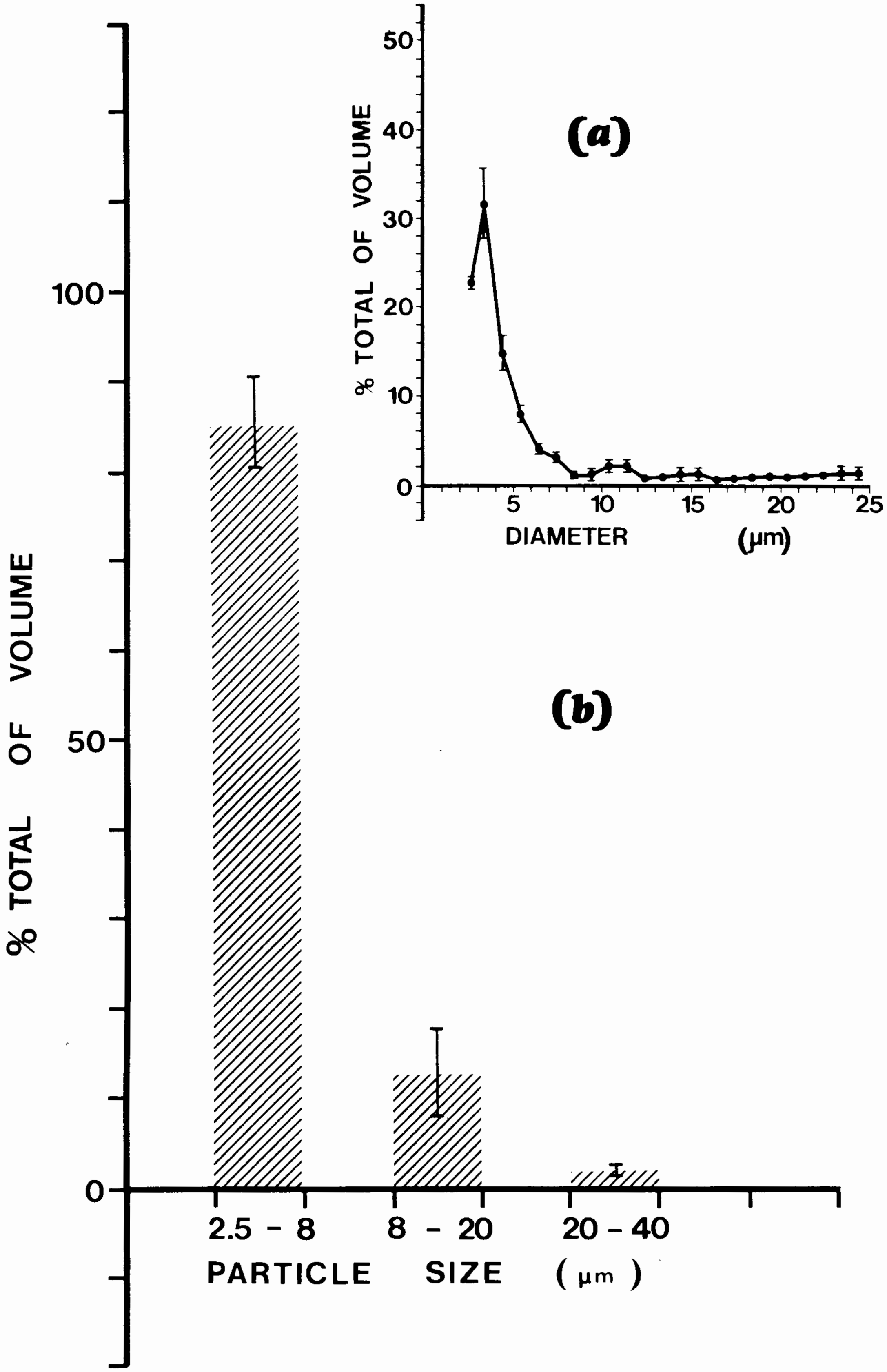
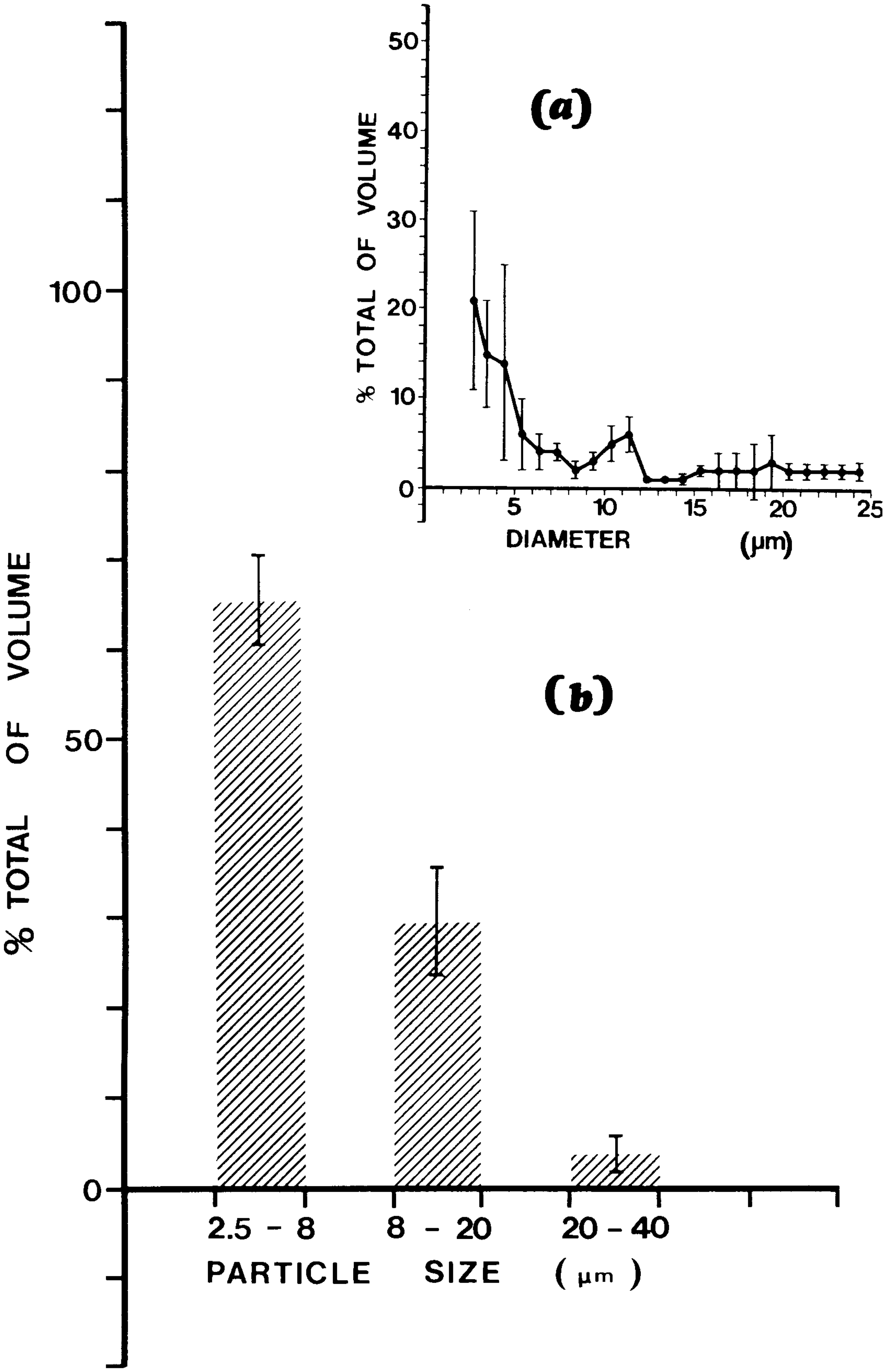
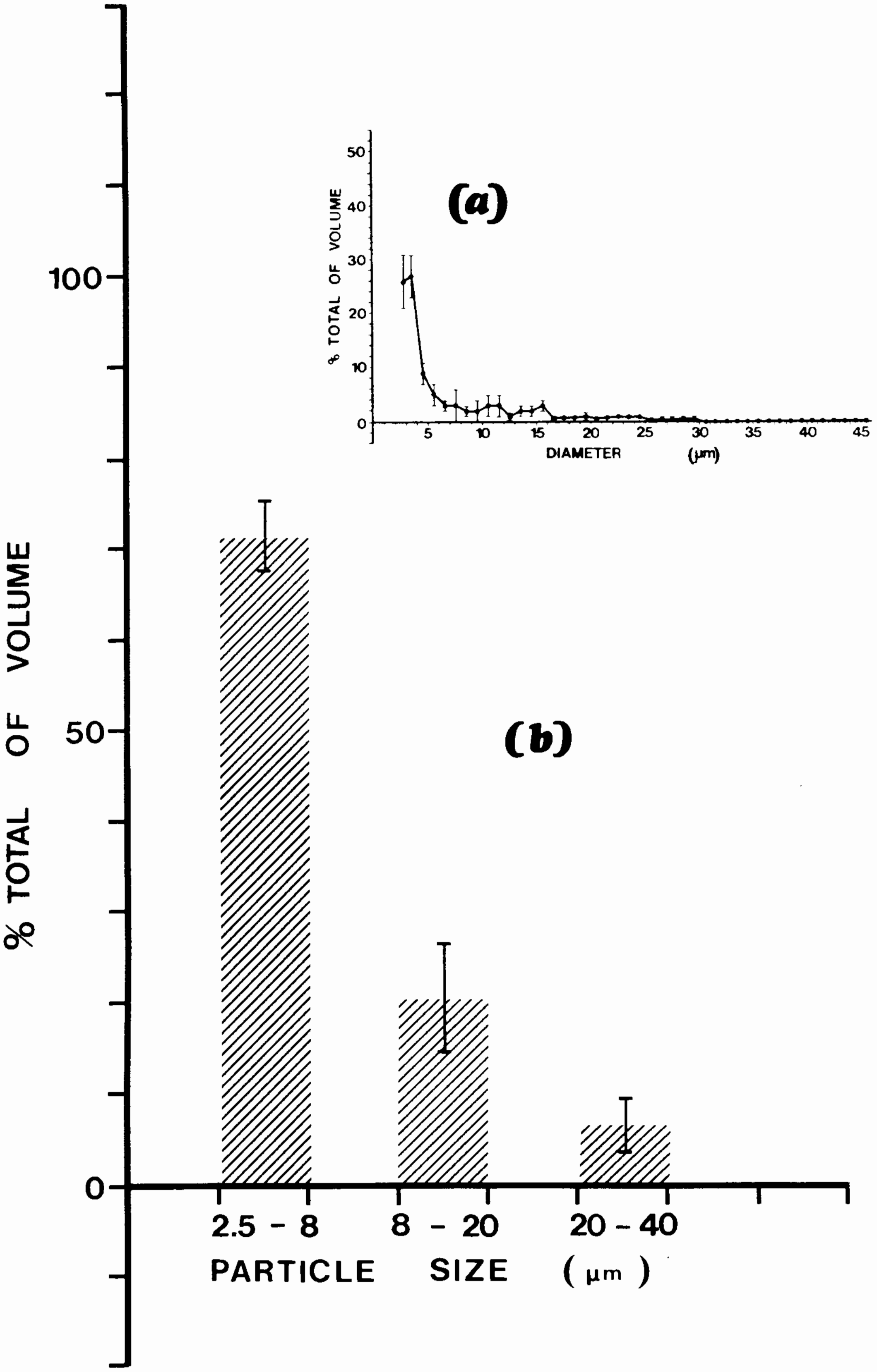


Figure 4.34 (a) and (b): THE PARTICLE SIZE DISTRIBUTION CURVE AND HISTOGRAM FOR WHITE MALE NORMALS (WMN).



<u>Figure 4.35 (a) and (b):</u> THE PARTICLE SIZE DISTRIBUTION CURVE AND HISTOGRAM FOR WMMMR 1.



<u>Figure 4.36 (a) and (b):</u> THE PARTICLE SIZE DISTRIBUTION CURVE AND HISTOGRAM FOR WMMMR 2.

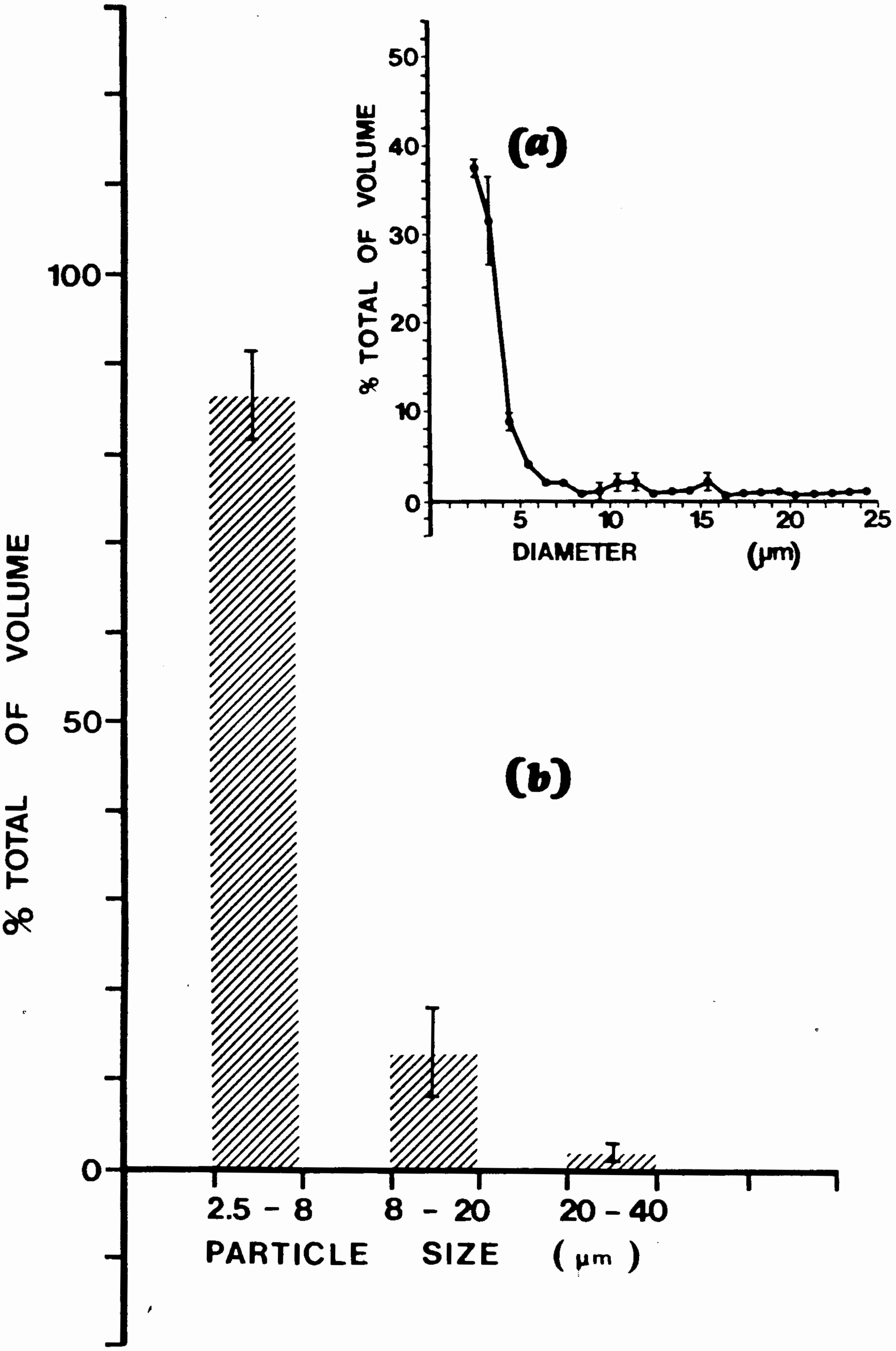


Figure 4.37 (a) and (b): THE PARTICLE SIZE DISTRIBUTION CURVE AND HISTOGRAM FOR BMNs.

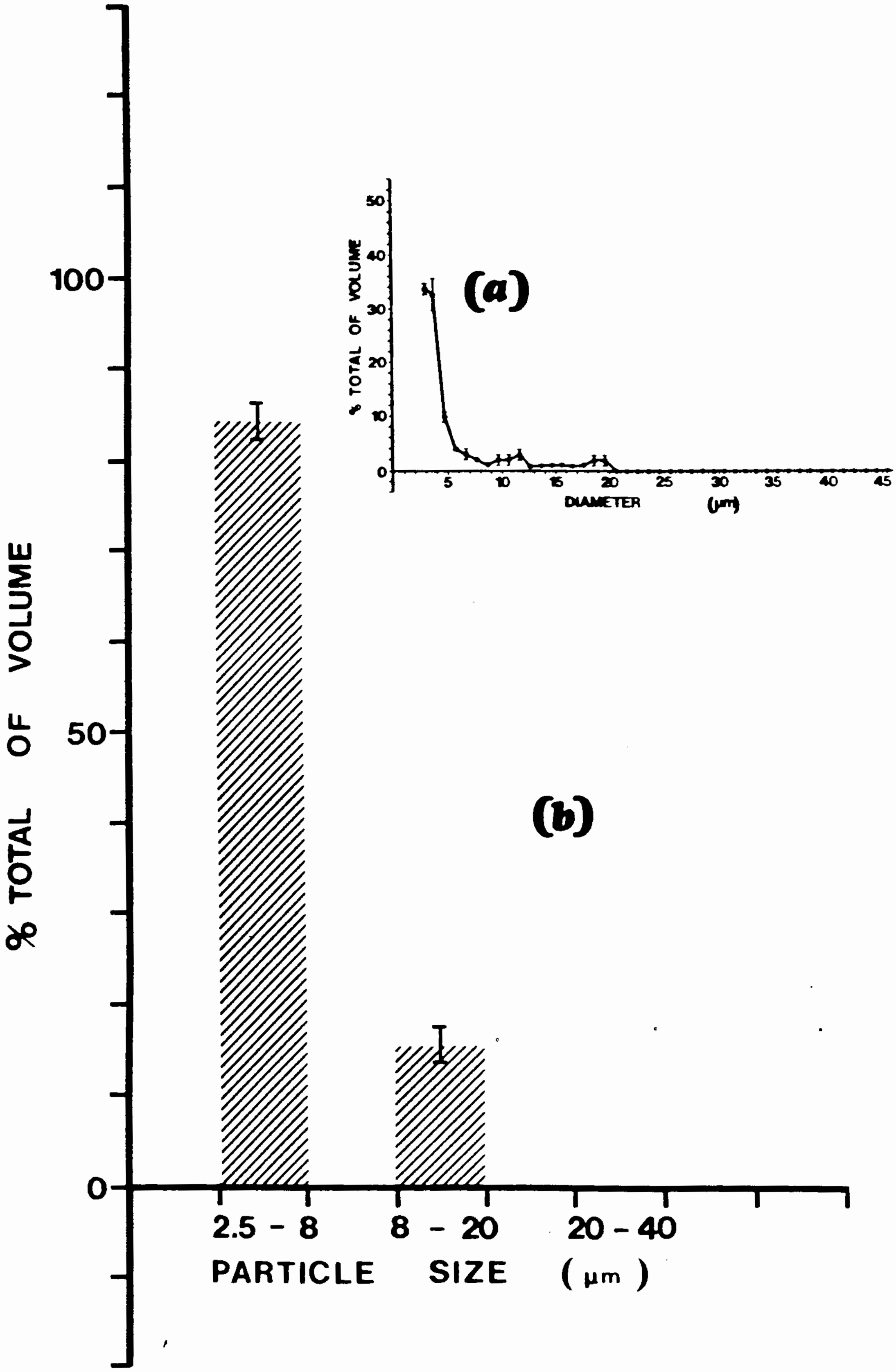
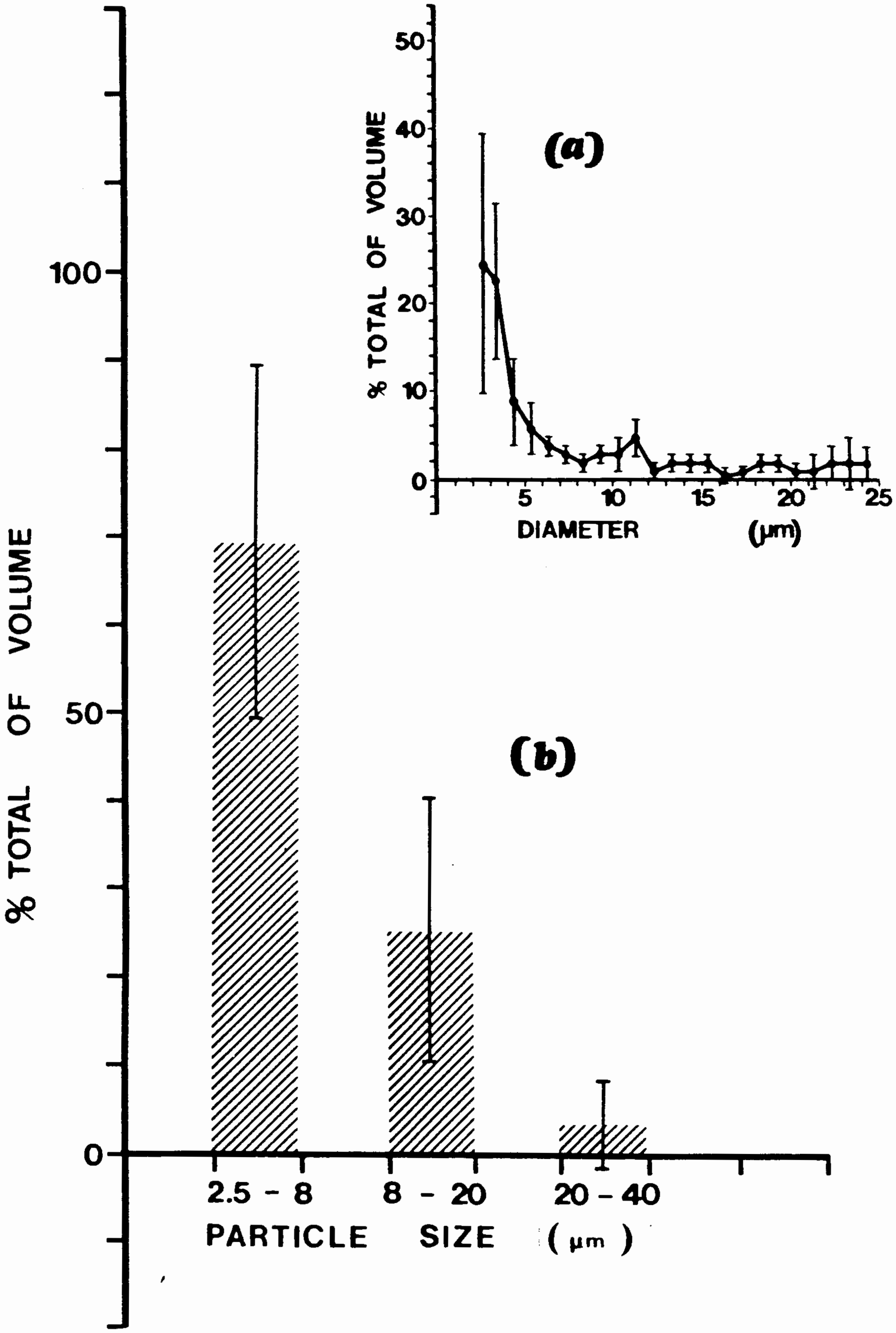


Figure 4.38 (a) and (b): THE PARTICLE SIZE DISTRIBUTION CURVE AND HISTOGRAM FOR BMMR 1.



<u>Figure 4.39 (a) and (b):</u> THE PARTICLE SIZE DISTRIBUTION CURVE AND HISTOGRAM FOR BMMR 2.

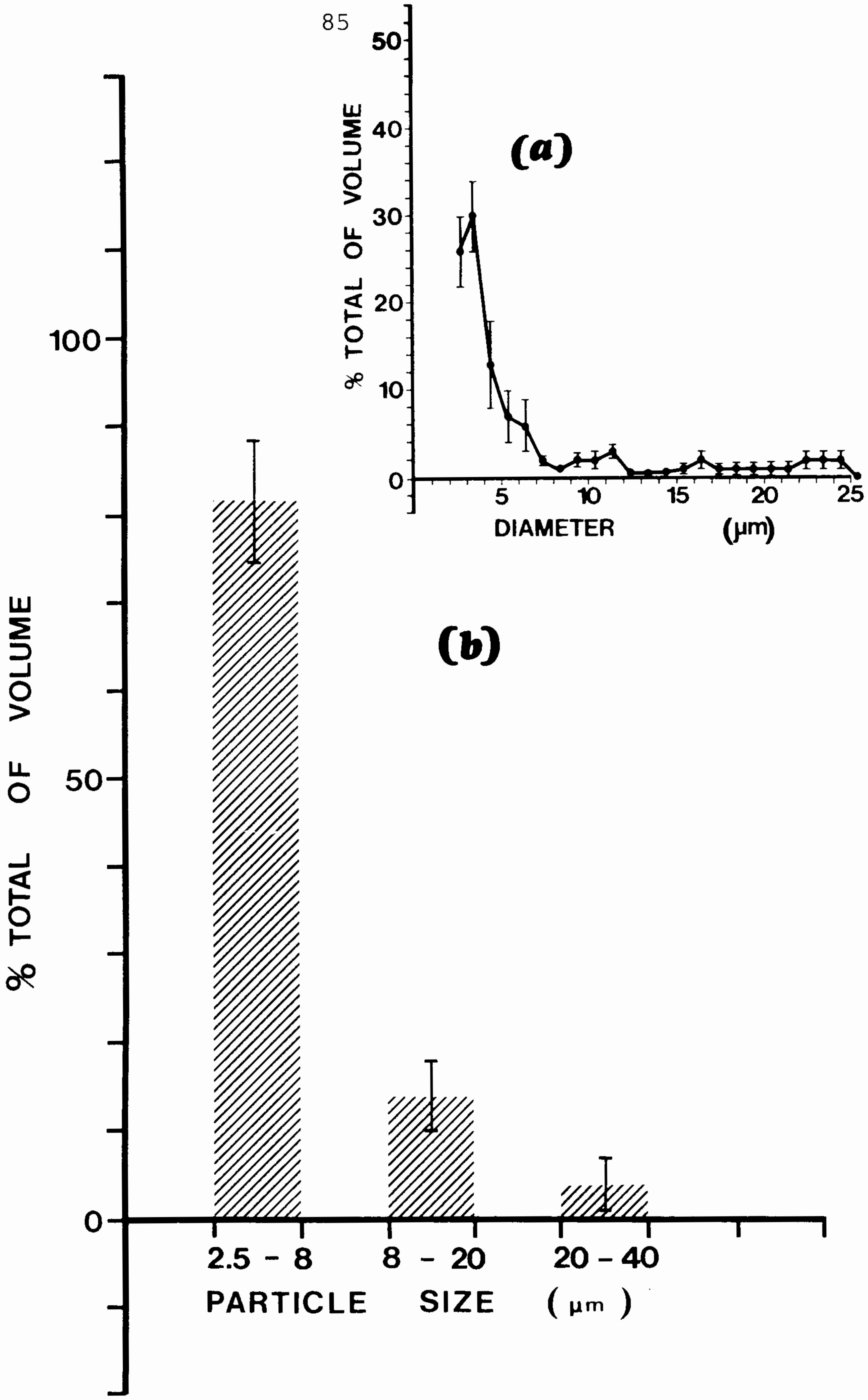


Figure 4.40 (a) and (b): THE PARTICLE SIZE DISTRIBUTION CURVE AND HISTOGRAM FOR WMC 1.

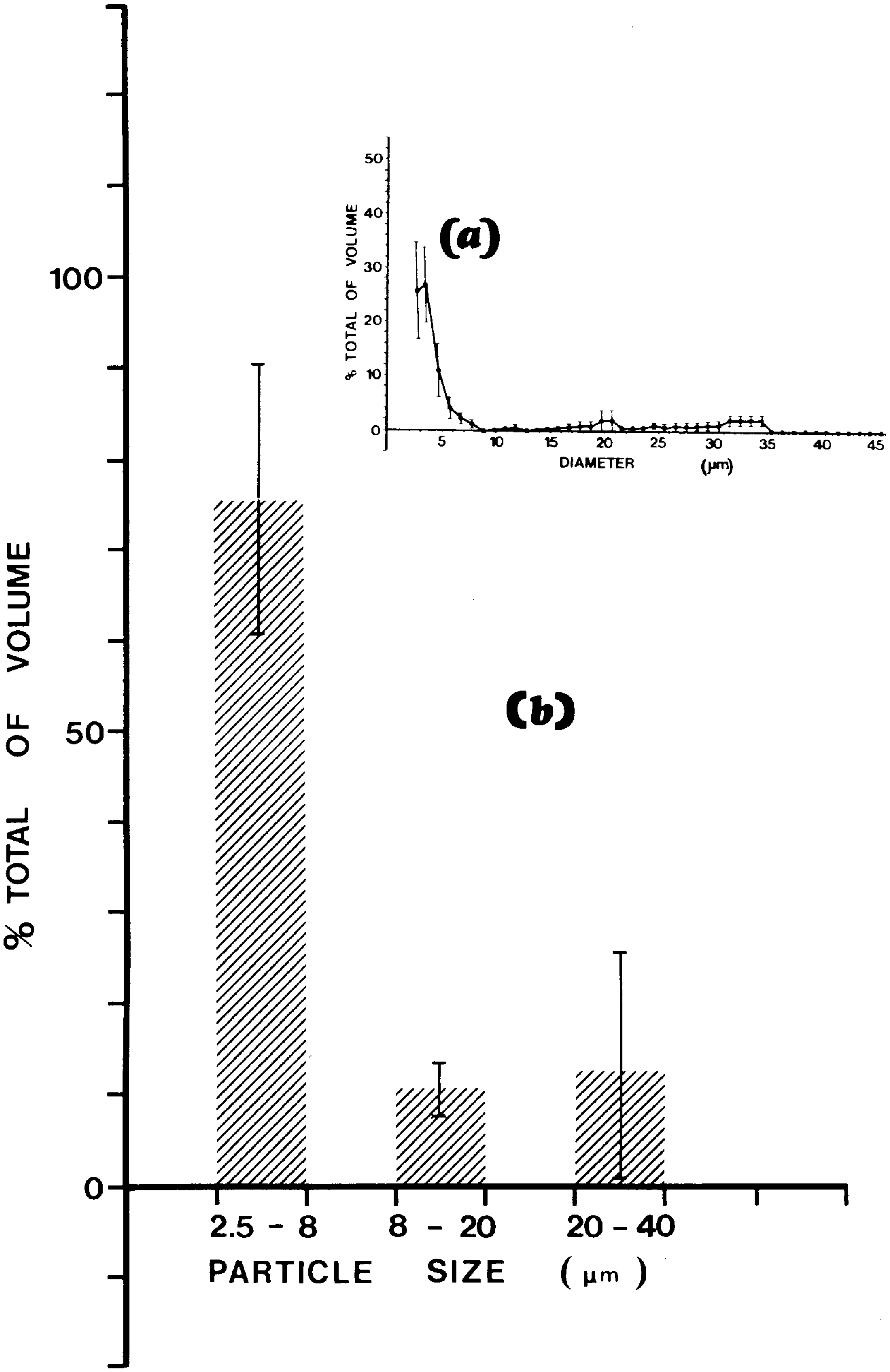


Figure 4.41 (a) and (b): THE PARTICLE SIZE DISTRIBUTION CURVE AND HISTOGRAM FOR WMC 2.

Attention is again drawn to the fact that these larger particles occur in the WMC 2 group as opposed to group WMC 1 and reflects a similar trend to that mentioned previously for WMMR 1 and WMMR 2 above. The final group, namely swimmers, showed similar particle size distribution characteristics. Here the distributions were different to the normal group with larger particles again manifesting themselves in urine samples of the group WMS 2. (Figures 4.42 (a) and (b); 4.43 (a) and (b))

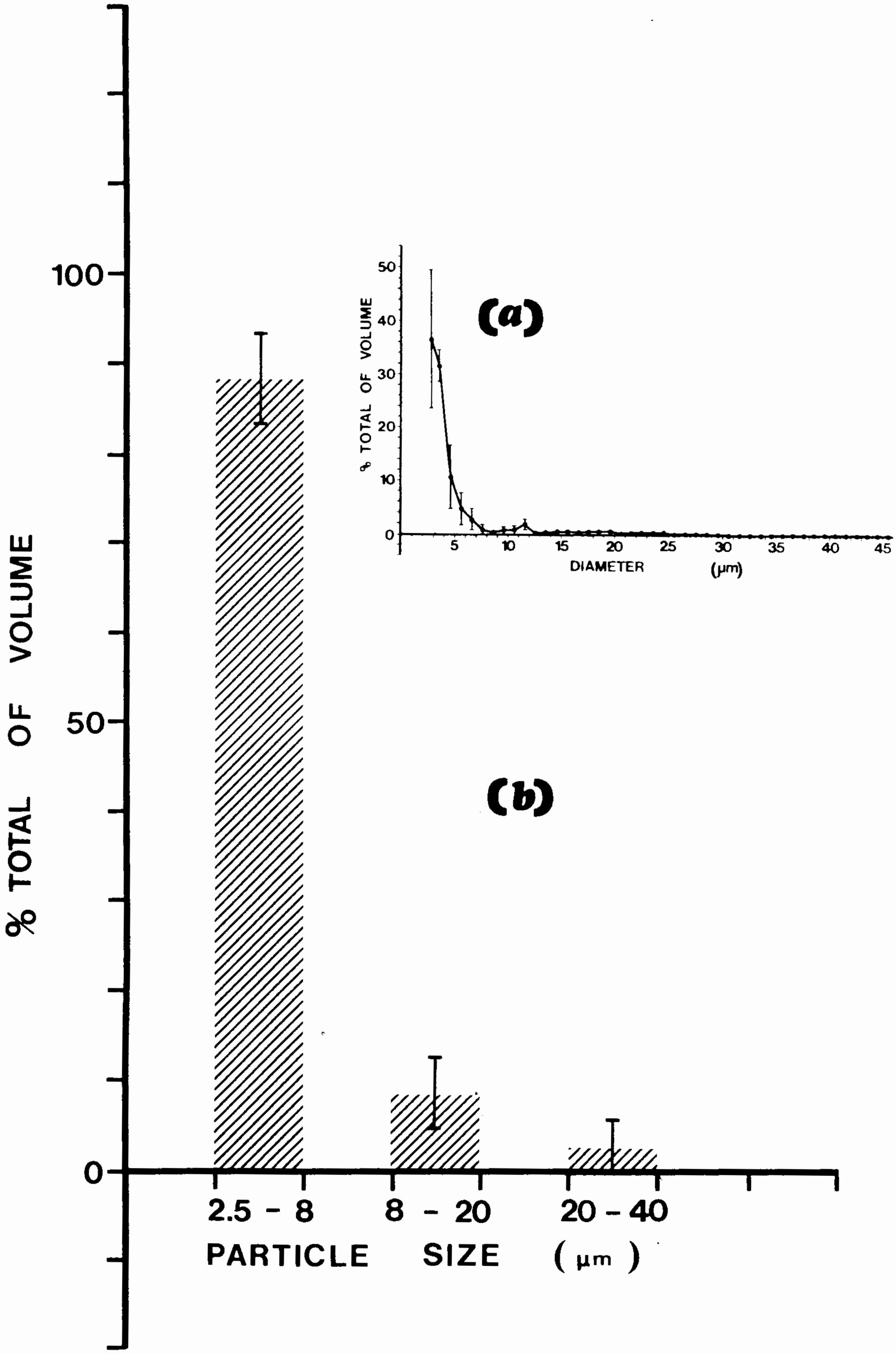


Figure 4.42 (a) and (b): THE PARTICLE SIZE DISTRIBUTION CURVE AND HISTOGRAM FOR WMS 1.

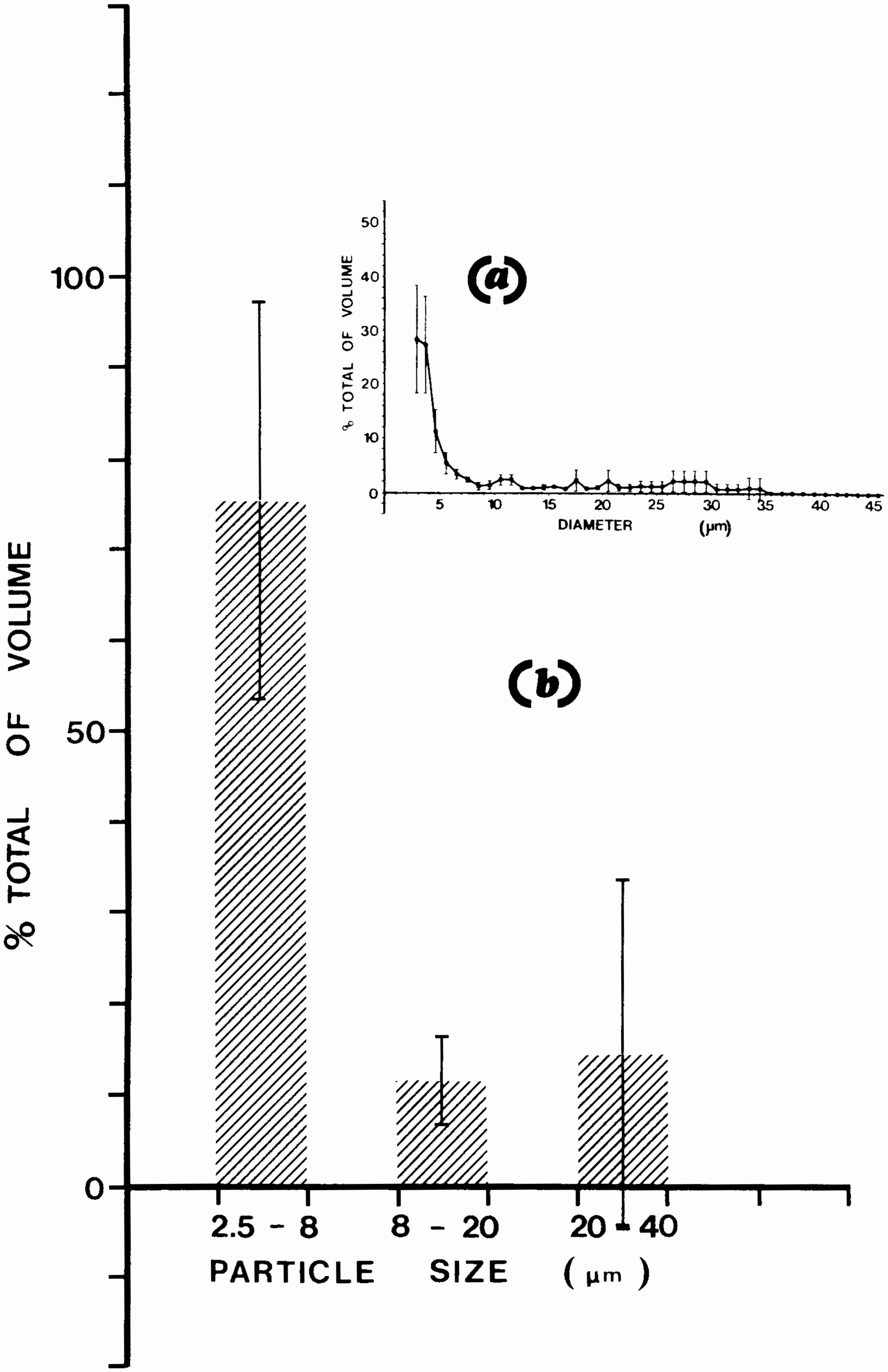


Figure 4.43 (a) and (b): THE PARTICLE SIZE DISTRIBUTION CURVE AND HISTOGRAM FOR WMS 2.

4.3 ATOMIC ABSORPTION SPECTROSCOPY

The urinary concentration levels of Na, Ca, Mg and K in the various groups are listed in tables 4.1, 4.2, 4.3, 4.4 respectively. The ratios Na/Ca, K/Ca, Mg/Ca are presented in tables 4.5, 4.6, 4.7 respectively.

Table 4.1: MEAN 24 HOUR URINARY Na^+ EXCRETION
(± STANDARD DEVIATION)

SUBJECTS	SAMPLE SIZE	Na LEVEL (mg)/24hr
BMMR 2	8	3624 ± 1104
BMMR 1	14	3609 ± 1655
WMC 2	10	3380 ± 1127
BMN	13	3265 ± 1265
WMC 1	21	3035 ± 1173
SWIM 1	8	2966 ± 1517
WMN	16	2828 ± 1173
SWIM 2	10	2805 ± 1012
WMMR 2	14	2230 ± 805
WMMR 1	18	2023 ± 1035

Table 4.2: MEAN 24 HOUR URINARY Ca^{2+} EXCRETION
(± STANDARD DEVIATION)

SUBJECTS	SAMPLE SIZE	Ca LEVEL (mg)/24hr
WMMR 1	18	200 ± 80
WMC 1	21	200 ± 80
WMC 2	10	200 ± 40
SWIM 2	10	160 ± 120
SWIM 1	8	160 ± 80
WMMR 2	14	160 ± 80
BMMR 2	8	160 ± 80
BMN	13	120 ± 80
WMN	16	120 ± 80
BMMR 1	14	120 ± 40

Table 4.3: MEAN 24 HOUR URINARY Mg EXCRETION
(± STANDARD DEVIATION)

SUBJECTS	SAMPLE SIZE	Mg LEVEL (mg)/24hr
SWIM 1	8	195 ± 122
WMMR 1	18	195 ± 73
BMMR 2	8	170 ± 97
SWIM 2	10	146 ± 73
WMC 2	10	146 ± 49
BMMR 1	14	122 ± 97
WMC	21	122 ± 73
WMMR 2	14	97 ± 73
WMN	16	97 ± 49
BMN	13	73 ± 24

Table 4.4: MEAN 24 HOUR URINARY K EXCRETION (± STANDARD DEVIATION)

SUBJECTS	SAMPLE SIZE	K LEVEL (mg)/24hr
SWIM 2	10	2581 ± 1408
WMN	16	2385 ± 1603
WMC 2	10	2268 ± 1212
WMC 1	21	1955 ± 860
WMMR 1	18	1916 ± 821
WMMR 2	14	1799 ± 939
BMN	13	1681 ± 860
BMMR 2	8	1681 ± 704
SWIM 1	8	1564 ± 899
BMMR 1	14	1330 ± 587

Table 4.5: MEAN 24 HOUR URINARY Na/Ca RATIOS (± STANDARD DEVIATIONS)

SUBJECTS	Na/Ca RATIO
BMMR 1	78 ± 51
BMN	69 ± 42
BMMR 2	53 ± 29
WMS 1	40 ± 16
WMS 2	40 ± 23
WMN	38 ± 10
WMMR 2	31 ± 17
WMC 1	29 ± 11
WMC 2	29 ± 10
WMMR 1	19 ± 13

Standard deviation values were calculated using the formula

$$SD = \sqrt{\frac{\sum (\bar{x}-x)^2}{(n-1)}}$$

where $\bar{x}$: mean Na/Ca ratio for each group

x : Na/Ca ratio for each subject within a particular group

n : no of subjects

Table 4.6: MEAN 24 HOUR URINARY K/Ca RATIOS (± STANDARD DEVIATION)

SUBJECTS	K/Ca RATIO
SWIM 2	27 ± 19
BMN	27 ± 14
WMN	24 ± 25
WMMR 2	16 ± 11
BMMR 1	16 ± 10
BMMR 2	15 ± 11
SWIM 1	14 ± 8
WMC 1	11 ± 5
WMC 2	10 ± 6
WMMR 1	10 ± 5

Standard deviation values were calculated using the formula

$$SD = \sqrt{\frac{\sum(\bar{x}-x)^2}{(n-1)}}$$

where $\bar{x}$: mean K/Ca ratio for each group

x : K/Ca ratio for each subject within a particular group

n : no. of subjects

Table 4.7: MEAN 24 HOUR URINARY Mg/Ca RATIOS
(± STANDARD DEVIATION)

SUBJECTS	Mg/Ca RATIO
BMN	2 ± 1
BMMR 1	2 ± 1
BMMR 2	2 ± 1
WMMR 1	2 ± 1
SWIM 1	2 ± 1
SWIM 2	2 ± 1
WMN	1 ± 1
WMMR 2	1 ± 1
WMC 1	1 ± 0.6
WMC 2	1 ± 0.5

Standard deviation values were calculated using the formula

$$SD = \sqrt{\frac{\sum_{1} (\bar{x}-x)^2}{(n-1)}}$$

where $\bar{x}$: mean Mg/Ca ratio for each group

x : Mg/Ca ratio for each subject within a particular group

n : no of subjects

4.4 STATISTICAL ANALYSIS

Significant differences were sought for the mean 24 hour urinary Na and Ca concentrations in numerous inter-group comparisons. Table 4.8 and 4.9 lists the significant differences occurring in these two elements respectively.

Table 4.8: SIGNIFICANT INTER-GROUP DIFFERENCES IN MEAN 24 HOUR URINARY Na LEVELS

SUBJECTS - SIGNIFICANCE AT P<0.05	
WMN/WMMR 1	BMN/WMMR 1
WMMR 1/WMC 1	BMMR 1/WMMR 1
WMMR 1/WMC 2	BMMR 1/WMMR 2
WMMR 2/WMC 1	BMMR 2/WMMR 1
WMMR 2/WMC 2	BMMR 2/WMMR 2
BMN/WMMR 2	

Table 4.9: SIGNIFICANT INTERGROUP DIFFERENCES (P<0.05) IN MEAN 24 HOUR URINARY Ca

SUBJECTS WITH SIGNIFICANT DIFFERENCES	
WMN/WMMR 1	BMN/WMC 1
WMN/WMC 1	BMN/WMC 2
WMN/WMC 2	BMMR 1/WMMR 1
WMMR 2/WMC 1	BMMR 1/WMMR 2
WMMR 2/WMC 2	BMMR 1/WMC 1
SWIM 1/WMC 2	BMMR 1/WMC 2
BMN/WMMR 1	

Computations for Mg and K levels were limited to comparisons of the WMN group with the other groups. Significant differences for these two elements are shown in Table 4.10.

Table 4.10: SIGNIFICANT DIFFERENCES ($p<0.05$) IN MEAN 24 HOUR URINARY Mg AND K LEVELS

SUBJECTS SHOWING SIGNIFICANT DIFFERENCES	
Mg	K
WMN/WMMR 1	WMN/BMMR 1
WMN/SWIM 2	
WMN/WMC 2	
WMN/BMMR 1	
WMN/BMMR 2	

The three ratios Na/Ca, K/Ca, Mg/Ca were also examined for significant inter-group differences. The results of these comparisons are presented in tables 4.11, 4.12, 4.13 respectively.

Table 4.11: MEAN 24 HOUR URINARY Na/Ca RATIOS SHOWING SIGNIFICANT DIFFERENCES BETWEEN WMNs AND VARIOUS GROUPS

SIGNIFICANT DIFFERENCES BETWEEN WMN AND VARIOUS GROUPS	
WMN/WMMR 1	WMMR 1/BMMR 2
WMN/WMC 1	WMMR 2/BMMR 1
WMN/WMC 2	WMMR 2/BMMR 2
WMN/BMN	WMC 1/BMMR 1
WMN/BMMR 1	WMC 1/BMMR 2
WMMR 1/WMMR 2	WMC 1/SWIM 1
WMMR 1/SWIM 1	SWIM 2/BMMR 1
WMMR 1/SWIM 2	BMN/WMMR 1
WMMR 1/WMC 1	BMN/WMMR 2
WMMR 1/WMC 2	BMMR 1/BMMR 2
WMMR 1/BMMR 1	

Table 4.12: SIGNIFICANT INTER-GROUP DIFFERENCES MEAN 24 HOUR URINARY K/Ca RATIOS

SUBJECTS SHOWING SIGNIFICANT DIFFERENCES	
WMN/WMMR 1	BMN/WMC 1
WMN/WMC 1	BMMR 1/WMMR 1

Table 4.13: SIGNIFICANT INTER-GROUP DIFFERENCES IN MEAN 24 HOUR URINARY Mg/Ca RATIOS

SUBJECTS SHOWING SIGNIFICANT DIFFERENCES	
WMN/BMMR 1	BMMR 1/SWIM 1
WMMR 1/WMC 1	BMMR 1/WMC 1
BMMR 1/WMMR 1	

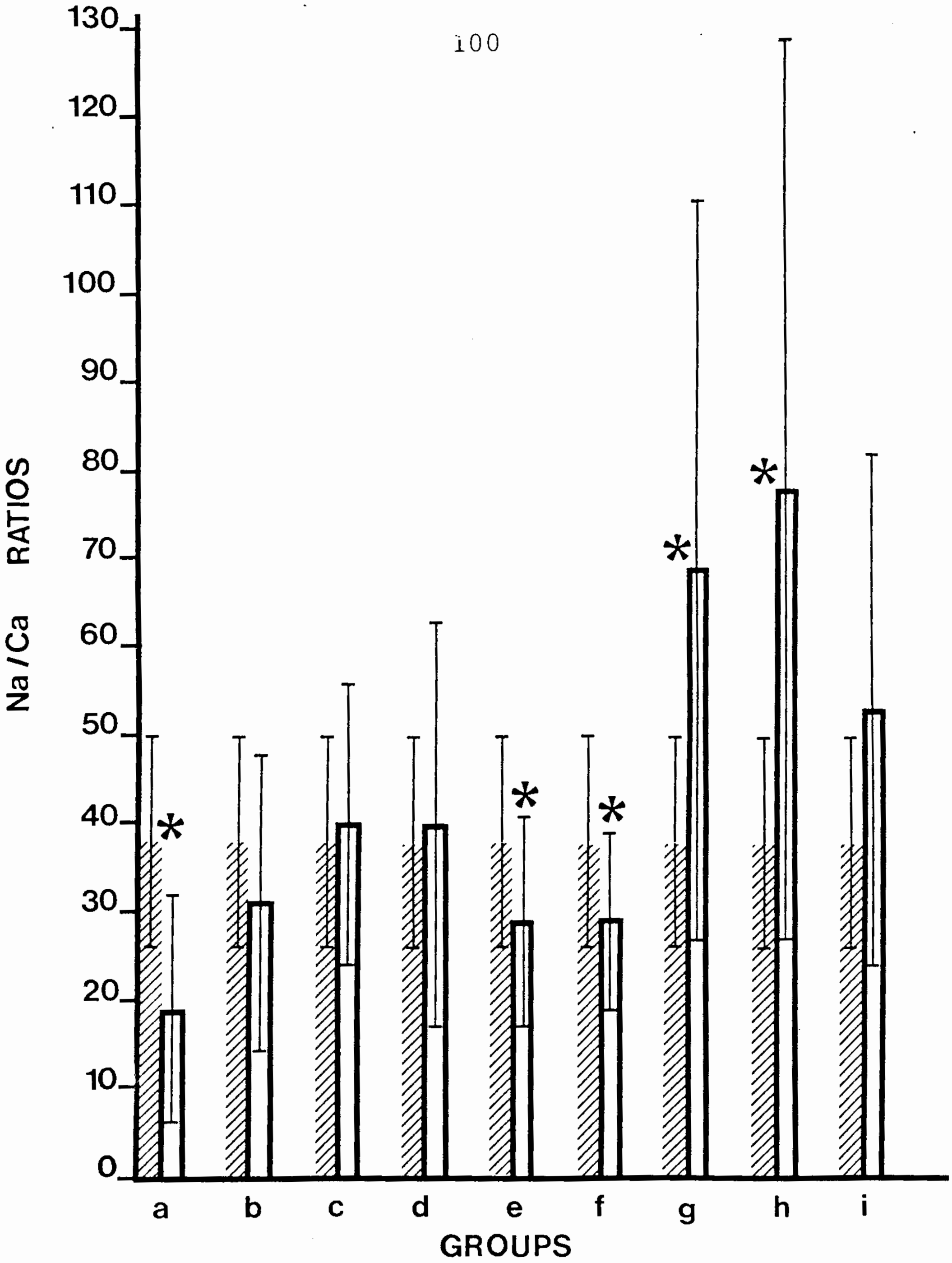


Figure 4.44: Histogram summary of Na/Ca ratios indicating comparison of the WMN group with all the other groups.

GROUPS REPRESENTED IN FIGURE 4.44

* INDICATES SIGNIFICANCE AT p<0.05

a	WMN/WMMR 1	d	WMN/WMS2	g	WMN/BMN
b	WMN/WMMR 2	e	WMN/WMC 1	h	WMN/BMMR 1
c	WMN/WMS 1	f	WMN/WMC 2	i	WMN/BMMR 2

▨ REPRESENTS WMN

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CHAPTER 5

DISCUSSION

CHAPTER 5

DISCUSSION

As previously mentioned in chapter one, motivation for this thesis arose from the results of Irving et al [1] and Rodgers et al [2] in their studies of the crystalluria in marathon runners. Their investigations based on Coulter counter data and SEM analysis showed that this group of sportsmen are at risk with respect to kidney stone formation. In order to establish "base lines", particle distribution analyses were conducted in the present project which overlapped the studies of the afore-mentioned workers. Thus the WMN size distribution shown in Figure 4.34 is in agreement with the results published by Robertson et al [3], Irving et al [1] and Rodgers et al [2]. Moreover the distribution for the WMMR 1 group confirms the presence of a second peak in the large diameter range (8-20μm, Fig 4.35) which has been interpreted as an indication of stone forming risk [1,2]. Thus the agreement of these results with those previously published lend confidence to the other Coulter counter studies of this thesis.

Of some interest is the follow-up study (post 3 weeks marathon) with the WMMR 2 group. This aspect was not previously investigated [1,2]. The present study shows that the large particle distribution is more pronounced in this particular group (20-40μm, Fig 4.36). This suggests that large particle crystalluria is a feature of marathon runners' urine and is possibly a result of the fact that long distance road running is routinely part of their daily activities. It might also be indicative of a delayed effect in the voiding of large particles after a marathon. Irving and Rodgers et al [1,2] suggested that crystals and/or debris could become entrapped at various sites in the urinary tract. It is thus reasonable to hypothesize that such particles might reside for long periods prior to being voided.

The particle distribution for BMNs as observed in the present study (Figure 4.37) and its resemblance to the WMN profile is in agreement with the curves reported by Rodgers and De Klerk [3]. The size distribution curves for black marathon runners (Figure 4.38) and black controls (Figure 4.37) showed no significant differences. However, the curves for white runners (Figure 4.35) and white controls (Figure 4.34) were significantly different. This would appear to suggest that black runners are not prone to the same risk as white runners and is consistent with the observation that stone disease in the black community of South Africa is extremely rare. Not-withstanding this observation the detection of large particles (some greater than 20μm) in the follow-up study (BMMR 2) indicates a similar trend to that observed with the WMMR 2 group and supports the hypothesis of a delayed effect.

The particle size distribution for WMC 1 (Figure 4.40) is not significantly different to that of the control group (WMN). However, WMC 2 clearly shows an increased incidence of large particles in the 20-40μm range. These data again lend support to a delayed effect mechanism.

Once again a similar trend in particle size distribution is seen for WMS 2 (Figure 4.43). The distribution is different to the normal group with large particles manifesting themselves in the urine samples in the larger diameter range (20-40μm).

Thus it is evident that regardless of the particular sport pursued, differences in the size distribution of crystalluria particles of the various groups of athletes do occur.

The absence of crystals in the control urines is contrary to that reported by Irving et al [1] who detected COD crystals of diameter less than 10μm. Since the Coulter counter does not distinguish between cellular debris and crystals, the peak reported earlier (Figure 4.34 WMN) is therefore probably due to urinary salts and epithelial debris which were observed in the SEM examination. The crystals observed in the SEM study for WMMRs revealed certain similar features to those previously reported in that salt and debris were often observed. However the absence of large COD crystals (SEM) particularly in the second group of runners is suprising in view of the enhanced peak in Figure 4.36 (WMMR 2).

The occurrence of COD crystals in BMNs is also suprising in that Rodgers and De Klerk [3] have reported the absence of recognisable crystals in black control urines. The crystals observed in the present study were of average size 3-5μm as well as several of size 10μm. This is in agreement with the particle size distribution (Figure 4.38, BMMR 1) which showed two distinct peaks in the above ranges and was also reported by Rodgers and De Klerk [3]. The presence of urinary salt deposits is also in agreement with the previous workers' results [3]. Despite the fact that the particle size distribution profile for BMMR 1 shows no difference to the WMN, the SEM investigations showed differences relating to the presence of crystals. As reported under RESULTS the black runners (BMMR 1 and 2) had COD crystals of size 10μm as well as aggregates in 20-25μm range. The larger particles observed by SEM is also shown to a certain extent in the histogram for BMMR 2 (Figure 4.39) where an increase incidence in the 8-20μm range is seen. In view of the fact that large particle crystalluria does not occur in black normals, the above mentioned observations strongly support the hypothesis that large COD crystalluria is associated with marathon running.

The observation in both groups of cyclists and swimmers of extensive COD deposits in the large diameter range indicates that these crystals are a feature in the crystalluria of these sportsmen as well. It seems reasonable that crystals in runners and cyclists might be due to a dehydration mechanism as a result of perspiration. However this does not apply in the case of swimmers where such effects would be minimal if indeed it occurs at all. McArdle et al [4] studied the thermal adjustment to cold water exposure in exercising men and women and found no statistically significant difference in the rectal temperatures of the athletes relative to their resting temperatures. It can therefore be concluded that perspiration in swimmers is non existent. It is interesting to note that when comparing arm exercises relative to leg exercises during which athletes are working at the same oxygen consumption rate, the heat rate and serum catecholamine levels are higher in the former, resulting in a lower blood flow to the splanchnic circulation [5]. This suggests that renal blood flow may be more reduced during arm exercise (swimming) [5]. However studies have shown that urine flow rate is unaffected by marathon and ultra-marathon running [6]. It is thus unlikely to be affected in swimming.

Thus, the appearance of extensive crystals in the urine of swimmers appears to occur via an unknown mechanism although a possible explanation will be offered later in this chapter. The occurrence of uric acid and brushite crystals as well as other deposits of unknown composition in the swimmers' urines supports the idea of a different mechanism in swimmers as these cystals were not observed in any of the other subjects. The struvite crystals observed in one of the swimmer's urine Figure 4.24 is an indication of urinary tract infection in this subject and is unrelated to the proposed mechanism. Furthermore the presence of apatite in this subject is associated with the struvite. The mucoid material in Figure 4.27 is also related to the infected urine.

The different morphologies of COD crystals as observed in some BMMR 1 and WMS 1 urines deserve comment. The commonly observed crystal habit of COD is bipyramidal (octahedral) as shown in Figure 4.7 (COD). Prismatic morphology arises as a result of unequal growth rates of some of the faces. Slower growth rates might possibly be due to surface contaminants and/or certain special components in the urine which might inhibit growth along a certain axis.

Na levels (table 4.1) show a rough trend in which the concentration is highest in the black runners and lowest in white runners. This is in agreement with Modlins'report of higher Na in blacks relative to whites [7].

The mean 24 hour urinary Na for WMMR 1 for this study (2023mg ≡ 88 mmoles) is in agreement with the value reported by Irving and associates for marathon runners on race day [6]. These workers have shown that on race day the Na excretion decreases and remains low for the next 48 hours after which it recovers to its pre-race value. The Na value for WMMR 2 (2230mg ≡ 97 mmoles) is not significantly different to the value for WMMR 1 and therefore does not show the recovery predicted by Irving and associates [6]. This is likely to be due to the fact that these workers measure Na in subjects immediately after the race under resting conditions whereas the present follow-up study was conducted 3 weeks after the marathon when runners are likely to have resumed normal training.

The mean 24 hour Ca urinary excretion levels show statistically significant elevated levels for WMMR 1, WMC 1, WMC 2 groups relative to normals (Tables 4.2 and 4.9). This observation is in agreement with the results of McDonald and co-workers [8] who reported that exercise increases urinary Ca. These workers concluded that the increase in urinary Ca was not a function of increased bone turnover and suggested that other metabolic factors such as the maintanance of acid-base balance are responsible. The evidence of the present study also does not support the bone-resorption mechanism (as might seem reasonable for marathon runners), since cyclists also displayed elevated urinary Ca levels and cycling is a non weight bearing sport.

The raised levels of Ca observed in the runners and cyclists are thus likely to be dietary related. Sportsmen in general consume a calorie rich diet which is likely to have a high Ca content. If on the other hand the Ca dietary intake is the same as in controls then it appears that there might be greater absorption through the gut in athletes.

The urinary K excretion values determined in the present study for the various groups are not significantly different with the exception of BMMR 1 relative to WMN. (Table 4.10). Modlin [75] reported a mean K excretion in whites of 88 mmoles per 24 hours and for blacks 82 mmoles per 24 hours. The values reported by Irving et al [6] for WMMR lie in this range. Table 4.4 shows that the WMN group has a mean K level (2385 mg per 24 hour ≡ 61mmoles per 24 hours) and is thus close to the reported values. The present finding of no significant difference between whites and blacks is thus in agreement with Modlin but the very low value for BMMR 1 might be indicative of an effect worthy of investigation.

There was no significant difference in the Mg excretion values in the present study between white and black normals which is in agreement with the observation of Modlin. The significant differences between WMN and the sportsmen (Table 4.10) is interesting. It is suggested that this trend is related to dietary factors which were not monitored in this study.

As indicated in the INTRODUCTION, low urinary Na/Ca ratios might be an indicator of stone risk in that high ratios are associated with immunity to the disease. Table 4.5 clearly show that this ratio decreases through the various groups. Black subjects have the highest values for this ratio. White marathon runners have the lowest value. This relative difference between blacks and whites is in agreement with that reported by Modlin [7]. Moreover the absolute values for BMN (69±42) and WMN (38±10) are in good agreement with Modlins values. The value of the Na/Ca ratio for WMMR 1 (19±13) is in dramatic agreement with the ratio reported by Modlin [7] for white stone formers. Thus in the first instance it can be reasonably proposed that the significant differences in Na/Ca ratios are indicative of stone-forming risk. In that event, the appearance of the WMMR 1 ratio at the bottom of the table would support the suggestion by Irving et al [1] that runners are at risk.

In the second instance the trend may be interpreted as indicating the <u>relative</u> risk of the various sporting activities. Table 4.11 and Figure 4.44 show that WMMR1 has the lowest Na/Ca ratio and this is significantly lower than the values for both groups of cyclists. The cyclists in turn (Table 4.5) have significantly lower Na/Ca ratios, compared to the WMNs. The ratios for both groups of swimmers (Table 4.5 and Figure 4.44) are not significantly different to the ratio for WMNs. Therefore this group (i.e. swimmers) would not appear to be at risk in the context of the Na/Ca ratio. The lowest risk groups would be the BMN and BMMR 1, both of which have signifcantly higher Na/Ca ratios compared to WMN (Table

4.11). It is of some interest to note that the BMMR 2 and WMMR 2 groups have ratios which are not significantly different to the WMN group. This would seem to suggest that in the context of Na/Ca ratios these groups are tending towards normality . Thus although the Coulter counter and SEM studies showed delayed effect in terms of the voiding of large particle crystalluria, the ratios stabilized over a three week period. However, it should be noted that the ratios represent Na/Ca levels in solution while crystalluria obviously concerns undissolved particles.

Thus if the hypothesis is accepted that low urinary Na/Ca ratios are indicative of high stone forming risk and that the high ratios are indicative of relative immunity then the results presented in Table 4.5 show that the risk associated with the three sports studied would be in the order:

WMMR 1 > WMC > WMMR 2

The swimmers would not be at increased risk while the immunity of black subjects would be evident from their high ratios.

An obvious question that needs to be addressed concerns the absolute values of urinary Na and Ca and their respective influence of Na/Ca ratios. As has been discussed earlier, Na levels in the sportsmen are depressed and correspond to the depressed Na excretion reported by Irving and associates for the 48 hour period following exertion [6]. On the other hand, Ca excretion is elevated perhaps due to dietary influences as mentioned earlier. These two factors combine to influence the Na/Ca ratio.

K/Ca ratios were calculated to examine whether they followed the same trend as Na/Ca ratios. Since K excretion values were not significantly different, the ratio is dependent on Ca values only. Therefore a low ratio can be expected in those subjects with high Ca values. An obvious similarity between these ratios and the Na/Ca ratios is the appearance of black normals at the top of the list and WMMR 1 at the bottom.

Mg is regarded as an inhibitor of Ca oxalate stone formation [9] and hence the relative concentrations of this constituent is of importance.

Low Mg/Ca ratios might be expected to be an index of stone risk. However such a trend is not obvious from the values presented in Table 4.7.

Runners and cyclists drink various "energy-supplying" fluids during their training or during an official race. Table 5.1 lists the constituent concentrations of some commonly used fluids. While these drinks are Ca free, variable Na concentrations are observed and these could influence urine compositions. However, urine collections in the present study were commenced 24 hour after the race to avoid such effects.

<u>Table 5.1:</u> Constituent concentrations for common fluid intake for athletes. [10,11]

	Na^+ (mmoles/l)	K^+ (mmoles/l)
COKE	2.1	0.5
DIET PEPSI	4.7	0.2
FRN	0.4	0.1
GAME	4.3	0.5
PEPSI	1.7	0.1

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CHAPTER 6

CONCLUSION

CONCLUSION

The results of this study show that the crystalluria of sportsmen is different to that of controls. The particle size distribution curves and SEM data indicate large particle crystalluria in all the groups of athletes and Finlayson has stated that this is a feature of kidney stone disease. Thus it may be concluded that whatever the mechanism, sportsmen are to some degree at risk. A possible explanation as far as runners and cyclists are concerned, involves dehydration with the additional factor of physical trauma playing a role in the runners. However the large particle crystalluria in swimmers can not arise in this way and must result from some other mechanism. This idea is supported by the observation of different crystal types in the urine.

Urine analysis established Na/Ca trends which may indicate "risk" for runners and cyclists. These sportsmen are subjected to effects (dehydration and trauma, the former being associated with cyclists only) not suffered by swimmers. Hence these results tend to support the mechanism proposed for runners and cyclists.

The data acquired independently for urinary concentrations and crystalluria show some degree of correlation in that all the athletes had raised Ca levels. Thus it is perhaps not surprising that their urines are characterized by Ca crystals. It is therefore suggested that Ca containing crystals are a consequence of physical exertion.

The results of this project show that Na levels are depressed after exercise while Ca levels are raised. The resulting Na/Ca ratio is therefore very low. There will thus be insufficient Na to compete with the Ca for sites in the crystal lattice. Thus athletes may be liable to show acute risk for stone formation.

This project has raised many new questions. Further investigations should address topics such as the role of diet and whether the same effects are likely to occur in females. Statistical surveys need to be conducted to establish the incidence of kidney stones in different sportsmen. The answers to these questions are likely to provide further insight into this phenomenon.

APPENDIX 1

APPENDIX 1.1

Table: SETTINGS USED FOR THE COULTER COUNTER

1. The calibration factor, Kd = 15.94
2. Stirrer set on 3.
3. Manometer volume = 500µl and 2000µl
4. Aperture diameter = 100µm
5. Electrolyte = ISOTON II

RANGE	ATTENUATION	CURRENT	FULL SCALE	TL	TU
2.5->	1	200	10	7.7	99.9
2.5-3	1	198	10	7.6	13.2
3-4	1	198	10	13.2	31.3
4-5	1	198	10	31.3	61.1
5-6	1	100	10	30.9	53.3
6-7	1	600	1	32.0	50.7
7-8	1	600	1	50.7	75.8
8->	1	600	1	75.8	99.9
12->	4	600	1	64.0	99.9
16->	8	600	1	75.9	99.9
20->	16	600	1	74.1	99.9
25->	32	600	1	72.4	99.9
30->	64	600	1	62.5	99.9
35->	128	600	1	49.7	99.9
40->	128	600	1	74.1	99.9
45->	128	400	1	70.3	99.9

A set of three readings for each diameter range was done for all subjects in the various groups.

ΔV X $\Delta \bar{N}$ was determined as follows:-

The mean of 3 readings x 4/3πr3, where r=radius.

TL: lower threshold limit } can be set manually on instrument
TU: upper threshold limit }
ΔV : $4/3 \pi r^3$, r = radius (particles are assumed to be spherical, radius r)
$\Delta \bar{N}$: mean no of particles for each sample (measured 3x)

WMN: 3 SUBJECTS

2½ ml urine sample in 250ml ISOTON II kept at 37°C in warm bath.

Manometer volume = 500 µm

Aperture diameter = 100 µm

Kd = Calibration factor = 15.94

SUBJECT A

	DIAMETER (µm)	$\Delta V\ X \Delta \bar{N}$	%TOTAL OF VOLUME
	2.5->	-	-
	1.4	168572	22
	1.8	224894	29
	2.3	117474	15
	2.8	69884	9
	3.3	40042	5
	3.8	27812	4
8 ->	4.3	8659	1
	4.8	12044	2
	5.3	16214	2
	5.8	21249	3
12 ->	6.3	4190	0.5
	6.8	5268	0.7
	7.3	6518	0.8
	7.8	7951	1
16 ->	8.3	2395	0.3
	8.8	2855	0.4
	9.3	3369	0.4
	9.8	3943	0.5
20 ->	10.3	4577	0.6
	10.8	5277	0.7
	11.3	6044	0.8
	11.8	6882	0.9
	12.3	7795	1
	TOTAL	773908	

SUBJECT B

	DIAMETER (μm)	ΔV X $\Delta\bar{N}$	% TOTAL OF VOLUME
	2.5->	-	-
	1.4	254582	23
	1.8	407623	37
	2.3	190558	17
	2.8	78435	7
	3.3	35827	3
	3.8	18618	2
8 ->	4.3	6661	0.6
	4.8	9265	0.8
	5.3	12472	1
	5.8	16346	2
12 ->	6.3	4190	0.4
	6.8	5268	0.5
	7.3	6518	0.6
	7.8	7951	0.7
16 ->	8.3	2395	0.2
	8.8	2855	0.3
	9.3	3369	0.3
	9.8	3943	0.4
20 ->	10.3	4577	0.4
	10.8	5277	0.5
	11.3	6044	0.6
	11.8	6882	0.6
	12.3	7795	0.7
	TOTAL	1097451	

SUBJECT C

	DIAMETER (µm)	ΔV X $\Delta \bar{N}$	% TOTAL OF VOLUME
	2.5->	-	-
	1.4	285638	23
	1.8	380800	30
	2.3	167777	13
	2.8	87906	7
	3.3	47418	4
	3.8	34707	3
8 ->	4.3	12655	1
	4.8	17603	1
	5.3	23697	2
	5.8	31057	2
12 ->	6.3	12569	1
	6.8	15805	1
	7.3	19554	2
	7.8	23854	2
16 ->	8.3	9580	0.8
	8.8	11418	0.9
	9.3	13477	1
	9.8	15770	1
20 ->	10.3	9154	0.7
	10.8	10553	0.8
	11.3	12088	1
	11.8	13765	2
	12.3	15590	2
	TOTAL	1272435	

MEAN % TOTAL OF VOLUME	DIAMETER (μm)
23 (0.7)	2.75
32 (4)	3.5
15 (2)	4.5
8 (1)	5.5
4 (0.6)	6.5
3 (0.6)	7.5
1 (0.4)	8.5
1 (0.7)	9.5
2 (0.7)	10.5
2 (0.7)	11.5
0.6 (0.3)	12.5
0.7 (0.3)	13.5
1 (0.8)	14.5
1 (0.7)	15.5
0.4 (0.3)	16.5
0.5 (0.3)	17.5
0.6 (0.3)	18.5
0.7 (0.3)	19.5
0.6 (0.2)	20.5
0.7 (0.2)	21.5
0.8 (0.2)	22.5
1 (0.8)	23.5
1 (0.7)	24.5

HISTOGRAM

PARTICLE SIZE (μm)	A	B	C	MEAN % TOTAL VOLUME
2.5-8	86	91	81	86 (5)
8-20	13	8	17	13 (5)
20-40	2	1	2	2 (0.7)

A : Subject A

B : Subject B

C : Subject C

WMMR 1 : 4 SUBJECTS

Manometer volume = 2000 µl

Aperture diameter = 100 µm

SUBJECT A

	DIAMETER (um)	ΔV X $\Delta\bar{N}$	% TOTAL OF VOLUME
	2.5->	-	-
	1.4	93194	31
	1.8	26724	9
	2.3	32720	11
	2.8	17655	6
	3.3	12494	4
	3.8	11952	4
8 ->	4.3	6994	2
	4.8	9728	3
	5.3	13096	4
	5.8	17163	6
12 ->	6.3	3142	1
	6.8	3951	1
	7.3	4889	2
	7.8	5963	2
16 ->	8.3	2395	0.8
	8.8	2855	0.9
	9.3	3369	1
	9.8	3943	1
20 ->	10.3	4577	2
	10.8	5277	2
	11.3	6044	2
	11.8	6882	2
	12.3	7795	3
	25->	0	0
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	302802	

SUBJECT B

	DIAMETER (μm)	ΔV X $\Delta \bar{N}$	% TOTAL OF VOLUME
	2.5->	-	-
	1.4	20777	9
	1.8	22323	9
	2.3	71045	29
	2.8	27954	12
	3.3	14602	6
	3.8	11033	5
8 ->	4.3	4663	2
	4.8	6486	3
	5.3	8731	4
	5.8	11442	5
12 ->	6.3	2095	0.9
	6.8	2634	1
	7.3	3259	1
	7.8	3976	2
16 ->	8.3	2395	1
	8.8	2855	1
	9.3	3369	1
	9.8	3943	2
20 ->	10.3	2746	1
	10.8	3166	1
	11.3	3626	2
	11.8	4129	2
	12.3	4677	2
	25->	0	0
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	241926	

SUBJECT C

	DIAMETER (μm)	ΔV X $\Delta\bar{N}$	% TOTAL OF VOLUME
	2.5->	-	-
	1.4	30160	26
	1.8	23867	20
	2.3	7645	7
	2.8	4046	3
	3.3	2710	2
	3.8	2758	2
8 ->	4.3	1665	1
	4.8	2316	2
	5.3	3118	3
	5.8	4086	4
12 ->	6.3	838	0.7
	6.8	1054	0.9
	7.3	1304	1
	7.8	1590	1
16 ->	8.3	4790	4
	8.8	5709	5
	9.3	6739	6
	9.8	7885	7
20 ->	10.3	915	0.8
	10.8	1055	0.9
	11.3	1209	1
	11.8	1377	1
	12.3	1559	1
	25->	0	0
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	118395	

SUBJECT D

	DIAMETER (µm)	ΔV XΔ$\bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1-4	89952	19
	1.8	96104	20
	2.3	34860	7
	2.8	20322	4
	3.3	16408	3
	3.8	18158	4
8 ->	4.3	16985	4
	4.8	23626	5
	5.3	31804	7
	5.8	41681	9
12 ->	6.3	4190	0.9
	6.8	5268	1
	7.3	6518	1
	7.8	7951	2
16 ->	8.3	2395	0.5
	8.8	2855	0.6
	9.3	3369	0.7
	9.8	3943	0.8
20 ->	10.3	9155	2
	10.8	10553	2
	11.3	12088	3
	11.8	13765	3
	12.3	15590	3
	25->	0	0
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	487540	

DIAMETER (μm)	MEAN % TOTAL OF VOLUME
2.75	21 (10)
3.5	15 (6)
4.5	14 (11)
5.5	6 (4)
6.5	4 (2)
7.5	4 (1)
8.5	2 (1)
9.5	3 (1)
10.5	5 (2)
11.5	6 (2)
12.5	0.9 (0.1)
13.5	1 (0.1)
14.5	1 (0.6)
15.5	2 (0.6)
16.5	2 (2)
17.5	2 (2)
18.5	2 (3)
19.5	3 (3)
20.5	2 (0.9)
21.5	2 (0.9)
22.5	2 (0.8)
23.5	2 (0.8)
24.5	2 (1)

HISTOGRAM

PARTICLE SIZE (μm)	A	B	C	D	MEAN % TOTAL OF VOLUME
2.5-8	61	68	72	62	66 (5)
8-20	33	27	24	36	30 (6)
20-40	6	5	4	2	4 (2)

A: Subject A
B: Subject B
C: Subject C
D: Subject D

WMMR 2 : 3 SUBJECTS

Manometer volume = 500 µl

SUBJECT A

	DIAMETER (µm)	ΔV X $\Delta\bar{N}$	% TOTAL OF VOLUME
	2.5->	-	-
	1.4	34896	30
	1.8	36375	31
	2.3	8307	7
	2.8	3218	3
	3.3	2409	2
	3.8	1609	1
8 ->	4.3	999	0.6
	4.8	1390	1
	5.3	1871	2
	5.8	2452	2
12 ->	6.3	1047	0.9
	6.8	1317	1
	7.3	1630	1
	7.8	1988	2
16 ->	8.3	1198	1
	8.8	1427	1
	9.3	1685	1
	9.8	1971	2
20 ->	10.3	915	0.8
	10.8	1055	0.9
	11.3	1209	1
	11.8	1377	1
	12.3	1559	1
25 ->	12.8	879	0.7
	13.3	986	0.8
	13.8	1101	0.9
	14.3	1225	1
	14.8	1358	1
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	117453	

SUBJECT B

	DIAMETER (µm)	$\Delta V \; X \Delta \bar{N}$	% TOTAL OF VOLUME
	2.5->	-	-
	1.4	36505	21
	1.8	40455	23
	2.3	16054	9
	2.8	9195	5
	3.3	6623	4
	3.8	9883	6
8 ->	4.3	4663	3
	4.8	6486	4
	5.3	8731	5
	5.8	11442	6
12 ->	6.3	2095	1
	6.8	2634	2
	7.3	3259	2
	7.8	3976	2
16 ->	8.3	1198	0.7
	8.8	1427	0.8
	9.3	1685	0.9
	9.8	1971	1
20 ->	10.3	915	0.5
	10.8	1055	0.6
	11.3	1209	0.7
	11.8	1377	0.8
	12.3	1559	0.9
25 ->	12.8	527	0.3
	13.3	591	0.3
	13.8	661	0.4
	14.3	735	0.4
	14.8	815	0.5
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	177726	

SUBJECT C

	DIAMETER (μm)	$\Delta V \text{ X } \Delta \bar{N}$	% TOTAL OF VOLUME
	2.5->	-	-
	1.4	29735	27
	1.8	29217	27
	2.3	10805	10
	2.8	5977	6
	3.3	3312	3
	3.8	2528	2
8 ->	4.3	999	0.9
	4.8	1390	1
	5.3	1871	2
	5.8	2452	2
12 ->	6.3	2095	2
	6.8	2634	3
	7.3	3259	3
	7.8	3976	4
16 ->	8.3	479	0.4
	8.8	571	0.5
	9.3	674	0.6
	9.8	789	0.7
20 ->	10.3	916	0.8
	10.8	1055	1
	11.3	1209	1
	11.8	1377	1
	12.3	1559	1
	25->	0	0
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	108879	

MEAN % TOTAL OF VOLUME	DIAMETER (μm)
26 (5)	2.75
27 (4)	3.5
9 (2)	4.5
5 (2)	5.5
3 (1)	6.5
3 (3)	7.5
2 (1)	8.5
2 (2)	9.5
3 (2)	10.5
3 (2)	11.5
1 (0.7)	12.5
2 (1)	13.5
2 (1)	14.5
3 (1)	15.5
0.7 (0.3)	16.5
0.8 (0.3)	17.5
0.8 (0.2)	18.5
1 (0.7)	19.5
0.7 (0.2)	20.5
0.8 (0.2)	21.5
0.9 (0.2)	22.5
0.9 (0.1)	23.5
1 (0.1)	24.5
0.3 (0.4)	25.5
0.4 (0.4)	26.5
0.4 (0.5)	27.5
0.5 (0.5)	28.5
0.5 (0.5)	29.5

HISTOGRAM

PARTICLE SIZE (μm)	A	B	C	MEAN % TOTAL OF VOLUME
2.5-8	74	67	75	72 (4)
8-20	16	28	19	21 (6)
20-40	10	5	6	7 (3)

A : Subject A
B : Subject B
C : Subject C

WMC1 : 6 SUBJECTS

Manometer volume = 500 μl

SUBJECT A

	DIAMETER (μm)	ΔV XΔ $\bar{N}$	% TOTAL OF VOLUME
	2.5 →	-	-
	1.4	27804	24
	1.8	29242	25
	2.3	10040	9
	2.8	5057	4
	3.3	3914	3
	3.8	1839	2
8 →	4.3	999	0.9
	4.8	1390	1
	5.3	1871	2
	5.8	2452	2
12 →	6.3	1047	0.9
	6.8	1317	1
	7.3	1630	1
	7.8	1988	2
16 →	8.3	1198	1
	8.8	1427	1
	9.3	1685	2
	9.8	1971	2
20 →	10.3	2746	2
	10.8	3166	2
	11.3	3626	3
	11.8	4129	4
	12.3	4677	4
	25→	0	0
	30→	0	0
	35→	0	0
	40→	0	0
	45→	0	0
	TOTAL	115215	

SUBJECT B

	DIAMETER (μm)	$\Delta V\ X\Delta \bar{N}$	% TOTAL OF VOLUME
	2.5 →	-	-
	1.4	19758	22
	1.8	23550	26
	2.3	11212	12
	2.8	5701	6
	3.3	3161	4
	3.8	2069	2
8 →	4.3	999	1
	4.8	1390	2
	5.3	1871	2
	5.8	2452	3
12 →	6.3	524	0.6
	6.8	659	0.7
	7.3	815	0.9
	7.8	994	1
16 →	8.3	719	0.8
	8.8	856	0.9
	9.3	1011	1
	9.8	1183	1
20 →	10.3	1831	2
	10.8	2111	2
	11.3	2418	3
	11.8	2753	3
	12.3	3118	3
	25→	0	0
	30→	0	0
	35→	0	0
	40→	0	0
	45→	0	0
	TOTAL	91155	

SUBJECT C

	DIAMETER (µm)	ΔV X Δ$\bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1.4	57114	13
	1.8	123269	28
	2.3	98159	22
	2.8	58114	13
	3.3	27999	6
	3.8	14021	3
8 ->	4.3	4663	1
	4.8	6486	2
	5.3	8731	2
	5.8	11442	3
12 ->	6.3	2095	0.5
	6.8	2634	0.6
	7.3	3259	0.7
	7.8	3976	0.9
16 ->	8.3	2395	0.5
	8.8	2855	0.6
	9.3	3369	0.8
	9.8	3943	0.9
20 ->	10.3	1831	0.4
	10.8	2111	0.5
	11.3	2418	0.5
	11.8	2753	0.6
	12.3	3118	0.7
	25->	0	0
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	446755	

SUBJECT D

	DIAMETER (µm)	ΔV X Δ$\bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1.4	58930	32
	1.8	63687	35
	2.3	20794	11
	2.8	8460	5
	3.3	3914	2
	3.8	2758	2
8 ->	4.3	1332	0.7
	4.8	1853	1
	5.3	2495	1
	5.8	3269	2
12 ->	6.3	524	0.3
	6.8	659	0.4
	7.3	815	0.5
	7.8	994	0.5
16 ->	8.3	2395	1
	8.8	2855	2
	9.3	3369	2
	9.8	3943	2
20 ->	10.3	0	0
	10.8	0	0
	11.3	0	0
	11.8	0	0
	12.3	0	0
	25->	0	0
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	183046	

SUBJECT E

2000 μl = Manometer volume

	DIAMETER (μm)	ΔV X Δ$\bar{N}$	% TOTAL OF VOLUME
	2.5 →	-	-
	1.4	145974	26
	1.8	154709	28
	2.3	52443	10
	2.8	27954	5
	3.3	15354	3
	3.8	11033	2
8 →	4.3	6328	1
	4.8	8802	2
	5.3	11849	2
	5.8	15528	3
12 →	6.3	3142	0.6
	6.8	3951	0.7
	7.3	4889	0.9
	7.8	5963	1
16 →	8.3	4790	0.9
	8.8	5709	1
	9.3	6739	1
	9.8	7885	1
20 →	10.3	9155	2
	10.8	10553	2
	11.3	12088	2
	11.8	13765	3
	12.3	15590	3
	25-	0	0
	30-	0	0
	35-	0	0
	40-	0	0
	45-	0	0
	TOTAL	554193	

SUBJECT F

	DIAMETER (μm)	ΔV X$\Delta\bar{N}$	% TOTAL OF VOLUME
	2.5 →	-	-
	1.4	394142	26
	1.8	537683	35
	2.3	212524	14
	2.8	99952	7
	3.3	56901	4
	3.8	40453	3
8 →	4.3	13322	0.9
	4.8	18530	1
	5.3	24945	4
	5.8	32691	2
12 →	6.3	4190	0.3
	6.8	5268	0.3
	7.3	6518	0.4
	7.8	7951	0.5
16 →	8.3	2395	0.2
	8.8	2855	0.2
	9.3	3369	0.2
	9.8	3943	0.3
20 →	10.3	9155	0.6
	10.8	10553	0.7
	11.3	12088	0.8
	11.8	13765	0.9
	12.3	15590	0.9
	25→	0	0
	30→	0	0
	35→	0	0
	40→	0	0
	45→	0	0
	TOTAL	1528783	

MEAN % TOTAL OF VOLUME	DIAMETER (µm)
24 (6)	2.75
30 (4)	3.5
13 (5)	4.5
7 (3)	5.5
6 (3)	6.5
2 (0.6)	7.5
1 (0.1)	8.5
2 (0.8)	9.5
2 (1)	10.5
3 (0.8)	11.5
0.5 (0.2)	12.5
0.6 (0.2)	13.5
0.7 (0.3)	14.5
1 (0.6)	15.5
2 (1)	16.5
1 (0.6)	17.5
1 (0.7)	18.5
1 (0.7)	19.5
1 (0.8)	20.5
1 (0.8)	21.5
2 (1)	22.5
2 (1)	23.5
2 (1)	24.5
0 (0)	25.5

HISTOGRAM

PARTICLE SIZE (µm)	A	B	C	D	E	F	MEAN %
2.5-8	74	77	86	87	78	90	82 (7)
8-20	18	16	13	13	16	8	14 (4)
20-40	8	7	1	0	6	2	4 (3)

A to F : Subjects A to F

WMC2 : 5 SUBJECTS

Manometer volume = 500 μl

SUBJECT A

	DIAMETER (μm)	ΔV X $\Delta\bar{N}$	% TOTAL OF VOLUME
	2.5 →	-	-
	1.4	102090	27
	1.8	89728	24
	2.3	44034	12
	2.8	23540	6
	3.3	14903	4
	3.8	7585	2
8 →	4.3	1998	0.5
	4.8	2790	0.7
	5.3	3742	1
	5.8	4904	1
12 →	6.3	2095	0.6
	6.8	2634	0.7
	7.3	2359	0.6
	7.8	3976	1
16 →	8.3	2395	0.6
	8.8	2855	0.8
	9.3	3369	0.9
	9.8	3943	1
20 →	10.3	1831	0.5
	10.8	2111	0.6
	11.3	2418	0.6
	11.8	2753	0.7
	12.3	3118	0.8
25 →	12.8	879	0.2
	13.3	986	0.3
	13.8	1101	0.3
	14.3	1225	0.3
	14.8	1358	0.4
30 →	15.3	6001	2
	15.8	6609	2
	16.3	7256	2
	16.8	7945	2
	17.3	8675	2

SUBJECT A

DIAMETER (µm)	ΔV X $\Delta\bar{N}$	% TOTAL OF VOLUME
30->	0	0
40->	0	0
45->	0	0
TOTAL	373196	

SUBJECT B

	DIAMETER (µm)	ΔV X $\Delta\bar{N}$	% TOTAL OF VOLUME
	2.5->	-	-
	1.4	41758	20
	1.8	38573	18
	2.3	14117	7
	2.8	5977	3
	3.3	2409	1
	3.8	1609	0.8
8 ->	4.3	666	0.3
	4.8	927	0.4
	5.3	1247	0.6
	5.8	1635	0.8
12 ->	6.3	838	0.4
	6.8	1054	0.5
	7.3	1304	0.6
	7.8	1590	0.8
16 ->	8.3	4790	2
	8.8	5709	3
	9.3	6739	3
	9.8	7885	4
20 ->	10.3	1831	0.9
	10.8	2111	1
	11.3	2418	1
	11.8	2753	1
	12.3	3118	2
25 ->	12.8	3514	2
	13.3	3942	2
	13.8	4403	2

SUBJECT B

	DIAMETER (μm)	$\Delta V \; X \Delta \bar{N}$	% TOTAL OF VOLUME
	14.3	4900	2
	14.8	5432	3
	15.3	6001	3
	15.8	6609	3
30 ->	16.3	7256	4
	16.8	7945	4
	17.3	8675	4
	35->	0	0
	40->	0	0
	45->	0	0
TOTAL		209735	

SUBJECT C

	DIAMETER (μm)	$\Delta V \; X \Delta \bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1-4	148066	43
	1.8	103677	30
	2.3	23087	7
	2.8	9931	3
	3.3	5118	2
	3.8	4597	1
	4.3	1332	0.4
	4.8	1853	0.5
8 ->	5.3	2495	0.7
	5.8	3269	0.9
	6.3	2095	0.6
	6.8	2634	0.8
12 ->	7.3	3259	0.9
	7.8	3976	1
	8.3	719	0.2
	8.8	856	0.3
16 ->	9.3	1011	0.3
	9.8	1183	0.3
	10.3	1831	0.5
	10.8	2111	0.6

SUBJECT C

	DIAMETER (µm)	ΔV X$\Delta\bar{N}$	% TOTAL OF VOLUME
20 ->	11.3	2418	0.7
	11.8	2753	0.8
	12.3	3118	0.9
25 ->	12.8	1757	0.5
	13.3	1971	0.6
	13.8	2202	0.6
	14.3	2450	0.7
	14.8	2716	0.8
30 ->	15.3	900	0.3
	15.8	991	0.3
	16.3	1088	0.3
	16.8	1192	0.3
	17.3	1301	0.4
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	347957	

SUBJECT D

	DIAMETER (µm)	ΔV X$\Delta\bar{N}$	% TOTAL OF VOLUME
	2.5->	-	-
	1.4	68321	24
	1.8	91267	33
	2.3	50048	18
	2.8	18574	7
	3.3	7828	3
	3.8	5976	2
8 ->	4.3	1665	0.6
	4.8	2316	0.8
	5.3	3118	1
	5.8	4086	2
12 ->	6.3	1047	0.4
	6.8	1317	0.5
	7.3	1630	0.6
	7.8	1988	0.7

SUBJECT D

	DIAMETER (µm)	ΔV X $\Delta \bar{N}$	% TOTAL OF VOLUME
16 ->	8.3	1916	0.7
	8.8	2284	0.8
	9.3	2695	1
	9.8	3154	1
20 ->	10.3	915	0.3
	10.8	1055	0.4
	11.3	1209	0.4
	11.8	1377	0.5
	12.3	1559	0.6
25 ->	12.8	879	0.3
	13.3	986	0.4
	13.8	1101	0.4
	14.3	1225	0.4
	14.8	1358	0.5
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	280894	

SUBJECT E

	DIAMETER (µm)	ΔV X $\Delta \bar{N}$	% TOTAL OF VOLUME
	2.5->	-	-
	1.4	62206	22
	1.8	99377	36
	2.3	44238	16
	2.8	14437	5
	3.3	6925	3
	3.8	4137	2
8 ->	4.3	1665	0.6
	4.8	2316	0.8
	5.3	3118	1
	5.8	4086	2
	6.3	1047	0.4

SUBJECT E

	DIAMETER (µm)	ΔV X $\Delta \bar{N}$	% TOTAL OF VOLUME
12	6.8	1317	0.5
	7.3	1630	0.6
	7.8	1988	0.7
16	8.3	2395	0.9
	8.8	2855	1
	9.3	3369	1
	9.8	3943	1
20	10.3	915	0.3
	10.8	1055	0.4
	11.3	1209	0.4
	11.8	1377	0.5
	12.3	1559	0.6
25	12.8	1757	0.6
	13.3	1971	0.7
	13.8	2202	0.8
	14.3	2450	0.9
	14.8	2716	1
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	278260	

HISTOGRAM

PARTICLE SIZE (µm)	A	B	C	D	E	MEAN % TOTAL
2.5-8	76	50	85	86	83	76 (15)
8-20	10	16	7	10	11	11 (3)
20-40	15	34	8	4	6	13 (12)

A to E: Subjects A to E

MEAN % TOTAL OF VOLUME	DIAMETER (µm)
27 (9)	2.75
28 (7)	3.5
12 (5)	4.5
5 (2)	5.5
3 (1)	6.5
2 (0.8)	7.5
0.5 (0.1)	8.5
0.6 (0.2)	9.5
0.9 (0.2)	10.5
1 (0.7)	11.5
0.5 (0.1)	12.5
0.6 (0.1)	13.5
0.7 (0.1)	14.5
0.8 (0.2)	15.5
0.9 (0.7)	16.5
1 (1)	17.5
1 (1)	18.5
2 (2)	19.5
2 (2)	20.5
0.6 (0.3)	21.5
0.6 (0.3)	22.5
0.7 (0.2)	23.5
1 (0.6)	24.5
0.7 (0.7)	25.5
0.8 (0.7)	26.5
0.8 (0.7)	27.5
0.9 (0.7)	28.5
1 (0.8)	29.5
1 (1)	30.5
2 (0.7)	31.5
2 (1)	32.5
2 (1)	33.5
2 (1)	34.5
0 (0)	35.5

WMS 1 : 5 SUBJECTS

Manometer volume = 500 μm

SUBJECT A

	DIAMETER (μm)	ΔV X Δ$\bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1.4	103964	48
	1.8	72237	33
	2.3	8002	4
	2.8	3494	2
	3.3	1806	0.8
	3.8	1609	0.7
8 ->	4.3	333	0.2
	4.8	463	0.2
	5.3	624	0.3
	5.8	817	0.4
12 ->	6.3	1047	0.5
	6.8	1317	0.6
	7.3	1630	0.8
	7.8	1988	0.9
16 ->	8.3	1198	0.6
	8.8	1427	0.7
	9.3	1685	0.8
	9.8	1971	0.9
20 ->	10.3	1831	0.8
	10.8	2111	1
	11.3	2418	1
	11.8	2753	1
	12.3	3118	1
	25->	0	0
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	217843	

SUBJECT B

	DIAMETER (μm)	ΔV X Δ$\bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1.4	80539	41
	1.8	70576	36
	2.3	16971	9
	2.8	6161	3
	3.3	3763	2
	3.8	2758	1
8 ->	4.3	999	0.5
	4.8	1390	0.7
	5.3	1871	1.0
	5.8	2452	1
12 ->	6.3	838	0.4
	6.8	1054	0.5
	7.3	1304	0.7
	7.8	1590	0.8
16 ->	8.3	1198	0.6
	8.8	1427	0.7
	9.3	1685	0.9
	9.8	1971	1
20	10.3	0	0
	10.8	0	0
	11.3	0	0
	11.8	0	0
	12.3	0	0
	25->	0	0
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	198547	

SUBJECT C

	DIAMETER (µm)	$\Delta V \; X \Delta \bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1.4	112044	18
	1.8	174203	29
	2.3	111461	18
	2.8	59309	10
	3.3	29504	5
	3.8	20686	3
8 ->	4.3	7660	1
	4.8	10655	2
	5.3	14343	2
	5.8	18798	3
12 ->	6.3	4190	0.7
	6.8	5268	0.9
	7.3	6518	1
	7.8	7951	1
16 ->	8.3	2395	0.4
	8.8	2855	0.5
	9.3	3369	0.6
	9.8	3943	0.6
20 ->	10.3	916	0.2
	10.8	1055	0.2
	11.3	1209	0.2
	11.8	1377	0.2
	12.3	1559	0.3
25 ->	12.8	1757	0.3
	13.3	1971	0.3
	13.8	2202	0.4
	14.3	2450	0.4
	14.8	2716	0.4
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	612364	

SUBJECT D

	DIAMETER (μm)	$\Delta V \; X \Delta \bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1.4	60148	28
	1.8	67571	31
	2.3	33637	16
	2.8	14988	7
	3.3	7075	3
	3.8	3907	2
8 ->	4.3	1665	0.8
	4.8	2316	1
	5.3	3118	2
	5.8	4086	2
12 ->	6.3	838	0.4
	6.8	1054	0.5
	7.3	1304	0.6
	7.8	1590	0.7
16 ->	8.3	1198	0.6
	8.8	1427	0.7
	9.3	1685	0.8
	9.8	1971	0.9
20 ->	10.3	196	0.4
	10.8	1055	0.5
	11.3	1209	0.6
	11.8	1377	0.6
	12.3	1559	0.7
	25->	0	0
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	215694	

SUBJECT E

	DIAMETER (μm)	ΔV XΔ$\bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1.4	191744	49
	1.8	127080	33
	2.3	34758	9
	2.8	13057	3
	3.3	6473	2
	3.8	847	0.2
8 ->	4.3	1332	0.3
	4.8	1853	0.5
	5.3	2495	0.6
	5.8	3269	0.8
12 ->	6.3	314	0.1
	6.8	395	0.1
	7.3	489	0.1
	7.8	596	0.2
16 ->	8.3	719	0.2
	8.8	856	0.2
	9.3	1011	0.3
	9.8	1183	0.3
20 ->	10.3	275	0.1
	10.8	317	0.1
	11.3	363	0.1
	11.8	413	0.1
	12.3	468	0.1
	25->	0	0
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	390307	

MEAN % TOTAL OF VOLUME	DIAMETER (µm)
37 (13)	2.75
32 (3)	3.5
11 (6)	4.5
5 (3)	5.5
3 (2)	6.5
1 (1)	7.5
0.6 (0.3)	8.5
0.9 (0.7)	9.5
1 (0.8)	10.5
2 (1)	11.5
0.4 (0.2)	12.5
0.5 (0.3)	13.5
0.6 (0.3)	14.5
0.7 (0.3)	15.5
0.5 (0.2)	16.5
0.6 (0.2)	17.5
0.7 (0.2)	18.5
0.7 (0.3)	19.5
0.3 (0.3)	20.5
0.4 (0.4)	21.5
0.4 (0.4)	22.5
0.4 (0.4)	23.5
0.4 (0.4)	24.5
0.1 (0.1)	25.5
0.1 (0.1)	26.5
0.1 (0.1)	27.5
0.1 (0.1)	28.5
0.1 (0.1)	29.5
0	30.5
↓	↓
0	45.5

HISTOGRAM

PARTICLE SIZE (µm)	A	B	C	D	E	MEAN % TOTAL
2.5-8	83	91	83	87	96	89 (5)
8-20	7	9	14	10	4	9 (4)
20-40	6	0	3	3	5	3 (3)

A to E : Subjects A to E

WMS 2 : 5 SUBJECTS

SUBJECT A

	DIAMETER (μm)	$\Delta V\ X \Delta \bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1.4	33930	20
	1.8	46733	27
	2.3	26757	16
	2.8	16000	9
	3.3	6172	4
	3.8	3678	2
8 ->	4.3	1665	1
	4.8	2316	2
	5.3	3118	2
	5.8	4086	2
12 ->	6.3	1047	0.6
	6.8	1317	0.8
	7.3	1630	1
	7.8	1988	1
16 ->	8.3	719	0.4
	8.8	856	0.5
	9.3	1011	0.6
	9.8	1183	0.7
20 ->	10.3	916	0.5
	10.8	1055	0.6
	11.3	1209	0.7
	11.8	1377	0.8
	12.3	1559	0.9
25 ->	12.8	1757	1
	13.3	1971	1
	13.8	2202	1
	14.3	2450	1
	14.8	2716	2
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	171418	

SUBJECT B

	DIAMETER (μm)	$\Delta V \; X \, \Delta \bar{N}$	% TOTAL OF VOLUME
	2.5 →	-	-
	1.4	15345	14
	1.8	13949	13
	2.3	5453	5
	2.8	2851	3
	3.3	1957	2
	3.8	1839	2
8 →	4.3	1665	2
	4.8	2316	2
	5.3	3118	3
	5.8	4086	4
12 →	6.3	84	0.1
	6.8	105	0.1
	7.3	130	0.1
	7.8	159	0.2
16 →	8.3	719	0.7
	8.8	856	0.8
	9.3	1011	0.9
	9.8	1183	1
20 →	10.3	1831	2
	10.8	2111	2
	11.3	2418	2
	11.8	2753	3
	12.3	3118	3
25 →	12.8	3514	3
	13.3	3942	4
	13.8	4403	4
	14.3	4900	5
	14.8	5432	5
30 →	15.3	3001	3
	15.8	3304	3
	16.3	3628	3
	16.8	3972	4
	17.3	4338	4

SUBJECT B

DIAMETER (µm)	ΔV X $\Delta\bar{N}$	% TOTAL OF VOLUME
35->	0	0
40->	0	0
45->	0	0
TOTAL	109491	

SUBJECT C

	DIAMETER (µm)	ΔV X $\Delta\bar{N}$	% TOTAL OF VOLUME
	2.5->	-	-
	1.4	40298	33
	1.8	34982	29
	2.3	9174	8
	2.8	4322	4
	3.3	3462	3
	3.8	2299	2
8 ->	4.3	666	0.5
	4.8	927	0.8
	5.3	1247	1
	5.8	1635	1
12 ->	6.3	524	0.4
	6.8	659	0.5
	7.3	815	0.7
	7.8	934	0.8
16 ->	8.3	719	0.6
	8.8	856	0.7
	9.3	1011	0.8
	9.8	1183	1
20 ->	10.3	916	0.8
	10.8	1055	0.9
	11.3	1209	1
	11.8	1377	1
	12.3	1559	1
25 ->	12.8	1757	1
	13.3	1971	2
	13.8	2202	2
	14.3	2450	2
	14.8	2716	2

SUBJECT C

DIAMETER (μm)	$\Delta V \times \Delta \bar{N}$	% TOTAL OF VOLUME
30->	0	0
35->	0	0
40->	0	0
45->	0	0
TOTAL	122925	

SUBJECT D

	DIAMETER (μm)	$\Delta V \times \Delta \bar{N}$	% TOTAL OF VOLUME
	2.5->	-	-
	1.4	157020	35
	1.8	168658	38
	2.3	56826	13
	2.8	21885	5
	3.3	9484	2
	3.8	4597	1
8 ->	4.3	1332	0.3
	4.8	1853	0.4
	5.3	2495	0.6
	5.8	3269	0.7
12 ->	6.3	838	0.2
	6.8	1054	0.2
	7.3	1304	0.3
	7.8	1590	0.4
16 ->	8.3	719	0.2
	8.8	856	0.2
	9.3	1011	0.2
	9.8	1183	0.3
20 ->	10.3	275	0.1
	10.8	317	0.1
	11.3	363	0.1
	11.8	413	0.1
	12.3	468	0.1
25 ->	12.8	879	0.2
	13.3	986	0.2
	13.8	1101	0.3
	14.3	1225	0.3
	14.8	1358	0.3

SUBJECT D

DIAMETER (μm)	ΔV X $\Delta \bar{N}$	% TOTAL OF VOLUME
30->	0	0
35->	0	0
40->	0	0
45->	0	0
TOTAL	443359	

SUBJECT E

	DIAMETER (μm)	ΔV X $\Delta \bar{N}$	% TOTAL OF VOLUME
	2.5->	-	-
	1.4	158514	38
	1.8	122536	30
	2.3	51220	12
	2.8	25655	6
	3.3	13247	3
	3.8	7815	2
8 ->	4.3	1665	0.4
	4.8	2316	0.6
	5.3	3118	0.8
	5.8	4086	1
12 ->	6.3	2095	0.5
	6.8	2634	0.5
	7.3	3259	0.8
	7.8	3976	1
16 ->	8.3	719	0.2
	8.8	856	0.2
	9.3	1011	0.2
	9.8	1183	0.3
20 ->	10.3	916	0.2
	10.8	1055	0.3
	11.3	1209	0.3
	11.8	1377	0.3
	12.3	1559	0.4

SUBJECT E

	DIAMETER (μm)	ΔV X $\Delta \bar{N}$	% TOTAL OF VOLUME
25 ->	12.8	527	0.1
	13.3	591	0.1
	13.8	661	0.2
	14.3	735	0.2
	14.8	815	0.2
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	415350	

MEAN % TOTAL OF VOLUME	DIAMETER (µm)
28 (10)	2.75
27 (9)	3.5
11 (4)	4.5
5 (2)	5.5
3 (0.9)	6.5
2 (0.5)	7.5
0.8 (0.7)	8.5
1 (0.8)	9.5
2 (1)	10.5
2 (1)	11.5
0.5 (0.3)	12.5
0.4 (0.3)	13.5
0.6 (0.4)	14.5
0.7 (0.4)	15.5
0.4 (0.2)	16.5
2 (2)	17.5
0.5 (0.3)	18.5
0.7 (0.4)	19.5
2 (2)	20.5
0.8 (0.7)	21.5
0.8 (0.7)	22.5
1 (1)	23.5
1 (1)	24.5
1 (1)	25.5
2 (2)	26.5
2 (2)	27.5
2 (2)	28.5
2 (2)	29.5
0.6 (1)	30.5
0.6 (1)	31.5
0.6 (1)	32.5
0.8 (2)	33.5
0.8 (2)	34.5
↓ 0	↓ 45.5

HISTOGRAM

PARTICLE SIZE (µm)	A	B	C	D	E	MEAN % TOTAL
2.5-8	78	38	77	94	91	76 (22)
8-20	12	14	9	4	7	12 (5)
20-40	10	48	14	2	2	15 (19)

A to E: Subjects A to E

BMN : 4 SUBJECTS

Manometer volume = 500 μl

SUBJECT A

	DIAMETER (μm)	ΔV X$\Delta \bar{N}$	% TOTAL OF VOLUME
	2.5 →	-	-
	1.4	106458	22
	1.8	154978	32
	2.3	78792	16
	2.8	43034	9
	3.3	22580	5
	3.8	14251	3
8 →	4.3	4996	1
	4.8	6949	1
	5.3	9354	2
	5.8	12259	3
12 →	6.3	3142	0.6
	6.8	3951	0.8
	7.3	4889	1
	7.8	5963	1
16 →	8.3	958	0.2
	8.8	1142	0.2
	9.3	1348	0.3
	9.8	1577	0.3
20 →	10.3	2289	0.5
	10.8	2638	0.5
	11.3	3022	0.6
	11.8	3441	0.7
	12.3	3897	0.8
	TOTAL	491908	

SUBJECT B

	DIAMETER (µm)	ΔV X Δ$\bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1.4	57091	45
	1.8	32564	25
	2.3	5963	5
	2.8	3678	3
	3.3	3161	3
	3.8	2758	2
8 ->	4.3	1332	1
	4.8	1853	2
	5.3	2495	2
	5.8	3269	3
12 ->	6.3	1047	0.8
	6.8	1317	1
	7.3	1630	1
	7.8	1988	2
16 ->	8.3	1198	0.9
	8.8	1427	1
	9.3	1685	1
	9.8	1971	2
20 ->	10.3	275	0.2
	10.8	317	0.3
	11.3	363	0.3
	11.8	413	0.3
	12.3	468	0.4
	TOTAL	128263	

SUBJECT C

	DIAMETER (μm)	ΔV X $\Delta\bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1.4	13977	23
	1.8	14144	23
	2.3	4638	8
	2.8	2759	5
	3.3	2108	3
	3.8	2299	4
8 ->	4.3	1332	2
	4.8	1853	3
	5.3	2495	4
	5.8	3269	5
12 ->	6.3	1047	2
	6.8	1317	2
	7.3	1630	3
	7.8	1988	3
16 ->	8.3	719	1
	8.8	856	1
	9.3	1011	2
	9.8	1183	2
20 ->	10.3	458	0.7
	10.8	528	0.9
	11.3	604	1
	11.8	688	1
	12.3	780	1
	TOTAL	61683	

SUBJECT D

	DIAMETER (μm)	ΔV X $\Delta\bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1.4	11172	9
	1.8	11995	10
	2.3	7339	6
	2.8	5977	5
	3.3	4817	4
	3.8	4827	4
8 ->	4.3	3330	3
	4.8	4633	4
	5.3	6236	5
	5.8	8173	7
12 ->	6.3	2095	2
	6.8	2634	2
	7.3	3259	3
	7.8	3976	3
16 ->	8.3	2395	2
	8.8	2855	2
	9.3	3369	3
	9.8	3943	3
20 ->	10.3	3662	3
	10.8	4221	4
	11.3	6044	5
	11.8	6882	6
	12.3	6236	5
	TOTAL	120070	

MEAN % TOTAL OF VOLUME	DIAMETER (µm)
25 (15)	2.75
23 (9)	3.5
9 (5)	4.5
6 (3)	5.5
4 (1)	6.5
3 (1)	7.5
2 (1)	8.5
3 (1)	9.5
3 (2)	10.5
5 (2)	11.5
1 (1)	12.5
2 (1)	13.5
2 (1)	14.5
2 (1)	15.5
0.6 (0.9)	16.5
1 (0.7)	17.5
2 (1)	18.5
2 (1)	19.5
1 (1)	20.5
1 (2)	21.5
2 (2)	22.5
2 (3)	23.5
2 (2)	24.5

HISTOGRAM

PARTICLE SIZE (µm)	A	B	C	D	MEAN % TOTAL
2.5-8	87	83	67	44	70 (20)
8-20	12	17	31	45	26 (15)
20-40	2	0·7	2	1	4 (4)

A to D : Subjects A to D

BMMR1 : 2 SUBJECTS

Manometer volume = 2000 μl

SUBJECT A

	DIAMETER (μm)	ΔV X$\Delta\bar{N}$	% TOTAL OF VOLUME
	2.5 →	-	-
	1.4	133124	38
	1.8	97254	28
	2.3	28235	8
	2.8	13149	4
	3.3	7376	2
	3.8	7355	2
8 →	4.3	3997	1
	4.8	5559	2
	5.3	7483	2
	5.8	9807	3
12 →	6.3	3142	0.9
	6.8	3951	1
	7.3	4889	1
	7.8	5963	2
16 →	8.3	1916	0.5
	8.8	2284	0.7
	9.3	2695	0.8
	9.8	3154	0.9
20 →	10.3	1831	0.5
	10.8	2111	0.6
	11.3	2418	0.7
	11.8	2753	0.8
	12.3	3118	0.9
	TOTAL	353562	

SUBJECT B

	DIAMETER (µm)	ΔV X Δ$\bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1.4	210525	37
	1.8	203616	35
	2.3	54431	10
	2.8	22436	4
	3.3	12494	2
	3.8	8734	2
8 ->	4.3	3330	0.6
	4.8	4633	0.8
	5.3	6236	1
	5.8	8173	1
12 ->	6.3	3142	0.5
	6.8	3951	0.7
	7.3	4889	0.9
	7.8	5963	1
16 ->	8.3	1916	0.3
	8.8	2284	0.4
	9.3	2695	0.5
	9.8	3154	0.6
20 ->	10.3	1831	0.3
	10.8	2111	0.4
	11.3	2418	0.4
	11.8	2753	0.5
	12.3	3118	0.5
	TOTAL	574833	

MEAN % TOTAL OF VOLUME	DIAMETER (µm)
38 (1)	2.75
32 (5)	3.5
9 (1)	4.5
4 (0)	5.5
2 (0)	6.5
2 (0)	7.5
0.8 (0.3)	8.5
1 (1)	9.5
2 (1)	10.5
2 (1)	11.5
0.7 (0.3)	12.5
0.9 (0.2)	13.5
1 (0.1)	14.5
2 (1)	15.5
0.4 (0.2)	16.5
0.6 (0.2)	17.5
0.7 (0.2)	18.5
0.8 (0.2)	19.5
0.4 (0.1)	20.5
0.5 (0.1)	21.5
0.6 (0.2)	22.5
0.7 (0.2)	23.5
0.7 (0.3)	24.5

HISTOGRAM

PARTICLE SIZE (µm)	A	B	MEAN % TOTAL OF VOLUME
2.5-8	83	90	87 (5)
8-20	16	9	13 (5)
20-40	2	1	2 (1)

A - B : Subjects A + B

BMMR2 : 2 SUBJECTS

Manometer volume = 500 μl

SUBJECT A

	DIAMETER (μm)	$\Delta V\ X\ \Delta\bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1.4	57252	33
	1.8	61121	35
	2.3	17022	10
	2.8	7540	4
	3.3	3914	2
	3.8	3907	2
8 ->	4.3	1332	0.8
	4.8	1853	1
	5.3	2495	1
	5.8	3269	2
12 ->	6.3	1047	0.6
	6.8	1317	0.8
	7.3	1630	0.9
	7.8	1988	1
16 ->	8.3	1916	1
	8.8	2284	1
	9.3	2695	2
	9.8	3154	2
20 ->	10.3	0	0
	10.8	0	0
	11.3	0	0
	11.8	0	0
	12.3	0	0
	25->	0	0
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	175736	

SUBJECT B

	DIAMETER (µm)	$\Delta V \, X \Delta \bar{N}$	% TOTAL OF VOLUME
	2.5 ->	-	-
	1.4	50057	35
	1.8	43679	31
	2.3	12181	9
	2.8	5885	4
	3.3	3914	3
	3.8	2758	2
8 ->	4.3	1665	1
	4.8	2316	2
	5.3	3118	2
	5.8	4086	3
12 ->	6.3	1047	0.7
	6.8	1317	0.9
	7.3	1630	1
	7.8	1988	1
16 ->	8.3	1198	0.8
	8.8	1427	1
	9.3	1685	1
	9.8	1971	1
	20->	0	0
	25->	0	0
	30->	0	0
	35->	0	0
	40->	0	0
	45->	0	0
	TOTAL	141922	

MEAN % TOTAL OF VOLUME	DIAMETER (µm)
34 (1)	2.75
33 (3)	3.5
10 (1)	4.5
4 (0)	5.5
3 (1)	6.5
2 (0)	7.5
1 (0.2)	8.5
2 (1)	9.5
2 (1)	10.5
3 (1)	11.5
0.7 (0.1)	12.5
0.9 (0.1)	13.5
1 (0.1)	14.5
1 (0)	15.5
0.9 (0.1)	16.5
1 (0)	17.5
2 (1)	18.5
2 (1)	19.5
0 ↓ 0	20.5 ↓ 45.5

HISTOGRAM

PARTICLE SIZE (µm)	A	B	MEAN % TOTAL OF VOLUME
2.5-8	86	83	85 (2)
8-20	14	17	16 (2)
20-40	0	0	

A, B : Subjects A+B

APPENDIX 2.1: CONSTRUCTION OF CALIBRATION CURVES FROM ATOMIC ABSORBANCE SPECTROSCOPY READINGS OF Ca; Na; Mg; K.

Table 1:- Specifications and parameters for the construction of calibration curves for AAS readings of Ca; Na; Mg; K.

ELEMENT	(nm)	SLIT WIDTH (nm)	CURRENT (mA)
Ca	422.7	0.5	3
Na	330.2	0.2	5
Mg	285.2	0.5	3
K	769.9	1.0	5

TABLE 2:- Dilution Specifications

ELEMENT	AMOUNT OF SAMPLE (µl)	DILUENT (ml) RELEASING AGENT
Ca	125	4.875 of La 5000
Na	100	5.000 of KCl
Mg	Double dil:100µl in	
	a) 1ml H_2O	
	b) 100µl	4.900 of La 5000
K	Double dil:100µl in	
	a) 1ml H_2O	
	b) 100µl	5.000 of La 5000

La 5000 $\mu g ml^{-1}$ releasing agent was made according to:-
50 ml La Spectrosol of 10% w/v BDH solution]1L H_2O
10 ml conc HCL |distilled

KCl solution was made according to:-
3.814g of dried KCl analar grade was dissolved in 1 litre of distilled water.

Due to these dilutions the concentration must be multiplied by the following to accomodate the dilution factor:
1) 11 x 1.1 for K and Mg
2) 1.1 for Na and Ca

When the concentration was calculated from an equation and not from the calibration curves, the following equation was used:

$$\text{Concentration} = \frac{\text{Mean - Blank}}{\text{Standard - Blank}} \times \text{concentration of standard}$$

The concentrations of the various standards used are as follows:-

Table 3: Concentration of standard used for calculations

ELEMENT	CONCENTRATION	DILUTION FACTORS
Ca	4.00mMl^{-1}	1.1
Na	200mMl^{-1}	1.1
Mg	0.412mMl^{-1}	11 x 1.1
K	4.26mMl^{-1}	11 x 1.1

1ST SET OF CALIBRATION CURVES

AAS READINGS FOR THE Ca CALIBRATION CURVE

Titisol Merck ART 9943 $CaCl_2$ was used to make up 1 mmolel^{-1} stock solution of Ca. Appropriate dilutions were made for the standards used.

STD mMl^{-1}	SAMPLE	REPEAT	MEAN	MEAN-BLANK
0.00	0.016	0.011	0.014	-
1.00	0.077	0.074	0.076	0.06
2.00	0.131	0.140	0.136	0.12
3.00	0.200	0.202	0.201	0.19
4.00	0.266	0.258	0.262	0.25
5.00	0.324	0.326	0.325	0.31
6.00	0.386	0.386	0.386	0.37
7.00	0.449	0.452	0.451	0.44
8.00	0.509	-	0.509	0.50

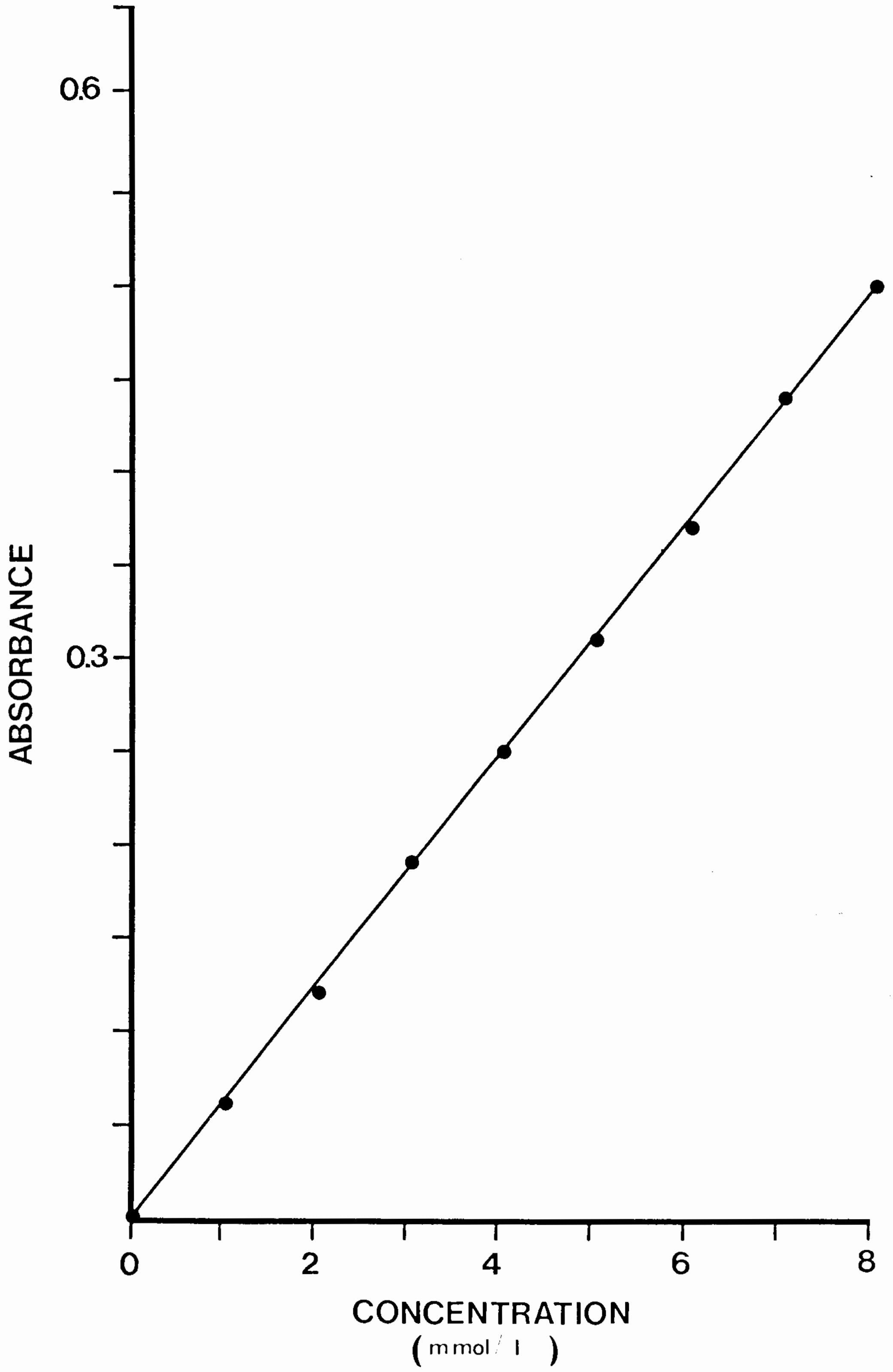
Refer to Graph 1 for the Calcium calibration curve.

AAS READINGS FOR THE Na CALIBRATION CURVE

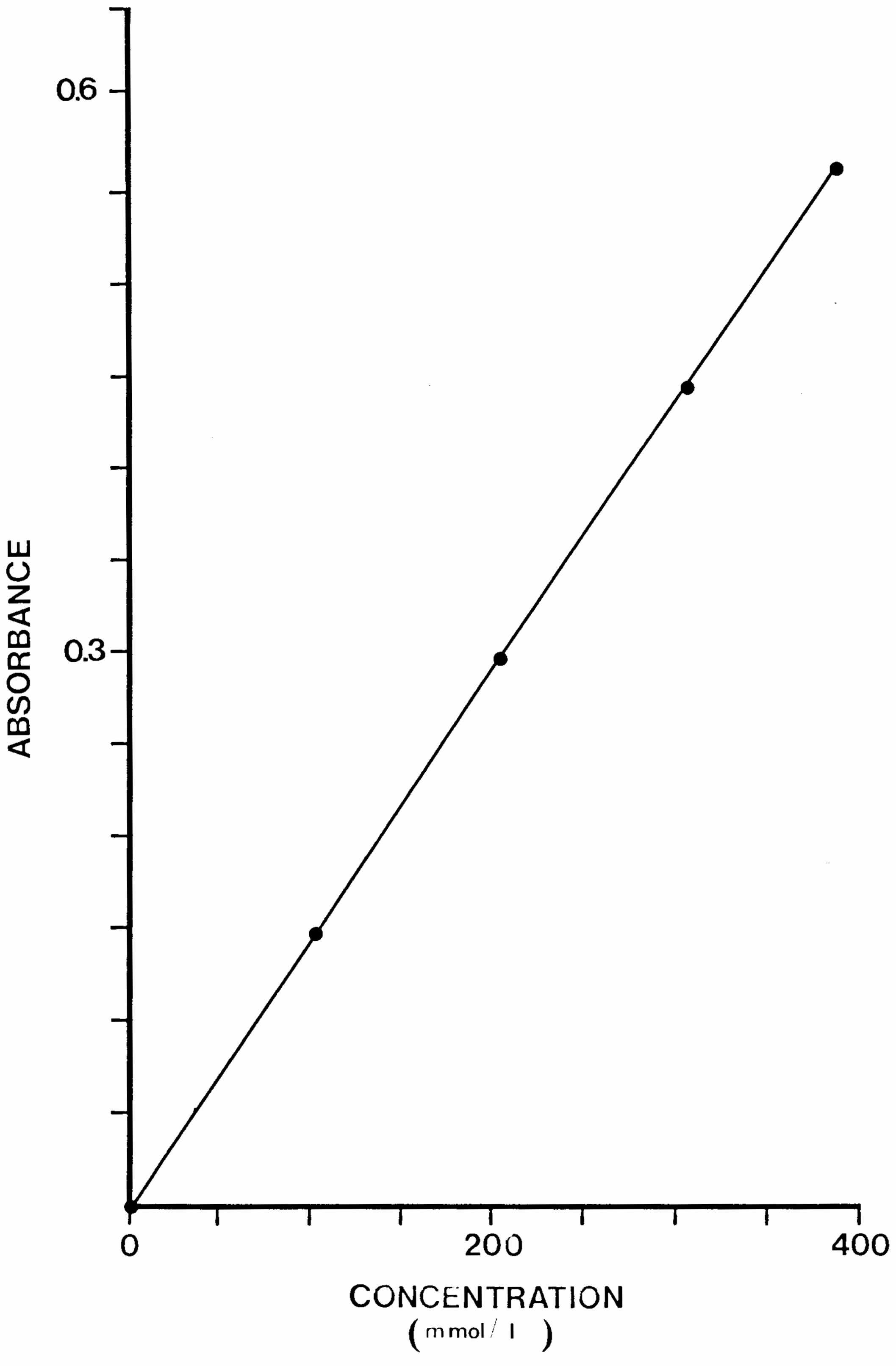
From a 1<u>M</u> stock solution of NaCl appropriate dilutions were made for the standards.

STD mMl^{-1}	SAMPLE	REPEAT 1	REPEAT 2	REPEAT 3	MEAN	MEAN-BLANK
0.0	0.002	0.002	0.001	0.001	0.002	0.000
100	0.152	0.153	0.155	0.155	0.154	0.15
200	0.304	0.306	0.307	0.307	0.306	0.31
300	0.447	0.448	0.452	0.448	0.449	0.45
380	0.568	0.566	0.568	0.566	0.567	0.57

Refer to Graph 2 for the Na calibration curve.



GRAPH 1: Ca CALIBRATION CURVE



GRAPH 2: Na CALIBRATION CURVE

AAS READINGS FOR THE Mg CALIBRATION CURVE

A stock solution of 1.000g in 1000ml of $MgCl_2$ was made up and appropriate dilutions were made for the standards.

STD mMl^{-1}	SAMPLE	REPEAT 1	 2	 3	MEAN	MEAN-BLANK
0.0	0.014	0.013	0.013	0.015	0.014	0.000
0.206	0.090	0.080	0.090	0.100	0.090	0.08
0.412	0.185	0.186	0.185	0.186	0.186	0.17
0.617	0.265	0.263	0.262	0.264	0.264	0.25
0.823	0.338	0.338	0.335	0.336	0.337	0.32

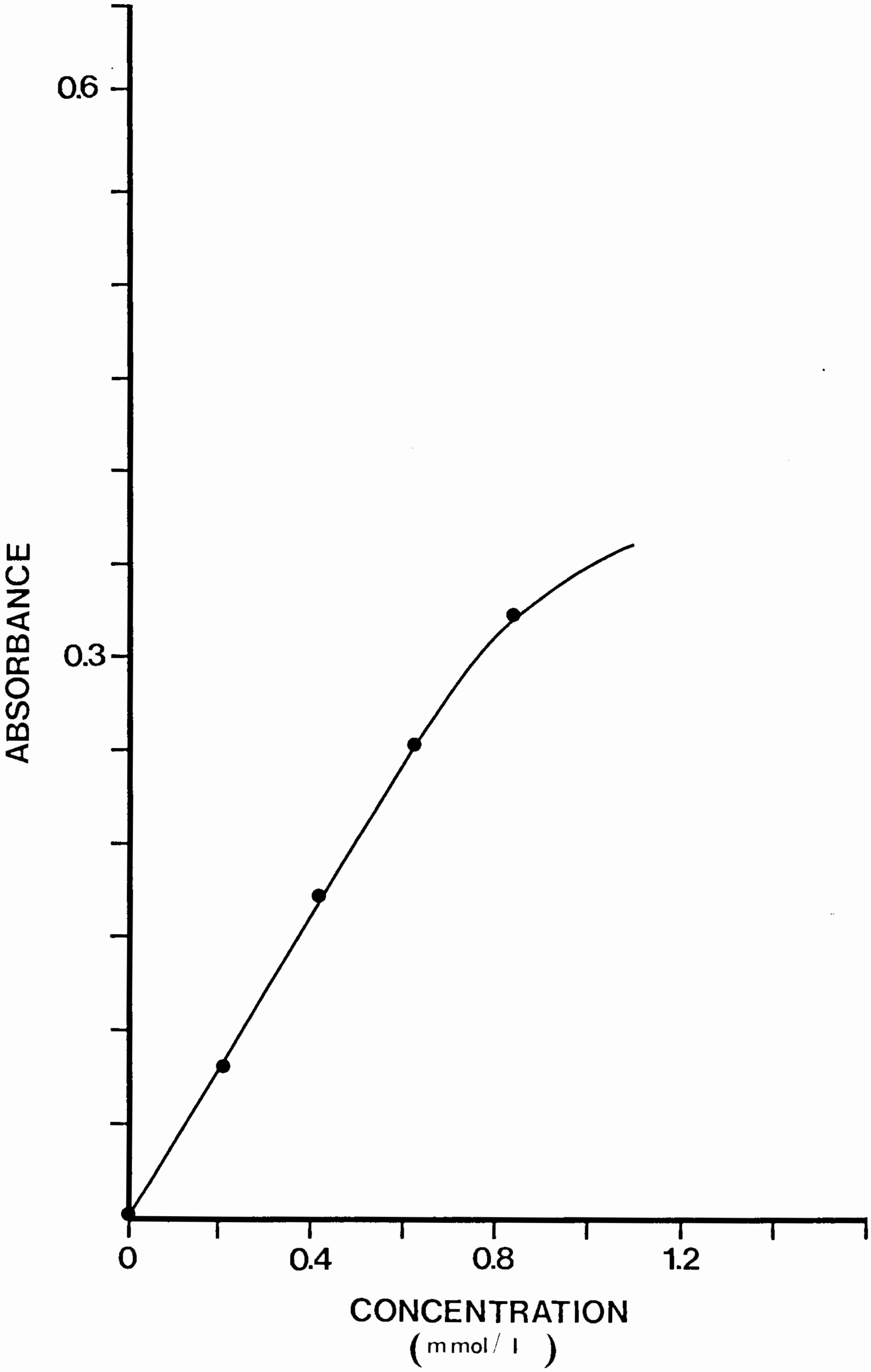
Refer to Graph 3 for the Mg calibration curve.

AAS READINGS FOR THE POTASSIUM CALIBRATION CURVE

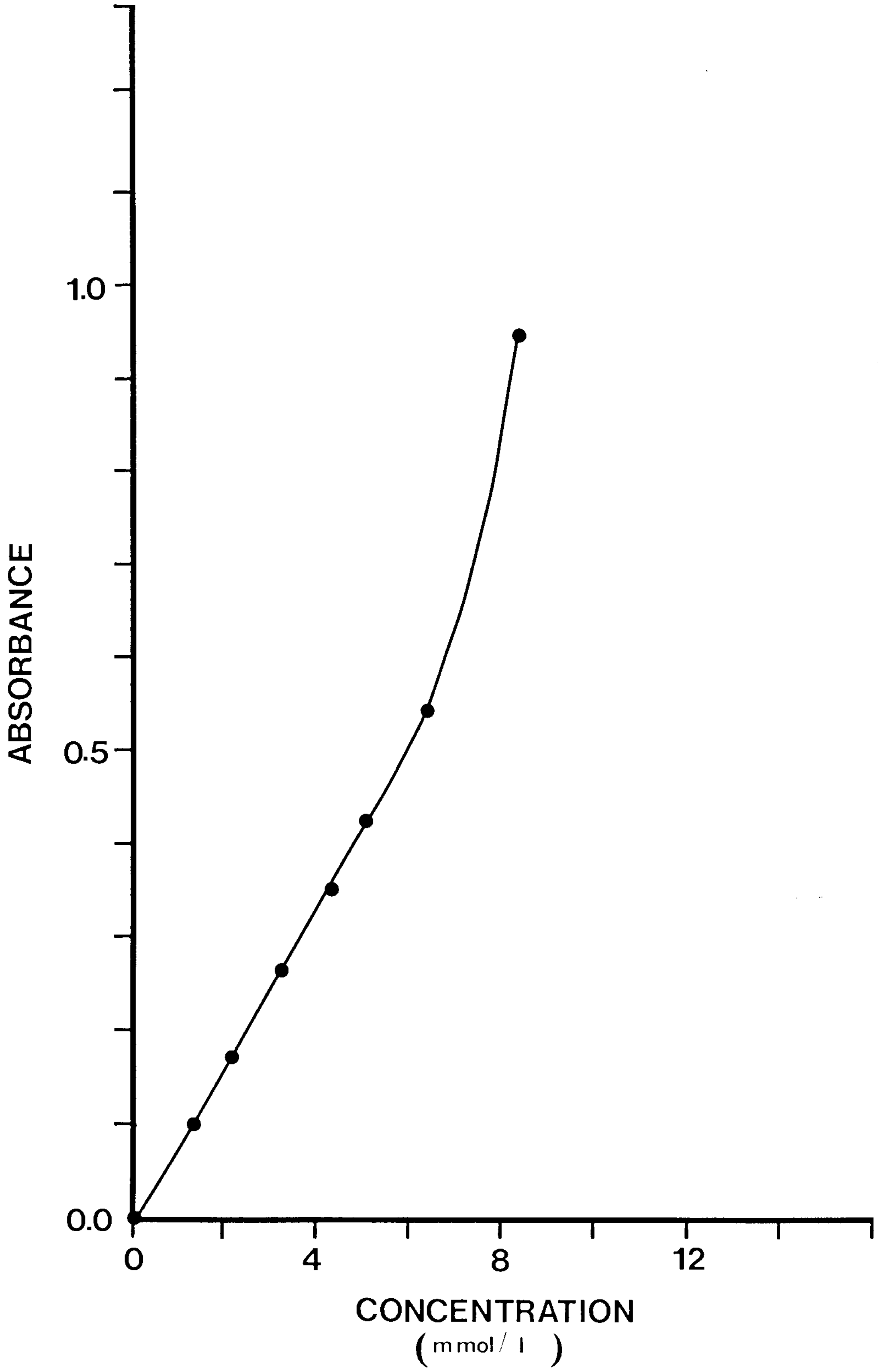
A stock solution of 1000µgml^{-1} of KCl was made up and appropriate dilutions were made for the standards.

STD mMl^{-1}	SAMPLE 1	REPEAT 2	 3	MEAN	MEAN-BLANK
0.00	0.032	0.033	0.033	0.033	0.000
1.28	0.132	0.130	0.135	0.132	0.100
2.13	0.205	0.206	0.205	0.207	0.17
3.20	0.292	0.289	0.295	0.292	0.26
4.26	0.380	0.380	0.387	0.382	0.35
5.12	0.454	0.454	-	0.454	0.42
6.40	0.566	0.569	-	0.568	0.54
8.53	0.970	0.973	0.977	0.973	0.94

Refer to Graph 4 for the Potassium calibration curve.



GRAPH 3: Mg CALIBRATION CURVE



GRAPH 4: K CALIBRATION CURVE

2ND SET OF CALIBRATION CURVES

AAS READINGS FOR THE Ca CALIBRATION CURVE

Previous standards were used and the calibration curve was rerun.

STD	SAMPLE	REPEAT			MEAN	MEAN-BLANK
mMl^{-1}		1	2	3		
BLANK	0.02	0.018	0.018	0.018	0.019	-
1.0	0.083	0.085	0.084	0.085	0.084	0.07
2.0	0.145	0.146	0.145	0.148	0.146	0.13
3.0	0.206	0.209	0.210	0.208	0.208	0.19
4.0	0.269	0.272	0.276	0.273	0.273	0.25
5.0	0.330	0.332	0.333	0.337	0.333	0.32
6.0	0.397	0.399	0.398	0.399	0.398	0.38
7.0	0.451	0.453	0.450	0.452	0.452	0.43
8.0	0.515	0.513	0.509	0.516	0.513	0.50

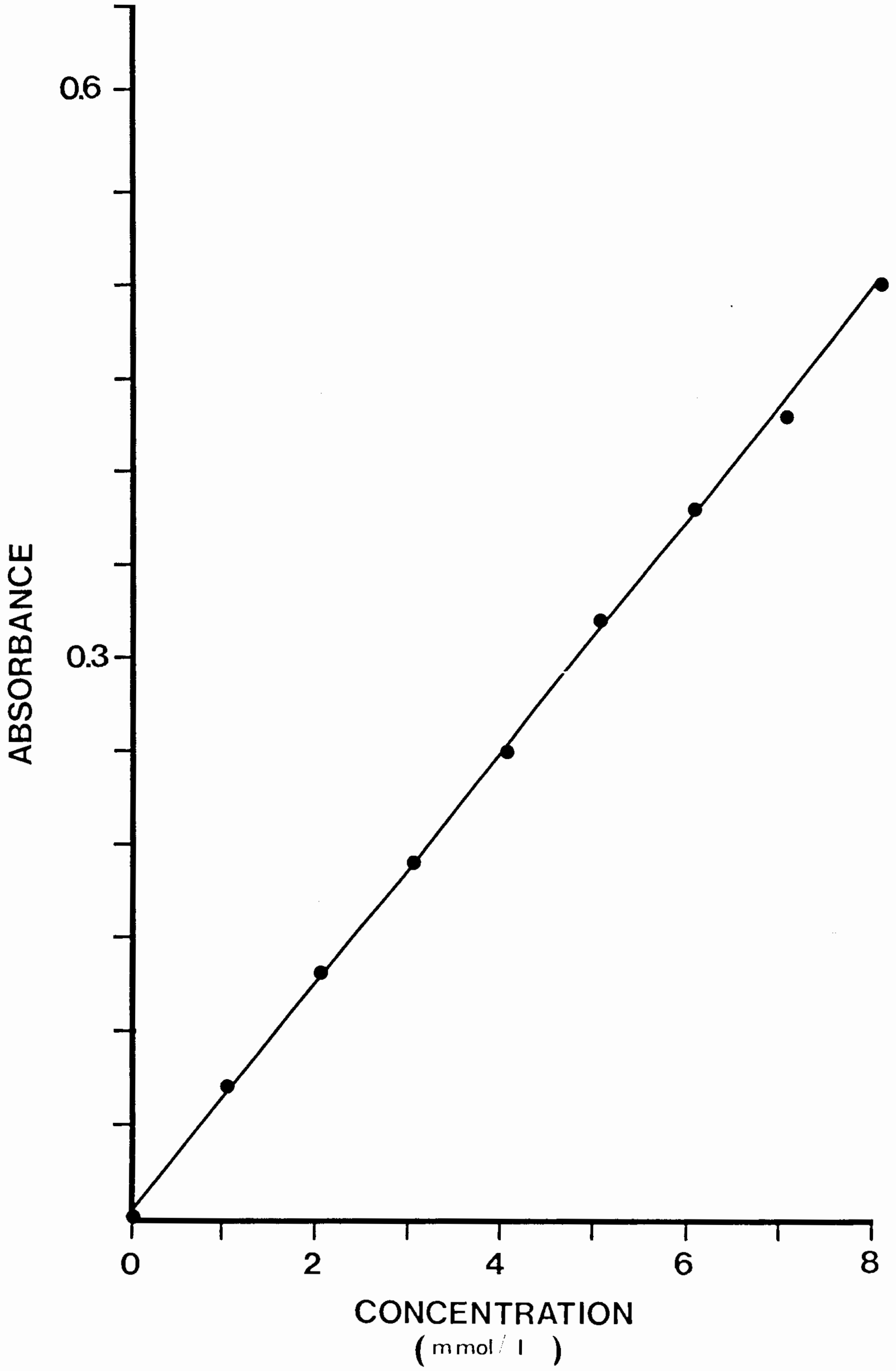
Refer to Graph 5 for the Ca calibration curve.

AAS READINGS FOR THE Na CALIBRATION CURVE

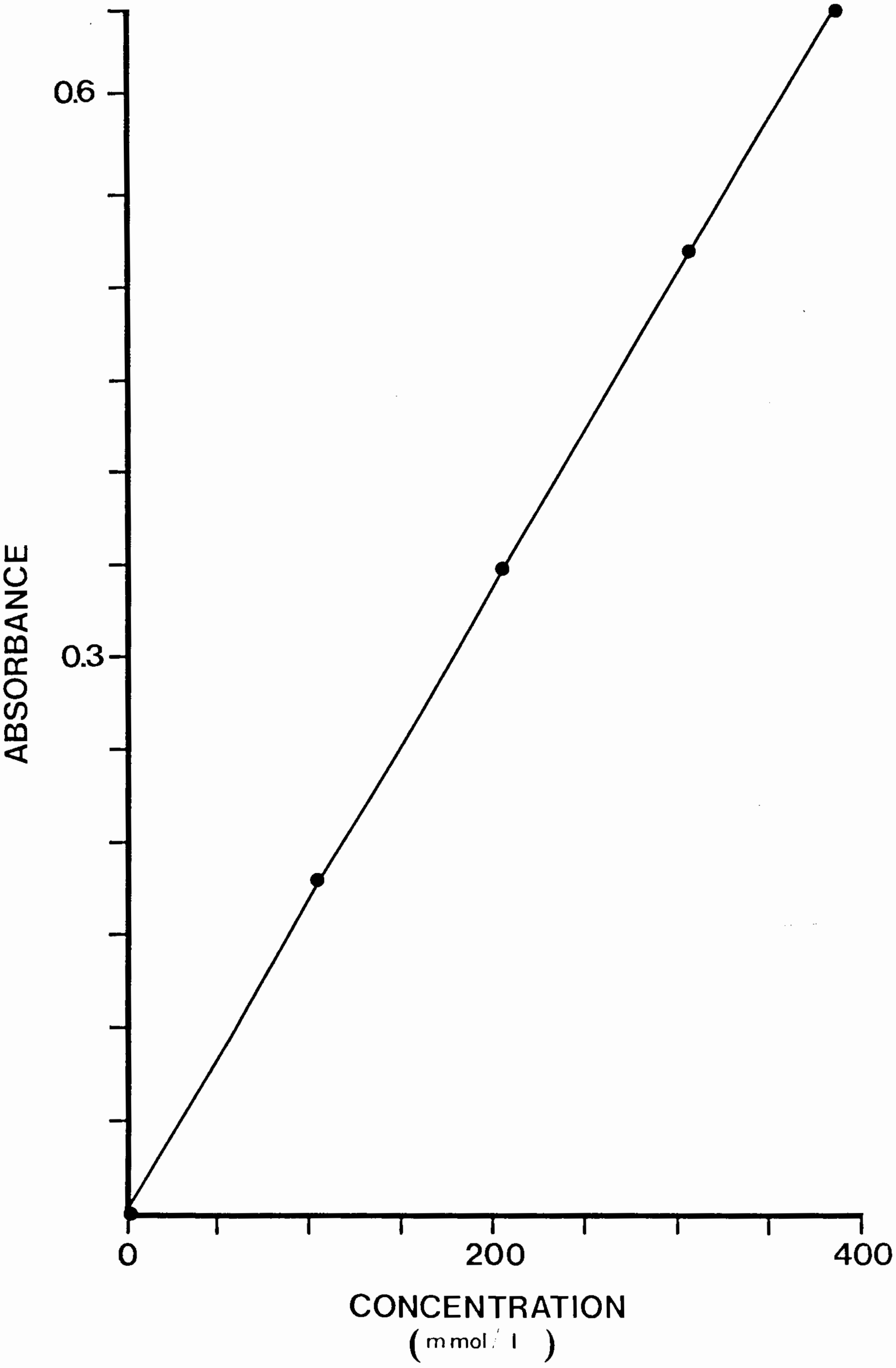
Previous standards were used and the calibration curve was reconstructed.

STD	SAMPLE	REPEAT			MEAN	MEAN-BLANK
mMl^{-1}		1	2	3		
BLANK	0.004	0.004	0.004	0.004	0.004	-
100	0.182	0.183	0.183	0.182	0.183	0.18
200	0.357	0.359	0.358	0.356	0.358	0.35
300	0.520	0.521	0.523	0.519	0.521	0.52
380	0.650	0.652	0.650	0.651	0.651	0.65

Refer to Graph 6 for the Na calibration curve.



GRAPH 5: Ca CALIBRATION CURVE



GRAPH 6: Na CALIBRATION CURVE

AAS READINGS FOR THE Mg CALIBRATION CURVE

Previous standards were used and the calibration curve rerun.

STD	SAMPLE	REPEAT			MEAN	MEAN-BLANK
mMl^{-1}		1	2	3		
BLANK	-	0.174	0.185	0.187	0.186	-
0.206	0.275	0.277	0.272	0.271	0.274	0.09
0.412	0.341	0.346	0.346	0.343	0.344	0.16
0.617	0.426	0.423	0.415	0.420	0.421	0.24
0.823	0.470	0.473	0.475	0.476	0.474	0.29

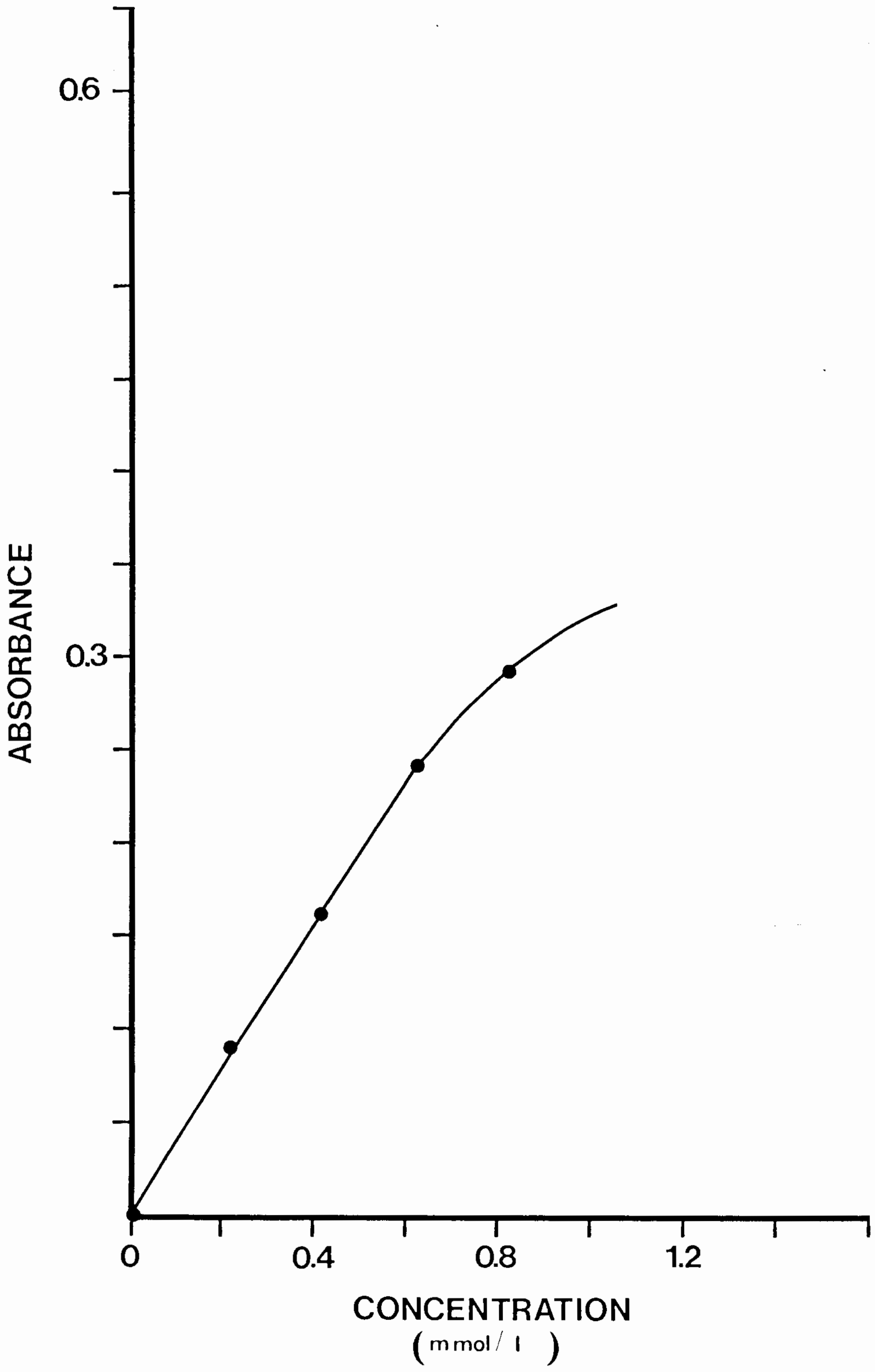
Refer to Graph 7 for the Mg calibration curve.

AAS READINGS FOR THE POTASSIUM CALIBRATION CURVE

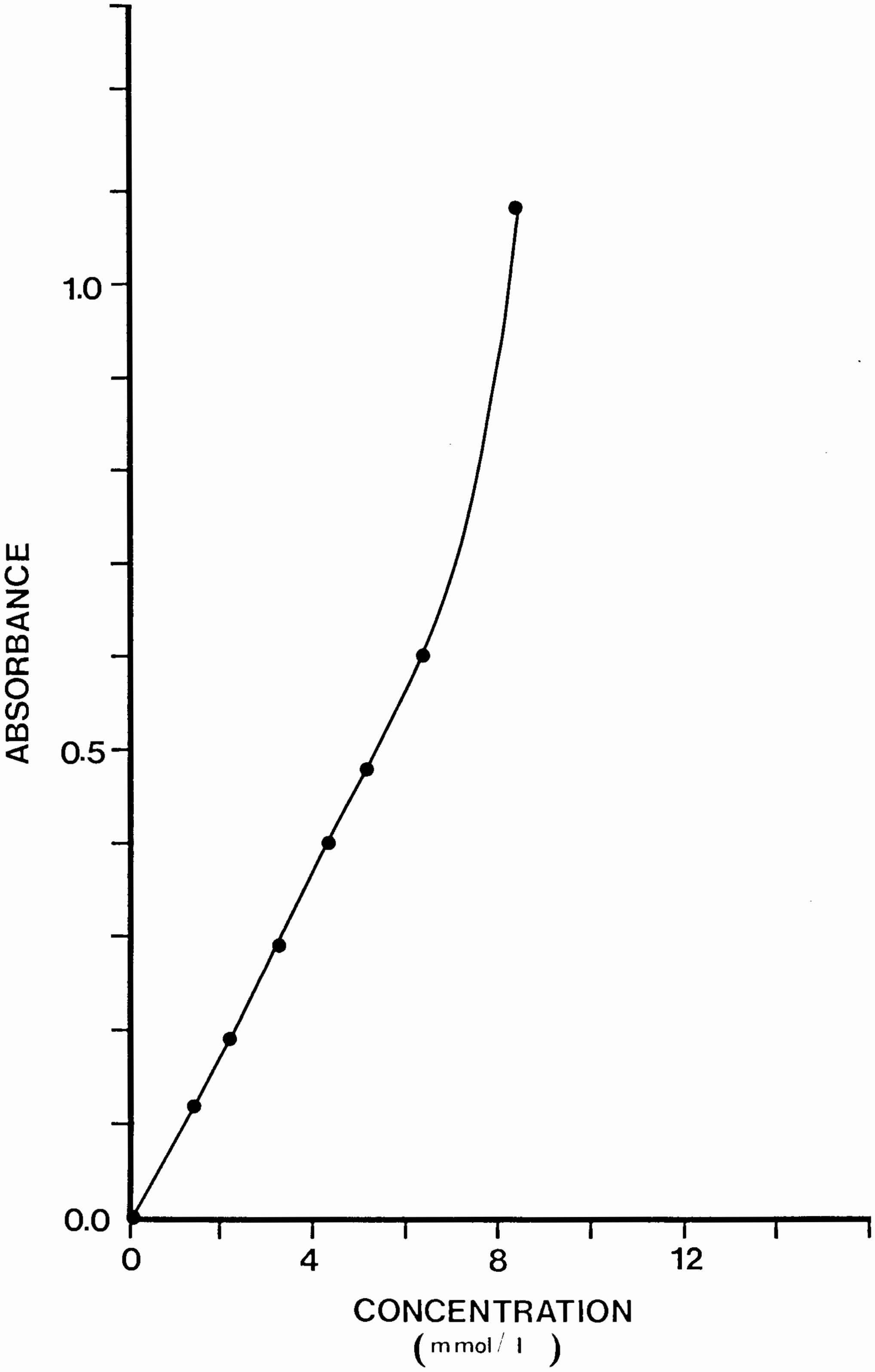
Previous standards were used and K calibration curve rerun.

STD	SAMPLE	REPEAT			MEAN	MEAN-BLANK
mMl^{-1}		1	2	3		
BLANK	0.013	0.012	0.013	0.014	0.013	-
1.28	0.120	0.120	0.124	0.121	0.121	0.11
2.13	0.199	0.201	0.201	0.203	0.201	0.19
3.20	-	0.304	0.303	0.305	0.304	0.29
4.26	0.411	0.410	0.409	0.415	0.411	0.40
5.12	0.494	0.495	0.491	0.495	0.494	0.48
6.40	0.614	0.618	0.619	-	0.617	0.60
8.53	1.086	1.094	1.096	1.092	1.092	1.08

Refer to Graph 8 for the Potassium calibration curve.



GRAPH 7: Mg CALIBRATION CURVE



GRAPH 8: K CALIBRATION CURVE

APPENDIX 2.2: ATOMIC ABSORPTION SPECTROSCOPY READINGS FOR THE VARIOUS SUBJECT GROUPS

Table 1: White male normals

NAME	AGE	VOLUME (ml)
P. Kyriacou	22	280
P. Mitchell	55	1260
I.E.Muller	21	1860
G. Hesselink	27	1010
E. Reyneke	55	560
Dr Torrington	55	980
Mr Harris	56	1760
M. Creech	38	260
L. Lavelle	23	1650
P. Stonestree	23	980
Prof. Orren	-	1020
Jasper	20	555
James Dean	22	1050
Clark Kent	23	225
Mr Myrtle	45	1085
Guy Anderson	24	1940

SUBJECT NUMBER = 16

Calcium Readings

Concentrations determined from the first set of calibration curves.
Mean blank reading = 0.013 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
PK	0.255	-	5
PM	0.258	-	5
IM	0.153	-	3
GH	0.044	-	1
ER	0.127	-	2
DrT	0.319	-	6
MrH	0.235	-	4
MC	0.134	-	2
LL	0.304	-	5
CONCENTRATION DETERMINED BY EQUATION Mean blank reading = 0.004mmoles l^{-1}			
GA	0.248	0.311	4
MM	0.315	0.309	5
CK	0.147	0.311	2
PS	0.265	0.309	4
PD	0.230	0.309	3
JD	0.065	0.308	1
J	0.297	0.310	4

Each mean reading was determined from 3 readings.
The mean blank was subtracted from the mean and standard reading before calculating or determining the concentration.

Mean Ca = 3 mmoles l^{-1} (2)
= 120 mg l^{-1} (80)

Sodium Readings

Concentrations were determined from the first set of calibration curves.
Mean blank reading = 0.002 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
PK	0.256	-	166
PM	0.274	-	201
IM	0.152	-	112
GH	0.053	-	44
ER	0.161	-	119
DrT	0.329	-	241
MrH	0.198	-	147
MC	0.162	-	120
LL	0.158	-	117

CONCENTRATION DETERMINED BY CALCULATION
Mean blank reading = 0.003mmoles l^{-1}

GA	0.154	0.327	104
MM	0.176	0.325	119
CK	0.150	0.331	100
PS	0.167	0.327	112
PD	0.111	0.326	75
JD	0.064	0.321	44
J	0.224	0.323	153

Mean Na = 123 mmoles l^{-1} (51)
= 2828 mg l^{-1} (1173)

Magnesium Readings

Concentrations determined from the first set of calibration curves.
Mean blank reading = 0.014 mmolesl-1

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
PK	0.109	-	3
PM	0.205	-	6
IM	0.067	-	2
GH	0.050	-	2
ER	0.092	-	3
DrT	0.159	-	5
MrH	0.073	-	2
MC	0.073	-	2
LL	0.131	-	4
CONCENTRATION DETERMINED BY CALCULATION			
Mean blank reading = 0.010mmolesl^{-1}			
GA	0.165	0.196	4
MM	0.197	0.197	5
CK	0.272	0.197	7
PS	0.185	0.196	5
PD	0.183	0.196	5
JD	0.031	0.197	1
J	0.209	0.197	5

Mean Mg = 4.0 mmoles l^{-1} (2)
= 97 mg l^{-1} (49)

Potassium Readings

Concentrations determined from the first set of calibration curves.
Mean blank reading = 0.033 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
PK	0.334	-	49
PM	0.206	-	30
IM	0.331	-	49
GH	0.449	-	73
ER	0.821	-	98
DrT	0.144	-	23
MrH	0.304	-	45
MC	0.240	-	35
LL	0.310	-	46
CONCENTRATION DETERMINED BY CALCULATION			
Mean blank reading = 0.006mmoles l^{-1}			
GA	0.435	0.399	56
MM	0.415	0.401	53
CK	1.417	0.403	18
PS	0.669	0.399	86
PD	0.338	0.401	44
JD	0.087	0.393	11
J	0.789	0.403	101

Mean K = 61 mmoles l^{-1} (41)
= 2385 mg l^{-1} (1603)

VARIOUS RATIOS

SUBJECTS	Na/Ca	K/Ca	Mg/Ca
PK	166/5 = 33	49/5 = 10	3/5 = 1
PM	201/5 = 40	30/5 = 6	6/5 = 1
IM	112/3 = 37	49/3 = 16	2/3 = 1
GH	44/1 = 44	73/1 = 73	2/1 = 1
ER	119/2 = 60	98/2 = 49	3/2 = 2
DrT	241/6 = 40	23/6 = 4	5/6 = 1
MrH	147/4 = 37	45/4 = 11	2/4 = 1
MC	120/2 = 60	35/2 = 18	2/2 = 1
LL	117/5 = 23	46/5 = 9	4/5 = 1
GA	104/4 = 26	56/4 = 14	4/4 = 1
MM	119/5 = 24	53/5 = 11	5/5 = 1
CK	100/2 = 50	181/2 = 91	7/2 = 4
PS	112/4 = 28	86/4 = 22	5/4 = 1
PD	75/3 = 25	44/3 = 15	5/3 = 2
JD	44/1 = 44	11/1 = 11	1/1 = 1
J	153/4 = 38	101/4 = 25	5/4 = 1
MEAN RATIO	38 (10)	24 (25)	1 (1)

Table 2: White male marathons runners 1

NAME	AGE	VOLUME (ml)
D. Yeo	24	1500
Dr Hutching	-	2040
G Arthur	24	1510
S. Whittingham	21	400
C. Bowen	25	2320
R. Sims	23	920
R. Vogel	25	840
S. Philips	24	670
P. Mannion	28	840
C.A.P. Bestel	23	1260
J. Innes	23	800
Barry Clark	35	845
Ivor Hill	-	980
Ian Bocook	38	840
John Brimble	39	760
Prof. Linder	-	2500
Nigel Evans	31	2940
Riel Hauman	39	1110

SUBJECT NUMBER = 18

Calcium Readings

Concentrations determined from the first set of calibration curves.
Mean blank reading = 0.013 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	CONC.
DY	0.147	3.0
RH	0.179	3.0
GA	0.335	6.0
SW	0.226	4.0
CB	0.166	3.0
RS	0.271	5.0
RV	0.439	8.0
SP	0.324	6.0
CAPB	0.225	4.0
PM	0.543	10.0
JI	0.353	6.0

CONCENTRATION CALCULATED FROM SECOND SET OF CALIBRATION CURVES.
Mean blank reading = 0.018mmoles l^{-1}

BC	0.578	9.0
IH	0.382	7.0
IB	0.341	6.0
JB	0.302	5.0
PL	0.257	4.0
NE	0.183	3.0
RH	0.227	4.0

Each mean reading was determined from 3 readings.
The mean blank was subtracted from the mean and standard reading before calculating or determining the concentration.

Mean Ca = 5.0 mmoles l^{-1} (2)
= 200 mg l^{-1} (80)

Sodium Readings

Concentrations were determined from the first set of calibration curves.
Mean blank reading = 0.002 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	CONC.
DY	0.105	77.0
RH	0.005	6.0
GA	0.101	75.0
SW	0.016	13.0
CB	0.183	135.0
RS	0.083	63.0
RV	0.186	138.0
SP	0.116	85.0
CAPB	0.196	144.0
PM	0.145	107.0
JI	0.145	107.0

CONCENTRATION CALCULATED FROM SECOND SET OF CALIBRATION CURVES.
Mean blank reading = 0.004mmoles l^{-1}

BC	0.084	53.0
IH	0.119	76.0
IB	0.109	70.0
JB	0.238	152.0
PL	0.072	45.0
NE	0.126	83.0
RM	0.244	155.0

Mean Na = 88.0 mmoles l^{-1} (45)
= 2023 mg l^{-1} (1035)

Magnesium Readings

Concentrations determined from the first set of calibration curves.
Mean blank reading = 0.014 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	CONC.
DY	0.254	8.0
RH	0.166	5.0
GA	0.204	6.0
SW	0.140	4.0
CB	0.180	5.0
RS	0.336	11.0
RV	0.346	12.0
SP	0.284	9.0
CAPB	0.270	8.0
PM	0.256	8.0
JI	0.262	8.0

CONCENTRATION CALCULATED FROM SECOND SET OF CALIBRATION CURVES.
Mean blank reading = 0.186mmoles l^{-1}

BC	0.289	10.0
IH	0.196	6.0
IB	0.323	15.0
JB	0.221	7.0
PL	0.148	5.0
NE	0.127	4.0
RH	0.197	6.0

Mean Mg = 8.0 mmoles l^{-1} (3)
= 195 mg l^{-1} (73)

Potassium Readings

Concentrations determined from the first set of calibration curves.
Mean blank reading = 0.033 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	CONC.
DY	0.326	47.0
PM	0.324	47.0
CAPB	0.458	67.0
SP	0.059	9.0
RV	0.382	56.0
RS	0.309	46.0
JI	0.459	68.0
CB	0.262	39.0
SW	0.072	11.0
GA	0.463	68.0
RH	0.307	45.0

CONCENTRATION DETERMINED FROM SECOND SET OF CALIBRATION CURVES.
Mean blank reading = 0.013mmoles l^{-1}

BC	0.464	60.0
IH	0.321	42.0
IB	0.566	73.0
JB	0.658	83.0
PL	0.166	22.0
NE	0.306	40.0
RH	0.492	64.0

Mean K = 49 mmoles l^{-1} (21)
= 1916 mg l^{-1} (821)

VARIOUS RATIOS

SUBJECTS	Na/Ca	K/Ca	Mg/Ca
DY	77/3 = 26	47/3 = 16	8/3 = 3
RH	6/3 = 2	45/3 = 15	5/3 = 2
GA	75/6 = 13	68/6 = 11	6/6 = 1
SW	13/4 = 3	11/4 = 3	4/4 = 1
CB	135/3 = 45	39/3 = 13	5/3 = 2
RS	63/5 = 13	46/5 = 9	11/5 = 2
RV	138/8 = 17	56/8 = 7	12/8 = 2
SP	85/6 = 14	9/6 = 2	9/6 = 2
CAPB	144/4 = 36	67/4 = 17	8/4 = 2
PM	107/10 = 11	47/10 = 5	8/10 = 1
JI	107/6 = 18	68/6 = 11	8/6 = 1
BC	53/9 = 6	60/9 = 7	10/9 = 1
IH	76/7 = 11	42/7 = 6	6/7 = 1
IB	70/6 = 12	74/6 = 12	15/6 = 3
JB	152/5 = 30	83/5 = 17	7/5 = 1
PL	45/4 = 11	22/4 = 6	5/4 = 1
NE	83/3 = 28	40/3 = 13	4/3 = 1
RH	155/4 = 39	64/4 = 16	6/4 = 2
MEAN RATIO	19 (13)	10 (5)	2 (1)

Table 3: White male marathon runners 2

NAME	AGE	VOLUME (ml)
D. Yeo	24	1905
R. Hutchings	-	1555
P. Mannion	28	1925
R. Vogel	25	960
C. Bowen	25	2185
S. Whittingham	21	720
G. Arthur	24	905
R. Sims	23	1620
C.A.P. Bestel	23	680
S. Philips	24	1260
J. Innes	23	2160
B. Clark	35	1080
R. Haumann	39	1140
N. Evans	31	3280

SUBJECT NUMBER = 14

Calcium Readings

Concentrations determined from calculations.
Mean blank reading = 0.013 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
DY	0.058	0.245	1
RH	0.144	0.245	3
SW	0.297	0.245	5
CB	0.111	0.245	2
RV	0.318	0.245	6
PM	0.229	0.245	4
GA	0.400	0.245	7
RS	0.183	0.263	3
CAPB	0.157	0.267	3
SP	0.246	0.267	4
JI	0.176	0.268	3
Mean blank reading = 0.019mmoles l^{-1}			
BC	0.319	0.248	6
RH	0.133	0.242	2
NE	0.099	0.242	2

Each mean reading was determined from 3 readings.
The mean blank was subtracted from the mean and standard reading before calculating or determining the concentration.

Mean Ca = 4 mmoles l^{-1} (2)
= 160 mg l^{-1} (80)

Sodium Readings

Concentrations were determined from calculations.
Mean blank reading = 0.002 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
DY	0.063	0.301	46
RH	0.065	0.301	48
GA	0.146	0.303	106
SW	0.167	0.301	122
CB	0.169	0.301	124
RV	0.156	0.298	115
PM	0.174	0.298	129
RS	0.121	0.297	90
CAPB	0.107	0.303	78
SP	0.139	0.303	101
JI	0.154	0.303	112

Mean blank reading = 0.004mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
BC	0.189	0.344	121
RH	0.217	0.346	138
NE	0.035	0.348	22

Mean Na = 97 mmoles l^{-1} (35)
= 2230 mg l^{-1} (805)

Magnesium Readings

Concentrations determined from calculations.
Mean blank reading = 0.186 mmoles l^{-1}

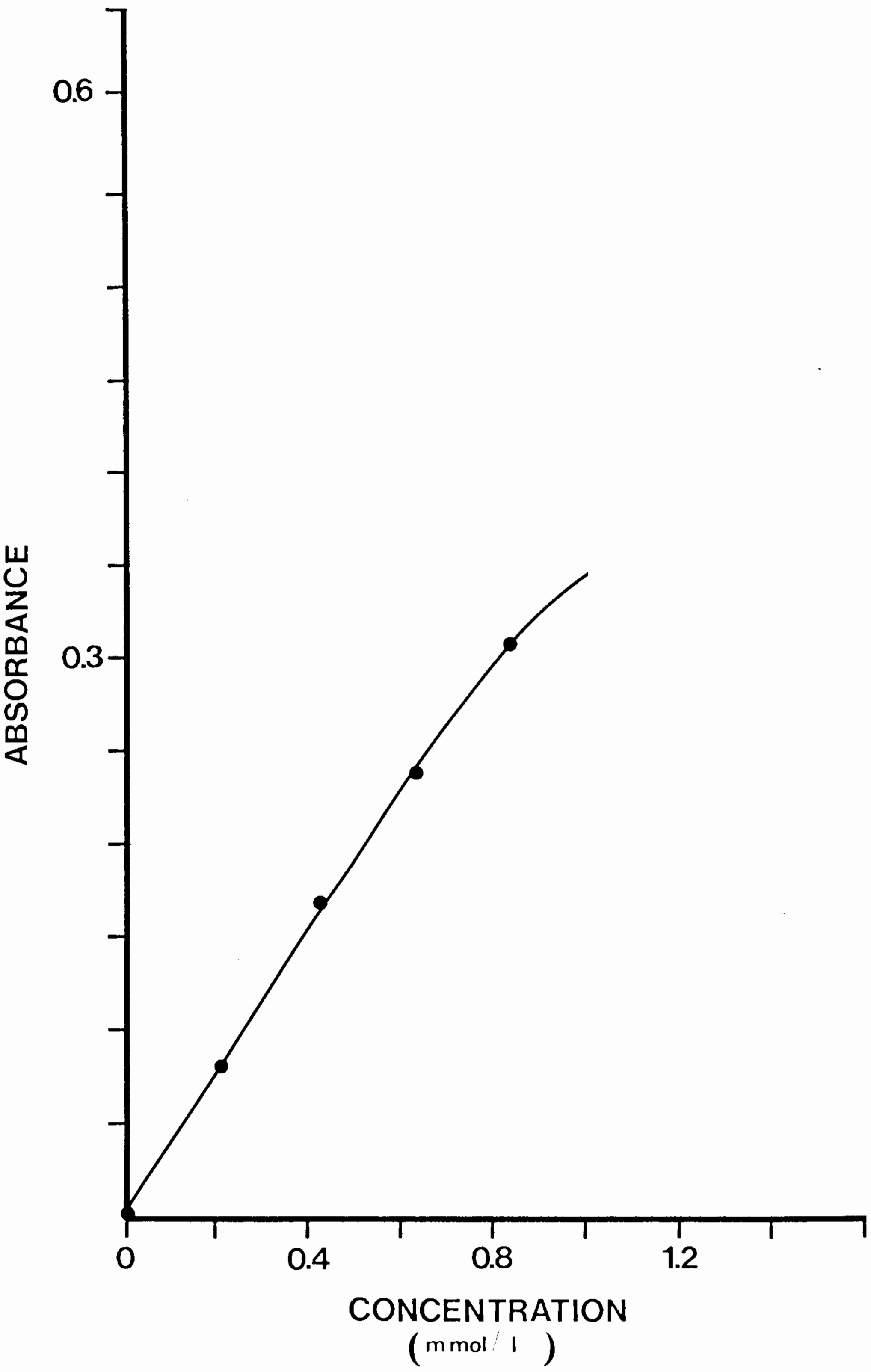
SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
BC	0.020	0.232	0.4
RH	0.038	0.232	0.8
NE	-	0.235	-

Mean blank reading = 0.014mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
DY	0.115	0.171	3.0
RH	0.132	0.171	4.0
GA	0.216	0.171	6.0
SW	-	0.172	-
CB	0.122	0.172	4.0
RV	0.204	0.172	6.0
PM	0.078	0.172	2.0

CALIBRATION CURVE WAS CONSTRUCTED FOR THE LAST FOUR SUBJECTS

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
RS	0.129	-	4.0
CAPB	0.306	-	10.0
SP	0.137	-	4.0
JI	0.108	-	3.0



GRAPH 9: Mg CALIBRATION CURVE

CONSTRUCTION OF Mg CALIBRATION CURVE

mml^{-1}	1	2	3	MEAN	M-B
0.000	0.008	0.008	0.008	0.008	0.000
0.206	0.091	0.092	0.093	0.092	0.08
0.412	0.173	0.177	0.176	0.175	0.17
0.617	0.246	0.246	0.243	0.245	0.24
0.823	0.318	0.311	-	0.315	0.31

Mean Mg = 4.0 mmoles l^{-1} (3.0)
= 97 mg l^{-1} (73)

Potassium Readings

Concentrations determined from calculations.
Mean blank reading = 0.013 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
BC	0.107	0.402	14
RH	0.068	0.401	9
NE	0.529	0.500	55
Mean blank reading = 0.033mmoles l^{-1}			
DY	0.323	0.371	45
RH	0.433	0.344	65
SW	-0.02	0.350	-
CB	0.168	0.350	25
RV	0.559	0.350	82
PM	0.260	0.350	38
GA	0.697	0.440	82
RS	0.257	0.317	42
CAPB	0.164	0.316	27
SP	0.375	0.319	61
JI	0.332	0.318	54

Mean K = 46 mmoles l^{-1} (24)
= 1799 mg l^{-1} (939)

VARIOUS RATIOS

SUBJECTS	Na/Ca	K/Ca	Mg/Ca
DY	46/1 = 46	45/1 = 45	3/1 = 3
RH	48/3 = 16	65/3 = 22	4/3 = 1
SW	122/5 = 24	-/5 = -	6/7 = 1
CB	124/2 = 62	25/2 = 13	-/5 = -
RV	115/6 = 19	82/6 = 14	4/2 = 2
PM	129/4 = 32	38/4 = 10	6/6 = 1
GA	106/7 = 15	82/7 = 12	2/4 = 1
RS	90/3 = 30	42/3 = 14	4/3 = 1
CAPB	78/3 = 26	27/3 = 9	10/3 = 3
SP	101/4 = 25	61/4 = 15	4/4 = 1
JI	112/3 = 37	54/3 = 18	3/3 = 1
BC	121/6 = 20	14/6 = 2	0.4/6 = 0.1
RH	138/2 = 69	9/5 = 2	0.8/2 = 0.4
NE	22/2 = 11	52/2 = 28	-/2 = -
MEAN RATIO	31 (17)	16 (11)	1 (1)

Table 4: Black male normals

NAME	AGE	VOLUME (ml)
C. Jacobs	35	1160
V. Mayekiso	18	670
G. Madyo	35	1570
G. Thame	41	1040
Kenneth	57	570
Erick	44	480
Noaa	67	240
William	31	180
Raymond	43	1040
Mandlenkosi	48	680
Leonard	41	40
Armstrong	53	140
Geoffrey	28	945

SUBJECT NUMBER = 13

Calcium Readings

The concentration for all these readings were determined using the calibration method.

Mean blank reading = 0.013 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
CJ	0.173	0.246	3
VM	0.336	0.247	6
GM	0.057	0.246	1
GT	0.206	0.249	4
Mean blank reading = 0.004mmoles l^{-1}			
MC	0.262	0.308	4
L	0.315	0.310	5
A	0.149	0.306	2
G	0.144	0.308	2
K	0.044	0.307	1
E	0.025	0.301	0.4
N	0.123	0.297	2
W	0.220	0.300	3
R	0.038	0.303	1

Each mean reading was determined from 3 readings.

The mean blank was subtracted from the mean and standard reading before calculating or determining the concentration.

Mean Ca = 3 mmoles l^{-1} (2)
        = 120 mg l^{-1} (80)

Sodium Readings

Mean blank reading = 0.002 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
CJ	0.207	0.274	166
VM	0.250	0.275	200
GM	0.133	0.276	106
GT	0.226	0.274	182
Mean blank reading = 0.003mmoles l^{-1}			
MC	0.287	0.322	196
L	0.260	0.324	177
A	0.236	0.325	160
G	0.230	0.325	156
K	0.142	0.317	99
E	0.102	0.317	71
N	0.184	0.316	128
W	0.257	0.309	183
R	0.023	0.325	16

Mean Na = 142 mmoles l^{-1} (55)
= 3265 mg l^{-1} (1265)

Magnesium Readings

Mean blank reading = 0.014 mmoles 1-1

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
CJ	0.077	0.245	2
VM	0.183	0.241	6
GM	0.039	0.229	1
GT	0.118	0.229	4
CONCENTRATION OF STANDARD = 0.412 mm L^{-1}			
Mean blank reading = 0.010mmoles l^{-1}			
MC	0.173	0.197	4
L	0.161	0.200	4
A	0.066	0.198	2
G	0.148	0.193	4
K	0.106	0.195	3
E	0.063	0.196	2
N	0.112	0.198	3
W	0.136	0.197	3
R	0.091	0.195	2

Mean Mg = 3.0 mmoles l^{-1} (1)
= 73 mg l^{-1} (24)

Potassium Readings

CONCENTRATION OF STANDARD FOR SUBJECTS CJ AND VM = 5.12 mm l^{-1}.

Mean blank reading = 0.033 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
CJ	0.229	0.451	32
VM	0.464	0.464	62
GM	0.230	0.349	34
GT	0.138	0.348	21
Mean blank reading = 0.006mmoles l^{-1}			
MC	0.424	0.391	56
L	0.676	0.400	87
A	0.191	0.403	24
G	0.312	0.401	40
K	0.175	0.395	23
E	0.430	0.395	56
N	0.542	0.395	71
W	0.272	0.395	36
R	0.134	0.395	18

Mean K = 43 mmoles l^{-1} (22)
= 1681 mg l^{-1} (860)

VARIOUS RATIOS

SUBJECTS	Na/Ca	K/Ca	Mg/Ca
CJ	166/3 = 55	32/3 = 11	2/3 = 1
VM	200/6 = 33	62/6 = 10	6/6 = 1
GM	106/1 = 106	34/1 = 34	1/1 = 1
GT	182/4 = 46	21/4 = 5	4/4 = 1
MC	196/4 = 49	56/4 = 14	4/4 = 1
L	177/5 = 35	87/5 = 17	4/5 = 1
A	160/2 = 80	24/2 = 12	2/2 = 1
G	156/2 = 78	40/2 = 20	4/2 = 2
K	99/1 = 99	23/1 = 23	3/1 = 3
E	71/0.4 = 178		2/0.4 = 5
N	128/2 = 64	71/2 = 36	3/2 = 2
W	183/3 = 61	36/3 = 12	3/3 = 1
R	16/1 = 16	18/1 = 18	2/1 = 2
MEAN RATIO	69 (42)	27 (14)	2 (1)

Table 5: Black marathon runners 1

NAME	AGE	VOLUME (ml)
Maxwell	-	820
Victor	(37)	590
Noel	(40)	1450
Johannes	(24)	620
Nase	(31)	880
Zola	-	500
Patrick	-	560
Solly	-	1020
Eric	35	400
Philip	19	610
Welkom	25	590
Nicholas	30	950
Jackson	31	140
Mqondeki	23	700

SUBJECT NUMBER = 14

Calcium Readings

Concentrations determined by calculation.
Mean blank reading = 0.004 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
SM	0.037	0.234	0.7
NA	0.139	0.240	3
ZOLA	0.147	0.239	3
P	0.088	0.235	2
VN	0.221	0.234	4
J	0.164	0.236	3
MAX	0.083	0.239	2
MASE	0.120	0.240	2

CONCENTRATION DETERMINED FROM SECOND SET OF CALIBRATION CURVES.
Mean blank reading = 0.018mmoles l^{-1}

EP	0.294	-	5
PI	0.113	-	2
WP	0.190	-	3
NT	0.128	-	2
JJ	0.081	-	1
MA	0.130	-	2

Each mean reading was determined from 3 readings.
The mean blank was subtracted from the mean and standard reading before calculating or determining the concentration.

Mean Ca = 3.0 mmoles l^{-1} (1)
= 120 mg l^{-1} (40)

Sodium Readings

Mean blank reading = 0.003 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
SM	0.051	0.257	44
NA	0.178	0.259	151
ZOLA	0.138	0.255	119
P	0.226	0.258	193
VN	0.092	0.260	78
J	0.090	0.260	76
MAX	0.309	0.255	267
MASE	0.159	0.257	136

CONCENTRATIONS WERE DETERMINED FROM SECOND SET OF CALIBRATION CURVES.

Mean blank reading = 0.004mmoles l^{-1}

EP	0.147	-	94
PI	0.306	-	191
WP	0.362	-	230
NT	0.399	-	255
JI	0.316	-	201
MA	0.246	-	157

Mean Na = 157 mmoles l^{-1} (72)
= 3609 mg l^{-1} (1655)

Magnesium Readings

Mean blank reading = 0.010 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
SM	0.047	0.145	2
NA	0.157	0.141	6
ZOLA	0.294	0.146	10
P	0.006	0.141	-
VN	0.249	0.141	9
J	0.235	0.136	9
MAX	0.140	0.135	5
MASE	0.216	0.135	8

CONCENTRATION WERE DETERMINED FROM THE 2ND SET OF CALIBRATION CURVES.

Mean blank reading = 0.186mmoles l^{-1}

EP	0.241	-	8
PI	0.006	-	0.1
WP	-	-	-
NT	0.155	-	5
JI	0.138	-	4
MA	0.147	-	3

Mean Mg = 5.0 mmoles l^{-1} (4)
= 122 mg l^{-1} (97)

Potassium Readings

Mean blank reading = 0.006 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
SM	0.158	0.318	26
NA	0.347	0.323	55
ZOLA	0.202	0.322	31
P	0.003	0.326	0.5
VN	0.197	0.320	32
J	0.133	0.326	21
MAX	0.314	0.324	50
MASE	0.366	0.323	58

CONCENTRATIONS WERE DETERMINED FROM THE 2ND SET OF CALIBRATION CURVES.

Mean blank reading = 0.013mmoles l^{-1}

EP	0.299	-	39
PI	0.166	-	22
WP	0.277	-	36
NT	0.295	-	39
JI	0.182	-	24
MA	0.273	-	36

Mean K = 34 mmoles l^{-1} (15)
= 1330 mg l^{-1} (587)

VARIOUS RATIOS

SUBJECTS	Na/Ca	K/Ca	Mg/Ca
SM	47/0.7 = 63	26/0.7 = 37	2/0.7 = 3
NA	151/3 = 50	55/3 = 18	6/3 = 2
ZOLA	119/3 = 40	31/3 = 10	10/3 = 3
P	193/2 = 97	0.5/2 = 0.3	-/2 = -
VN	78/4 = 20	32/4 = 8	9/4 = 2
J	76/3 = 25	21/3 = 7	9/3 = 3
MAX	267/2 = 134	50/2 = 25	5/2 = 3
MASE	136/2 = 68	58/2 = 29	8/2 = 4
EP	94/5 = 19	39/5 = 8	8/5 = 2
PI	191/2 = 96	22/2 = 11	0.1/2 = 0.1
WP	230/3 = 77	36/3 = 12	-/3 = -
NT	255/2 = 128	39/2 = 20	5/2 = 3
JI	201/1 = 201	24/1 = 24	4/1 = 4
MA	157/2 = 79	36/2 = 18	3/2 = 2
MEAN RATIO	78 (51)	16 (10)	2 (1)

Table 6: Black male marathon runners 2

NAME	AGE	VOLUME (ml)
Maxwell	(27)	790
Victor	-	465
Noel	-	2490
Mase	-	745
Zola	(32)	-
Solly	(25)	660
Weldom	(25)	340
D. Sibara	(26)	640

SUBJECT NUMBER = 8

Calcium Readings

Mean blank reading = 0.009 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
MAX	0.133	0.229	3
VK	0.273	0.229	5
NOEL	0.126	0.231	2
MASE	0.064	0.230	1
ZOLA	0.271	0.231	5
SOLLY	0.095	0.233	2
Mean blank reading = 0.013mmoles l^{-1}			
WP	0.471	0.285	7
DS	0.315	0.285	5

The concentrations for BMMR 2 were calculated using the equation method. The mean reading was determined from three readings and the mean blank reading was subtracted from the mean and standard readings.

Mean Ca = 4 mmoles l^{-1} (2)
= 160 mg l^{-1} (80)

Sodium Readings

Mean blank reading = 0.000 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
MAX	-	0.257	226
VIC	-	0.261	166
NOEL	-	0.262	108
MASE	-	0.259	113
ZOLA	-	0.259	166
SOLLY	-	0.260	95
Mean blank reading = 0.002mmoles l^{-1}			
WP	0.290	0.309	207
DS	0.250	0.306	180

Mean Na = 158 mmoles l^{-1} (48)
= 3624 mg l^{-1} (1104)

Magnesium Readings

Mean blank reading = 0.000 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
MAX	-	0.152	3
VIC	-	0.149	9
NOEL	-	0.152	2
MASE	-	0.151	4
ZOLA	-	0.150	9
SOLLY	-	0.150	4
Mean blank reading = 0.014mmoles l^{-1}			
WP	0.487	0.187	13
DS	0.360	0.187	10

Mean Mg = 7.0 mmoles l^{-1} (4.0)
= 170 mg l^{-1} (97)

Potassium Readings

Mean blank reading = 0.012 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
MAX	0.105	0.350	16
VIC	0.453	0.350	67
NOEL	0.199	0.348	30
MASE	0.251	0.349	37
ZOLA	0.243	0.348	36
SOLLY	0.320	0.349	47
Mean blank reading = 0.033mmoles l^{-1}			
WP	0.803	0.591	70
DS	0.479	0.596	41

Mean K = 43 mmoles l^{-1} (18)
= 1681 mg l^{-1} (704)

VARIOUS RATIOS

SUBJECTS	Na/Ca	K/Ca	Mg/Ca
MAX	226/3 = 75	16/3 = 5	3/3 = 1
VIC	166/5 = 33	67/5 = 13	9/5 = 2
NOEL	108/2 = 54	30/2 = 15	2/2 = 1
MASE	113/1 = 113	37/1 = 37	4/1 = 4
ZOLA	166/5 = 33	36/5 = 7	9/5 = 2
SOLLY	95/2 = 48	47/2 = 24	4/2 = 2
WP	207/7 = 15	70/7 = 10	13/7 = 2
DS	180/5 = 36	41/5 = 8	10/5 = 2
MEAN RATIO	53 (29)	15 (11)	2 (1)

Table 7: White male swimmers 1

NAME	AGE	VOLUME (ml)
D. Preece	21	440
W. Spiro	14	630
G. Hignett	19	650
C. Biggs	14	300
D. Hadjiandreou	16	1035
B. Melville	13	610
E. Miller	14	1060
C. Hammond	18	700

SUBJECT NUMBER = 8

Calcium Readings

All the concentrations were determined from calculations. Mean blank reading = 0.005 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
EM	0.060	0.334	1
DH	0.137	0.336	2
CB	0.261	0.338	3
BM	0.318	0.334	4
CHAM	0.587	0.346	8
GH	0.320	0.351	4
WS	0.223	0.347	3
DP	0.221	0.342	3

Each mean reading was determined from 3 readings. The mean blank was subtracted from the mean and standard reading before calculating or determining the concentration.

Mean Ca = 4 mmoles l^{-1} (2)
= 160 mg l^{-1} (80)

Sodium Readings

Mean blank reading = 0.001 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
EM	0.063	0.295	47
DH	0.202	0.293	152
CB	0.128	0.290	97
BM	0.227	0.290	172
CH	0.332	0.287	255
GH	0.189	0.295	141
WS	0.128	0.289	97
DP	0.096	0.286	74

Mean Na = 129 mmoles l^{-1} (66)
= 2966 mg l^{-1} (1517)

Magnesium Readings

Mean blank reading = 0.013 mmoles l-1

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
EM	0.022	0.161	1
DH	0.124	0.162	4
CB	0.143	0.158	5
BM	0.123	0.158	4
CH	0.193	0.159	6
GH	0.108	0.157	3
WS	0.196	0.158	6
DP	0.063	0.157	2

Mean Mg = 8.0 mmoles l^{-1} (5)
= 195 mg l^{-1} (122)

Potassium Readings

Mean blank reading = 0.000 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
EM	0.077	0.295	14
DH	0.305	0.296	53
CB	0.165	0.291	29
BM	0.123	0.288	22
CH	0.218	0.288	39
GH	0.513	0.304	87
WS	0.268	0.298	46
DP	0.166	0.297	29

Mean K = 40 mmoles l^{-1} (23)
= 1564 mg l^{-1} (899)

VARIOUS RATIOS

SUBJECTS	Na/Ca	K/Ca	Mg/Ca
EM	47/1 = 47	14/1 = 14	1/1 = 1
DH	152/2 = 76	53/2 = 27	4/2 = 2
CB	97/3 = 32	29/3 = 10	5/3 = 2
BM	172/4 = 43	22/4 = 6	4/4 = 1
CH	255/8 = 32	39/8 = 5	6/8 = 1
GH	141/4 = 35	87/4 = 22	3/4 = 1
WS	97/3 = 32	46/3 = 15	6/3 = 3
DP	74/3 = 25	29/3 = 10	2/3 = 1
MEAN RATIO	40 (16)	14 (8)	2 (1)

Table 8: WHITE MALE SWIMMERS 2

NAME	AGE	VOLUME (ml)
G. Hignett	19	975
P. Van Niekerk	20	900
C. Hammond	18	480
A	-	520
B. Melville	14	980
G. Kagan	14	675
W. Spiro	14	360
D. Hadjiandreou	16	920
E. Miller	14	1060
C. Biggs	13	200^+

SUBJECT NUMBER = 10

Calcium Readings

Concentrations determined by calculation.
Mean blank reading = 0.008 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
GK	0.202	0.267	3
WS	0.170	0.333	2
DH	0.033	0.333	0.5
EM	0.110	0.331	2
CB	0.386	0.268	6
CH	0.578	0.270	9
PVN	0.442	0.270	7
A	0.286	0.271	5
GH	0.183	0.270	3
BM	0.078	0.270	1

Each mean reading was determined from 3 readings. The mean blank was subtracted from the mean and standard reading before calculating or determining the concentration.

Mean Ca = 4.0 mmoles l^{-1} (3)
= 160 mg l^{-1} (120)

Sodium Readings

Mean blank reading = 0.000 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
GK	-	0.296	104
WS	-	0.285	179
DH	-	0.288	75
EM	-	0.290	115
CB	-	0.296	139
CH	-	0.292	186
PVN	-	0.298	144
A	-	0.291	98
GH	-	0.290	135
BM	-	0.292	45

Mean Na = 122 mmoles l^{-1} (44)
= 2805 mg l^{-1} (1012)

Magnesium Readings

Mean blank reading = 0.021 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
GK	0.282	0.157	9
WS	0.168	0.152	6
DH	0.051	0.150	2
EM	0.093	0.155	3
CB	0.234	0.154	8
CH	0.329	0.151	11
PVN	0.266	0.151	9
A	0.174	0.152	6
GH	0.103	0.158	3
BM	0.071	0.158	2

Mean Mg = 6.0 mmoles l^{-1} (3)
= 146 mg l^{-1} (73)

Potassium Readings

Mean blank reading = 0.009 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
GK	0.778	0.398	101
WS	0.980	0.399	127
DH	0.130	0.391	17
EM	0.405	0.391	53
CB	0.190	0.392	25
CH	0.477	0.390	63
PVN	0.725	0.390	96
A	0.689	0.388	92
GH	0.310	0.395	41
BM	0.379	0.400	49

Mean K = 60 mmoles l^{-1} (36)
= 2581 mg l^{-1} (1408)

VARIOUS RATIOS

SUBJECTS	Na/Ca	K/Ca	Mg/Ca
GK	104/3 = 35	101/3 = 34	9/3 = 3
WS	179/2 = 90	127/2 = 64	6/2 = 3
DH		17/0.5 = 34	2/0.5 = 4
EM	115/2 = 58	53/2 = 27	3/2 = 2
CB	139/6 = 23	25/6 = 4	8/6 = 1
CH	186/9 = 21	63/9 = 7	11/9 = 1
PVN	144/7 = 21	96/7 = 14	9/7 = 1
A	98/5 = 20	92/5 = 18	6/5 = 1
GH	135/3 = 45	41/3 = 14	3/3 = 1
BM	45/1 = 45	49/1 = 49	2/1 = 2
MEAN RATIO	40 (23)	27 (19)	2 (1)

Table 9: White male cyclists 1

NAME	AGE	VOLUME (ml)
I. Gallard	19	590
J. Herbstein	16	2180
S.A. Knoesen	21	3520
T. Thring	19	940
C.R. Norris	19	680
T. Brink	18	395
P. Segar	25	900
C. Deary	31	-
V. Eisermaan	20	820
D.C. Linde	32	965
R.A.S. Lotter	41	945
A. Buratovich	26	1880
I. Buratovich	27	900
P. Doherty	34	1020
C. McClune	18	480
A.G. MacDonald	39	-
R. Fish	-	630
R. Philips	33	1020
K. Stevenson	25	1540
D. Kruger	33	840
D.A. Lloyd	34	600

SUBJECT NUMBER = 21

Calcium Readings

All the concentrations were determined from calculations.
Mean blank reading = 0.013 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
IG	0.455	0.241	8
JH	0.348	0.243	6
SK	0.101	0.242	2
TT	0.378	0.241	7
CH	0.268	0.243	5
TB	0.531	0.243	10
Mean blank reading = 0.006mmoles l^{-1}			
AB	0.134	0.240	3
IB	0.240	0.241	4
CMcC	0.238	0.242	4
RF	0.194	0.241	4
K	0.167	0.244	3
RP	0.431	0.246	8
OL	0.323	0.247	6
DK	0.417	0.250	7
PD	0.161	0.249	3
AMACD	0.066	0.250	1
Mean blank reading = 0.018mmoles l^{-1}			
PS	0.289	0.245	5
CD	0.480	0.240	9
VE	0.245	0.242	5
DL	0.195	0.243	4
RL	0.264	0.247	5

The mean reading was determined from three readings and the mean blank reading was subtracted from the mean and standard readings.

Mean Ca = 5 mmoles l^{-1} (2)
= 200 mg l^{-1} (80)

Sodium Readings

Mean blank reading = 0.002 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
IG	0.271	0.299	199
JH	0.272	0.300	199
SK	0.101	0.300	74
TT	0.296	0.300	217
CN	0.145	0.304	105
TB	0.157	0.320	114
Mean blank reading = 0.003mmoles l^{-1}			
AB	0.093	0.279	73
IB	0.178	0.278	141
CMcC	0.137	0.277	109
RF	0.204	0.277	162
K	0.169	0.277	134
RP	0.154	0.279	121
OL	0.185	0.279	146
DK	0.281	0.278	222
PD	0.076	0.278	60
AD	0.074	0.278	59
Mean blank reading = 0.004mmoles l^{-1}			
PS	0.237	0.351	149
CD	0.138	0.347	88
VE	0.123	0.342	79
DL	0.215	0.345	137
RL	0.298	0.354	185

Mean Na = 132 mmoles l^{-1} (51)
= 3035 mg l^{-1} (1173)

Magnesium Readings

Mean blank reading = 0.014 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
IG	0.305	0.176	9
JH	0.173	0.174	5
SK	0.067	0.173	2
TT	0.246	0.173	7
CH	0.324	0.171	10
TB	0.277	0.171	8
Mean blank reading = 0.011mmoles l^{-1}			
AB	0.104	0.174	3
IB	0.277	0.176	6
CMcM	0.263	0.172	8
RF	0.243	0.171	7
K	0.165	0.173	5
RP	0.235	0.169	7
OL	0.257	0.170	8
DK	0.282	0.174	8
PO	0.149	0.171	4
AD	0.049	0.172	1
Mean blank reading = 0.185mmoles l^{-1}			
PS	0.200	0.232	4
CD	0.187	0.233	4
VE	0.042	0.234	1
DL	0.109	0.233	2
RL	0.009	0.233	0.2

Mean Mg = 5.0 mmoles l^{-1} (3.0)
= 122 mg l^{-1} (73)

Potassium Readings

Mean blank reading = 0.033 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
IG	0.322	0.346	48
JH	0.359	0.351	53
SK	0.118	0.345	18
TT	0.361	0.347	54
CN	0.610	0.343	92
TB	0.534	0.444	62
Mean blank reading = 0.006mmoles l^{-1}			
AB	0.444	0.437	52
IB	0.500	0.438	59
CMcC	0.237	0.438	28
RF	0.694	0.435	82
K	0.398	0.441	47
RP	0.494	0.434	59
OL	0.663	0.439	78
DK	0.198	0.437	23
PD	0.368	0.433	44
AD	0.118	0.433	14
Mean blank reading = 0.013mmoles l^{-1}			
PS	0.558	0.487	59
CD	0.608	0.487	64
VE	0.061	0.401	8
DL	0.606	0.492	64
RL	0.430	0.492	45

Mean K = 50 mmoles l^{-1} (22)
= 1955 mg l^{-1} (860)

VARIOUS RATIOS

SUBJECTS	Na/Ca	K/Ca	Mg/Ca
IG	199/8 = 25	48/8 = 6	9/8 = 1
JH	199/6 = 33	53/6 = 9	5/6 = 1
SK	74/2 = 37	18/2 = 9	2/2 = 1
TT	217/7 = 31	54/7 = 8	7/7 = 1
CN	105/5 = 21	92/5 = 18	10/5 = 2
TB	114/10 = 11	62/10 = 6	8/10 = 1
AB	73/3 = 24	52/3 = 17	3/3 = 1
IB	141/4 = 35	59/4 = 15	6/4 = 2
CMcC	109/4 = 27	28/4 = 7	8/4 = 2
RF	162/4 = 41	82/4 = 21	7/4 = 2
K	134/3 = 45	47/3 = 16	5/3 = 2
RP	121/8 = 15	59/8 = 7	7/8 = 1
OL	146/6 = 24	78/6 = 13	8/6 = 1
DK	222/7 = 32	23/7 = 3	8/7 = 1
PD	60/3 = 20	44/3 = 15	4/3 = 1
AD	59/1 = 59	14/1 = 14	1/1 = 1
PS	149/5 = 30	59/5 = 12	4/5 = 1
CD	88/9 = 10	64/9 = 7	4/9 = 0.5
VE	79/5 = 16	8/5 = 2	1/5 = 0.2
DL	137/4 = 34	64/4 = 16	2/4 = 0.5
RL	185/5 = 37	45/5 = 9	0.2/5 = 0.1
MEAN RATIO	29 (11)	11 (5)	1.0 (0.6)

Table 10: White male cyclists 2

NAME	AGE	VOLUME (ml)
R. Philips	33	805
R. Fish	-	640
A. Buratovitch	26	740
P. Doherty	34	800
D. Kruger	33	940
H. Van Andel	39	695
K. Stevenson	25	1460
I. Buratovitch	27	800
O. Lloyd	34	630
C. McClune	18	360

SUBJECT NUMBER = 10

Calcium Readings

Mean blank reading = 0.008 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
IB	0.298	0.253	5
K	0.256	0.256	4
PO	0.380	0.256	7
RF	0.263	0.255	5
AB	0.332	0.256	6
CMcC	0.350	0.256	6
RP	0.292	0.256	5
OL	0.311	0.256	5
HVA	0.308	0.253	5
DK	0.250	0.251	4

All the concentrations were calculated using the equation method.
Each mean reading was determined from 3 readings.
The mean blank was subtracted from the mean and standard reading before calculating or determining the concentration.

Mean Ca = 5.0 mmoles l^{-1} (1)
= 200 mg l^{-1} (4.0)

Sodium Readings

Mean blank reading = 0.001 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
IB	0.115	0.293	86
K	0.195	0.291	147
PO	0.306	0.292	230
RF	0.242	0.293	182
AB	0.233	0.291	176
CMcC	0.211	0.287	162
RP	0.088	0.292	66
OL	0.157	0.290	119
HVA	0.162	0.293	122
DK	0.236	0.290	179

Mean Na = 147 mmoles l^{-1} (49)
= 3380 mg l^{-1} (1127)

Magnesium Readings

Mean blank reading = 0.012 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
IB	0.185	0.176	5
K	0.214	0.177	6
PD	0.245	0.175	7
RF	0.140	0.176	4
AB	0.220	0.174	6
CMcC	0.302	0.178	9
RP	0.256	0.177	7
OL	0.152	0.177	4
HVA	0.112	0.176	3
DK	0.190	0.177	5

Mean Mg = 6.0 mmoles l^{-1} (2)
= 146 mg l^{-1} (49)

Potassium Readings

Mean blank reading = 0.009 mmoles l^{-1}

SUBJECTS	MEAN-BLANK	STANDARD-BLANK	CONC.
IB	0.201	0.340	31
K	0.341	0.338	52
PO	0.317	0.342	48
RF	0.480	0.342	72
AB	0.522	0.343	79
CMcC	0.283	0.338	43
RP	0.514	0.342	78
OL	0.139	0.340	21
HVA	0.814	0.338	124
DK	0.205	0.340	31

Mean K = 58 mmoles l^{-1} (31)
= 2268 mg l^{-1} (1212)

VARIOUS RATIOS

SUBJECTS	Na/Ca	K/Ca	Mg/Ca
IB	86/5 = 17	31/5 = 6	5/5 = 1
K	147/4 = 37	52/4 = 13	6/4 = 2
PO	230/7 = 33	48/7 = 7	7/7 = 1
RF	182/5 = 36	72/5 = 14	4/5 = 1
AB	176/6 = 29	79/6 = 13	6/6 = 1
CMcC	162/6 = 27	43/6 = 7	9/6 = 2
RP	66/5 = 13	78/5 = 16	7/5 = 1
OL	119/5 = 24	21/5 = 4	4/5 = 1
HVA	122/5 = 24	124/5 = 25	3/5 = 1
DK	179/4 = 45	31/4 = 8	5/4 = 1
MEAN RATIO	29 (10)	10 (6)	1 (0.5)

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