ZINC NUTRITIONAL STATUS OF PRESCHOOL CHILDREN IN SELECTED COMMUNITIES OF SOUTHERN GHANA

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DECLARATION

This study was undertaken by me. as presented, under the supervision of Dr. Ebenezer Asibey-Berko, of the Department of Nutrition and Food Science.

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DEDICATION

1. To God Almighty, for His love, protection, and enablement which made it possible for me to study at this rather advanced age.
2. To my family for their help and everything that they have sacrificed to enable me complete this study.
3. To all my lovely cohorts who without least hesitation willingly participated in the study. During my recent visits to them, they were very happy to see me and I feel I can hear them telling me 'Please, come again'. I shall always remember them when I pick up this thesis to read.

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SUMMARY

Zinc is a micronutrient indispensable for growth, development, reproduction, and for the activities of over 200 enzymes embracing all physiological activities. Zinc deficiency in the preschool age group therefore leads to growth faltering, wasting, stunting and general degeneration in physiological activities.

In rural areas of Egypt and Iran where zinc deficiency was first identified, dietary factors of plant origin (especially phytic acid and fibre), which impair zinc absorption, were the main etiological factors of zinc deficiency. For the fact that recent studies have suggested that zinc deficiency might be as alarming as iron deficiency and the fact that zinc deficiency predominates in populations which subsist largely on vegetable diet with little animal protein, it was felt that this study, is both relevant and necessary in the Ghanaian context.

Studies were carried out in 4 communities on 200 preschool (nursery) children aged 3 to 5 years, to determine if zinc deficiency occurs within this age group; and if it does, whether it relates in anyway to any of the anthropometric indices of the cohorts.

The four communities were Ashalley Botwe, Kwabenya,(typical rural southern villages), Dome (periurban) and New Achimota (urban), all located in Greater Accra region, southern Ghana.

In the study, age and the anthropometric indicators of nutritional status (weight, height, mid upper arm circumference, triceps, and subscapular skin-folds), and the biochemical

indicators of zinc nutriture [hair zinc, plasma zinc, red blood cell (rbc) zinc, and alkaline phosphatase activity], as well as indicators of protein nutriture (plasma protein, albumin, and A/G ratio) were determined.

Results of anthropometric measurements indicated that the mean percentage of pre-schoolers affected by Wasting, Stunting and Wasting plus stunting in the four communities combined were 3.5%, 16.5%, and 1.5% respectively, with 78.5% of normal status. When the results were considered for each community, 69.6% (Ashalley Botwe), 81.2% (Kwabenya), 80.9% (Dome), and 84.1% (New Achimota), respectively, were of normal stature. Percentage wasting was 3.6%, 0%, 4.4% and 4.5% while stunting levels were 25%, 18.8%, 13.2%, and 9.1% respectively. The percentage wasting plus stunting was 1.8%, 0%, 1.5%, and 2.3%, respectively.

Statistical analysis using Duncan's and Least significant difference (LSD) multiple comparison tests, indicated that there were no significant differences ($p>0.05$) in the mean values for the indicators of zinc nutriture (plasma zinc, rbc zinc, hair zinc, and plasma alkaline phosphatase activity) in the different nutritional states (normal, wasted, stunted, wasted plus stunted).

Further comparison with reference values indicated that there was no zinc deficiency in any of the groups- eg. the mean plasma zinc values obtained for the normal, wasted, stunted, and wasted plus stunted groups (for the 200 cohorts) were 1.13 ± 0.35, 1.16 ± 0.37, 1.04 ± 0.23 and 0.95 ± 0.29 ppm, respectively, as compared to a normal range of 0.50-1.50ppm. The corresponding values for the

hair zinc were 247.0 ± 101.6, 200.9 ± 65.2, 220.0 ± 83.8 and 157.6 ± 40 ppm, as compared to a normal level of >70 ppm. Also, the mean plasma, red blood cell, and hair zinc values for all the cohorts were normal.

Analysis of indicators of protein nutritional status (total plasma protein, albumin, and A/G ratio) revealed that the Plasma Protein values for the various anthropometric states were within the normal reference ranges - eg. the mean Albumin/Globulin ratio for the normal, wasted, stunted and wasted plus stunted groups (for the 200 cohorts) were 1.6 ± 0.5, 1.2 ± 0.3, 1.6 ± 0.5, and 1.5 ± 0.5, respectively, as compared to a normal range of 1-2.5. This indicates that the protein nutritional status was adequate.

It was concluded that there was no zinc deficiency in the cohorts, and that zinc nutritional status did not differ in the various nutritional states of the cohorts.

CHAPTER ONE

INTRODUCTION

In line with the generally accepted idea that the nutritional status of a child is perhaps the most important determinant factor in the survival and/ or health status of that child, a lot of efforts have been made to address this problem.

Despite these efforts, malnutrition continues to worsen in the developing world. The lack of progress is due to the many varied and complex factors which affect the nutritional status of children but which can not be easily controlled or met, mainly due to adverse socio-economic factors.

There are four principal nutritional deficiency diseases known in the world today[1]. These are:

1. Protein-energy malnutrition (PEM)- caused by deficiency of protein (leading to kwashiorkor) or energy (leading to marasmus) or deficiency of both protein and energy (leading to marasmic-kwashiorkor).
2. Anaemia- due mainly to iron deficiency.
3. Goitre and hypothyroidism- caused by iodine deficiency.
4. Xerophthalmia- caused by vitamin A deficiency.

Whereas iodine and vitamin A deficiency diseases are found in definite areas with inadequate iodine in the soil, and vitamin A in the diet, PEM and nutritional anaemias are rather widespread.

Iron deficiency anaemia is the most prevalent nutritional deficiency disease in the world and it is estimated that about 29%

of the world population or 43% of people in Africa suffer from iron deficiency anaemia[2]. In fact, it is the only nutritional deficiency showing a general deterioration in many parts of the world, such as south Asia and sub-saharan Africa[2].

Iodine deficiency, which causes endemic goitre and hypothyroidism leading (if untreated) to retarded growth, mental retardation, deafness and deaf-mutism (in infants) affects about 400 million people world-wide with a further 400 million people at risk[3]. Vitamin A deficiency affects about half a million children globally each year leading to partial or total blindness with about two-third of this number eventually dying, within a few months of loosing their sight[4].

It is now becoming clear that many nutritionally-important trace elements may become deficient in the diets of apparently healthy individuals, thus a lot of attention is now being focused on this area.

As at now, fourteen elements have been shown to be required by the human body in quantities less than a few micrograms per day. These are chromium, cobalt, copper, fluorine, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium and zinc[5]. Most nutritionists believe that the list would be extended in future as more and more evidence become available. However, deficiency states which have responded to specific replacement or supplementation have been described for only iron, iodine, copper, chromium, manganese and zinc[6].

These micronutrients are important nutritionally because they

are mostly components of many enzymes[7]. The metals function in the enzymes by (a)direct participation in catalysis, (b) combination with substrate to form a complex upon which the enzyme acts (c)formation of metalloenzyme which binds substrate (d) combination of metal with a reaction product and / or (e) maintenance of quaternary structure of the enzyme[7]. Thus,in the absence of these micronutrients, vital physiological processes are inhibited.

Even though the biological importance of zinc has been recognised for over a 100 years now, human deficiency states were not described until 1963[8]. As at now, about 200 zinc-metalloenzymes have been identified[9]; all key metabolic pathways have zinc metalloenzymes[5].

When an animal is given a zinc-deficient diet, plasma zinc levels fall in the first day and within three days the animal becomes anorexic (loss of appetite) and stops growing[5]. Forced feeding, particularly of a high-protein diet, leads to severe metabolic disturbances. As the deficiency continues, skin lesions develop, particularly around the body orifices. These progress to form ulcers which quickly become colonized with bacteria. At this stage, there is immuno-deficiency, particularly of the cell-mediated immune system. Diarrhoea ensues and the animal enters a phase of gross wasting and finally death[5]. In the early stages, zinc supplementation leads to reversal of the condition within an hour, in the latter stages within a day or so[5]. This pattern has been observed in at least fifteen species of animals, including man[5].

It has been shown that zinc is an essential micronutrient, implicated in many biological processes including growth, enzyme function, protein synthesis, sexual maturation, wound healing and host defence reactions[10-14]. In conditions of zinc deficiency or inadequacy therefore the above processes are retarded or abolished, depending on the level of deficiency.

The etiology of growth retardation is unknown but it is suggested to be related to nutrition or genetics or both[15]; however it is believed that,in the pre-school age, nutritional factors predominate over genetic factors[16].

Features of nutritional zinc deficiency in animals and man include anorexia, hypogonadism, (under-development of sex organs),lethargy, behavioral changes, increased susceptibility to infection and impaired development of the immune system[10-14]. Diminished height has been described in infants and children with poor zinc nutriture[15]. In fact zinc deficiency has been described as the cause of pronounced growth retardation, dwarfism and impairment of sexual maturation in young people living in Egypt and Iran who,consume diets of very low zinc bioavailability[17]. A milder form of zinc deficiency with slight growth retardation, poor appetite and impaired taste acuity was described over two decades ago in children of middle or upper-income families in Denver, Colorado. These children were presumed to be of a good nutritional status[18]. Since then, more studies have identified marginal zinc deficiency in preschool and school children of several countries[19-21].

Due to the ubiquitous presence of zinc in foods, classical symptoms of severe zinc deficiency are rarely observed. However, nutritional surveys and recent clinical findings with human patients suggest that marginal or mild zinc deficiency may be widespread in developed nations[22]. Consequently, human zinc deficiency may represent health concern throughout the world, particularly during preschool, adolescent, pregnancy, old age, stress or in disease.

Evidence is also slowly accumulating that deficiencies of other trace elements may be as prevalent as that of iron and that alteration of their metabolism may be the cause of many diseases bothering mankind[5].

In Ghana, both protein-energy malnutrition and micronutrient deficiency abound. A national nutrition survey in 1961 reported that about 40-50% of all preschool children were underweight[23]. A similar survey in 1986 reported that 58.4% of children aged 0-5 years were underweight. A World bank report in 1988 indicated that 8.2% of preschool children were suffering from a severe form of PEM in the form of marasmus or kwashiorkor. This figure of 8.2% is about twice that usually found in low income countries[24].

Nutritional surveys in 1988 have also revealed that about 28% of preschool children in Ghana were stunted[25]. The exact cause(s) of stunting have not been clearly identified; however, PEM and micronutrient deficiencies are implicated.

In the past, research in Ghana has concentrated largely on finding the causes and /or management of protein-energy

malnutrition, vitamin A,iron and lately iodine deficiencies. In contrast, nothing is known about the zinc status of any age group in Ghana. In fact Golden and Golden[5] believe that we must ask ourselves critically if children with malnutrition are indeed zinc deficient since malnutrition and experimental zinc deficiency have clinical signs in common. Anorexia, diarrhoea, stunting growth, wasting, skin desquamation with ulceration, a reduction in lymphoid tissue and increased susceptibility to infection are common to the two conditions[5].

This study was therefore planned to ascertain if zinc deficiency is one of the factors associated with malnutrition in preschool children in some selected communities in Ghana.

Zinc deficiency was considered a likely etiological factor in malnutrition in preschool children due to the fact that Ghanaians of all age groups consume cereals and vegetables as their staple food with only a little amount of animal protein. Zinc in foods is primarily associated with proteins and nucleic acids[26]. It is particularly abundant in red meats, some sea foods and the embryo portions of grains. However, zinc in plant products is generally less available than that supplied by animal proteins[27]. A major contributing factor is the presence of phytic acid, which forms an insoluble zinc-chelate complex (zinc phytate) that is not absorbed from the gastrointestinal tract[27]. High dietary calcium has been shown to enhance the adverse effect of phytic acid. Fibre also reduces zinc bioavailability while food processing can also markedly alter zinc bioavailability from that present in the

unaltered food[28].

It has been shown in rural Iran and Egypt where zinc deficiency was first identified that dietary factors especially phytic acid and fibre were the main etiological factors of zinc deficiency[17]. These plant materials impair zinc absorption. Also in the USA, 10 out of 338 middle and upper class children consuming diets consisting of little meat and a great deal of cow milk - which is a relatively poor source of zinc - had very low hair zinc levels[17].

Thus the bioavailability of zinc in the largely vegetable Ghanaian diet would be further reduced in the body with the result that the RDA of zinc (10-15 mg) might not be met in majority of people. This is especially in preschoolers(where zinc requirement is increased as a result of rapid growth rates) leading to zinc deficiency with its attendant consequences.

1.2. General objective.

To determine if zinc deficiency exists in preschool children in selected communities in Ghana; and if so, whether the zinc levels relate to the various nutritional states of the cohorts .

1.3. Specific objectives

i. To determine the extent of malnutrition in preschool children in the selected communities using anthropometric measurements.

ii. To study the correlation between zinc status and growth in normal and malnourished preschool children in the selected communities.

iii. To determine the level of hair zinc, plasma zinc erythrocyte zinc and alkaline phosphatase activity in the subjects.

iv. To determine plasma total protein, albumin, and Albumin/Globulin ratio as indicators of protein nutritional status in the cohorts.

CHAPTER TWO

LITERATURE REVIEW

2.1. Elements essential to man.

Of the ninety naturally occurring elements, twenty six are known to be essential to animal life[6]. Eleven of these are regarded as major elements because of their relative abundance in the body - each is present in the body at concentration of more than 0.005% body weight[7] - these are: carbon, hydrogen, oxygen, nitrogen, sulphur, calcium, phosphorus, potassium, sodium, chlorine and magnesium.

The remaining essential elements are known as trace elements because they are present in animal tissues in such relatively small amounts (each is less than 0.005% body weight), that the early researchers were unable to measure their concentrations accurately.

Trace elements which are essential in various animal species are iron, chromium, copper, fluorine, iodine, manganese, molybdenum, nickel, selenium, tin,cobalt silicon,vanadium and zinc[5]. In man, however, deficiency states which have responded to specific replacement or supplementation have been described for only iron, copper, chromium, manganese, iodine and zinc[6,29-34](table 1)

2.2. Recognized trace element deficiencies in man- Table 1 [16,29-34]

Element	Some effects of deficiency
Iron	Anaemia, impaired learning ability
Iodine	Endemic goitre, congenital hypothyroidism leading to retarded growth, mental retardation, deafness and deaf-mutism.
Copper	Falling levels of serum copper and ceruloplasmin, failure of iron absorption, neutropenia, leucopenia, bone demineralization, failure of erythropoiesis and death.
Chromium	Impaired glucose tolerance, poor growth, decreased nitrogen retention, ataxia, defective peripheral nerve conduction.
Zinc	Impaired appetite, growth retardation, delayed closure of epiphyses, pica, hypogeusia and delayed sexual maturation.
Manganese	Low clotting factor

2.2.1. Iron

Iron deficiency and associated anaemia remain the most common and widespread nutritional disorder in the world today[5], and affect over one billion people worldwide[35]. There is concern that the adverse effects of inadequate iron may not be limited to anaemia. It has been found that the function of haem proteins and the morphology of some subcellular structures can be disturbed and the future learning ability may be adversely affected by iron deficiency during infancy[34].

Although iron is essential for the existence of all plants and

animals, only trace amounts are present in living cells. These small but biologically indispensable amounts are related to the relative insolubility of iron and to the specialised systems required for its transport and incorporation into iron proteins: haemoglobin, myoglobin, haem enzymes(cytochromes),non-haem iron enzymes (flavoproteins), ferritin, transferrin,and hemosiderin[36].

The body carefully controls its stores of iron by limiting absorption and by reusing iron derived from breakdown of haemoglobin and catabolizing of nonhaem iron proteins. Although there is no planned iron excretion as such, there is a daily physiological loss of about 1 mg iron that must be replaced with intestinal absorption of 5-10% of the dietary 10-30 mg/ day[37].

The iron needs must be satisfied by absorption from haem and nonhaem food iron. In infants and children, the daily requirements are relatively high: from birth to 6 months, 10 mg/day; from 6 months to 3 years, 15 mg/day; for boys 11 to 18 years, 18 mg/day, dropping to 10 mg for older men, while it is 18-23 mg/day for girls/women aged 10 to 50 years, dropping to 10 mg in the older ones[7].

Iron deficiency is the most common cause of anaemia and is significantly more frequent in women of childbearing age[37]. In the developing countries anaemia is frequent in both sexes and in children of low socioeconomic background[36].

The classical symptoms of anaemic-hypoxia such as weakness, palpitation, pallor, and dizziness may be absent in even moderate to severe anaemia (less than 100g haemoglobin/L blood), whereas

persons with latent iron deficiency (low serum iron, depleted iron stores,but no anaemia), may complain of these symptoms[38].

More recent investigations show a significant relationship between iron deficiency and physical fitness and work capacity in adults[39] and between iron deficiency and mental performance and irritability in children[40]. In iron deficiency, monoamine oxidase activity is decreased leading to increased excretion of norepinephrine in urine. Norepinephrine is believed to influence behaviour in humans[40,41].

2.2.2. Copper

Despite evidence dating back to Josephs since 1931[42], it has repeatedly been stated that copper deficiency did not or could not exist in man[43]. Deficiency states of copper have now been found in a number of conditions such as severely malnourished infants rehabilitated on milk-based, low copper diets[44]; in untreated malnourished infants[45], premature infants[46], malnourished infants alimented exclusively by the intravenous route[47], and in adults[48].

Copper is essential for life because of its presence in certain metalloenzymes[29,30,32]. For example, as a component of cytochrome oxidase, copper is important in mitochondrial oxidative phosphorylation which produces Adenosine triphosphate[6].

Copper is essential for the function of lysyl oxidase, an enzyme required in the cross-linking that occurs in collagen and elastin. Tyrosinase, a copper-dependent enzyme, is required for formation of melanin.

As a component of superoxide dismutase, copper may be important in protecting newborn lungs from oxygen damage since this enzyme protects cells from damage by superoxide radicals.

Copper is also an important component of ceruloplasmin, a metalloenzyme synthesised in the liver in response to increased dietary copper, estrogens, androgens and leucocyte endogenous mediator[6]. Ceruloplasmin has many important functions[6,30,32]; these include oxidation of ferrous iron to ferric iron, the form in which iron is bound by apotransferrin for iron transport to the bone marrow; it oxidises amines such as epinephrine, and may serve as a transport protein for copper[49].

In copper deficiency, anaemia could result. This is attributable to a lack of copper-containing ferroxidase, including ceruloplasmin that are required for oxidation of ferrous iron to ferric iron and this prevents transport of iron to bone marrow. Deficiency of copper-containing amine oxidases required for cross-linking of elastin and collagen leads to defects in the walls of blood vessels; in animals, death may result from rupture of a major artery. Melanin synthesis is also impaired by copper deficiency[50].

In a study of copper-deficient infants hospitalised in Lima, Peru, it was found that neutropenia and leucopenia were the prime early indicators of copper deficiency. Anaemia due strictly to copper deficiency occurred only late in the course of the deficiency and bone disorders still later[50].

There are two well-defined genetic diseases, Menkes' syndrome and Wilson's disease in which copper metabolism is disrupted and

hypocupremia occurs inspite of adequate dietary copper intake[51]. Menkes' syndrome is a sex-linked, genetic disease in human male, characterised by slow growth, cerebral degeneration and early death. The pathology is similar to that observed for copper-deficient animals; patients have low levels of copper in serum, liver, and brain and depressed levels of serum oxidase activity. It is believed that the basic defect is the failure to transport copper across membranes[51].

Wilson's disease is also characterised by low plasma copper associated with excessive accumulation of copper in tissues such as liver, brain, kidney and cornea. Patients suffer from neurologic symptoms, liver cirrhosis and corneal degeneration. Treatment involves use of chelating agents such as penicillamine, to aid in the elimination of excess copper especially from the central nervous system[51].

Etiological factors that contribute to copper deficiency include general malnutrition, diarrhoea, prolonged feeding with milk-based diets, intestinal malabsorption syndromes, inherited defects in copper metabolism, prolonged perinatal feeding and prematurity[34,52].

Normally, healthy term infants do not appear to be at risk of copper deficiency even if fed with relatively low-copper milk products since neonatal copper stores are usually adequate to tide over the young infant until he is fed other foods rich in copper[34].

In contrast, premature infants do not start post-natal life with similar copper stores, thus low- birth weight infants are at

risk from severe symptomatic copper deficiency by three months after birth[46,52].

Wilson and Lahey[53] reported that a daily intake of 15μg copper/ kg body weight for low birth weight infants during the first two months of post-natal life was sufficient to avoid any gross signs of copper deficiency; Cordano[54] has suggested a level of 90μg/ kg body weight.

2.2.3. Chromium, manganese and iodine

Chromium is essential as a component of nucleic acids and as a component of a factor that potentiates the action of insulin[29,32,55]. Chromium is biologically active when it is complexed in a naturally-occurring, organic compound known as glucose tolerance factor. In this form, chromium absorption from the small intestine and its action as an insulin cofactor are several times greater than inorganic trivalent chromium compounds[55]. Current hypothesis concerning mechanism of action of glucose tolerance factor is that it facilitates the interaction between insulin and its receptors.

Chromium deficiency in man is characterised by impaired glucose tolerance, decreased nitrogen retention, ataxia and defective peripheral nerve conduction[56,57]. Animals fed chromium-deficient diet develop glycosuria, fasting hyperglycaemia, corneal opacities and aortic plaques[58,59].

Plasma chromium concentration is also lower during pregnancy and it has been explained that the mild glucose intolerance during

pregnancy may be due in part to decreased maternal chromium, since the fetus accumulates chromium at the expense of maternal chromium[6].

It has been shown that in the US., tissue chromium decreases with age and it has also been found that one of the causes of glucose intolerance in the elderly is chromium deficiency[60]. The recommended daily allowance has not been established for pregnant women nor for newborns[6].

Manganese is important for life due to the fact that manganous ion is known to be an activator of many enzymes[7].

In 1972,the first report of manganese deficiency in man appeared[61]. Until then there was doubt as to whether manganese deficiency could occur in man. The symptoms were weight loss, transient dermatitis, occasional nausea and vomiting, changes in the colour of hair and beard and slow growth of hair and beard[61].

Manganese deficiency has also been observed in an adult man in association with vitamin K deficiency[62]. The clotting factor of this patient was low until given manganese and vitamin K. In other species, manganese deficiency is associated with impaired growth, skeletal abnormalities, disturbed reproductive function, ataxia in the newborn and defects in lipid and carbohydrate metabolism[29], which may relate to its role with pyruvate carboxylase[7].

Iodine is essential for the synthesis of thyroid hormones that are important in cellular oxidation, growth and development,and gonadal function[33]. In conditions of iodine deficiency,a spectrum of symptoms referred to as Iodine Deficiency Disorders (IDD) occur. These include goitre, endemic cretinism characterised most

commonly by mental deficiency, deaf-mutism and spastic diplegia, and some neurological defects, impaired mental function, increased still births, increased perinatal and infant mortality[33].

From the May 1993 conference on iodine deficiency at the Franklin Institute, Philadelphia, USA, came the following consensus: ' The employment of prevention of iodine deficiency is urgent because even a modest degree of it has a deleterious effect on cognition and neuromotor functions'. IDD therefore threatens the health, wellbeing, social and economic productivity of the sufferers.

In 1960, WHO estimated a population of 200 million as suffering from IDD. Most recent estimates greatly exceeded this figure in spite of extensive iodation programmes. There is a consensus that 800 million people are at risk of suffering from IDD worldwide[33].

IDD is a good example of a major nutritional disorder for which the techniques for treatment, control, and prevention are easily available and affordable. All that it takes is a strong will, wider awareness and cooperation among those who hold the key to the solution of the problem.

2.2.4. Zinc

There are forty or more substances known to be essential in the human diet, but of these only three micronutrients- vitamin A, iron and iodine are thought to be commonly deficient[9]. There is a

growing suspicion however that zinc might also be included in this category. This is based on two separate considerations. On the one hand, the pervasive nature of zinc-dependent enzymes in metabolic processes. On the other hand, zinc supplementation is beneficial in many disease states; for example, in malnourished children, vitamin A status has been improved and immune response corrected, and even the duration of diarrhoeal diseases seem to be reduced by zinc supplementation[9].

The biological importance of zinc was established first in plants in 1869[63] . In 1934, Todd et al[64] reported that zinc was necessary for animal life and suggested that this might be the case of humans too. This was confirmed by Prasad et al[65]. Today a number of zinc-responsive syndromes are known, many of which reflect underlying zinc deficiency states.

2.2.4.1. Distribution of zinc in human body.

In the late forties, McCance and Widdowson[9] showed that the adult human body contains about two grams of zinc. Sixty percent of body zinc is in the muscle, 20% in bone, 5% in blood and liver and 3% in skin and the gastrointestinal tract.

Scoular and Macy did balance studies in preschool children in the early forties to show that five milligram of zinc were retained out of an intake of 16mg a day[9]. Such a retention, five times greater than that of iron for example, seemed to speak against the classification of zinc as a trace element. This rethinking has been made possible with the introduction of atomic absorption

spectroscopy in the investigation of the importance of zinc in human nutrition[9].

Zinc is present in virtually all cells, but certain tissues in animals have a higher abundance[22]. Moreover, zinc distribution among tissues is similar in different animal species. Concentrations typically are between 10 and 100 μg/g wet weight[22]. The zinc concentrations of most soft tissues such as muscle, brain, lung,and the heart are relatively stable and unresponsive to amounts of dietary zinc over most ranges of intake, however zinc concentrations in other tissues such as bone, testes, hair, and blood tend to reflect dietary zinc intake[22].

Zinc is the most abundant trace element inside most cells, with the exception of the erythrocytes where iron has its oxygen-carrying functions. Even the macro- element calcium is less abundant than zinc in all other cells except in bone cells[9].

2.2.4.2. Biochemistry and the physiological roles of zinc

The biological importance of zinc appears to be related primarily to its role in many enzyme systems. Zinc is not limited as are calcium and iron to a few functional cells but is present in practically all cells of the body[22].

Zinc is a functionally essential component of more than 200 enzymes[9], pervading all metabolic pathways and at least one zinc metalloenzyme is known in every major enzyme classification[5].
The role of zinc in such enzymes can be either catalytic and/ or structural. Enzymes which contain zinc or require adequate zinc

nutriture in human and/ or experimental systems for activity include alkaline phosphatase, alcohol dehydrogenase, carbonic anhydrase, lactic dehydrogenase, glutamic dehydrogenase, carboxypeptidase, RNA and DNA polymerase, thymidine kinase and delta aminolevulinic acid dehydratase[66].

Some non-enzyme ligands with which zinc forms complexes include alpha$_2$-macroglobulin glycoprotein in plasma which firmly binds about 30% of plasma zinc, albumin, which loosely binds about 66% of plasma zinc and amino acids such as histidine and cysteine with which about 2% of circulating zinc is reported to be complexed. Zinc can also bind to transferrin, metallothionin and nucleoproteins[66].

Zinc also helps to stabilize membrane structures and protects membrane integrity by the reduction of free radical formation,thus preventing lipid peroxidation[9].

Thus zinc has been shown to be important in a variety of physiological processes[10-14]. In its deficiency, many clinical manifestations become apparent. These include anorexia, growth retardation, delayed closure of epiphysis, hypogeusia, dysgeusia, delayed sexual maturation, alopecia, diarrhoea[17]. Other symptoms are delayed healing of wounds, mental depression and lethargy, increased susceptibility to infections, eye lesions such as retinitis and congenital malformations[17]. These symptoms and signs are to some extent dependent on age, acuteness of onset, duration and severity of zinc depletion and on the cause of zinc deficiency[17]. In the paediatric age however, retarded growth is an

early, consistent and prominent feature[17].

The vital importance of zinc for normal growth and development may be explained at least in part by its central role in nucleic acid metabolism. Zinc is needed in the synthesis of both DNA and RNA and may also have a role in the structure of nucleic acids[17]. Zinc therefore has a functional role in gene replication, activation and repression, is critical for transcription and translation, and affects nucleic acid metabolism[9].

Growth of young rats on a zinc-deficient diet stops within 24 hours, probably due to the lack of gene regulatory proteins[9]. These proteins contain a common structure - "Zinc finger" which are loops of chains of amino acids held together at the base by a zinc atom. Gene regulatory proteins may contain eleven such "fingers" which reach down into the grooves of DNA helix and promote transcription[9].

The critical participation and importance of zinc in the synthesis of nucleic acids, proteins and in the structure and functions of biomembranes account for its roles in immunity and wound healing[66].

Zinc also mediates the activity of growth hormone[9]. When growth hormone attaches to its receptor sites on a cell membrane, zinc atom is needed to make the connection. The resulting complex is called "zinc-sandwich".

2.2.4.3. Zinc deficiency

Due to the critical importance and roles of zinc in metabolic

processes, all systems of the body can be adversely affected by zinc deficiency. This is more so if the deficiency occurs at a time when cells of the particular system are rapidly dividing, growing or synthesising proteins[66]. Processes which are profoundly affected by experimental zinc deficiency include RNA metabolism, DNA metabolism, protein metabolism, and mucopolysaccharide metabolism[66]. These effects of zinc deficiency are reflected in the intact animal by poor utilization of dietary nitrogen and sulphur and prompt arrest of growth[66]. In fact it has been suggested that zinc deficiency causes a block in protein and nucleic acid synthesis[5].

Reproduction is severely affected by zinc deficiency. Gonadal maturation is severely retarded in animals and humans. In adult animals, atrophy and fibrosis of gonads occur. If severe zinc deficiency is induced in rats in early pregnancy, there is a very high incidence of teratology and abortion. If induced later, parturition is severely impaired and fetal growth is retarded but no gross teratology[66].

The teratogenic effects of congenital zinc deficiency on the nervous system is particularly pronounced. When deficiency is induced during the last trimester, adverse effects on the fetal brain are limited to abnormal composition with a decrease in total DNA and an increased ratio of protein to DNA[66]. When such rats are raised to adulthood, the males have decreased tolerance to stress; the females show increased aggression. Thus zinc deficiency during critical period of brain growth and development appears to cause irreversible damage which adversely affects behaviour in adult

life. It is unknown whether this effect holds true in humans[66].

The effect of zinc deficiency on carbohydrate metabolism is less clear. However,it has been noticed that in zinc deficiency, there is intolerance to parenteral glucose in rats and humans, while the oxidation of glucose does not seem to be impaired. It has also been suggested that zinc in some ways participate in the uptake of glucose by cells[66].

It has been shown that experimental zinc deficiency is associated with decreased synthesis of retinol-binding protein (RBP) and release of vitamin A from its hepatic stores. Such mechanisms could explain low levels of vitamin A and RBP in alcoholics with liver diseases[67].

In experimental zinc deficiency, the activity of zinc metalloenzymes is decreased in certain tissues, but not in others. For example, a decrease in serum alkaline phosphate activity has been used clinically to diagnose zinc deficiency in humans[68].

Another system particularly sensitive to zinc deficiency is the integument[66]. Zinc-deficient swine develop dermatitis with abnormally high hyaluronic acid content of the skin. Zinc-deficient rats also develop dermatitis and alopecia (baldness). Histologically, hair follicles are decreased and collagenous. Subcutaneous tissues appear less dense. Electron microscopic examination of these tissues show abnormal appearance of collagen and fibroblasts while chemical studies of collagen suggest impaired synthesis and cross-linking, impaired incorporation of methionine into proteins and of thymidine into DNA, decreased thymidine kinase

activity in collagen and abnormal RNA metabolism. The gross effects of these abnormalities is impaired wound-healing. The clinical equivalents of these animal abnormalities in humans include impaired wound-healing, acrodermatitis enteropathica, and dermatitis in infants alimentated parenterally without adequate zinc[66].

Zinc deficiency also has adverse effects on the gustatory system. In rats, a loss of taste acuity, as measured by preference for sodium chloride, occurs within three days and anorexia is evident in two days. Abnormal taste and smell acuity, responsive to zinc supplementation, have also been described in humans. In humans, however, abnormal taste and smell can occur as a result of other unrelated stresses[66].

2.2.4.4. Human zinc nutrition and deficiency.

Etiological factors:

The etiological factors responsible for human zinc deficiency have been identified and these are presented in table 2[17]:

Table 2. Etiological factors in human deficiency[17]

State	Cause
Inadequate dietary intake	Diets low in animal protein, zinc lost in food processing.
Dietary factors inhibiting zinc absorption	Phytic acid, fibre.
Disease states inhibiting zinc absorption	Steatorrhoea (cf. some body zinc is bound to fat *Acrodermatitis enteropathica
Genetic defects in zinc metabolism	Acrodermatitis enteropathica
Excessive loss of zinc	Hyperzincuria eg.in chronic alcoholics, diaphoresis, chronic blood loss.
Increased zinc requirement	Rapid growth, pregnancy and lactation, wound healing.

It now appears that a marginal zinc deficiency may be quite common in paediatric populations - especially in 0 to 6 month babies. It also occurs in infants and children who are ingesting adequate quantities of other nutrients[66], since the risk of zinc

* A lethal inherited zinc deficiency which results in eczematoid skin lesions, diarrhoea, low plasma zinc levels, poor growth and development, malnutrition, intercurrent bacterial and yeast infections and eventual death if left untreated. It usually shows up in infancy upon changing from breast milk to cow's milk. It has been postulated that cow milk contains a peptide that these children can not digest and that chelates zinc and prevents its absorption[69]. The treatment is to provide zinc sulphate in amounts large enough to overcome this chelating effects and allow zinc absorption. Neldner and Hambidge[70] found that zinc supplementation at a level of 22 mg of elemental zinc was enough to cause remission of the disease in an adult.

deficiency is enhanced at the time of increased requirement such as periods of rapid growth, pregnancy and lactation.

In rural areas of Egypt and Iran where zinc deficiency was first identified, dietary factors, especially phytic acid and fibre,that impair zinc absorption, were the major etiological factors[17]. Studies of rats support this concept[66].

Other pathogenic factors that contributed to zinc deficiency are hookworm and schistosomiasis in Egypt and geophagia in Iran and Turkey. High environmental temperatures may be another factor since sweat is rich in zinc- as much as 1 mg. per liter[66].

Conditioned zinc deficiency appears to be a potential problem in patients with alcoholism and/ or cirrhosis, patients with chronic renal disease , patients with severe trauma, patients with chronic infections or inflammatory diseases of the bowel and malabsorption syndrome. The causes of the zinc deficiency are multiple. They include inadequate intake of zinc in diets or parenteral fluids as well as increased losses from the body in association with catabolism, protein deprivation, and PEM in infants[66].

Other potential causes include abnormal loss of zinc due to proteinuria or aminoaciduria and impaired intestinal absorption[66]. It appears that the rapidity of onset of symptoms on zinc-deficient diet is due to the fact that there is no functional body store of zinc since virtually all body store is locked up in bone or protein[5].

Zinc and foetus

Animal studies have emphasised the nutritional importance of adequate maternal zinc nutrition for normal growth and development of the foetus. The Food and Nutrition Board of the National Academy of Science, USA, has recommended a daily intake of 20mg zinc during pregnancy or about 5 mg more than for non-pregnant adult[17]. It has been found that human fetal size correlated with zinc concentrations in amniotic fluid.

A teratogenic role for zinc deficiency in humans has also been suggested on account of high incidence of congenital malformations in the offsprings of women with acrodermatitis enteropathica. In fact the striking effects of experimental zinc deficiency in rats prompted speculations that the greater incidence of congenital abnormalities in certain developing countries is due to habitual consumption of diets low in available zinc[17].

Studies on zinc nutrition in pregnancy are hampered by lack of established biochemical indices of zinc status. Plasma zinc levels are normally depressed during pregnancy due to raised levels of circulating estrogens[17]. It is also known that the causes of maternal mortality in developing countries include excessive bleeding and hypertension, both of which are associated with zinc deficiency[66].

Zinc and the infants.

In well nourished populations,neonatal tissue concentrations of zinc are similar to those of adult even though the young infant

does not have stores of zinc in contrast to those of iron and copper. The Food and Nutrition Board recommended a daily dietary intake of 3 mg zinc from 0-6 months, and 5 mg from 7-12 months[17].

There is reason for concern that zinc requirements may not be met adequately in formula-fed infants, at least after the first two months or so of lactation. This is because most of the zinc in milk is associated with the protein fraction. Thus when the protein content in cow milk is reduced during the preparation of infant milk formulas, to approximate that of human milk,there is a corresponding reduction in zinc content. Hence the final zinc concentration of these formulas are generally less than 2mg/L unless supplemented with additional zinc[71].

It has been shown that zinc levels of breast-fed infants were significantly greater than those of formula-fed infants even when the concentration of zinc in the formulas was about three times that of breast milk[71]. Zinc in infant formulas is added as inorganic salts while in breast milk the zinc is bound mostly to high molecular weight proteins; thus it is not only the amount of zinc but also the molecular localisation of zinc in milk that affects the extent of absorption[71].

Zinc deficiency in infants may also occur in association with protein-energy malnutrition. Hypozincemia in the acute state may be due to hypoproteinemia and to superimposed infections[17].

Zinc and preschool children

This is an age group that both merits and has received

considerable priority in nutrition surveys and nutrition intervention programmes but it is only recently that studies have included biochemical indices of zinc nutritional status.

It has been observed that the levels of zinc in plasma and hair in preschoolers in some countries such as USA were low as compared to those of older children and adults[72]. Though these lower levels may be in part physiological at a time of rapid growth, similar low levels had not been observed in countries of Europe and Far East[73]. One factor responsible for the low zinc in American children may be the low zinc content of some popular infant formulas, prior to their recent supplementation with zinc through legislation.The majority of the children with low zinc status were also of low height percentile.

The reduced zinc intake particularly among growing children is accompanied by low hair zinc content, anorexia, stunting and lowered taste acuity[20]. Functional disorders appear in children with hair zinc content below 70 ppm[20]. It has also been suggested that plasma zinc levels below 70 μg per 100 mL indicate hypozincemia[67].

Results of study on zinc nutrition of preschool children in Denver Head Start Programme indicated that inadequate zinc nutrition may be common among preschool children from low income families[16].

Zinc in School children and adolescents.

Many studies have revealed an association between low zinc levels and low growth percentiles in school children considered as

healthy. Some of these children had impaired taste acuity. Dietary supplementation with 0.2 mg zinc per kg body weight per day was followed within a few months by normalisation of taste perception and a suboptimal increases in hair zinc content. Thus it appears that suboptimal zinc deficiency may not be uncommon in otherwise healthy school-age children[16,72].

The first report of human zinc deficiency was related to the syndrome of adolescent nutritional dwarfism in Egypt and Iran. Since then, additional studies have identified marginal zinc deficiency in preschool and school children in several countries[20,71,74,75]. The main features of this syndrome were growth retardation, and failure of sexual maturation.

Numerous tests have shown that these adolescents were zinc-deficient and zinc therapy under controlled conditions led to the conclusion that zinc deficiency was a major factor responsible for retarded growth and delayed sexual maturation. Other features of zinc depletion included anorexia, lethargy, dry and roughened skin[17].

It is possible that a variety of factors are responsible for zinc deficiency states in different areas of the world. In Iran, the major factor is the large quantities of phytic acid and fibre present in the unleavened bread that is a major dietary staple in rural areas. These dietary components inhibit zinc absorption through formation of insoluble zinc phytate[17].

Zinc and the adult.

Once growth and development are complete, zinc requirements are comparatively lower and the effects of zinc deficiency, except during pregnancy, are likely to be less severe[17]. Nevertheless, a number of zinc-responsive syndromes have been reported in adults . For example, zinc supplementation has been widely used in surgical patients to promote wound healing. Conflicting reports on the efficacy of such therapy are probably due to the fact that the patients were not selected on the basis of their zinc status[17].

Wound healing is impaired in the zinc-deficient animals and in humans with low plasma levels and healing is accelerated with zinc therapy. Similarly improvements in the rate of healing of venous leg ulcers have been observed with zinc therapy, but only in patients with hypozincuria. Zinc therapy has also been reported to accelerate the rate of healing of benign gastric ulcers[17].

In a study of the zinc status of elderly black Americans from urban low-income house-holds, Wagner et al found that eleven percent of 135 subjects had hair zinc levels less or equal to 70 μg/g or serum zinc concentration less or equal to 70μg/g and suggested that the zinc status of this elderly population may be less than ideal[76].

In summary , it is apparent that zinc is not only essential, but because it is involved in so many important physiological processes, it may even be the "first limiting factor" in these processes. This means that it could be the critical limiting factor in the diet and the consequences of zinc deficiency are likely to

be extensive, if not catastrophic for the organism.

2.2.4.5. Zinc Toxicity

Although the consequences of zinc deficiency have been recognised for many years, it is recently that attention has been directed to the potential consequences of excessive zinc intakes.

Zinc is considered to be relatively non-toxic, particularly if taken orally; however, manifestations of overt toxicity symptoms such as nausea, vomiting, epigastric pain, lethargy and fatigue will occur in high intakes[77]. At low intakes, but at concentrations well in excess of RDA (ie. 100-300 mg zinc/day vrs.an RDA of 15 mg/day) evidence of induced copper deficiency with attendant symptoms of anaemia and neutropenia as well as impaired immune functions and elevated ratio of high density lipoprotein(HDL) to low density lipoprotein(LDL) have been reported[77]. Even low levels of supplementation, close to RDA, have been suggested to interfere with the utilization of copper and iron and to adversely increase HDL[77].

It has also been shown that the form of zinc ingested may influence the ensuing symptoms. For example, an emetic dose of 1-2g. zinc sulphate (corresponding to 225-450mg elemental zinc) caused nausea, vomiting, epigastric pain, abdominal cramps and diarrhoea or dysentery while ingestion of 12g of elemental zinc produced lethargy,light-headedness, slight staggering of gait and difficulty of writing; all symptoms disappearing with chelating therapy; interestingly, no gastrointestinal symptoms were

reported[77].

2.2.4.6. Assessment of zinc status in man

Several analytical methods involving the use of flame or flameless atomic absorption spectrometry have been used to determine body zinc status from biological tissues and fluids such as plasma, serum, whole blood, erythrocytes, urine, hair,and saliva[78-82].

1. Plasma and serum: Plasma and serum have been most widely used samples, but such results are greatly affected by various states such as acute infection, stress, myocardial infarction, and physical exertion; thus a low plasma or serum zinc concentration per se, does not necessarily reflect the actual body zinc status[83-85]. It is therefore desirable to carry out determinations on a variety of biological tissues or fluids such as plasma or serum, erythrocytes, hair and urine to arrive at the zinc status in an organism[84].

2. Erythrocytes and hair: The turnover of zinc in erythrocytes and hair is slow and as such lowered values in these materials would be indicative of a long-term deficient state. On the other hand the turnover in plasma or serum is relatively fast hence a low value here would reflect a current zinc deficiency , but as explained earlier, the level is affected by a variety of factors[84].

3. Neutrophils: Neutrophils have a high zinc content and a short life-span, so acute zinc status might be expected to be reflected more promptly in these cells[84].

4. Urine zinc: A decreased excretion of zinc in urine appears to be a valuable indicator of zinc deficiency in man; however, hypozincuria may be indicated in other conditions such as liver cirrhosis and in sickle cell disease[85].

5. Metabolic balance studies: Measurements such as metabolic balance study, turnover rates and 24-hour exchangeable pool for zinc using zinc-65 may provide additional tools for assessing zinc status in man[17].

Zinc balance studies also provide a good basis for assessing the zinc status. A positive retention of zinc would be indicative of zinc deficiency. Using zinc-65, it was shown that in zinc deficiency, plasma turnover was increased, the 24-hour exchangeable pool was decreased and the cumulative excretion of zinc-65 in urine and stool was low[85].

6. Zinc metalloenzymes: Certain zinc metalloenzymes in blood may be used for assessment of zinc status in man. The activity of Alkaline phosphatase was found to be significantly decreased in serum or plasma of zinc-deficient rats, pigs, dairy cows, calves, chicks and swine[85]. Consequently, serum Alkaline phosphatase activity has been shown to increase following zinc supplementation to zinc-deficient dwarfs in the middle East[8].

7. Carbonic anhydrase: Studies of sickle cell disease subjects revealed that a significant number of these patients are zinc deficient and show an increased activity of ribonuclease in the plasma[86].

8. Plasma ribonuclease: Studies of sickle cell patients revealed that a significant number of these are zinc deficient and show an increased activity of ribonuclease in the plasma. Zinc is an inhibitor of ribonuclease and an increased activity of this enzyme has been observed in zinc-deficient tissues of experimental animals; thus, measurements of plasma ribonuclease may provide another parameter for assessing zinc status in man[86].

9. Urinary sulphate excretion following an ingestion of S^{35}-cysteine to zinc-deficient rats is enhanced due to the fact that rats utilise sulphur-containing amino acids for proteosynthesis[87]. It remains to be tested whether such an approach will be fruitful in man[87].

2.2.4.7. Estimation of zinc concentration

Zinc has been determined colorimetrically using dithizone[88,89] and fluorimetrically as a 8-hydroxyquinoline complex stabilised by gum arabic at ph 8 with activation at 375nm and emission at 517nm[90].

The method of choice now is atomic absorption spectrometry[91,92] which has several advantages, such as, simplicity, increased sensitivity, and accuracy, over the old traditional methods. A characteristic absorption at 213.8nm provides sensitivity to < 1.5 μmol/ L (0.1mg/L) with excellent accuracy and precision[22]. Also, provided the instrument is fitted with a high solid burner such as the Boling 3 slot burner which allows direct aspiration of high solid-containing samples without clogging, determination can be carried out directly on suitably diluted samples of serum or urine.

The increased viscosity of the test compared to the aqueous standards can cause some matrix problems. Parker et al., and Hackley et al., overcame this problem by preparing standards in albumin (40g/L)[91] and dextran (30g/L)[92], respectively.

Preparation of other samples for analyses involves combustion of organic material (wet or dry ashing) ,followed by solubilization of the ash in acid. Cells and organelles can be hydrolysed in base such as 0.2 mol. NaoH/L before atomic absorption analysis[22].

Fluids are usually diluted with water or dilute acids to appropriate concentrations, without ashing, before analysis. X-ray emission spectroscopy is gaining increased attention as a semi-quantitative method to subcellular zinc localization and measurement[22]. Atomic absorption spectrophotometry has been used in the present work to determine zinc concentration in hair, erythrocytes and plasma.

2.2.5. Variables to be measured and justification for their choice

2.2.5.1. Variables

i. hair zinc
ii. alkaline phosphatase activity
iii. total plasma protein
iv. albumin
v. plasma and erythrocyte zinc

2.2.5.2. Justification

Right now, there is no single laboratory measurement that

reliably reflects zinc status. However, it has been suggested that the determination of the zinc concentration in two or more tissues, such as serum, plasma, erythrocytes, hair and/ or urine should provide a better data-base from which zinc nutriture can be evaluated[93,94].

(i). Zinc status based on hair zinc erythrocyte and plasma zinc analysis:

The use of hair for the evaluation of human zinc nutriture remains somewhat controversial[94-96]. It has been suggested that contamination from exogenous sources[94], beauty treatments[95,96], and variable hair growth rates, may complicate the interpretation of hair zinc data. However, evidence from animal studies demonstrates that hair zinc concentration reflects chronic or long-term zinc nutriture and that it correlates with the zinc content of bone and diet[9,97].

Flynn et al[98]., have emphasised that hair can be very useful in nutritional assessment if attention is paid to proper sampling techniques and appropriate statistical analysis.

It is generally agreed that homogeneity in the distance of the sample from the scalp is an important factor and that the hair closest to the scalp should be sampled for zinc analysis. This newly-grown hair is presumably more reflective of current zinc metabolic status and is less subject to contamination from exogenous sources[98].

Since grease, dust and other foreign bodies on the surface of

hair may contribute most of the zinc of hairs, the hair must be washed. Even though the importance of a standardised washing procedure is widely acknowledged, different laboratories use different washing procedures and there is no consensus on how the washing should be done[99]. For example. the following washing solutions with variable volumes and washing times, have been used: distilled water, ionic or nonionic detergents, combinations of aqueous detergents and organic solvents, chelating agents and even mineral acids[99]. The effects of these washing agents/ procedures have been stated to differ. Moreover, mammalian hair may continue to loose material for an indefinite number of washes. Thus the problem in hair analysis is not the reliability of the measurements but preparation of the sample ie. sampling, washing agents, length (time) of washing, and number of successive washings. These could be the source of discrepancy between results of various workers[99].

The use of scalp hair has the advantage of non-invasiveness, ease of collection, preservation and transport (including airmail postage to distant specialised laboratories)[99].

A depressed level of circulating zinc in plasma or serum has been associated with human zinc deficiency[16,100,101], but circulating zinc levels are not always a reliable index of zinc nutriture[93,94,100]. One must rule out any haemolysis, hypoproteinemia, heritable hyperzincemia, or redistribution of zinc from plasma to tissues or from tissues to plasma, in order for this parameter to be an accurate indicator of zinc status[93,94]. Due to the high zinc content of the erythrocytes, any slight haemolysis would add zinc to serum.

Beside, numerous factors, as indicated earlier are known to influence the concentration of zinc in plasma and for that matter serum[94,102-105]. Despite these drawbacks, serum/plasma zinc and hair analysis are the indicators most often employed in zinc status assessments[16].

Whitehouse et al[84],have proposed that zinc in other tissues such as erythrocytes and leucocytes may more closely reflect body-zinc status. Since erythrocytes have an average lifespan of 120 days, data on zinc concentrations in erythrocytes have mainly been useful for indicating long-term zinc status.

(ii). Plasma total protein and albumin:

Zinc in plasma is distributed between two major fractions-about 60% are loosely bound to albumin and the rest firmly bound to globulin and other cellular components.

It has been shown that as a result of modifications of cow's milk to produce infant formulas in which the original high protein level is reduced to equivalent amounts present in human breast milk, these infant formulas become zinc-deficient. In animal tissues, zinc is carried as a zinc-protein complex, so any deficiency of protein could affect the amount or availability of zinc to tissues. Indeed, work by Lonnerdal et al.[104], revealed that in human milk, 14% of zinc is complexed with casein, 28% with serum albumin, 29% is associated with low molecular weight proteins such as metallothionein[105], while the rest, that is 29%, is associated with fat.

Following oral administration of zinc-65, the isotope is associated exclusively with albumin which suggests that albumin transports zinc from the intestinal cells[17]. Thus any deficiency of protein and/ or albumin could lead to zinc deficiency.
It is therefore important to measure plasma proteins and albumin levels in any studies on zinc nutriture. The determination of albumin/globulin ratios will also give an indication of the protein nutrition status of the subjects.

(iii). Alkaline phosphatase activity:

Many studies have shown that serum Alkaline phosphatase activity responds rapidly to zinc depletion through a significant reduction of its activity[17,106]. In the studies by Roth and Kirchgessner[106], this enzyme lost 27% and 48% of its activity within two and four days, respectively, after the zinc-deficient diet was initiated.

In limited studies, however, either there was no reduction in activity or there was no significant difference in the enzyme activity in zinc-deficient or zinc-supplemented pair-fed control rats[107,108].

An accurate determination of Alkaline phosphatase activity may therefore give invaluable clue about the zinc nutritional status of the cohorts. It must be noted that alkaline phosphatase activity can be affected by many factors unrelated to zinc status.

CHAPTER THREE

METHODOLOGY

3. Study design and plan of work.

3.1. Study population:

Children attending nursery/kindergarten school in four communities: Kwabenya village, Ashalley Botwe village, Dome and New Achimota, constituted the reference population. These villages were chosen because they are easily accessible by road. Most of the target group attend the nursery schools in which the studies were carried out. These conditions made the project manageable within the limit of the available resources.

3.2. Subjects selected for the study:

Children aged 3 to 5 years attending nursery/ kindergarten in the four communities were recruited for the study. All the children were used since none showed any overt signs of infection such as pneumonia, measles, or upper respiratory tract infection.

Informed consent of chiefs/assembly men/parents/ guardians/teachers were sought for children participating in the study.

3.3. Study areas:

The study was carried out in the nursery/ kindergarten schools in the four communities with different socio-economic status, judged by the number of sandcrete houses, aluminium/zinc-roofed houses and infracstructural developments such as places of

convenience, and potable water. This was to determine if zinc status was dependent on socio-economic status. Accordingly, two of these communities (Ashalley Botwe and Kwabenya) are typical villages; Dome is peri-urban while New Achimota is almost urban.

The socio-economic status can therefore be ranked as follows: New Achimota > Dome > Ashalley Botwe > Kwabenya village.

The estimated total population of the 4 communities was about 3500 of which about 15% are within the age group of 3-5 years. Depending on the community, the adult inhabitants (ie, parents/ guardians of the cohorts) are either farmers, artisans, taxi drivers, teachers, architects, engineers, and medical officers, who work in Accra. There are no health centres in the first three communities (ie. Ashalley Botwe, Kwabenya, Dome); the inhabitants depend on native doctors as well as the clinics at the Ghana Atomic Energy Commission, Madina or Legon.

3.4. Plan of work:

The correct ages of the subjects were mostly ascertained from health or weighing cards (99%) or from mothers' interview(1%). Children who fell within the ages of 3-5 years, were recruited for the study:

3.4.1. Anthropometric measurements.

Standing heights were measured, using a stadiometer. Height was measured twice - the second measurement being after repositioning of the subjects.

Weights were measured using a bathroom scale. Scales were calibrated daily with a 10kg standard weight and zeroed before each

measurement, when necessary. Mid-upper arm circumference (MUAC) was measured (to the nearest 0.1 cm) on the left arm using tapes. The tape measure used was a non-stretchable polyvinyl-coated fibreglass. Triceps and subscapular skin fold thicknesses (in duplicate to the nearest 0.1mm) on the left arm were measured with Harpenden skinfolds calipers. Triceps skinfolds and MUAC were both measured half-way down the left arm between the tip of the acranial process of the scapular and the olecranon process of the ulna.

3.4.2. Sampling of hair:

Hair samples (about 200-400mg) were cut from all the participating children for analysis for hair zinc concentration.

3.4.3. Blood collection:

Five (5) mL of blood were collected via venipuncture of all the participating children for the following analyses: plasma total protein, albumin, alkaline phosphatase activity, erythrocyte and plasma zinc.

3.5. Laboratory analyses:

3.5.1. Determination of hair zinc

3.5.1.1. Sampling of hair:

Head hair was combed out and the top cut off uniformly until it was easy to comb using a selected gauge of fine comb. About 200- 400mg of the hair were then cut close to the scalp, in the occipital region, onto white sheets of paper (using a pair of stainless steel scissors), and collected into thin plastic bags, labelled, sealed, and brought back to the laboratory. Samples were

stored in a desiccator until processed for analysis.

3.5.1.2. Washing of Hair

The hair samples were individually treated as follows[109]: Samples were introduced into labelled 100 mL acid-washed plastic containers. Hexane (100 mL) was added and tubes transferred to mechanical flask shaker(Yamato shaker model SA-31), and shaken for 20 minutes, using the highest speed. After pouring out the hexane washings, the hair was washed in succession with 100 mL of 95% ethanol and three changes of reagent water. The water was an ultra pure deionised water obtained by passing tap water through millepore filters. The hair samples were then removed onto filter papers in acid-washed plastic petri dishes , blotted with acid-washed filter paper and air- dried at room temperature for 30 mins. The samples were finally dried in an oven at 70°C for 10 mins. After cooling in a desiccator over silica gel , the samples were transferred into thin plastic bags, labelled and stored in a desiccator until used for assay.

3.5.1.3. Wet-ashing of hair and analysis by AAS:

Dried hair samples, weighing between 15 to 15.7 mg, were weighed for each sample in duplicate into acid-washed test- tubes and 1.5mL of a digest mixture (of 1 part of 60-70% perchloric acid and two parts of 30% hydrogen peroxide) added and samples placed in an oven at 70°C for one hour, to solubilise the hair[110]. After cooling, 5 mL of reagent water were added, vortex-mixed and stored at 4°C until used for zinc analysis by atomic absorption

spectrometry (Shimadzu Atomic Absorption/ Flame emission spectrophotometer AA-630-12: AAS) at the following settings:

Wavelength =213.9 nm

Slit width: 1.9 A°

Acetylene gas flow rate: 10 L $min.^{-1}$

Air flow rate: 2.4 L $min.^{-1}$

Burner height: 4 mm.

All the necessary precautions, including an overnight soaking of containers in 2M hydrochloric acid, use of special deionised water and preferential use of plastic containers to glass containers, were taken.

3.5.1.4. Quantification of zinc:

A calibration curve was prepared from a commercial zinc standard (1000ppm) containing 0, 20, 40, 60, 80 and 100 μg Zn/ 100 mL in perchloric acid/hydrogen peroxide mixture. The best line was fitted by regression and used to calculate hair zinc concentration. Each determination was in duplicate on two samples of the same hair for each child. The coefficient of variation (CV) was determined by analyzing 10 replicates of the same hair sample.

3.5.2. Determination of plasma and erythrocyte zinc:

3.5.2.1. Preparation of plasma and erythrocytes[84]

Blood (5 mL) was withdrawn by venipuncture with a disposable needle(Nipro;21G x 1.5) fitted to polypropylene syringe (10 mL).

The needle was removed and blood introduced into acid-washed polypropylene tubes, containing 60 units of zinc-free sodium heparin. The tube was inverted gently several times to mix and then placed on ice and transported to the laboratory, where it was centrifuged at 2250 x g for 15 minutes at 4°C.

The plasma was transferred into 2 mL polypropylene tubes using acid-washed Pasteur pipette, capped, and promptly frozen at -30-40°C. The erythrocytes were resuspended in 10 mL of 9g/L cold saline and stored at 4°C for subsequent washing, the next day.

3.5.2.2. Erythrocyte zinc analysis:

The packed erythrocytes were washed three times by resuspending the cells in 10 mL of cold 9 g/L saline and centrifuging (2250 x g for 15 min. at 4°C). After each centrifugation, the saline was aspirated with pasteur pipette and 10 mL of fresh cold saline added and cells resuspended by gentle inversion several times[84]. After the final washing, the tubes were flicked gently about four times. A volume (200 μL) of washed cells was wet- ashed in 6 mL of the digest mixture for 1 hr. at 70°C. Thereafter, the clear solution was transferred into acid-washed 10 mL volumetric flask, and the volume made up to the mark with the reagent water and used for zinc determination using Shimadzu Atomic Absorption Spectrometer, as in 3.5.1.3. Triplicate determinations were made as well as the coefficient of variation.

3.5.3. Determination of Plasma zinc levels.

Plasma was diluted with distilled water in ratio of 1:4 and the zinc levels determined using AAS as outlined earlier. A

standard graph was prepared as before with the exception that zinc solutions were made up in 5% glycerol,to cater for matrix effect[76]. Duplicate determinations were made.

3.5.4. Determination of Plasma total protein:

This was done using Biuret reagent method of Weischselbaum[111]. Determination were done in triplicate using the following scheme:

	blank	standard	test
Biuret reagent(mL)	4.0	4.0 m	4.0
Water	1.0	-	-
Plasma(1:9 dilution)	-	-	1.0
Standard protein-BSA	-	1.0	-
Total volume (mL)	5.0	5.0	5.0

The content of each tube was mixed using vortex mixer and allowed to stand at room temperature, with occasional mixing, for 30 minutes and the optical density measured at 550nm using a Shimadzu spectrophotometer against the reagent blank, which was used to zero the instrument. Determinations were carried out in triplicate.

A standard graph was drawn from measurements of solutions containing 2.0, 4.0, 6.0, 8.0, and 10.0 mg BSA/mL and the best line fitted by regressing analysis and then used to compute the concentration of the test plasma. Percentage recoveries and CV were determined.

3.5.5. Albumin determination

This was done using Bromo Cresol Green method (BCG)[112]: Plasma (25μL) was added to 5mL of the working BCG dye solution, mixed and the optical density measured within 30 seconds, at 630nm using a Shimadzu spectrophotometer against a reagent blank. Standard graph using BSA (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0g/dL) was prepared and best line fitted by regressing equation and used to compute albumin levels of the test plasma. Determinations were carried out in triplicate. The percentage recovery as well as the CV, were determined.

3.5.6. Measurement of Alkaline phosphatase activity (ALP):

This was determined using the recommended method of the German Society on enzymes[36]. The conditions of reaction were:

Buffer: Diethanolamine(DEA), 1.0 mmol L^{-1}

ph: 9.8

Reaction temperature: 30 $\pm$ 1^{0}C

Substrate concentration: p-Nitrophenol phosphate, disodium salt, (p-NPP), 10 mmol L^{-1}

Mg^{+2} : 0.5 mmol L^{-1}

Reaction volume: 3.027 mL

Fraction volume: 0.009

Plasma was removed from storage and kept overnight at 4°C for 10- 12 h. to reactivate alkaline phosphatase[113]. Three (3) mL of freshly-prepared substrate buffer, prewarmed to 30°C were introduced into a cuvette and used to zero the spectrophotometer

set at 405 nm.

Plasma (27 μL) was added and quickly mixed by placing cellophane seal at the mouth of the cuvette and inverting it for about 4 times. A timer(stopwatch) was simultaneously started. The outside of the cuvette was quickly wiped dry and then returned into the spectrophotometer. The absorbance was immediately taken and then at 30 seconds interval for a total of six minutes.

For the sample blank, 3 mL of the neat buffer and 27 μL of plasma were mixed in a cuvette and the absorbance measured. For the solvent blank, the absorbance of substrate-buffer and 27 μL of deionised water was measured. Duplicate measurements were initially made(for about thirty samples), but since it was found out that there were no significant difference between the readings, single determinations were made, thereafter. This was also necessitated by the fact that the quantities of p-NP, p-NPP and DEA, which had to be borrowed, were limited. The cv was measured as before.

Calculations:

Catalytic activity of ALP (U L^{-1}),

$$= \frac{V}{\epsilon \times l \times v} \times (\Delta A/\Delta t) \quad ....1$$

where V is reaction volume (L), ϵ is the micromolar absorbtivity (L x μmol^{-1} x cm^{-1}), l is pathlength of cuvette (cm), v is sample volume (L), Δ A is the change in absorbance, and t is the reaction time (min).

Since the micro molar absorptivity must be determined in each laboratory in order to obtain good results[114], the following procedures were used[115]:

p-Nitrophenol was crystallised from boiling water, air-dried at room temperature for 30 min. and then stored in a desiccator over silica gel for a week to obtain dry crystals.

1 mmol/L solution of p-nitrophenol(pNP) was prepared by dissolving 139.1 mg of dried pNp in distilled water and making up the volume to 1L with distilled water. Twenty (20) mL of this solution were transferred into a 500mL volumetric flask and the volume made up with 10 mmol L^{-1} sodium hydroxide solution. A second solution was made using DEA buffer to make up the volume, just for comparison. The absorbance of the two solutions were measured at 405nm using either neat sodium hydroxide or neat DEA as blank.

3.5.7 Statistical analysis of results.

Lotus 123 was used to calculate the values of all the biochemical parameters measured using standard graphs. This was done for protein, albumin, plasma zinc, hair zinc and rbc zinc.

Data were entered into the computer using D-base. For the anthropometric classification of the cohorts, WHO/NCHS EPINFO/EPINUTR. programme was used to classify the cohorts into the various nutritional states. Thereafter, SPSS statistical package was used to calculate the various values of the parameters measured, and then used to group these into the appropriate nutritional states.

Multiple range (comparison) tests , using Duncan's and LSD methods were used to compare means. SPSS packages were used to derive regression equations as well as establishing correlation coefficients. Harvard graphics (hg3) was used to draw the bar (histograms) as well as the line graphs.

CHAPTER FOUR

RESULTS

4.1. Age distribution of the cohorts

The age distribution of the cohorts used in the present study is depicted in figure 1a. Of the 200 cohorts, 3% (6/200) were below 36mo; 20% (40/200) were 36-47mo; 44.5% (89/200) were 48-60mo; while the rest, 32.5% (65/200) were above 60mo., but below 72mo.

4.2. Anthropometric classification of cohorts:

The basis of classification of the cohorts was as follows: Normal= weight-for height and height-for Age are both > -2SD of the NCHS reference standards; Wasted= weight-for-height is < -2SD of the reference standards; Stunted= Height-for age < -2SD of reference standards; Wasted plus stunted= weight-for-height and height-age are both <-2SD of reference standards. This scheme is similar to the classification scheme proposed by Waterlow[116]. The results are indicated in Table 1 (see appendix).

Of the 200 cohorts screened, 78.5% (157/200) were of a normal nutritional status, 3.5% (7/200) wasted, while 16.5% (33/200) were stunted and 1.5% (3/200) both wasted and stunted.

The breakdown by communities indicated that 69.6% (39/56), 81.2% (26/32), 80.9% (55/68) and 84.1% (37/44) of the cohorts in Ashaley Botwe, Kwabenya, Dome and New Achimota, respectively had normal nutrition. In the same sequence, 25% (14/56), 18.8% (6/32), 13.2% (9/68) and 9.1% (4/44) were stunted, while 3.6% (2/56), 0%, 4.4% (3/68) and 4.5% (2/44) were wasted. In Ashalley Botwe, 1.8%

(1/56) of the children were wasted plus stunted; the corresponding figures for Kwabenya, Dome, and New Achimota were 0%, 1.5% (1/68) and 2.3%(1/44). These are presented graphically in figure 1b.

When the growth status was assessed by age (fig 2a), it was seen that in children below 3 years (n=6), none was wasted or wasted plus stunted; 83.3% had normal growth, and 16.7% were stunted. In the 36-47mo. old children,(n=40), 87.5% were of normal growth and 12.5% stunted. In the oldest children,ie those above 60mo.(n=65), 66.2%, 3.1%, 26.6% and 4.1%, respectively were normal, wasted, stunted and wasted plus stunted. The corresponding figure was 83.1%, 5.6%, 11.2%, and 0% for the 48-60mo. old group.

Fig 2b depicted the trends in the skinfolds thicknesses (triceps and subscapular) and the mid- upper arm circumference. The mean triceps skinfolds, for the 200 cohorts, was 8.2 $\pm$ 1.8mm with values ranging from 7.2 $\pm$ 1.0mm in New Achimota to 8.8 $\pm$ 1.8mm in Ashalley Botwe. Subscapular skinfolds was 5.7 $\pm$ 1.2mm, with values ranging from 5.2 $\pm$ 0.8mm in New Achimota to 6.0 $\pm$ 1.4mm in Ashalley Botwe. The mean MUAC was 16.1 $\pm$ 1.0cm, ranging in value, from 16.1 $\pm$ 1.0cm in Ashalley Botwe to 16.2 $\pm$ 1.0cm in New Achimota.

Fig 1a Age distribution of cohorts in the various communities

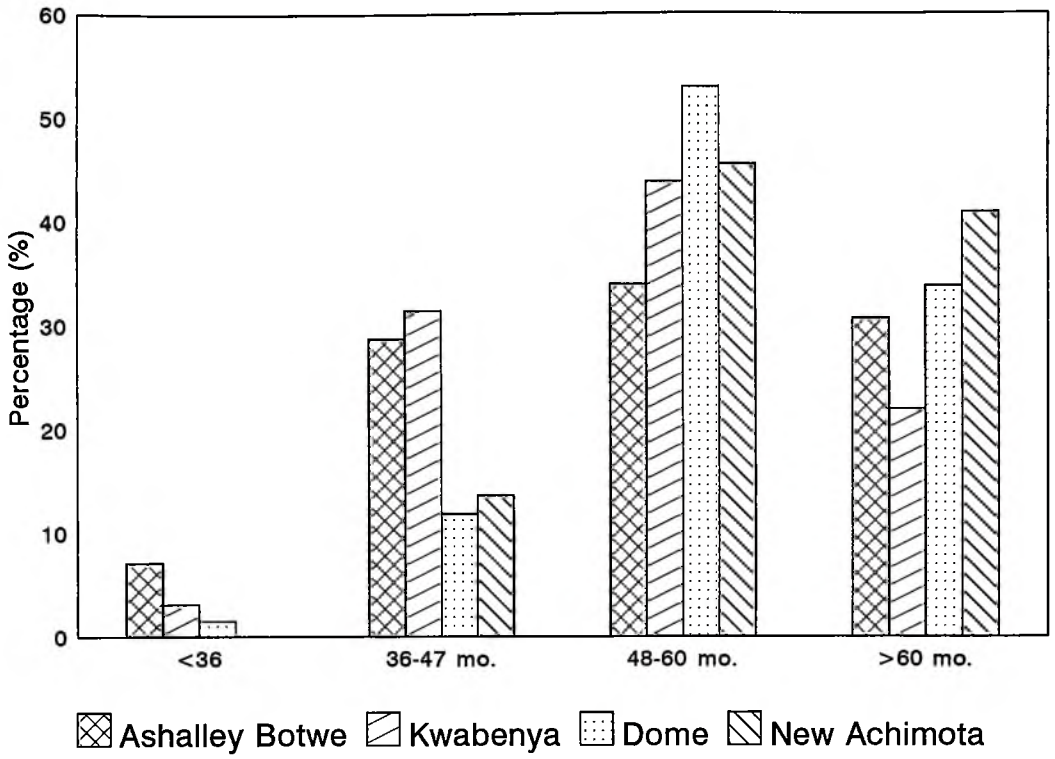
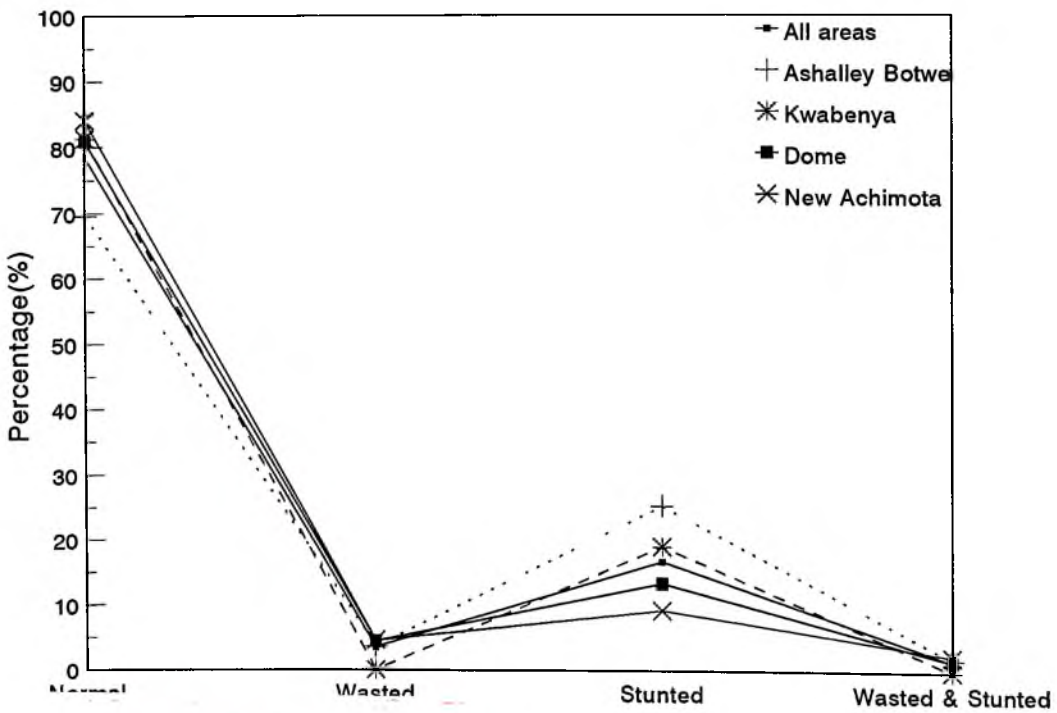


Fig 1b Level of Malnutrition (wasting, stunting, wasting plus stunting) among preschoolers in communities studied



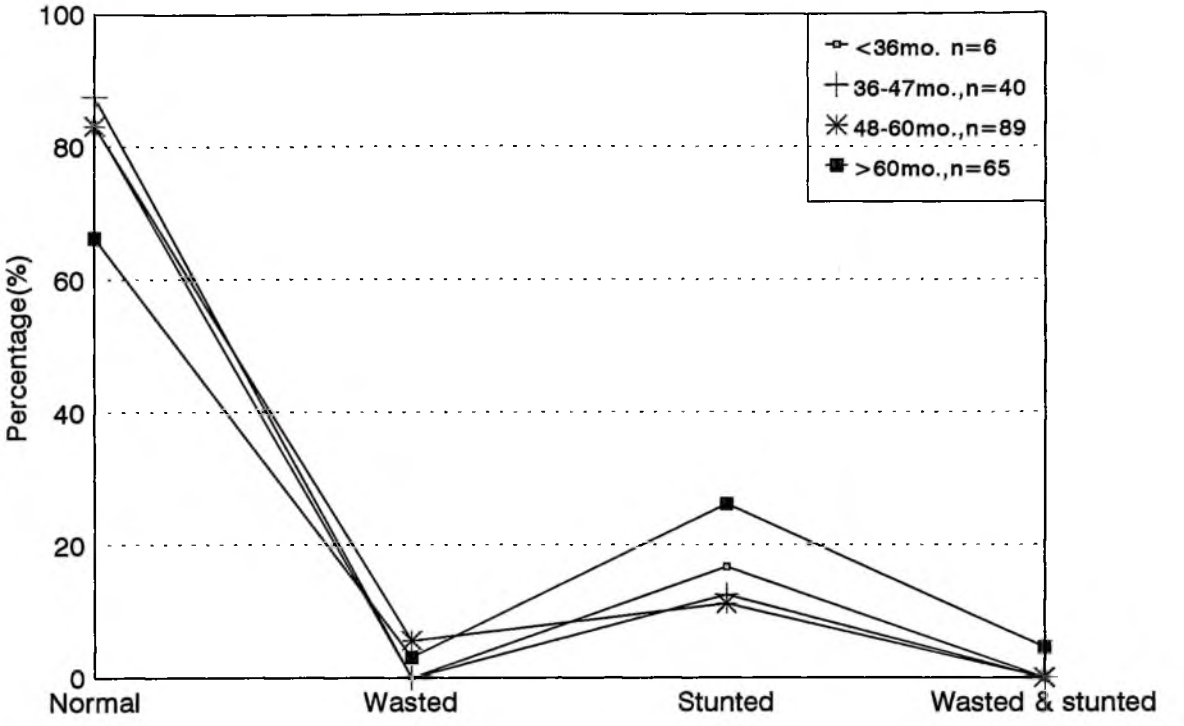
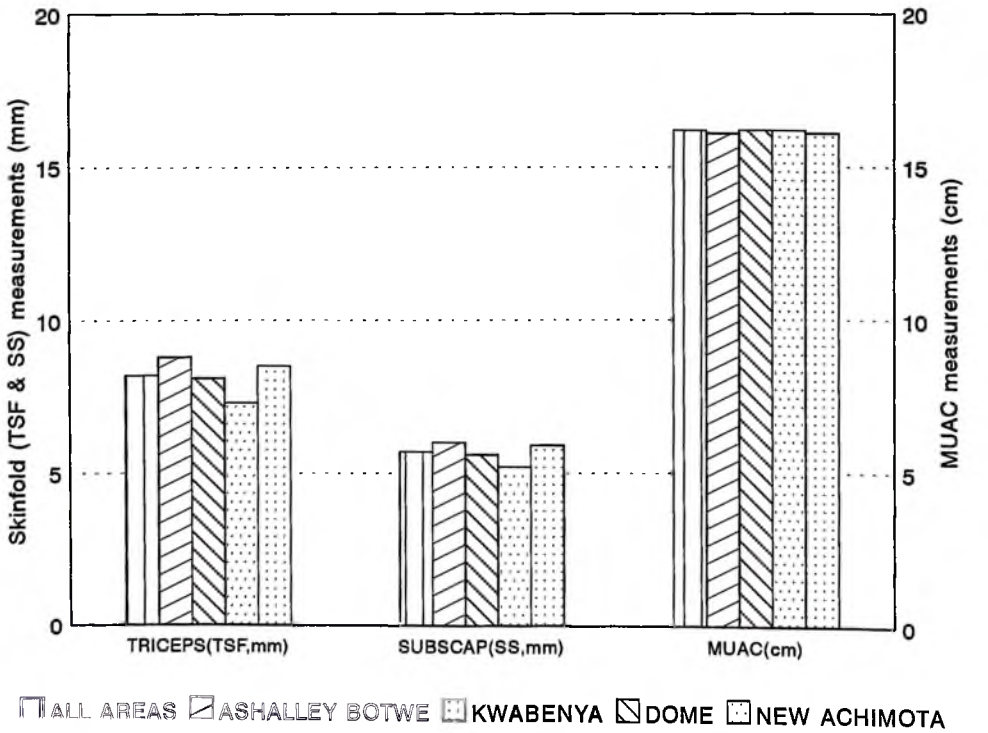


Fig 2b Skinfold and mid-upper arm circumference measurements of cohorts



4.3. Biochemical data based on nutritional status:

The biochemical data of the cohorts were matched for the various types of growth/nutritional status to indicate whether there were any significant differences in the biochemical data that could explain differences in the growth/nutritional status. These values are shown in table 2 and figs. 3a-6b.

When the mean values of the indicators of zinc nutriture (hair zinc, rbc zinc, plasma zinc, and alkaline phosphatase activity) were compared to the reference values, (table 2), it was seen that they all fell within their reference range except for the rbc zinc for the stunted groups of Kwabenya and Dome and the wasted plus stunted group in New Achimota, where they fell slightly below their reference values. No significant differences ($p>0.05$) were however found between them and those of the normal groups. This indicated the absence of zinc deficiency in all cohorts studied.

The analyses of the data for the "All areas combined" group, using Duncan's and LSD multiple comparison tests indicated that there were significant differences ($p< 0.05$) in the weight, height,triceps and mid upper arm circumference (MUAC) levels in the various types of growth; however, there was no significant difference ($p>0.05$) in the case of proteins, albumin, alkaline phosphatase activity (ALP), rbc zinc levels, plasma zinc, hair zinc or A/G ratios.

The trend was basically the same when the data were broken down into communities. Thus, in Ashalley Botwe, Dome and New

Achimota, there was a significant difference ($p<0.05$) in the weight, height, triceps, and MUAC levels, while no significant differences existed in the case of proteins, albumin, A/G ratios, ALP, rbc zinc, plasma zinc, and hair zinc levels.

In the determination of the various biochemical parameters, the accuracy of the analytical procedures were evaluated using basically two approaches. For hair, plasma and rbc zinc determinations, a standard bovine liver (# 1577b; kindly donated by the National Institute of Standards and Technology, USA) was analyzed alongside these analyses anytime analyses were done. The average zinc level in this sample was 130$\pm$ 5ppm which compared favourably with certified value of 127$\pm$16 ppm.

For the protein and albumin, percentage recoveries were carried out. Recovery ranged from 95-101% for protein and 96-100% for albumin. For the precision, the coefficient of variation ,(cv), of 10 replicate determinations was carried out. The respective values for protein, albumin, hair zinc,plasma zinc, rbc zinc and alkaline phosphatase activity were 9.4%, 7.7%, 5.1%, 8.1%, 7.0%, and 10.0%.

Fig 3a Trends in the mean plasma protein, albumin, and A/G ratios in the various communities

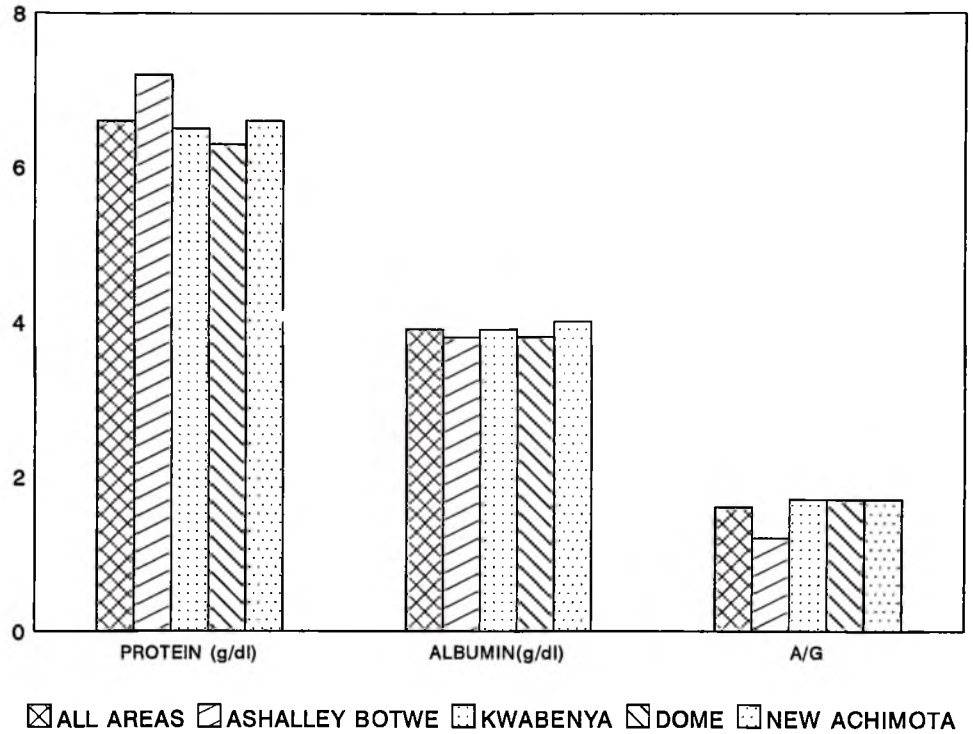
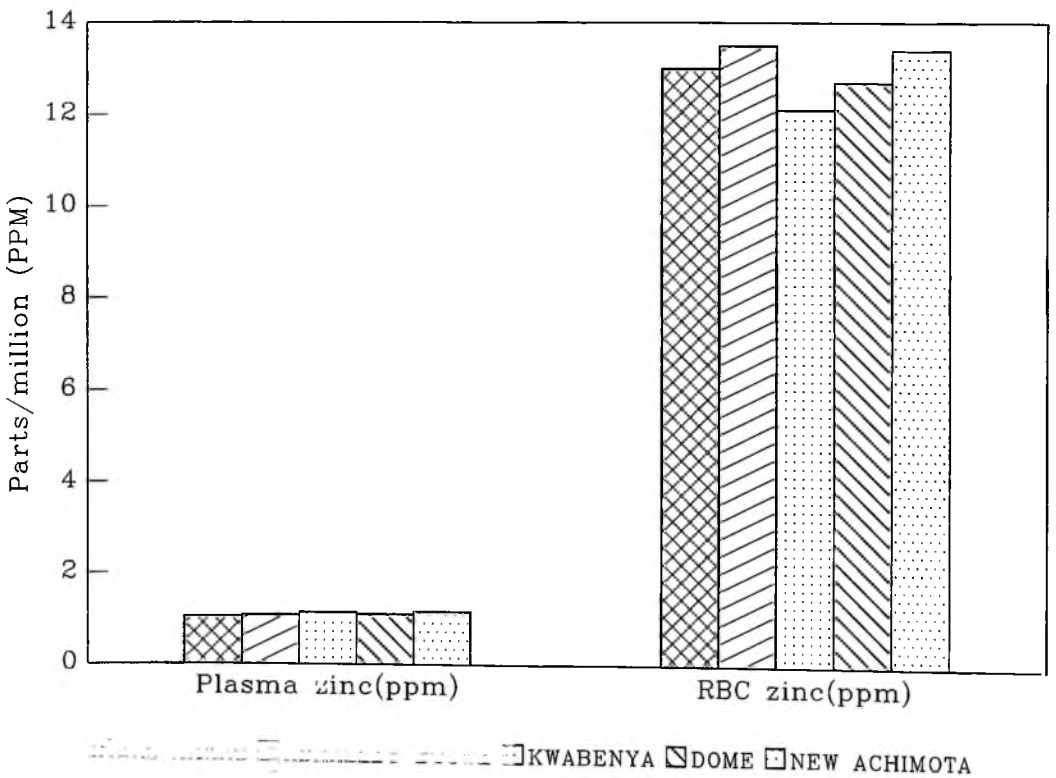
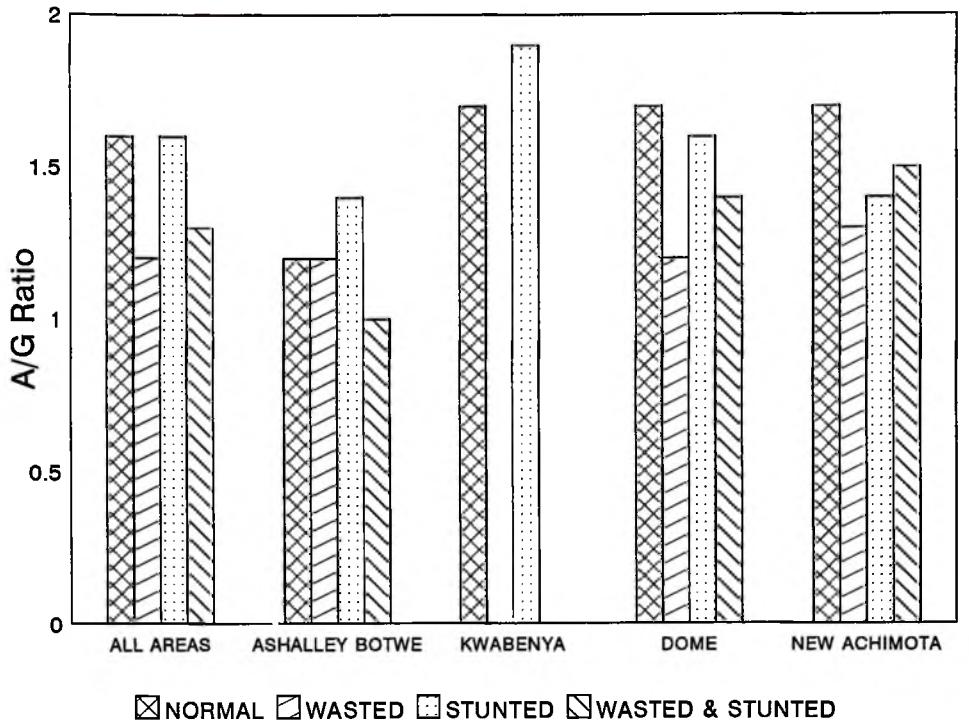


Fig 3b Plasma zinc and rbc zinc levels in the various communities

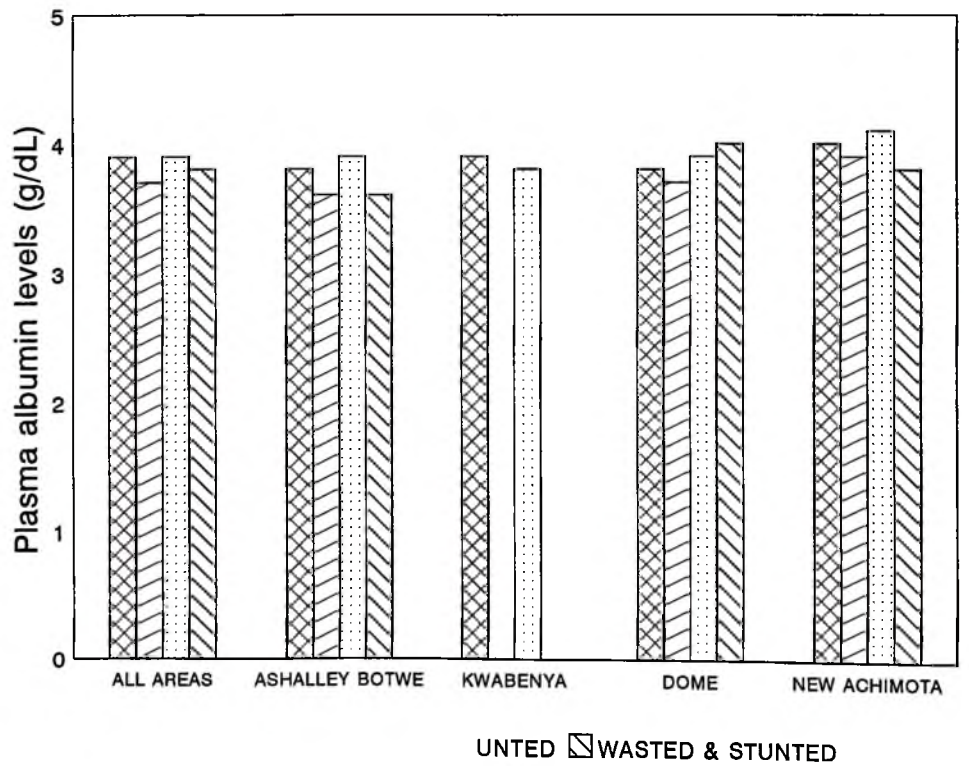


ulin ratio in the different nutritional states



Note the absence of wasting and wasting plus stunting in Kwabenya

Fig 4b Albumin levels in the different nutritional states



5a Trends in the mean alkaline phosphatase activity in the different nutritional states

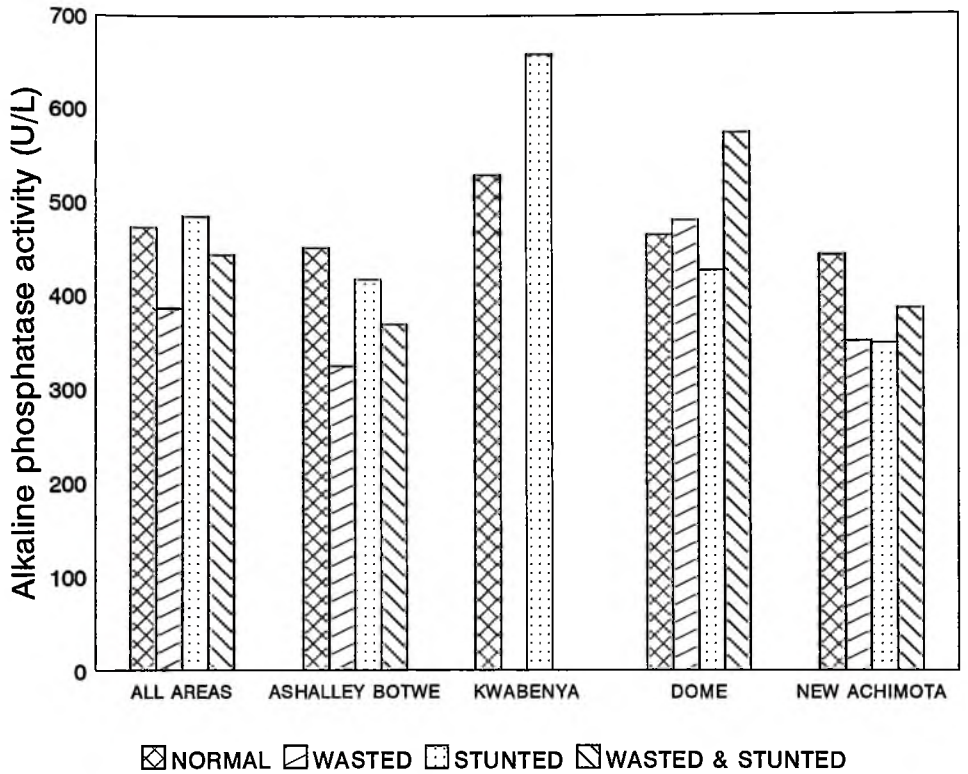


Fig 5b Plasma zinc levels in the various nutritional states in the communities

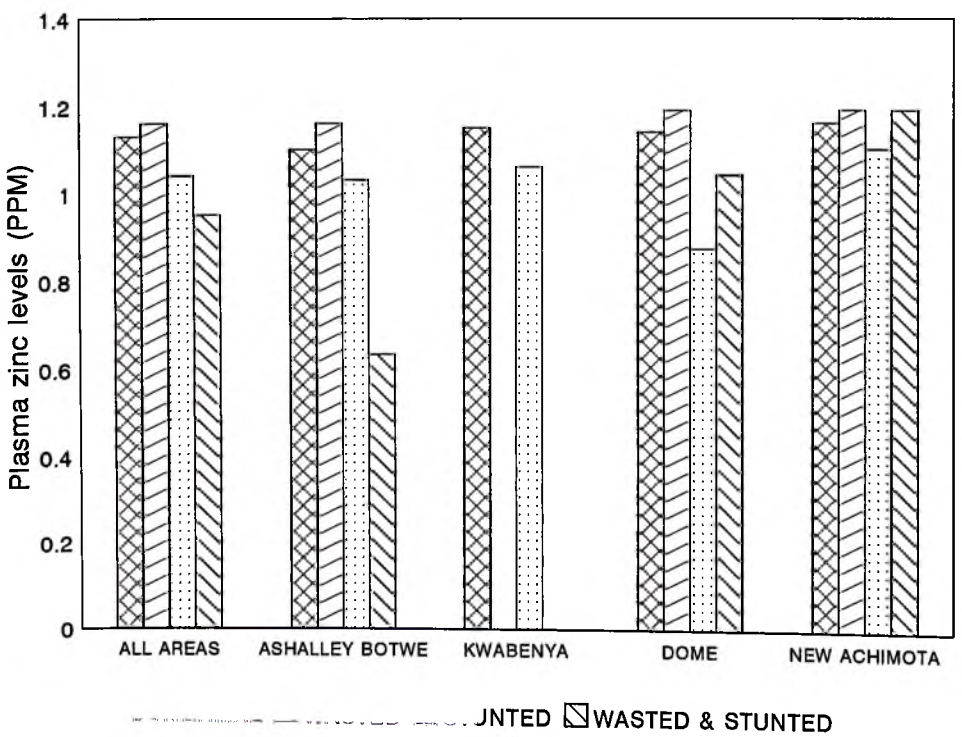


Fig 6a TRENDS IN THE MEAN HAIR ZINC IN VARIOUS NUTRTIONAL STATES IN THE COMMUNITIES.

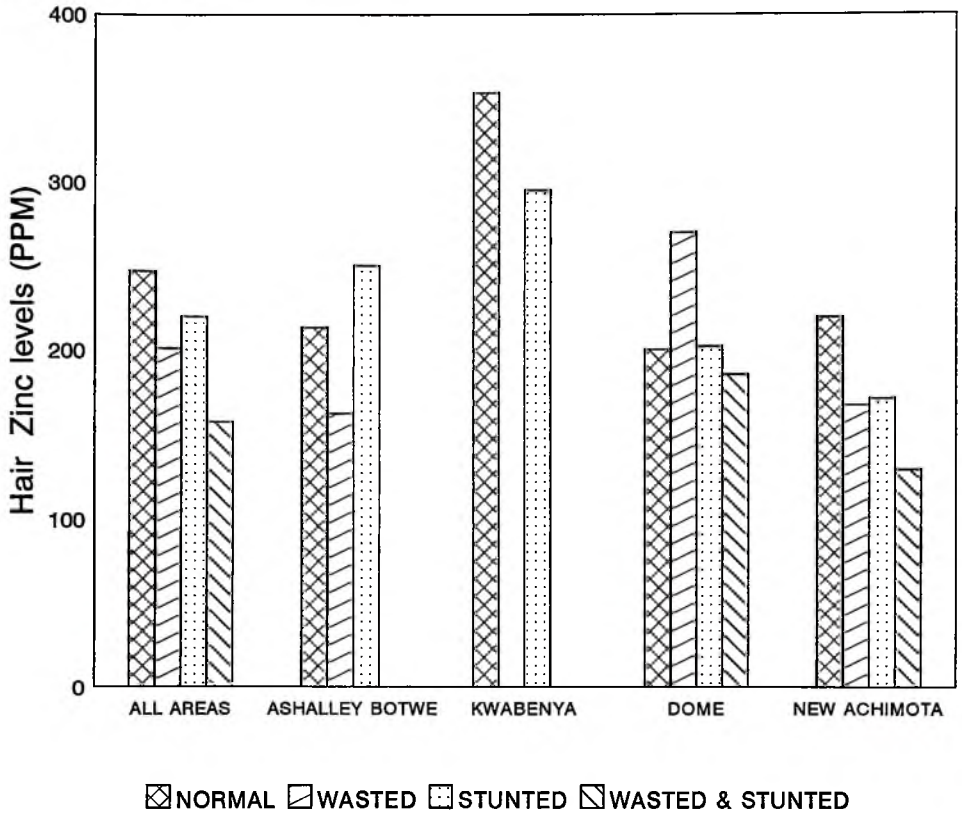
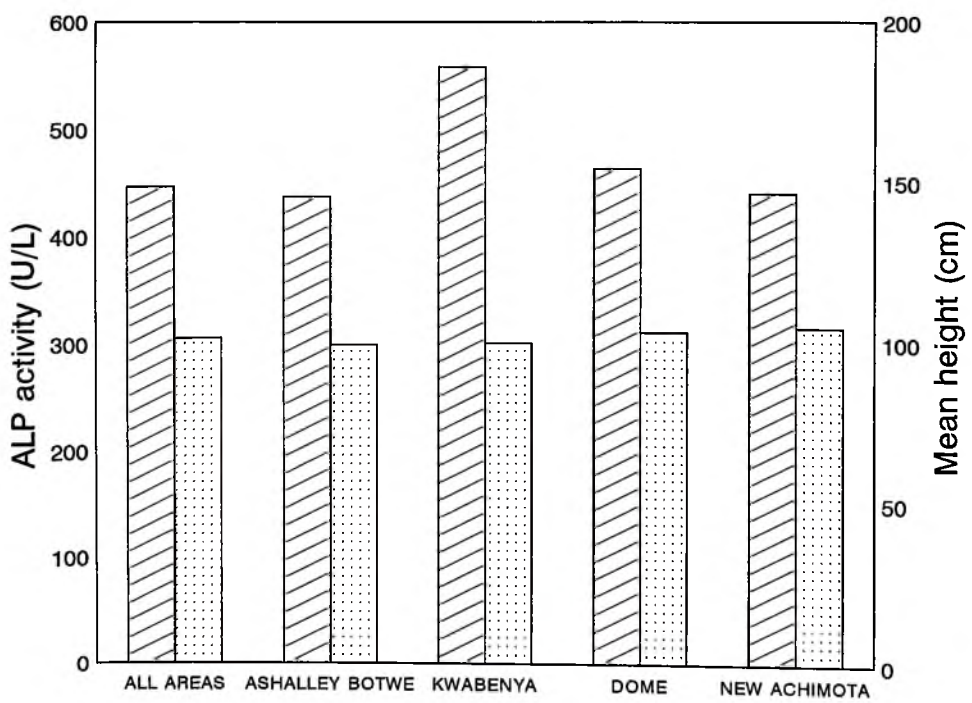


Fig 6b MEAN HEIGHT AND ALKALINE PHOSPHATASE ACTIVITY IN THE COMMUNITIES



MEAN HEIGHT ALKALINE PHOS.

4.4. Correlation among some indicators of zinc nutriture and anthropometric parameters.

1. The equation for regression line between hair zinc concentration and height of subjects for the various nutritional states was:

$$Y = 0.008x + 99.612, \quad r=0.092$$

2. The equation for regression line between plasma zinc concentrations and height of subjects for the various nutritional states was: $Y= 23.222x + 76.400, \quad r=0.675$

3. The equation for regression line between rbc zinc concentrations and height of subjects for the various nutritional states was:

$$Y= 2.569x + 67.790, \quad r=0.615$$

4. The relationship between hair zinc levels and

(i) triceps, $Y =0.007x +6.264, \quad r=0.297$

(ii) subscapular, $Y =0.017x +2.711, \quad r=0.699$

(iv) MUAC. $Y =0.019x +11.393, \quad r= 0.850$

CHAPTER FIVE

DISCUSSION OF RESULTS

The present study was undertaken essentially to ascertain whether or not zinc deficiency occurs within preschool children in the selected communities. If it does, whether the zinc nutritional status relates in any way to the various anthropometric states of the cohorts. The 4 communities studied were Ashalley Botwe, Kwabenya, Dome and New Achimota. They were chosen because of easy access by road from the research station and for the fact that they represent typical Ghanaian set-ups desired in the study. Thus Ashalley Botwe and Kwabenya villages represent typical southern rural villages, Dome represents a typical periurban cosmopolitan township while New Achimota is almost urban.

In Ashalley Botwe and Kwabenya, over 90% of the children within the desired age group (3-5 years) attend the nursery school in which the study was conducted. For Dome and New Achimota, other nurseries existed so the percentage of the cohorts assessed in these two settings was about 50% in each case.

The socioeconomic strata, based on the number of sandcrete houses, zinc/aluminium roofed houses, and general infrastructural developments such as availability of places of convenience, health posts, and potable water are as follows: New Achimota> Dome > Ashalley Botwe > Kwabenya.

In the study, age, anthropometric measurements (weight, height, triceps, subscapular and MUAC) and biochemical indicators of protein and zinc nutritional status(total plasma protein,

albumin, A/G ratio, plasma zinc, rbc zinc, alkaline phosphatase activity and hair zinc) were determined in the 200 cohorts. Their ages ranged from 35 to 62mo.

Based on a cut-off point of -2SD on the NCHS reference standard, 16.5% of the 200 cohorts screened were chronically malnourished,(stunted); 3.5% were acutely malnourished (wasted), 1.5% were chronically and acutely malnourished (wasted plus stunted), while 78.5% were of a normal nutritional status (fig. 1a). The wasted plus stunted group represents cohorts which have acute (i.e. recent) malnutrition Superimposed on a background of Chronic malnutrition.

The Ghana National Demographic Health Survey of 1988[25] on preschool children reported a chronic malnutrition rate of about 28% and wasting rate of 7.4%. A 1987-1988 Agroecological nutrition survey[117] based on z-score cut-off point of -2SD. indicated that malnutrition was particularly high in the savannah agroecological zone,ie northern sections of Ghana. The rates were 36% (chronic malnutrition) and 9.5% (acute malnutrition). The Greater Accra region was said to have the lowest levels of both chronic and acute malnutrition, being 22% and 6.5%, respectively.

When these figures are compared with the values obtained in the present study, (ie. 22% versus 16.5% for chronic malnutrition, and 6.5% versus 3.5%, for acute malnutrition), using tests of proportion, indicated that there is no significant difference ($p>0.05$) between the levels of chronic or acute malnutrition obtained in 1988 and the present study. It means that both forms of

malnutrition are still with us and of the same magnitude as in 1988. This is rather sad in view of numerous intervention programmes mounted by various agencies.

It is evident (from fig.1b) that in Ashalley Botwe, Dome and New Achimota, the cohorts suffer from both acute and chronic malnutrition, while in Kwabenya, they suffer from chronic malnutrition only.

Based on the percentage of cohorts of normal nutritional status, the nutritional adequacy in the four communities can be ranked as follows. New Achimota> Kwabenya> Dome > Ashalley Botwe. The differences in the nutritional states are however not significant ($p>0.05$), except in the case of Ashalley Botwe. Analysis using test of proportions (table 1), indicated that the percentage of cohorts with normal nutritional status was significantly greater ($p< 0.05$) in New Achimota than in Ashalley Botwe. Also the percentage stunting in Ashalley Botwe was significantly higher ($p< 0.05$) than in either Dome or New Achimota.

In order to ascertain whether there were any significant differences in the zinc level in the various nutritional states, the biochemical data of the cohorts have been grouped based on anthropometric classification. (table 2). In this way, the biochemical indicators, such as protein, albumin, A/G ratio, plasma, rbc and hair zinc are compared in the various nutritional states. It must be noted that this type of comparison is valid because cohorts in the age-group of 35-60mo. are regarded as a homogenous group with virtually similar biochemical values.

When the mean biochemical values were compared with standard values (table 2), it was seen that only the plasma zinc in the wasted plus stunted group in Ashalley Botwe fell below the accepted level. The value was 0.63ppm as against a standard quoted value of 0.70 ppm[67,76]. Considering the fact that the normal plasma zinc range (determined by AAS) is 0.50-1.50ppm[118], the level of 0.63ppm is acceptable.

The mean plasma alkaline phosphatase activity obtained in this work is 447±131 U/L (with a range of 243-879 U/L) as compared to a normal range of 164-984 U/L[119]. The mean ± SD plasma and hair zinc values are 1.07 ± 0.33 ppm (range of 0.50-2.25 ppm) and 206.4± 101.6 ppm (range of 73.4-632.7 ppm), respectively as compared to normal levels of 0.50-1.50ppm[118] and >70 ppm[20,67], respectively. The mean ± SD for rbc zinc is 13.0 ± 2.6 ppm, with a range of 11.8-14.2 ppm. as compared to a normal range of 12-14 ppm[17].

It must be noted that even though the accepted lower limit for hair zinc is 70ppm[17,20,76] , the upper limit has not been established. However, literature values indicate values up to 426ppm for elderly black Americans[76]. It might be expected that zinc levels in hair samples of normal African preschool children will be higher than the level in elderly people due to faster growth rate. Thus, using both plasma zinc (indicator of acute zinc status) as well as hair and rbc zinc (indicators of chronic zinc status), as indicators of zinc nutritional status, the mean values obtained in this study, are within the normal physiological range.

The analysis of the biochemical data in the normal, wasted,

stunted and wasted plus stunted groups (table 2), using Duncan's and LSD multiple comparison tests indicate that there is no significant difference among any of the biochemical indicators in the various nutritional states. This also indicates that the protein nutritional status was adequate.

A visual inspection of the hair zinc values in the various groups (table 2) would suggest that values for the wasted plus stunted groups are significantly less than that for the other groups. The lack of any statistical significance is due to the rather large standard deviations, reflecting the differences in the respective hair zinc values in the cohorts.

It is therefore clear from the analyses that there is no significant difference ($p>0.05$) in the indicators of zinc nutriture (plasma zinc, hair zinc, rbc zinc and alkaline phosphatase activity) in the various anthropometric states. In other words, there is no zinc deficiency in the cohorts and also the zinc status does not relate in any way to the various anthropometric states, including stunting.

It must be noted that even though growth retardation is one of the earliest manifestations of zinc deficiency[66], studies have shown that low zinc levels are not a characteristic feature of short stature per se. In a typical study[30] , hair zinc concentrations were determined in 40 children, aged 4-16 years, referred to the pediatric endocrine clinic at Colorado General Hospital, USA, for investigation of severe growth retardation. Final diagnosis included growth hormone deficiency, familial short stature,

primordial short stature, constitutional short stature, and hypothyroidism. The mean hair zinc levels did not differ from that of the control group and only one child, diagnosed as having constitutional short stature, had levels less than 70 ppm[30]. The results of the present study also show that hair zinc levels can be adequate even in the case of stunted growth, hence low zinc levels are not a characteristic feature of short stature per se.

The only work reported in the literature on the zinc status in preschool children in Ghana was by Ferguson et al.[120]. In their work, hair zinc levels in 76 preschool children, consuming cereals or starchy foods, were assessed in 2 rural southern villages. It was found that 83% of the children in one village (Sleper) and 39% in another village (Gidantuba), had 'low' hair zinc of <1.68μmol/g (109.8μg/g). This study clearly shows that there can be very wide variations in the percentage of subjects affected by 'low' levels of zinc within the various preschool populations in different communities in Ghana.

It is also noteworthy that the subjects used in the present study consumed mixed diet, as compared to the cereal/starchy diet in Ferguson's work, hence the absence of zinc deficiency in the present cohort, is not surprising. It is well known that cereals are a rich source of phytic acid, which is the most potent dietary inhibitor of zinc absorption in the small intestine[120].

The starchy diets (as in Fergeson's work) will be a fairly rich source of fibre, which is also a potent inhibitor of zinc absorption. It is therefore apparent that the results of the two

primordial short stature, constitutional short stature, and hypothyroidism. The mean hair zinc levels did not differ from that of the control group and only one child, diagnosed as having constitutional short stature, had levels less than 70 ppm[30]. The results of the present study also show that hair zinc levels can be adequate even in the case of stunted growth, hence low zinc levels are not a characteristic feature of short stature per se.

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The starchy diets (as in Fergeson's work) will be a fairly rich source of fibre, which is also a potent inhibitor of zinc absorption. It is therefore apparent that the results of the two

studies can not be compared, due to some differences in the experimental design.

Equations for regression between hair, plasma and rbc zinc and the height for the various nutritional states, ie. normal, wasted, stunted and wasted plus stunted groups, indicate correlation coefficients, r , ranging from 0.092, for hair zinc and the corresponding heights to an r value of 0.675 for regression line between rbc zinc and the corresponding heights. This indicates a fairly strong positive correlation between height and either plasma zinc (r=0.675), or rbc zinc (r=0.615), but a low correlation between the height and hair zinc (0.092).

Even though there was no statistical difference between plasma, hair, and rbc zinc levels in the various nutritional states for the 200 cohorts (due to the large standard deviations, resulting from differences in the individual values), the regression equations between height and these indicators (which do not take account of standard deviations) show that there is a positive linear correlation between plasma, hair or rbc zinc levels and heights in the various nutritional states.

Although these results showed that there is no zinc deficiency in these cohorts, this does not necessarily mean that zinc metabolism is normal in the cohorts. Studies have shown beneficial effects of zinc supplementation even though plasma zinc levels are normal[121,122]. Beside, circulating and tissue levels of zinc do not necessarily reflect zinc status; for example, high hair zinc has been found in both replete and severe deficiency states[9].

Perhaps, this vindicates the fact that we are yet to find sensitive indicators for assessing zinc nutritional states.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS.

The results of the present study indicated quite a high level of chronic malnutrition (stunting) 16.5% (33/200) and a lower level of acute malnutrition (wasting)-3.5% (7/200) among the preschoolers studied. As discussed earlier, these figures are comparable to those found in the 1987-1988 agroecological survey carried out in the Greater Accra region[117].

The present study has also shown that there is no zinc deficiency in these cohorts, based on the parameters used, and that the zinc status did not differ significantly ($p>0.05$) in the various the anthropometric states, even in the case of stunting.

6.2. RECOMMENDATIONS FOR FUTURE WORK.

1. The study should be repeated in other areas, especially in the northern sector of the country, where malnutrition is generally higher than in the south.
2. Since zinc deficiency is also associated with excessive maternal bleeding at birth, and hypertension during pregnancy[66], both of which are common in Ghana, a proper study should be designed to measure the zinc status of pregnant women at regular intervals, especially in areas or populations where these conditions are prevalent.
3. Zinc supplementation trials should be done, with appropriate controls, in normal and malnourished preschool children and other vulnerable groups,such as the elderly, to test its efficacy in our populations and to demonstrate any deficiencies in zinc nutrition in various populations.

7. APPENDIX

(a) Determination of micro molar absorptivity ie. ϵ

Mean absorbance reading = 0.731 ; $\epsilon = A_{4Np\,(0.04mmol/L)} \times 25 \times 10^{-3}$

$= 0.731 \times 25 \times 10^{-3}$

$= 18.28 \times 10^{-3}$

Substituting this value into equation 1, we have

Catalytic activity (U L^{-1}) = 3.027×10^{-3}

$$\frac{}{(18.28 \times 10^{-3}) \times 1 \times 0.027 \times 10^{-3}} \times (\Delta A/\Delta t$$

$$= 6133 \times (\Delta A/\Delta t) \quad \ldots\ldots\ldots\ldots 2$$

Equation 2 was used to calculate the catalytic(enzyme) activity of all the plasma.

(b) Sample calculation:

From measurement of a plasma sample, the change in absorbance/ min. ie.($\Delta A/\Delta t$) = 0.052

Substituting this value into equation 2 above, the enzyme activity of this plasm sample = (0.055 x 6133) U L^{-1}

= 337 U L^{-1}

TABLE 1. ANTHROPOMETRIC CLASSIFICATION OF THE COHORTS

GROWTH	COMMUNITY	MALE		FEMALE		TOTAL	
	All areas combined	n	%	n	%	n	%
Normal		87[a]	43.5	70[a]	35.0	157	78.5
Wasted		5[b]	2.5	2[b]	1.0	7	3.5
Stunted		21[c]	10.5	12[c]	6.0	33	16.5
Wasted &stunted		2[d]	1.0	1[d]	0.5	3	1.5
Total		**115**	**57.5**	**85**	**42.5**	**200**	**100.0**
	Ashalley Botwe						
Normal		23	41.0	16	28.6	39[e]	69.6
Wasted		2	3.6	0	0	2[f]	3.6
Stunted		7	12.5	7	12.5	14[g]	25.0
Wasted & stunted		0	0	1	1.8	1[h]	1.8
Total		**32**	**57.1**	**24**	**42.9**	**56**	**100.0**
	Kwabenya						
Normal		14	43.8	12	37.5	26[e]	81.2
wasted		0	0	0	0	0[f]	0
Stunted		6	18.8	0	0	6[g]	18.8
Wasted & stunted		0	0	0	0	0[h]	0
Total		**20**	**62.5**	**12**	**37.5**	**32**	**100.0**

Table 1 continued

	Dome						
Wasted		30	44.1	25	36.8	55[i]	80.9
Stunted		2	2.9	1	1.5	3[k]	4.4
Wasted & stunted		4	5.9	5	7.4	9[L]	13.2
Total		1	1.5	0	0	1[m]	1.5
		37	**54.4**	**31**	**45.6**	**68**	**100.0**
	New Achimota						
Normal		20	45.5	17	38.6	37[i]	84.1
Wasted		1	2.3	1	2.3	2[k]	4.5
Stunted		4	9.1	0	0	4[L]	9.1
Wasted & stunted		1	2.3	0	0	1[m]	2.3
Total		**26**	**59.1**	**18**	**40.9**	**44**	**100.0**

Normal = weight-for-height and height-for-age are both >- 2SD of NCHS reference standards
Wasted = weight-for-height is <-2 SD of NCHS reference standard,
Stunted = Height-for-age < -2SD of NCHS reference standard;
Wasted & stunted = weight-for-height and height-for-age are both <-2SD of NCHS reference standard.
Analysis using test of proportion indicated that numbers marked with the same superscript are not significantly different (p>0.05).

TABLE 2. MEAN VALUES OF THE ANTHROPOMETRIC AND BIOCHEMICAL DATA OF THE COHORTS

Growth/ Community	Weight (kg)	Height (cm)	Triceps (mm)	Sub-scapular (mm)	Mid upper arm circ (cm)	Protein (g/dL)	Albumin (g/ dL)	Albumin/ globulin ratio	Alkaline phosphatase activity (U/L)	Plasma zinc (ppm)	Hair zinc (ppm)	RBC zinc (ppm)
Reference ranges						5.8-8.3	3.5-5.5	1-2.5	164-984	0.50-1.50	>70	12-14
All areas combined												
Normal n=157	15.7 (1.7)	102.9 (5.5)	8.2 (1.7)	5.7 (1.1)	16.3 (0.9)	6.6[a] (0.7)	3.9[b] (0.3)	1.6[c] (0.5)	473[d] (129)	1.13[e] (0.35)	247.7[f] (101.6)	13.1[g] (2.6)
Wasted n=7	13.4 (1.5)	104.6 (5.5)	6.4 (0.9)	4.4 (0.5)	14.6 (0.8)	6.9[a] (1.1)	3.7[b] (0.5)	1.2[c] (0.3)	386[d] (82)	1.16[e] (0.37)	200.9[f] (65.2)	14.0[g] (2.6)
Stunted n=33	14.3 (1.7)	97.1 (6.1)	8.1 (2.1)	5.6 (1.4)	15.9 (1.1)	6.7[a] (0.6)	3.9[b] (0.3)	1.6[c] (0.5)	485[d] (152)	1.04[e] (0.23)	220.0[f] (83.8)	12.9[g] (2.4)
Stunted & wasted n=3	12.6 (0.6)	100.4 (2.1)	7.8 (0.3)	4.8 (0.4)	14.7 (0.5)	7.1[a] (0.3)	3.8[b] (0.5)	1.3[c] (0.7)	444[d] (114)	0.95[e] (0.29)	157.6[f] (40)	12.1[g] (1.0)
Ashalley Botwe												
Normal n=39	15.5 (1.4)	101.6 (5.8)	8.6 (1.6)	6.0 (1.3)	16.3 (0.7)	7.2 (0.9)	3.8 (0.4)	1.2 (0.4)	452 (126)	1.10 (0.28)	213.5 (56)	13.5 (3.1)
Wasted n=2	13.0 (0)	101.3 (1.5)	6.4 (1.4)	4.6 (1.1)	14.2 (1.1)	7.0 (0.9)	3.6 (0.3)	1.2 (0.2)	325 (50)	1.06 (0.20)	162.5 (10)	16.2 (3.1)
Stunted n=14	13.6 (1.8)	93.1 (6.7)	9.7 (2.3)	6.5 (1.6)	15.9 (1.3)	6.9 (0.7)	3.9 (0.4)	1.4 (0.5)	418 (125)	1.03 (0.24)	250.6 (71.5)	13.0 (3.6)
Wasted & stunted n=1	12.0 (0)	101.3	8.0	4.6	15.0	7.4 (0.3)	3.6 (0.3)	1.0 (0.2)	370	0.63 (0.10)	-	13.4

Table 2 continued

Kwabenya												
Normal n=26	15.2 (1.6)	101.1 (6.7)	8.6 (1.5)	5.8 (1.0)	16.3 (0.7)	6.5 (0.8)	3.9 (0.3)	1.7 (0.7)	530 (180)	1.15 (0.38)	353.7 (139.1)	12.5 (2.9)
Wasted n=0	0	0	0	0	0	0	0	0	0	0	0	0
stunted n=6	13.3 (1.5)	94.6 (5.6)	8.0 (2.0)	6.0 (0.8)	15.6 (1.1)	6.1 (0.5)	3.8 (0.3)	1.9 (0.5)	659 (199)	1.06 (0.08)	295.8 (121.3)	10.9 (1.1)
Wasted & stunted n=0	0	0	0	0	0	0	0	0	0	0	0	0
Dome												
Normal n=55	15.8 (1.9)	104.0 (4.8)	8.2 (2.0)	5.7 (1.2)	16.3 (1.1)	6.3 (0.6)	3.8 (0.4)	1.7 (0.5)	466 (108)	1.14 (0.41)	200.7 (66.3)	13.0 (2.3)
Wasted n=3	13.7 (2.1)	105.4 (6.1)	6.7 (0.8)	4.4 (0.3)	15.0 (0.5)	6.7 (0.6)	3.7 (0.5)	1.2 (0.2)	482 (33)	1.19 (0.51)	270.6 (43.5)	12.2 (2.4)
Stunted n=9	14.2 (0.9)	98.4 (3.6)	8.0 (1.5)	5.5 (0.9)	16.0 (0.6)	6.6 (0.5)	3.9 (0.2)	1.6 (0.4)	428 (86)	0.87 (0.21)	202.6 (64.9)	11.9 (4.6)
Wasted &stunted n=2	13.3 (0.4)	101.6 (0.8)	7.6 (0.3)	5.4 (0.8)	14.3 (0.4	7.2	4.0 (0.1)	1.4	575	1.04	185.9	12.1 (0.1)
Reference ranges						5.8-8.3	3.5-5.5	1-2.5	168-984	0.50-1.5	>70	12-14

Table 2 continued

New Achimota												
Normal n=37	16.1 (1.8)	104.9 (4.4)	7.3 (1.0)	5.4 (0.8)	16.4 (0.9)	6.6 (0.5)	4.0 (0.3)	1.7 (0.4)	444 (115)	1.16 (0.32)	220.1 (109.5)	13.3 (2.0)
Wasted n=2	13.5 (2.1)	106.7 (8.3)	5.9 (0.6)	4.2 (0.3)	14.4 (1.1)	7.1 (0.6)	3.9 (0.8)	1.3 (0.4)	352	1.19	167.9 (32.9)	13.6 (2.0)
Stunted n=4	15.9 (2.4)	102.2 (1.6)	6.7 (1.4)	4.4 (0.5)	16.0 (1.2)	7.3 (0.3)	4.1 (0.2)	1.4 (0.2)	435 (76)	1.10 (0.32)	171.7 (49.6)	15.9 (4.9)
Wasted &stunted n=1	12.5	98.3	7.8	4.4	15.1	6.7	3.8	1.5	387	1.19	129.3	10.9
Reference ranges						5.8-8.3	3.5-5.5	1-2.5	164-984	0.50-1.5	>70	12-14

Values are means for both sexes. SD values are shown in parenthesis. Values with the same superscript are not significantly different (P>0.05). Test for significance was done using LSD and Duncan's multiple range test. Sources of reference ranges: Reference # 36,111 & 118

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