# ZINC AND COPPER IN SYMPTOM-FREE NIGERIANS, THOSE WITH SICKLE CELL DISEASE AMD OTHER PATHOLOGICAL CONDITIONS

by

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# A B S T R A C T

Zinc and copper concentrations were estimated by atomic absorption spectrophotometry in serum, erythrocytes and scalp hair from inhibitants of northern Nigeria. Other measurements performed were serum caeruloplasnIN total protein, albumin and alkaline phosphatase, and blood haemoglobin and haematocrit.

The mean serum zinc was significantly lower in non-elite Nigerians (Hausa rural farmers presenting as blood donors) (105.30 + 7.30 ug/100 ml) than in elite Nigerians (University senior staff) (128.90 + 5.80 ug/100 ml) ($P<0.01$). The lower serum zinc in non-elite Nigerians was associated with lower mean albumin. Other measurements showed no significant differences. It is suggested that lower serum albumin in non-elite reflected a lower protein intake and the lower serum zinc resulted from poor absorption of the metal due to dietary ligands.

Intake of food was shown to lower the serum zinc, but had no consistent effect on other measurements. Therefore, it is important that specimens for measurements of serum zinc should be collected at a standardized time, and with the subject fasting.

The decrease of serum zinc and increase of copper during pregnancy are confirmed in Nigerians. It is possible that the reduced serum zinc in pregnancy may be a reflection of rapid protein

metabolism resulting in a shift into the intracellular compartment, and an increased mobilization >ecause of foetal demand. The raised serum copper in pregnancy may be due to increased erythropoiesis.

There was no significant difference in measurements between Nigerians with normal haemoglobin pattern (Hb. AA.) and those individuals with sickle cell trait (Hb. AS.). Patients with sickle cell anaemia (Hb. SS.) were observed to have lower mean serum and hair zinc concentrations than elite Nigerians ($P<0.01$). The erythrocyte zinc levels did not differ significantly between these two groups. There was no significant difference found in measurements between Hb. *SS* and non-elite groups, but it is probably true that patients with Hb. *SS,* were nearer the elite families than non-elite families. Patients with Hb. SS. in Nigeria probably do not have clinically significantly zinc deficiency. An elevated serum copper in Hb. *SS,* is thought to be the result of increased erythropoietic activity.

Patients with leg ulcers but not Hb. S.S., showed comparable serum zinc levels to patients With Hb. *SS.*. probably due to zinc immobiligation from chronic tissue damage.

Significantly decreased serum zinc concentrations have been demonstrated in the following conditions: primary liver cancer, hookworm anaemia, active pulmonary

tuberculosis and leukaemias. The serum copper was elevated in these conditions. The pathophysiological mechanisms underlying these changes may be increased urinary excretion and loss of body stores of zinc. These changes in serum zinc and copper may result in part from a redistribution of the metals within the body initiated by a hormone-like protein factor which is released from phagocytic cells. This factor, leukocytic endogenous mediator (LEM), stimulates the liver to take up zinc and iron from serum into the liver and to synthesize additional quantities of caeruloplasmin.

This work has established the normal ranges of zinc and copper concentrations in the northern Nigerian population with relation to social class and haemoglobin patterns, and that zinc deficiency is not a common feature in Nigeria.

The estimation of trace element concentrations may be a useful means of predicting the health status of various community groups in the general population; and the changes occuring in trace element concentrations may be a useful tool in following the progress of patients with malignant disease during the course of treatment.

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## ABBREVIATIONS USED IN THIS THESIS

| | |
|---|---|
| Zn | Zinc |
| Cu | Copper |
| HCl | hydrochloric acid |
| TCA | trichloroacetic acid |
| pH | hydrogen ion concentration |
| Hb | haemoglobin |
| Hb. AA | normal haemoglobin |
| Hb. AS | sickle cell trait |
| Hb. SS | sickle cell anaemia |
| Hb. SC | Haemoglobin S/Hb C disease |
| PCV | packed cell volume |
| K.A.U. | King Armstrong Unit |
| ml | millilitre |
| L/l | litre |
| V/V | Volume over volume |
| p.p.m. | parts per million |
| p.p.b. | parts per billion |
| % | is used interchangeably with per cent |
| g/ | gram |
| kg | kilogram |

| | |
|---|---|
| mg | milligram |
| µg | microgram |
| h | hour |
| °C | degree Celsius |
| r.p.m. | revolution per minute |
| r | correlation coefficient |
| p | probability |
| < | less than |
| > | greater than |
| S.E. | standard error |
| S.D. | standard deviation |
| / | per volume or weight |
| / | or |
| FIG. | Figure |
| ( ) | parenthesis indicates important statement |
| ∝2 | alpha 2 |
| wt. | weight |
| ß | beta |
| W.H.O. | World Health Organization |
| U.S.A. | United States of America |
| D.C. | District of Columbia |

# CHAPTER 1

# INTRODUCTION

## 1.1. Definition of trace elements.

A great number of elements, including zinc and copper, occur in living tissues in such small amounts that their precise concentrations could not be measured with the analytical methods formerly available. Such elements were therefore reported (WHO, 1973) to be present in "traces" and the term "trace elements" arose to describe them. This term with its connotation of imprecision, has continued in popular usage. Other popular designations such as "minor elements" have found favour with plant nutritionists and "oligo elements" (from the Greek oligos meaning scanty) is a term frequently used by French workers. None of these terms is fully acceptable because, with the introduction of the atomic absorption spectrophotometer, most of the elements present in biological materials can now be measured with reasonable degree of precision. Nor is the more scientifically respectable "micronutrients elements" entirely satisfactory, since it implies that all the elements present in living organisms in low concentrations and, therefore, of biological interest, function as nutrients. The term "trace elements" is retained here because it has historical associations, and because it has become hallowed by time and custom.

Classification of the trace elements into essential, non-essential and toxic groups can be inaccurate and

misleading. All the elements become toxic at sufficiently high intakes and the margin between levels that are beneficial and those that are harmful may be small (WHO, 1973).

According to Cotzias (1967), a trace element can be "dignified" with the formal title "essential" if it meets the following criteria:

1. it is present in healthy tissues of living organisms;
2. its concentration from one animal to the next is fairly constant;
3. its withdrawal from the body induces reproducibly the same structural and physiological abnormalities regardless of the species studied;
4. its addition either prevents or reverses these abnormalities;
5. the abnormalities induced by deficiency are always accompanied by pertinent, specific biochemical changes;
6. these biochemical changes can be prevented or cured when the deficiency is prevented or cured.

At the present time 14 trace elements are believed to be essential for mammalian life. These are iron, iodine, copper, zinc, manganese, cobalt, molybdenum, selenium, chromium, nickel, tin, silicon, fluorine, and vanadium. It has been the usual practice to express concentrations of trace elements as per wet weight in an animal tissue, but as per dry weight in

plant tissue. This convention will be followed unless otherwise stated.

In mammals including man, a trace element is defined as one that makes up less than 0.01% of the body's mass. Together, the 50 or more trace elements found in the body, both normal and abnormal, make up less than 0.2% of the total weight of the body. Iron, fluorine and zinc, the most abundant trace elements, comprise 0.006%, 0.0037% and 0.0033% of the body respectively. By comparison, the least abundant major element magnesium makes up 0.027% of the body.

There are two general classes of abnormalities associated with trace elements (1) specific deficiency resulting from dietary inadequacies, imbalance or secondary to other diseases and (2) accumulation of innately toxic trace elements from the environment, which can either displace essential elements from their metabolically active sites and cause conditioned deficiency or act directly as cellular toxins. Both kinds of abnormalities can be diagnosed by analyses of trace elements in plasma or serum, red blood cells and urine (Schroeder and Nason, 1971). Also, secondary changes occur as a result of systemic disease.

## 1.2. History of the research problem.

Zinc and copper are two of the essential elements necessary for optimal function of the mammalian organism, for growth, healing, and activity of many metabolic processes.

The concentrations of zinc, copper and most other trace

elements in plasma or serum are indices of metabolism of the body cells and may be increased or decreased depending upon the physiological and pathological states of the organism (Schroeder and Nason, 1971; Halsted et al., 1974; Mateo et al., 1975; Kapu, 1975a; Prasad et al., 1976). Man provides a useful system for the study of essential trace elements, because variations occur in association with a number of clinical conditions (Halsted and Smith, 1970; Underwood, 1977a,b; Halsted et al., 1974; Kapu et al., 1976a and 1976b; Olatunbosun et al., 1978).

Because of growing need for the application of trace elements as diagnostic aids and indices for therapy in clinical conditions, it was found necessary to investigate the range of concentrations of serum zinc and copper in various socio-economic groups, and the association of the concentrations with various diseases in Nigerians.

## 1.3. Review of the literature.

### 1.3.1. Zinc in man and other mammals.

#### 1.3.1.1. Historical Introduction.

In 1869, Raulin (Halsted et al., 1974), a pupil of Pasteur, discovered that zinc was indispensable for the growth of a black bread mould, Aspergillus niger. This finding was confirmed in 1911 by Bestrand and Javillier (Halsted et al., 1974). According to these authors Aspergillus niger requires zinc in order to synthesize phenylalanine, tryptophan, and tyrosine as well as several enzymes. Growth of penicillin-producing fungi is greatly retarded by zinc deficiency of the culture medium. Evidence

indicating that zinc is essential to the growth of micro-organisms has been summarized by Zajic (1969).

Todd et al. (1934) showed that zinc is an essential trace element for the rat. Interest in zinc in humans followed the findings of Pories and Strain (1966) on the relation of zinc to wound healing and of Prasad et al. (1961 and 1963 a) on the relation of zinc deficiency to dwarfism and hypogonadism in boys in Syria, Iran, Egypt, and Turkey (Cavdar et al. 1977).

1.3.1.2. Distribution in nature.

1.3.1.2.1. Earth.

Zinc makes up 0.004 to 0.01% of the earth's crust but is unevenly distributed, averaging about 50 ppm (Schroeder et al. 1967). Rocks average 16 to 95 ppm (Bowen, 1966). Shales have the highest concentration and sandstone has the lowest.

Analysis of moon samples brought back revealed a much lower concentration of zinc in both the rocks and soils than in materials found on earth. Igneous rock samples (basalt and gabbro) contained from 0.2 to 3.0 ppm zinc (Apollo 11). Eight rocks returned by Apollo 12 contained between 0.2 and 4.3 ppm zinc with a mean of about 4.0 ppm. Lunar soil samples showed a range of 4.0 - 8.2 ppm with a mean of 6.7 ppm, less than 15% the mean concentration of the earth soil (Halsted et al. 1974).

1.3.1.2.2. Natural water.

Sea water contains 9 - 21 ppb of zinc while river water in the United States of America contains 10 - 200 ppb, with larger amounts in acidic than alkaline waters (Schroeder et al., 1967).

1.3.1.2.3. Air.

Tabor and Warren (1958) found zinc over a number of American cities to be 0.3 - 49 µg/cubic metre, with the largest amounts coming from industrial areas.

1.3.1.2.4. Plants.

The zinc content of certain plant and grass species on the savanna of northern Nigeria has been reported to be around a mean of 46 and 43 ppm respectively in early growth (Kapu, 1975b). The contents of land plant species found in the United States of America vary widely around a mean of 23 ppm (Halsted et al., 1974). Vegetables and fruits have low contents (0.2 - 0.9 mg/100 g), except legumes which contain in the region of 3 mg/100 g.

1.3.1.2.5. Animals.

Marine animals have a wide range of zinc concentrations with oysters having the highest around 74.7 mg/100 g (Bowen, 1966).

Typical normal values for zinc concentration in organs of several mammals are shown in Table 1.1. The total amount of zinc in the human body is in the range 1.3 - 2.3 g (Widdowson et al., 1951). Zinc is concentrated in certain specialized tissues, especially in the prostate of the rat, rabbit and man and in its fluids (Vallee, 1962; Underwood, 1977a). The choroid of the eye in all species examined have high levels of zinc (Schroeder et al. 1967). Carnivores have 14,600 - 91,000 ppm dry weight, and herbivores 139 - 277 ppm. The iris also contains high concentrations (246 - 536 ppm). Although the reasons for such high concentrations of zinc in the eye have not been advanced, it is possible that zinc is needed in very high concentrations in association with its metalloprotein, retinal dehydrogenase, for normal regulation of visual functions, especially in the utilization of Vitamin A.

Hair zinc concentration has been reported to be affected by zinc intake in rats, swine, cattle, goats and in man (Halsted et al., 1974). Schroeder and Nason (1969) have reported a mean hair zinc of 167 ppm for healthy males and a value of 172 ppm for females. Butler et al. (1978) obtained higher values in the hair of normal American Negroes (220 ppm zinc) and American Caucasians (228 ppm zinc). A lower hair value was obtained by Baumslag et al. (1975) for newly delivered mothers (136 ppm zinc) in North America. These workers discovered that the

mean values of zinc in hair decreased in mothers as parity increased, and that zinc content of infants' hair (225 ppm) wa higher than mothers' hair (136 ppm). The finding of a decrease in zinc with parity seems to indicated a tendency to a nutritional insufficiency of zinc in mothers who go through many pregnancies. Further investigation is needed to identify the need for supplementation for such mothers. Strain et al. (1966) obtained a mean hair zinc value of 119.6 ppm for North Americans and corresponding value of 103.3 ppm for normal Egyptians. Nigerians living in western Nigeria (Ibadan) had mean zinc value of 150 ppm ranging 75-286 ppm (Olatunbosun et al. 1977). Inter-laboratory variations of technique may have contributed considerably to the reported differences.

Early studies (Underwood, 1977a) found in the zinc content of the nails of 18 normal subjects to range from 93 to 292 ppm and to average 151 ppm. Sohler et al. (1976) found fingernail zinc levels in healthy subjects to range from 68 to 145 ppm with a lower range of 108 ppm in one series, and an identical mean fingernail zinc to be 150 ppm with a range from 104 to 427 ppm in another series.

The concentrations of zinc in plasma or serum, blood cells, sweat, saliva and semen have been investigated. The values for concentrations of zinc in human body fluids are presented in Table 1.2. Semen of men, boar and rams contain high concentrations, 500 - 2930 ppm dry weight (Schroeder et al. 1967). Zinc concentration of spermatozoa can be

affected by time sampling after ejaculation, conditions of storage, and membrane integrity (Underwood, 1977). Thus Mauson and Fischer (Underwood, 1977a) have reported the following average values: 2930, 1400, 910 and 1990 ppm dry weight for first fraction of ejaculate, second fraction, third fraction (human semen) and human spermatozoa, respectively. And boar spermatozoa accumulate zinc when stored in vitro in contact with boar seminal plasma (Underwood, 1977a).

The results of Vallee and Gibson (1948) indicate that 75 - 88% of the total zinc of normal human blood is contained in the red cells, 12 - 22% in the plasma, and 3% in the leukocytes. The individual leukocyte contains 25 times as much zinc as the individual erythrocyte (Table 1.2).

1.3.1.3. Zinc metalloenzymes in mammals.

Many metalloenzymes contain zinc, while other enzymes are activated by zinc (Parisi and Vallee, 1969). Zinc metalloenzymes are of basic importance to many intracellular biochemical mechanisms; in particular they regulate various stages of protein and nucleic acid synthesis (Fell and Burns, 1976).

Carbonic anhydrase was the first metalloenzyme to be identified (Keilin and Mann, 1940), and since then over 80 such enzymes have been recognized (Lancet, 1978). The common

zinc metalloenzymes in mammalian tissues include carbonic anhydrase (Parisi and Vallee, 1969; Vallee and Altschule, 1949; Vallee et al., 1949; Keilin and Mann, 1939), carboxypeptidases (Anon, 1973; Prasad, 1969), cytochromes (Mendiola and Price, 1969; Parisi and Vallee, 1969), alkaline phosphatases (Reinhold and Kfoury, 1969; Parisi and Vallee, 1969; Prasad, 1969), RNA polymerase (Weismann, 1976), metallothionine (Davies, 1972) and rhodanase (Volini et al., 1967).

Three forms of carbonic anhydrase occur in human erythrocytes named carbonic anhydrase A, B, and C (Amstrong, et al., 1965; Laurent et al., 1965; Nyman, 1961). They are also located in the kidney, gastric mucosa and pancreas (Weismann, 1976). These different forms have many characteristics in common: the same principal role in catalyzing the reversible hydration of carbon dioxide, about the same molecular weight, and one atom of zinc in the molecule (Coleman, 1965; Nyman, 1961).

Addink (1965) examined the effect of X-rays on metalloprotein and found that only firmly bound zinc was affected by the releasing process. As the activity of the enzyme after irradiation with X-rays diminished, the activity of the enzyme was correlated with the content of firmly bound zinc.

Carboxypeptidases A and B occur in pancreatic juice. They participate in digestion. The activity of the enzymes was lowered in zinc deficient rats (Mills et al., 1967).

Glumatic dehydrogenase occurs in the liver and leukocytes. It is the principal enzyme catalyzing the metabolism of amino acids. Retinol dehydrogenase is located in the retina of the eye, it converts vitamin A alcohol into vitamin A aldehyde, and influences the visual function (Weismann, 1976). Alcohol dehydrogenase occurs in the liver, converts alcohols into aldehydes and participates in the metabolism of ethanol (Weismann, 1976). This enzyme is possibly identical to retinal dehydrogenase.

Animal experiments have shown that the role of zinc in nucleic acid metabolism is connected with RNA polymerase activity (Terhune and Sandsted, 1972), as well as with DNA sythesis. Lieberman and Ove (1962) demonstrated in vitro that zinc ions are required for DNA synthesis by rat kidney cells. Thus, inhibition of not only DNA synthesis, but also of DNA polymerase and thymidine kinase activity, occured when zinc was removed from the growth medium. In controlled experiments, Prasad et al. (1971) found a reduced zinc and RNA content in the tissue cells of pigs fed on a low zinc diet. The concentrations of certain zinc metalloenzymes were likewise reduced.

Fernandez-Madrid et al. (1973) examined the significance of zinc for the formation of collagen in rats that had polyvinyl sponges implanted subcutaneously. In the newly formed connective tissue among the sponges, rats on a zinc-restricted diet showed a reduction of the contents of RNA, total collagen and non-collagenous protein, as well as of the number of collagen-synthesizing polyribosomes. The interpretation of the results was that zinc deficiency has a direct effect on the metabolism of nucleic acids. with a resulting inhibition of the collagen and protein synthesis. In rats fed extremely high as well as low zinc diets, Chvapil (1974) found a reduced activity of lysyloxidase - an enzyme mediating the collagen synthesis.

### 1.3.1.4. Zinc metabolism in man

#### 1.3.1.4.1. Dietary requirements for zinc

The dietary requirements for zinc are related to the necessity for growth, tissue repair and excretion. The amounts required changes with growth. The amounts of zinc which must be retained by the body for growth can be estimated from analysis of the chemical composition of the body at various ages (WHO, 1973). On the basis of sweat, urine and the availability of zinc for absorption, it is possible to make a provisional estimate of the amount of the element that the diet must contain in order to provide for growth

and inevitable loss by excretion. Such provisional estimated levels would depend upon zinc availability for absorption, which is influenced by the type of diet ingested.

The apparent entero-enteric circulation of zinc makes precise estimation of absorption and excretion impossible by a simple measurement of intake and output. Nevertheless, the balance method has been the principal technique used in estimating zinc requirement for man. All of the balance studies with presumably healthy subjects indicated that urinary zinc excretion was low and not apparently related to intake. In studies with adults, the intakes varied from relatively low (about 5 mg/day) to relatively high (about 22 mg/day) (McCance and Widdowson, 1942; Tribble and Scoular, 1954; Osis et al., 1969; Tipton et al., 1969; Fox, 1970; Gommican and Catli, 1971; White and Gynne, 1971; Abdulla and Norden, 1974). This variation is probably due in part to the increasing use of ready-made food, when appreciable loss of zinc takes place during the course of preparation. In addition, the zinc content of many crops seems to be falling owing to an increasingly acidic precipitation and the use of lime fertilizers in modern agronomic practices (Underwood, 1977a).

The recommended daily dietary zinc allowances of the Food and Nutrition Board, National Academy of Sciences -

National Research Council (Harper, 1974) has been estimated to be 3 - 5 mg for infants (under the age of 1 year), 10 mg for adult males and females (11 - 15 years plus), 20 mg for pregnant and 25 mg for lactating women. According to Harper (1974), these are recommendations for nutrients that should be consumed and, therefore, do not allow for amounts lost during processing or preparation of foods. They are recommendations for the maintenance of health and do not take into account the special needs due to illness.

These estimates are of known physiological growth needs to ensure satisfactory rate of growth of children and maintenance of weight and prevention of nutrient depletion of adults as a population. They cannot be a satisfaction basis for an "ideal" diet for individuals because of differences in individual requirements. These allowances were calculated for a population in the United States of America and cannot be extrapolated justifiably to populations in Nigeria or Africa, generally because of the differences in culture, dietary habits, agronomic practices, economic status and geographical area.

The utilization of dietary zinc depends upon the contents of calcium, phytate, and copper in the intestines (Weismann, 1976).

On the whole, more information is required on factors affecting availability and utilization of dietary zinc in order to make sound recommendations on the requirements and dietary allowances for different population groups.

1.3.1.4.2. Dietary sources of zinc.

There is a wide range of variability of the zinc contents of different diets probably because of the composition of the diet, particularly the dietary nitrogen content, as most foods that contain a substantial amount of zinc are also high in protein (Osis et al., 1972). The wide range of values reported for the same food may be due to differences in analysis, source and variety. The zinc content of selected foods is shown in Table 1.3.

Good dietary sources are seafoods (700 - 1600 µg/g), meats (30 - 50 µg/g), and whole grains, peas and beans (20 - 30 µg/g). Bread, fat and sugar are poor in zinc and may contain less than 10 µg/g (Anon, 1975). Leafy vegetables and fruits are also usually poor sources of dietary sources of zinc (less than 2 µg/g fresh weight).

Human milk is reported to contain 3 - 5 ug/g (wet weight) which is comparable to cow's milk (Table 1.3. and WHO, 1973). Human milk contains about 10 µg/g during the first two weeks after parturation, with the concentration dropping sharply thereafter to about 0.5 ppm

(Anon, 1975).

The refining of foods usually results in decrease in zinc content. Thus, bread made from white flour has a lower zinc content than the whole wheat bread (Table 1.3.). Similarly, cornstarch contains less zinc than the whole kernel.

Food preparation methods also affect zinc concentrations. For example, the cooking water may contain a wide range of levels of zinc, while corrosive action of acid foods in contact with galvanized metal increases the zinc concentration. This source of contamination has decreased with the greater use of stainless steel and non-metalic coated utensils.

1.3.1.4.3. Absorption of zinc.

Early work on zinc absorption in man showed absorption to be approximately 20% of intake (McCance and Widdowson, 1942). Later studies suggested that the absorption of dietary zinc may be higher, probably in the region of 40% from a mixed western diet (WHO, 1973).

Zinc absorption from the gut is dependent upon a variety of factors. Among the factors that increase the absorption and utilization of zinc are the levels of trace elements in the body, vitamin D, certain chelates, pregnancy and androgens. Scott and Zeigler (Davies, 1972) have shown that certain natural foods, such as casein and liver extract, contain chelates which improve zinc absorption and utilization.

Lowering the pH of the intestines, and the changes occuring during the final period of pregnancy both result in increased zinc absorption (Feaster et al., 1955). Androgens markedly increase the uptake of radioactive zinc by the prostrate and presumably will increase intestinal absorption (Davies, 1972). Homeostatic control of zinc absorption operates in respect to dietary level of zinc. Absorption of $Zn^{65}$ is enhanced in zinc deficiency and declines at higher zinc intakes (Underwood, 1977a).

Many factors decreasing the absorption of zinc from the intestines include phytic acid, dietary calcium and soy protein.

Phytate (inosital hexaphosphate), which is present in all cereal grains and most vegetables, can bind zinc and reduce the biological availability of zinc (O'Dell and Savage, 1960). Later, Oberleas et al. (1962) showed that phytic acid added to an animal protein diet depressed growth in swine. Using rats, Hatcock and Abrahim (1978) reported that sodium phytate decreased the absorption of zinc without changing the distribution pattern in the rest of the body. In human subjects, Sandstrom et al. (1978) found that the absorption of zinc from a whole meal bread made from wheat flour was lower than from white bread. This finding might be related to earlier studies by Reinhold (1971 and 1972) which showed that unleavened bread consumed by Iranian villagers contains higher amounts of

phytate than urban bread, which is leavened and allowed to ferment.

Comparative absorption studies with $Zn^{65}$ in rats showed that zinc is absorbed more by females than by males (Strain et al., 1978).

There are at least two proteins in the intestinal mucosa that bind zinc (Van Campen and Kowalski, 1971), but the exact nature of this zinc-binding ligands has not been proven equivocally. However, the observations of Evans and Johnson (1978) implicate prostaglandin E2. Evans (1973) showed that zinc metabolism may be regulated by the metal binding protein metallothionein. When plasma concentration is decreased by altered metabolism or low dietary zinc intake, intestinal metallothionein synthesis is minimal. When plasma zinc concentrations become elevated, zinc is deposited in the intestinal cells which results in the induction of metallothionein synthesis. Metallothionein sequesters newly absorbed dietary zinc and thereby regulates the quantity of zinc which enters the body (Evans and Johnson, 1978).

Methfessel and Spencer (1973) using $Zn^{65}$ studied specific absorption sites in rats by means of ligated intestinal sacs. They concluded that the absorption of $Zn^{65}$ was significantly greater from the duodenum than from the more distal segments of the small intestine over a 2-hour observation period, absorption was much less from the mid jejunum and ileum. Only minimal amounts were observed from the stomach, caecum and

colon. The site of maximal absorption in humans has also been shown to be from the duodenum (Elmes, 1978).

1.3.1.4.4. Zinc transport and storage.

Absorbed or injected zinc is incorporated at differing rates into different tissues. Uptake of zinc by the bones and central nervous system is relatively slow. Zinc entering the hair is not available to the tissues (Underwood, 1977). The most rapid accumulation and turnover rate of absorbed zinc occurs in the pancreas, liver, kidney and spleen (Feaster et al., 1955). The zinc in these organs and the more slowly exchanging zinc in the muscles and the red cells constitute a soft tissue zinc pool of varying exchange rates.

In normal plasma in the post absorptive state, zinc is reported to be about one-third tightly bound to $\alpha_2$-macroglobulin and two-thirds loosely bound to albumin, with traces bound to the amino acids, histidine and cysteine (Frieden, 1978). However, in the absorptive process, two-thirds of the zinc was observed in a transferrin fraction in portal plasma (Evans & Winter, 1975). Thus zinc appears to be carried by transferrin to the liver prior to becoming bound to albumin or incorporated into $\alpha_2$-macroglobulin.

1.3.1.4.5. Zinc excretion.

Zinc loss occurs from the body mainly by way of the faeces. Greater part of the zinc in faeces is non-absorbed zinc from the food, whereas a smaller part comes with the pancreatic juice or

to a modest degree, with the bile (Weismann, 1976). In humans, daily faecal zinc excretion increases with dietary intake (Greger et al., 1978).

The normal 24-hour urinary zinc excretion is very small and varies between 0.1 and 0.9 mg (Weismann, 1976 and Table 1.2). And unlike faecal zinc excretion urinary zinc excretion is unaffected by dietary variations (Greger et al., 1978).

Sifnificant quantities of zinc are excreted in sweat, especially in hot climates, and in milk, especially colostrum (Table 1.2.). The daily loss of zinc via the skin, nails, and hair is approximately 0.5 mg which is similar to that in urine (Table 1.2., Schroeder et al., 1967).

1.3.1.5. Zinc deficiency in man.

Zinc deficiency has been reported in children living in North America (Hambridge et al., 1972) with low hair zinc levels of less than 70 ppm, a concentration similar to that reported for Egyptians adults suffering from symptomatic zinc deficiency (Strain et al., 1966). Daily dietary supplementation for 1 to months with 1 to 3 mg $ZnSO_4$/kg body weight restored appetite to normal and hair zinc concentrations increased in 50% of the children.

1.3.1.5.1. Nutritional zinc deficiency.

1.3.1.5.1.1. Absolute zinc deficiency

Insufficient zinc may be present in synthetic amino acid mixtures used both orally and intravenously in total

parenteral nutrition and consequently supplementation with zinc and other trace elements becomes obligatory when using these products to avoid abnormal nutritional effects (Fell and Burns, 1976).

1.3.1.5.1.2. Conditioned zinc deficiency.

Absolute dietary zinc deficiency is unlikely under natural conditions since zinc occurs in most foodstuffs. However, the reduced availability of zinc in a cereal diet rich in phytates can lead to occasional zinc deficiency (Parisi and Vallee, 1969; Prasad, 1969; Halsted et al., 1972; Reinhold et al., 1973) or from the habit of clay-eating (geophagia) (Prasad et al., 1961; Anon, 1973; Cavdar et al., 1977). These authors theorized that the ingestion of clay resulted in chelation of both iron and zinc.

1.3.1.5.2. Malabsorption.

Malabsorption of zinc can also be a feature of gastro-intestinal disease and an inborn error of metabolism (acrodermatitis enteropathica) in which reduced the intestinal absorption of zinc has been postulated (Lombeck et al., 1975). The defective absorption of zinc in acrodermatitis enteropathica can be compensated for by administration of about 50 mg zinc a day (Nelner and Hambidge, 1975; Weismann and Wadskov, 1975). The excretion of zinc in urine and faeces remains normal (Lombeck et al., 1975).

1.3.1.5.3. Increased losses of zinc.

Zinc is a component part of all biological fluids, and

excessive sweating, wound and skin exudates, loss of blood, gastrointestinal fluid, faeces and urine can result in zinc deficiency (Fell and Burns, 1976).

1.3.1.5.3.1.Excess sweat loss.

Excessive sweating could increase zinc loss up to 2 - 5 mg per day (Prasad et al. 1963b; Prasad, 1969; Anon, 1973), but may be preserved in the deficient subject (Prasad et al., 1963b).

1.3.1.5.3.2.Loss of blood.

Blood contains about 10 µg/ml (Table 1-2.), so that hookworm and S. haematobium infestations can contribute to body depletion (Prasad, 1969; Halsted et al., 1972; Anon, 1973).

1.3.1.5.3.3.Excess urine loss.

The metabolic response to injury and accompanying tissue catabolism, results in an increased excretion in zinc in urine, and this increase is in direct proportion to the severity of the original injury (Fell and Burns, 1976).

Zinc excretion in urine may be increased up to 2 - 8 mg per day in a large number of acute and chronic illnesses (Prasad, 1969; Halsted and Smith, 1970; Anon, 1973; Fell and Burns, 1976), hepatic cirrhosis (Vallee, 1959; Fredricks et al., 1964; Boyett and Sullivan, 1969; Prasad, 1969; Halsted and Smith, 1970; Halsted et al., 1972; Underwood, 1977a),

renal disease (Halsted and Smith, 1970; Halsted et al., 1972; Fell and Burns 1976; Underwood, 1977a), tissue damage from any cause (including surgery, trauma, myocardial infarction, and burns) (Bottomley, 1969; Halsted and Smith, 1970; Fell et al., 1973), protein deprivation (Smit and Pretorius, 1964), chronic skin ulcers (Halsted and Smith, 1970; Halsted et al., 1972), and sickle cell disease (Prasad et al., 1976) although Butler et al. (1978) were unable to confirm increased urinary zinc in sickle cell disease.

1.3.1.5.4. Metabolic alteration.

Disturbances of zinc metabolism are known to occur during a variety of other disease conditions leading to alterations in plasma zinc and may result in induced zinc deficiency secondary to the original disease process. The plasma zinc is low in patients with neoplasms (Vallee, 1959; Fredricks et al., 1964; Davies et al., 1968; Mathur et al., 1978), unless the site is in an organ which is rich in zinc, such as bone, when levels are high (Addink and Frank, 1959). Plasma zinc is low in leukaemias of all types (Vallee, 1959; Fredricks et al., 1964; Mendiola and Price , 1969), but red cells zinc is reported to be elevated (Talbot and Rose, 1960).

Plasma or serum zinc concentrations are reported to be diminished in megaloblastic anaemias (Fredricks et al., 1964) and β-thalassaemia (Prasad et al., 1965b; Askoy and Erdem, 1977), a finding not confirmed by Butler et al. (1978). Erythrocyte zinc levels are reported to be low in children with β-thalassaemia

(Aksoy and Erdem, 1977). In Michigan, New York and Jamaica, sickle cell anaemia patients were found to have depressed levels of zinc in plasma or serum (Serjeant et al., 1970; Karayalcin et al., 1974; Prasad et al., 1976). However, Kapu et al. (1976a) reported from northern Nigeria that sickle cell anaemia patients have a good zinc status, comparable to healthy controls in plasma. These workers postulated that Nigerian patients suffering from sickle cell anaemia may have a better zinc status than patients in North America and the West Indies, and this might explain why leg ulcers are less frequent in sickle cell anaemia in West Africa than in the New World. Butler et al. (1978) from Washington D.C., have also reported that sickle cell patients showed zinc levels equal to healthy Negro control groups in plasma. Red cell zinc is generally unaltered (Vallee and Gibson, 1949) or slightly raised (Vallee and Altschule, 1949) in most anaemias, except in megaloblastic anaemia, when it is elevated in parallel with high carbonic anhydrase activity (Vallee and Altschule, 1949; Vallee and Gibson, 1949; Talbot and Rose, 1960; Fredricks et al., 1964). Red cell zinc in sickle cell anaemia presents a special case similar to that of plasma zinc in sickle cell anaemia. Some workers have reported elevated levels (Vallee and Altschule, 1949; Talbot and Rose, 1960), and this may have been due to complicating folate deficiency; normal levels have been reported (Vallee and Gibson, 1949; Butler et al., 1978), but Gelshlegel et al. (1973) reported abnormally low red cell zinc in 10 out of 16

sickle cell patients, and pointed out that this could have bearing on a low haemoglobin oxygen affinity, stunted growth and indolent ulcers. This has been confirmed by Prasad et al. (1976) in 72 out of 84 sickle cell patients. These workers related their findings to the role of zinc in decreasing haemoglobin-binding to red blood cell membranes and antagonizing the effect of calcium on sickling in vitro (Prasad et al., 1976; Dash et al., 1974). Zinc and copper compete with each other for similar binding sites on proteins, and in zinc-deficient tissues an increase in copper has been observed (Prasad et al., 1967). Plasma serum copper has also been found to be elevated in sickle cell disease (Olatunbosun et al., 1975a; Butler et al., 1978; Kapu et al., 1976b; Prasad et al., 1976).

Hair zinc analysis has been shown to be a reliable, simple and atraumatic method of assessing body zinc stores (Strain et al., 1966). Subsequently, Prasad et al. (1976) determined hair zinc in sickle cell patients and reported significantly decreased levels in comparison with normal controls and suggested that the sickle cell patients may have had chronic zinc depletion, a finding that was not confirmed by Olatunbosun et al. (1977) and Butler et al. (1978). These findings further lend support to the earlier suggestion by Kapu et al. (1976a) that Nigerian patients suffering from sickle cell anaemia may have a better zinc status than patients in North America and the West Indies. This might explain why leg ulcers are less frequent in sickle cell anaemia in West Africa than in the West Indies, where improvement in

the rate of healing of leg ulcers has been reported after the administration of oral zinc (Sergeant et al., 1970).

### 1.3.2. Copper in man and other mammals.

#### 1.3.2.1. Historical introduction.

Copper was first shown to be essential in animal nutrition by Hart et al. (1928). They reported that when rats and rabbits were fed a milk diet an anaemia developed that iron supplementation did not correct and was relieved only when copper-containing ash of certain foods was fed to the animals. This work established the necessity of copper, in addition to iron, in the formation of haemoglobin in these animals. Haematopoiesis was considered to be the principal physiological activity involving copper until field studies with sheep and cattle revealed wider functions for this element (Underwood, 1977b).

Neal et al. (1931) first showed that copper deficiency occurs naturally in grazing cattle, in Florida. Sjollema (1933) discovered that copper deficiency was a casual factor in a disease of sheep and cattle locally known as lechsucht occuring in Holland. In 1937, Bennetts and Chapman found that a disease of lambs in Western Australia designated enzootic neonatal ataxia, was due to inadequate copper in the grazing fodder. During this period, laboratory studies disclosed a variety of other physiological functions involving copper. Defects in the process of pigmentation, keratiniza-

tion of wool, bone formation, reproduction, myelination of the spinal cord, cardiac function, and connective tissue formation, in addition to those of growth and haematopoiesis in several species including the rat, rabbit, cat, dog, goat, and sheep are found to be associated with copper deficiency (Underwood, 1977b).

A copper deficiency in humans has only recently been observed. Sturgeon and Brubaker (1956) demonstrated the use of copper sulphate in relieving an anaemic condition in five young children. However, adult man does not develop a copper deficiency even during long periods of malnutrition (Cartwright and Wintrobe, 1946). Two genetic defects adversely affecting copper metabolism in man are Wilson's disease, in which excessive copper is retained characterized by a deficiency of copper carrying protein caeruloplasmin (Cummings, 1962), and Menke's steely hair disease (Danks et al., 1972; Henkin and Grover, 1978).

1.3.2.2. Distribution of copper in nature.

1.3.2.2.1. Earth.

Soils contain on the average 20 ppm copper ranging from 2 to 100 ppm. Rocks contain 45 ppm on the average, with igneous rocks having the highest concentration (55 ppm) and sandstones the lowest (4 ppm) (Bowen, 1966).

1.3.2.2.2. Natural water.

In sea water, copper concentration (0.003 ppm) has changed very little due to precipitation in sea water (Schroeder et al., 1966). Fresh river water contains 0.01 ppm,

with higher levels occuring in some rivers possibly as a result of industrial contamination.

1.3.2.2.3. Air.

Air is a major source of a number of trace metals (Tipton and Schafer, 1964). Normal copper values in air is 0.02 $\mu g/m^3$ (Bowens, 1966). Distribution of copper in the air over some United States cities varied from 0.09 $\mu g/m^3$ to about 2.4 $\mu g/m^3$ (Schroeder et al., 1967).

1.3.2.2.4. Plants.

Marine plants contain copper in concentrations (11 ppm) greater than in sea water (Bowens, 1966; Schroeder et al., 1966). The average copper content of some of the plant and grass species on the savannas of northern Nigeria were reported to be 15.10 and 11.80 ppm, respectively during the beginning of the rainy season in April (Kapu, 1975b). Other workers elsewhere have reported similar figures (14 ppm) for land plants (Bowen , 1966).

1.3.2.2.4. Animals.

Marine animals concentrate copper from sea water up to 5000 ppm and American oysters concentrate copper up to 1500 ppm, green oysters having as much as 3300 ppm copper (Schroeder et al., 1966). Wide variations reflect the proximity of oyster beds to rivers containing factory effluents.

On a normal diet the average human body contains 100 - 150 mg of copper (Sass-Kortsak, 1965; Davies, 1972; Underwood

1977b; Butler and Daniel, 1973) although a lower range has been reported to be 80 to 120 mg (Burch et al., 1975). Young and newborns contain a higher tissue concentration of copper than adults.

The copper content of adult human organs and tissues is shown in Table 1.4. The liver, brain, kidneys, and heart are tissues of relatively high copper concentrations; the spleen, pancreas, muscles, skin, and prostate represent organs of intermediate copper concentrations; and the organs (bone, pituitary, thyroid, and thymus) are examples of tissues low in copper (Underwood, 1977b). In laboratory mice or rats and most wild animals studied high copper concentration occurs in the spleen, brain, liver and ovary (Schroeder et al., 1966). This has been confirmed in cattle and sheep, with higher copper concentrations in the liver than other animals (Underwood, 1977b). In man (Schroeder et al.,1966), the brain was the only organ with concentrations increasing from birth to about double the level at maturity. In most other tissues the copper concentration decreased from birth to maturity, indicating that the high total body copper of the newborn is a reflection of high copper levels of several organs and not merely of the liver (Underwood, 1977b). The pigmented parts of the eye contain exceptionally high concentrations of copper, although there are differences among species (Underwood, 1977b). Copper is associated particularly with the melanins and is largely bound in ionic form to protein.

Schroeder and Nason (1969) have shown that mean copper levels in natural hair from healthy females (55.6 µg/g) was higher than that from males (16.1 µg/g), declining with age in females to normal male values after the age of 40. These workers observed that natural hair from both sexes below the age of 30 years contained more copper than depigmented hair. Baumslag et al. (1974) obtained lower hair copper values (17.9 µg/g) for parturient mothers below 39 years of age. In that study, it was reported that as the age of the mother increased, the copper levels in the infants' hair decreased. However, the infants' hair copper values increased as the parity of the mothers increased only in blacks. Normal neonate hair copper values were reported to be 10.9 µg/g.

The concentrations of copper in biological fluids of man reported by different workers are shown in Table 1.5. In normal pregnancy there is a gradual increase of both serum copper and caeruloplasmin levels, culminating in levels about twice the non-pregnant normal at term (Scheinberg et al., 1958)

Serum copper levels vary markedly with age, with contents of newborn infants (36 µg/100 ml) much lower than adult values (Table 1.5.), and the average value obtained in parturient mothers (216µg/100 ml)(Sass-Kortsak, 1965). Cartwright and Wintrobe (1964) reported significantly higher levels of serum copper in normal adult females (114 µg/100 ml) compared to males (105 µg/100 ml). There is no significant sex differences in whole blood or plasma of most animals. Plasma copper does not increase following meals or decrease during fasting.

Physical exertion in man produces no change, but violent exercise induces a significant increase in whole blood copper in sheep (Underwood, 1977b).

1.3.2.3. Copper metalloenzymes in mammals.

Copper is known to be a constituent of a number of enzymes found in mammals. The active metal is firmly bound in a constant stoichiometric ratio to the protein in these enzymes. There are several ways of classifying metalloenzymes, but the simplest one, under individual metals, fails because several examples are known to contain more than one metal. Some copper enzymes like those of zinc have no prosthetic group and the metal is bound directly to the protein, so that one function of the metal may be that of maintaining the spatial arrangement of the protein chain. Table 1.6. shows the major copper metalloenzymes found in mammals.

Caeruloplasmin is an $\alpha$-globulin. It can act as an enzyme, acting as an oxidase towards serotonin and ascorbic acid *in vitro*. The physiological role of caeruloplasmin is partly to serve as a transport vehicle and to release copper at specific cellular sites. This role is related to the catalytic activity as an oxidase in which the copper cycles from Cu (II) to Cu (I) and back again (Freiden, 1978). The availability of iron and the rate at which it ultimately ends up as Fe $(III)_2$-transferrin complex depends on caeruloplasmin. Thus, caeruloplasmin is a molecular link between copper and iron metabolism.

Cytochrome C oxidase is an enzyme found in all aerobic cells, and is mainly responsible for the introduction of oxygen into the oxidative machinery that produces the energy which we need for physical activity and biochemical synthesis. When cytochrome oxidase action is blocked, as in cyanide poisoning, all cellular activity grinds to a quick halt, in only a few minutes. Cytochrome C oxidase may be regarded as the ultimate in the integration of the function of iron and copper in biological systems. A chemically similar enzyme, tryptophan - 2, 3 - dioxygenase, catalyzes the insertion of moleculer oxygen into the pyrrole ring of tryptophan yielding compound, N-formylkynurenine (Freiden, 1978).

Monoamine oxidase catalyzes the oxidative deamination of lysine residues of elastin peptide chains to produce desmosine, the compound that forms cross-links between elastin fibres in elastic tissue (Burch et al., 1975).

Lahey et al. (1958) first demonstrated that tyrosinase plays an integral role in skin pigmentation. Tyrosinase catalyzes the conversion of tyrosine to 3-L- alanine (dopa). Dopa is subsequently converted to melanin.

Both metallothionein and superoxide dismutase are implicated in the mechanisms of copper absorption from the stomach and the upper gut by providing binding sites for copper which is then released to the plasma transport mechanisms (Evans, 1973).

1.3.2.4. Copper metabolism in man.

Wintrobe (1967) summarized the process of ingestion, absorption, transport and storage, and excretion of copper in a schematic diagram (Figure 1.1.).

1.3.2.4.1. Dietary requirements for copper

The average North American diet has been shown to contain 2 - 5 mg copper per day (Cartwright and Wintrobe, 1964a and b). The minimum daily copper requirement for the adult human has been estimated by Chou and Adolph (1935) and by De (1949) as 2.0 and 2.02 mg, respectively, for the male, and by Leverton and Binkely (1944) as 2.0 mg for the female. Butler and Daniel (1973) suggested higher daily requirement of 4.5 to 5.0 mg for young women, allowing a safety factor of 2.0 mg and an allowance of 6.5 to 1.0 mg for sweat and menstrual losses, depending upon the climatic conditions, added to the minimum requirement of 2.0 mg.

Estimates of copper requirements of different ages of children have been made from balance studies. Children between the ages of 3 to 6 years weighing up to 20 kg require 1.0 to 1.6 mg copper daily (Underwood, 1977b). Engel et al. (1967) estimated, from balance studies carried out on 36 preadolescent girls aged 6 to 10 years, that 1.3 mg copper per day is necessary for equilibrium.

Copper balance studies reported by Sass-Kortack (1965) in full term newborn infants showed minor over-all loss or only

a minimal degree of retention. Premature infants, without exception, were found to have a slightly negative balance. These findings suggest that in this age group there may not be a dependance on dietary supply of copper. The increased copper depots present in newborn and premature infants are apparently being used preferentially for growth requirements in this age group.

1.3.2.4.2. Dietary sources of copper.

The amount of copper ingested daily from the diet is dependent upon the choice or selection of individual foods making up the total diet. It can also be influenced by the locality in which the foods are grown and by the degree of contamination from extraneous copper during processing, storage, and treatment with copper fungicides.

Schroeder et al. (1966) has stressed the importance of drinking soft water as a further source of copper. These workers observed a progressive increase in the copper in water from brook, to reservoir, to hospital tap, as well as a considerable increment in soft water compared to hard water from private homes. In their report, it was claimed that soft waters, because of their capacity to corrode metallic copper, could raise copper intakes by as much as 1.4 mg/day, whereas hard waters would reduce this source of copper to 0.05 mg/day.

The different classes of human foods differ greatly in copper content, with wide individual variation within particular food items (Table 1.7).

The richest sources of copper are crustaceans and shellfish, especially oysters, and the organ meats (liver, kidney, and brain), followed by nuts, dried legumes, dried vine and stone fruits and cocoa. The poorest sources of copper are dairy products, milk, butter and cheese, and white sugar and honey. These foods rarely contain more than 0.5 µg/g copper. The non-leafy vegetables, most fresh fruits, and refined cereals including white flour and bread, generally contain up to 2 µg/g copper (Schroeder et al.,1966).

The refining of cereals for human consumption results in a significant loss of copper, as with most other minerals. Thus, the mean copper content of whole grain of North American wheats was reported to be 5.3 µg/g, whereas the copper content of the white flour made from these wheats averaged only 1.7 µg/g (Underwood, 1977b).

The tolerance of most monogastric animals for copper is high (WHO, 1973). However, copper poisoning can occur as an industrial hazard in workers engaged in mining processing. And the report of probable copper poisoning in a 15-month-old infant suggests that continuous ingestion of copper from water supplies containing more than 8 mg/l may constitute a potential hazard if exposure is prolonged (WHO, 1973). Public Health measures in most developed countries effectively control the use of copper compounds in the colouring and preservation of foods and copper compounds are not now so prominent among available fungicides

and insecticides as they were several decades ago.

1.3.2.4.3. Absorption of copper.

Studies in man have given diverse figures for the amount of copper normally absorbed from an average diet. As little as 1% of the dietary intake was absorbed in one study (Cartwright et al., 1948) and about 38.6 and 49.9% on intakes of 3.4. and 4.2 mg, respectively (Butler and Daniel, 1973), which were comparable to the results of Leverton and Binkley (1944). Copper absorption and retention is affected by the chemical forms in which the metal is ingested, by the dietary levels of several other minerals and organic substances, and by the acidity of the intestinal contents in the absorptive area.

According to Tompsett (1940) an acid pH of the intestinal content facilitates dietary copper absorption in mice. This finding has been confirmed in human studies (Underwood, 1977; Davies, 1972). The more soluble copper salts or the carbonate are more easily absorbed in the human than the less soluble or the oxides (Underwood, 1977b).

High intestinal contents of other trace elements such as cadmium, mercury, silver, and zinc decrease copper absorption because they compete with copper for binding sites in the intestinal mucosa or on the metallothionein protein (Burch et al., 1975). Calcium salts depress copper absorption (Tompsett, 1940), possibly because they form a more alkaline medium in the upper intestines. This would

discourage the formation of acidic amino acids which are known to promote copper absorption (Underwood, 1977b). Dietary molybdenum and sulphate are known to adversely affect the storage and availability of copper. Both molybdenum and sulphate reduce the uptake of radioactive copper by the liver and reduce its incorporation into caeruloplasmin as well as reducing copper solubility in the gastrointestinal tract in animals (Underwood, 1977b; Davies, 1972; Burch et al., 1975), but appear unlikely to produce significant antagonistic effects upon copper metabolism in man (WHO, 1973). Sulphide anions also react with copper in the gut to form cupric sulphide, which is insoluble. This reaction is used therapeutically in Wilson's disease to diminish the absorption of dietary copper (Sass-Kortsak, 1965; Burch et al., 1975).

Organic substances, particularly phytates in the diet can reduce intestinal absorption of copper (Underwood, 1977b). Buttler and Daniel (1973) studied copper metabolism in young women fed on peanut flour and casein-lactalbumin sources of protein and concluded that the lower apparent absorption observed in those fed with peanut flour might have been due to the relatively high content of phytic acid in peanut flour, which is absent in casein-lactalbumin. This is due to the formation of nonabsorbable complexes in the gut (Burch et al., 1975).

Kirchgessner and Grassman (Underwood, 1977b) have shown that the affinity of copper ions for inorganic and organic ligands in

the food can reduce the rate of absorption, depending on the size and stability of the resulting complexes. With essential amino acids forming complexes, absorption is faster than from complexes formed by ligands with non-essential amino acids, in the gut wall.

Copper is absorbed mainly from the stomach and the duodenum in man (Sachs et al., 1943; Underwood, 1977b; Burch et al., 1975) by at least two mechanisms. One, an energy dependent process, is facilitated by amino acids and probably represents the absorption of copper complexes of amino acids. A larger portionof the copper is absorbed by the second mechanism and is bound to two protein fractions in the intestinal mucosa. One of these proteins is probably the copper enzyme, superoxide dismutase. The second protein involved in copper absorption is rich in sulphydryl groups and has the characteristics of metallothionein. This protein may act to provide binding sites for copper, which is then slowly released into the plasma transport mechanisms (Underwood, 1977b; Burch et al., 1975; Freiden, 1978).

1.3.2.4.4. Transport and storage of copper.

Absorbed copper is transported in the blood by Cu-albumin, Cu-histidine and caeruloplasmin (Freiden, 1978). Cu-albumin and Cu-histidine are transient compounds on their way to the liver for storage and for the biosynthesis of caeruloplasmin or released for incorporation into erythrocuprein and various other copper-containing enzymes of the cells (Underwood, 1977b; Freiden, 1978).

Injection of radioactive copper into the blood causes a rapid increase in plasma radioactivity as the copper is quickly bound to albumin, this is followed by a decline in radioactivity as radioactive copper attached to albumin is distributed to the copper proteins in the liver and elsewhere in the body. This decline is followed by a further rise in plasma radioactivity as copper incorporated into caeruloplasmin is released from the liver into the blood (Davies, 1972). After equilibrium has occured, copper bound to caeruloplasmin constitutes about 95% of that in the plasma (and is firmly bound). The remaining 5% is loosely bound to albumin (Bush et al., 1956a; Davies, 1972; Underwood, 1977b; Freiden, 1978) (see Figure 1.1.).

1.3.2.4.5. Excretion of copper.

Most of the copper ingested is excreted by way of the faeces (see Figure 1.1.). A high proportion of this normally consists of unabsorbed copper but active excretion via the bile occurs, and in man the biliary system is the major pathway of excretion (Underwood, 1977b) Of the 2-5 mg copper ingested daily by adult man, 32% is absorbed, slightly less than this is excreted in the bile, and a small amount passes directly into the bowel (Wintrobe, 1967).

The kidneys play a minor role in excretion, eliminating only ionic or loosely bound copper (Schroeder et al., 1966). Reported values of copper excreted daily are extremely variable (Table 1.5.). This variability may partly reflect analytical difficulties in estimating the comparatively low copper concen-

-trations normally present in urine.

Negligible amounts of copper are lost in the sweat. Under hot, humid conditions, Mitchell and Hamilton (1945) found high loses of 1.0 to 1.9 mg Cu per day. Under moderate conditions, smaller losses in the magnitude of 0.05 to 0.07 mg/day occured (Butler and Daniel, 1973).

Normal menstrual losses range from 0.19 to 0.72 mg/period (Ohlson and Daum, 1935; Leverton and Binkley, 1944) which was equivalent to an average of 0.5 mg Cu per period and estimated to be slightly less than 0.2 mg per day (Underwood, 1977b).

Salivary copper excretion is probably dependent upon the volume of saliva spat out, since most of the saliva produced in the mouth is often swallowed. However, substantial amounts of Cu may be lost during fasting periods during which a majority of those fasting spit out their saliva (personal observation).

The loss of copper in the milk at the height of human lactation is much higher (0.4 mg/per day), imposing no known nutritional hazards (Underwood, 1977b).

1.3.2.5. Copper deficiency in man.

1.3.2.5.1. Nutritional copper deficiency.

Copper deficiency has been reported in four infants who had fed exclusively on milk and developed malnutrition. Copper supplementation reversed the condition (Sturgeon, 1956).

Naturally occuring copper deficiency has not been proven to occur in adult man (Sass-Kortsak, 1965), most probably because of the low copper minimum requirements (2 mg/per day) and wide distribution in the foods (Table 1.7.). However, the possible effects on the human population of a lowered soil copper content, a lowered copper status in animals and consequent inadequate concentration in foods has been studied in several ways.

Guthrie et al. (1978) have shown that in New Zealand, the daily intakes of copper averaged only 1.5 mg, and that most residents studied had inadequate copper status as estimated by their toenail, serum, erythrocyte, hair and organ levels. A report (Klevay, 1978) from the U.S.A. stated that most of the diets contained lower copper contents (0.8 mg). Therefore, unless foods are chosen very wisely for their mineral contribution to the diet, adults may be at some risk in their intakes of copper (Harland et al., 1978).

1.3.2.5.1.1. Absolute copper deficiency.

Cases of copper deficiency anaemia (hypocupremia) have been described in children. In kwashiokor, the hypocupremia appears to arise from the low protein and the frequently low copper intakes of the diets. The hypocupremia of infants results from an inability of the infants to obtain sufficient copper from their copper-low milk-diets to prevent copper depletion in the face of increased loss of the plasma protein into the bowel (Cartwright and Wintrobe, 1964a; 1964b). Also, normal breast-fed infants are unable to obtain sufficient copper from the milk

to prevent some body copper depletion (Underwood, 1977b). However, hypocupremia of childhood does not necessarily arise in infants on exclusive milk diets (Wintrobe et al., 1953). A more oonvincing evidence of copper deficiency in infants has been obtained in Peru, where severely malnourished infants aged 8 - 15 months were receiving a high-calorie milk diet deficient in copper (Cordano et al., 1964; 1966). In most cases of infant copper deficiency, copper supplementation reversed the conditions (Cardano et al., 1966; Underwood, 1971b).

Naturally occuring copper deficiency has not been proven to occur in the adult human. However, with the advent of intravenous hyperalimenation for protracted periods, we can expect an increased number of adults to develop anaemia because of copper deficiency. The copper protein, caeruloplasmin, appears to be involved in the catalysis of absorbed or mobilized iron from the ferrous to the ferric state for transport of the iron to be used in haemoglobin synthesis (Evans, 1973).

1.3.2.5.1.2. Conditioned copper deficiency

Copper deficiency reported in infants and young children may be conditioned by the unavailability of copper in phytate rich cereal preparations from "milk formula" diets (Graham and Cordano, 1969; WHO, 1973). In improverished communities clay eating is common among many infants (Prasad et al., 1961; Anon, 1973 Cavdar et al., (1977). The chelation of iron by

clay may be responsible, in part, for the disturbed synthesis of caeruloplasmin and lead to conditioned impaired copper utilization.

1.3.2.5.2. Malabsorption.

Malabsorption of copper is a feature of Menke's "Kinky hair" syndrome in the infant (WHO, 1973; Burch et al., 1975 ). Menke's kinky hair syndrome is a progressive brain disease associated with subnormal serum copper and caeruloplasmin levels, and nervous tissue is almost devoid of the copper enzyme cytochrome C oxidase. Failure to absorb copper may lead to sprue and coeliac diseases in infants (Davies, 1972).

1.3.2.5.3. Increased losses of copper.

1.3.2.5.3.1. Excess sweat loss.

Copper, like zinc is present in all biological fluids. Heavy Sweating can result in increased excretion up to 1.9. mg copper per day (Mitchell and Hamilton, 1949), which is greater than sweat losses under moderate climatic conditions (0.05 to 0.07 mg/day) reported by the same authors and that reported (0.02 mg/day) by Schroeder et al. (1966).

1.3.2.5.3.2. Loss of blood.

From Table 1.5. blood contains more copper than the other biological fluids studied. Therefore, in women the problem of copper balance is much more precarious, because they are subject to regular additional copper losses in the menstrual blood, ranging 0.19 to 0.72

mg/period) (Ohlson and Daum, 1935; Leverton and Brinkley, 1944), apart from that lost from time to time with the newborn infant and its adnexa (Underwood, 1977b). Loss of at least 40 ml of blood a day from heavy hookworm infestation is seen not infrequently in adults in Nigeria (Gilles, et al., 1964; Ball, 1966); this could lead to loss of up to 40 µg of copper per day.

1.3.2.5.3.3. Excess urine loss.

Excessive excretion of copper occurs in Wilson's disease, with levels of urinary copper of about 1500 µg/day (Sass-Kortsak, 1965; Burch et al., 1975; Menke et al., 1978), and in Menke's kinky (steely) hair disease (3.8 - 5.4. µg/24 h./kg body weight) and above that (1.1 µg/24 h./kg body weight) of age-matched control infants (Henkin and Grover, 1978). In nephrotic syndrome 60 - 80% of the urinary copper is excreted bound to caeruloplasmin and the remainder to albumin (Davies, 1972). Urinary copper is also elevated in the presence of proteinuria (Davies, 1972). An increased excretion of copper in the urine during hepatitis (Henkin and Smith, 1972) was ascribed to a postulated increase in the glomerular filtration of microligands that consisted of copper bound to histidine, glutamine, threonine, or cysteine. However, the studies of Beisel et al. (1973) showed that the concentrations of histidine, glutamine, threonine, and cysteine in urine were generally depressed during symptomatic illness.

1.3.2.5.4. Metabolic alteration.

Constant alteration of copper metabolism have been reported in a variety of pathological states. These observations are restricted in most cases to a description of the alterations in the plasma or serum levels of copper and caeruloplasmin in the patients. The biochemical explanations for these changes are still obscure, although logical reasons can be advanced in a number of cases.

Hypocupremia and hypocaeruloplasminemia of marked degree are found regularly in patients with kwashiorkor and marasmus (Lahey et al., 1958; Reiff and Schnieden, 1959; Lehmann et al., 1971). The copper content of the liver is significantly reduced in these children (Underwood, 1977b). Most of these changes have been ascribed to nutritional copper deficiency (conditioned or absolute), decreased absorption from the gastrointestinal tract, or failure of the synthesis of caeruloplasmin. But according to Underwood (1977b), the hypocupremia of marasmus and cystic fibrosis are often associated with low protein intakes. The low serum copper and caeruloplasmin levels reported in coeliac disease (Axtrup, 1946; Lahley, 1957) and in tropical (Butterworth et al., 1958) and non-tropical sprue (Cartwright, 1955) have been shown to result from impaired copper absorption from the gut (Sternlieb and Janowitz, 1964; Davies, 1972). Nephrotic syndrome is constantly characterised by excess urine excretion of caeruloplasmin and loss of albumin-bound copper

(Cartwright et al., 1954), which might probably explain the development of hypocaeruloplasminemia and hypocupremia of nephrosis.

Hypocupremia and low liver copper occurs in Menke's kinky (steely) hair syndrome, known to be associated with a genetically determined defect in copper absorption (WHO, 1973). The hypocupremia of Wilson's disease is probably due to the failure to form caeruloplasmin. There might be increased absorption of copper which is retained because of increased binding of copper to hepatic protein, or decreased biliary excretion (Cartwright et al., 1960; Sternlieb et al., 1973).

Hypercupremia is more commonly found in disease states than hypocupremia. Hypercupremia is evident in most chronic infections of man from bacterial and viral organisms (Beisel et al., 1973) and rheumatoid arthritis, leukaemia, Hodgkin's disease, acute and chronic liver disease, multiple sclerosis, myocardial infarction, schizophrenia, and epilepsy (Sunderman, 1973; Olatunbosun et al., 1975b; Schwartz, 1975; Sorenson and DiTommaso, 1976; Underwood, 1977b; Olatunbosun et al., 1978). The elevations in liver disease were attributed to interference with the excretion of copper in the bile. The increases in concentration of both serum copper and its binding protein,

caeruloplasmin, develops at the onset of symptomatic illness during an infection and most connective tissue diseases. They are associated with a redistribution of the metal from the liver to the blood. This redistribution is thought to be initiated by a leukocytic endogenous mediator (LEM) which stimulates the liver to synthesize additional quantities of caeruloplasmin (Beisel et al., 1973).

In Hodgkin's disease, the raised serum copper varies with the activity of disease, increasing with progression, decreasing with improvement, and returning to within normal limits at complete remission (Thorling, 1976).

Elevated plasma or serum copper and caeruloplasmin concentrations have been reported in patients with sickle cell disease (Sachs et al., 1935), and other haemolytic anaemas including thalassaemia, aplastic anaemia, and pernicious anaemia (Cartwright et al.. 1948; Prasad et al., 1965b; Butler et al., 1978). From Michigan, Prasad et al. (1976) have reported raised plasma copper but normal erythrocyte copper concentrations in patients suffering from sickle cell disease. This was confirmed in similar patients from the Washington D.C. area (Butler et al., 1978). However, the report from Texas (Butler et al., 1978) showed elevated erythrocyte levels of sickle cell patients double the control values. From Nigeria, Olatunbosun et al. (1975a) reported raised plasma

copper levels in sickle cell patients from Western Nigeria. Kapu et al. (1976b) have confirmed this finding in sickle cell anaemia patients from the savannah areas in northern Nigeria.

The diverse findings in erythrocyte copper levels in American sickle cell patients may reflect differences in the local dietary levels of copper as well as the ratio of copper to other metals. The intakes in Texas averaged 8.1 mg/day Cu, which is higher than 1.09 mg/day in Iowa, 0.58 mg/day in the Chicago area (both in the Midwest United States of America), (Butler et al., 1978). The raised plasma or serum copper in sickle cell anaemia patients is mostly due to the increase in caeruloplasmin activity, because of increased need for copper in the utilization of iron during haemosynthesis (Wintrobe, 1967). The accumulation of copper in plasma or serum and red blood cells in sickle cell anaemia could be due to disturbed iron metabolism in the mitochondria of normoblasts. The hypercupraemia of thalassaemia, aplastic anaemia and pernicious anaemia is likely to have a similar mechanism.

However, there are no reports on copper and other trace element erythrocyte studies in relation to dietary intakes in this environment.

## JUSTIFICATION FOR THE INVESTIGATION

Sickle cell anaemia occurs in about 2% of newborn infants and sickle cell trait in about 29% of the population of Garki, Kano State, northern Nigeria (Fleming et al., 1979). Some complications of sickle cell disease, such as growth retardation, hypogonadism in man, and poor wound healing, have been related to zinc deficiency in these patients (Prasad et al., 1976). Zinc supplementation has been said to be beneficial to some individuals with sickle cell disease (Prasad et al., 1976). It was considered necessary to assess the zinc status of Nigerians with sickle cell anaemia and to see whether zinc deficiency contributed to stunted growth, poor development, and leg ulcers. It was also necessary to compare results with respect to sickle cell disease with those obtained from patients with leg ulcers without sickle cell disease.

Alterations in metal metabolism in sickle cell disease may be a consequence of intercurrent infection rather than sickle cell disease. Therefore, a group of patients with chronic infection (pulmonary tuberculosis) was studied.

Hookworm anaemia is a common and serious problem among Hausa farmers in northern Nigeria (Ezem et al., 1977). It seemed necessary to discover whether the trace element metabolism of these patients was altered as a result of severe chronic blood loss.

The changes which have been reported in trace metal concentration in malignant conditions may be of value in the prognosis and control of treatment (Thorning and Thorning,

1976). Primary liver cancer is a problem common in Africa (Edington and Gilles, 1969). Because the development of hepatomas in African cirrhotic livers is different from that in cirrhrosis elsewhere in the world (Versieck et al., 1974), it is possible that the trace metal metabolism might be different in primary liver cancer from that obtained in the rest of the world. Measurements of zinc and copper in serum, red cells and hair is likely to be useful in the management and treatment of cancer patients.

As a preliminary it was necessary to establish the normal range of zinc and copper in a symptom free population living in northern Nigeria. This may vary with socio-economic class due to differences in food and hygiene. There might be physiological differences due to intake of food and pregnancy.

## 1.4. Objectives of the Investigation.

The objectives of this investigation were:

1. to establish the range of normal serum zinc and copper concentrations in elite and non-elite Nigerians and to discover if there were any socio-economic differences. Ajaegbu (1976) defines the elite Nigerian population as those people who have moved up the social and economic ladder, achieving a position in the society as well as a combination of traits at given levels which are above, and distinct from, those of the mass (non-elite) of population. On this basis, "elite" and "non-elite" are used in this study to refer to University senior staff and rural farmers, respectively.

2. to determine the changes occuring in the serum zinc and copper after a meal.

3. to determine the effect of pregnancy on the serum zinc and copper in Nigerians.

4. to report levels in serum, erythrocyte and hair zinc and copper in Nigerians, classified by their haemoglobin types, that is, normal haemoglobin (HbAA), sickle cell

trait (Hb.AS), sickle cell anaemia (Hb.SS) and others.

5. to report the changes in serum zinc and copper in variety of diseased conditions in Nigerians, including hookworm anaemia, pulmonary tuberculosis, leukaemia and primary liver cancer.

## 1.5. Methods.

### 1.5.1. Selection of subjects.

Details of subjects studied in this thesis are given under each result chapter.

### 1.5.2. Precautions against contamination.

All glass and plastic-ware used in this investigation, except the plain venoject tubes used in the collection of blood samples, were washed with Teepol liquid detergent, rinsed thoroughly, immersed for at least 2 hours in 1% disodium ethylonediamine etra acetate ($Na_2EDTA$) and finally rinsed 3 times with deionized water.

Reagents and chemicals were analytical grades or certified highest reagent grades (British Drug Houses, Ltd.). Deionized water was used throughout for dilution.

### 1.5.3. Collection and processing of blood for serum for metallic analyses.

10 ml of venous blood was collected in plastic disposeble syringes with needles and transferred into plain venojet

tubes (Jintan Terumo Co. Ltd.; Tokyo, Japan) and allowed to clot at room temperature. The clotted blood was centrifuged at 3,000 r.p.m. for 10 minutes, and specimen showing even minimal signs of haemolysis were discarded. The serum for elemental analysis was stored in plastic vials with screw caps, and refrigerated or frozen depending on when the analysis was to be performed. When the blood could not be collected after an overnight fast (usually at 09.00 h. ) the time relation to the last meal was noted, making certain that no samples were collected within an hour of the last meal, except during the investigation of serum zinc response to food when blood was collected 60 minutes after the usual mid-day meal (13.00 h. ).

1.5.4. Preparation of red blood cells.

For metallic analysis, the red blood cells were prepared after the procedure outlined by Prasad et al. (1965a) except that 3.8% trisodium citrate was used as the anticoagulant. After separation of plasma, the red blood cells washed 3 times with 20 ml of normal saline. A measured amount (usually 2 ml) of red cells was transferred to a 25 ml volumetric flask and made to volume with deionized water. 2 ml of this haemolysate was transferred to a 10 ml glass test-tube and the following were added to the content in order: (1) 2 ml deionized water, (2) 1 ml. 6N HCl, and (3) 1 ml 20% TCA. The sample was then heated at 90°C for 5 minutes. The supernatant after centrifuging was decanted and saved in a plastic

vial. To the precipitate, 1 ml 10% TCA was added again. The sample was heated and centrifuged as described before. The precipitation with TCA was performed for a third time and all the supernatants were combined for determination of zinc and copper.

1.5.5. Collection and preparation of hair samples.

Hair samples were taken from the head with stainless steel clippers. Each sample was placed loosely in a paper envelope, transported to the laboratory and washed carefully (usually 3 times) with carbon tetrachloride to remove lipids and dust. The samples were dried overnight at 65°C and ashed in porcelain crucibles at 450°C for up to 24 hours, until a white powder remained. They were then dissolved in 5 ml or 10 ml HCl and stored in polyethylene bottles.

1.5.6. Haematological techniques.

Red cell indices were measured on the Coulter ZF counter system (Dacie and Lewis, 1975). Hb. electrophoresis was performed on cellulose acetate, following the techniques of Lehmann and Huntsman (1975).

1.5.7. Determination of zinc and copper.

Analyses were performed with a Perkin-Elmer Model 306 atomic absorption spectrophotometer and used in accordance with the manufacturer's operating manual (Perkin-Elmer Corporation, Norwalk, Connecticut, U.S.A.).

For assay of serum zinc, serum was diluted four-fold in deionized water and aspirated directly by using standards in glycerol solutions of 5% (v/v) in deionized water. For copper, serum was diluted 1:1 with deionized water and assayed directly by using standards in glycerol solution of 10% (v/v) in deionized water.

1.5.8. Chemical methods.

Total protein, albumin, alkaline phosphatase and caeruloplasmin were determined either on the day of collection or within three days after the collection of blood. Total protein, albumin and alkaline phosphatase were determined according to standard methods (Varley, 1969) and caeruloplasmin after a modified procedure of Ravin's method described by O'Brien et al. (1968).

1.5.9. Statistical analysis.

Statistical analyses were carried out at the Ahmadu Bello University Computer Centre, Zaria. The programme used was the SPSS-Statistical Package for the social sciences.

Descriptive summary statistics carried out included measures of central tendencies such as the:

Maximum value

Minimum Value

Mean (Arithmetic Average)

Range (Minimum - Maximum)

Measures of variability such as variance, standard deviation and standard error were considered. Students "t" test was

carried out as required between two groups*. The correlation coefficient between any two parameters was determined, and the level of significance in each statistical test was set at 0.05 or 95% confidence interval.

1.5.10. Ethical considerations

Informed consent was obtained according to a protocol approved by the Ethical Committee on Human Research of the Faculty of Medicine of the Ahmadu Bello University, Zaria. The subjects or their guardians were made to understand the purpose and procedures of the investigation.

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* It was more difficult to recruit elite than non-elite Nigerians. The HbAA is about 4 times more numerous in the population than HbAS. The number of patients with various pathological conditions studied was limited to the number of patients presenting to the hospital during the time of study.

Table 1.1. Zinc Concentrations of Normal Mammalian Tissues, Wet Weight*

| Tissue | Human (µg/g) | Monkey (µg/g) | Rat (µg/g) | Pig (µg/g) |
|---|---|---|---|---|
| Adrenal | 12 | 16 | - | 23 |
| Brain | 14 | - | 18 | - |
| Heart | 33 | 22 | 21 | - |
| Kidney | 55 | 29 | 23 | 40 |
| Liver | 55 | 51 | 30 | 40 |
| Lung | 15 | 19 | 22 | - |
| Muscle | 54 | 24 | 13 | - |
| Pancreas | 29 | 48 | 33 | 45 |
| Prostate | 102 | - | 223 | - |
| Spleen | 21 | 21 | 24 | 28 |
| Testis | 17 | 17 | 22 | - |

*from Underwood, 1977a

Table 1.2. Zinc Concentrations of Body Fluids in Normal Humans

| Fluid | Mean µg/100 ml | References |
|---|---|---|
| Blood | | |
| Whole | 880 | Vallee & Gibson (1948) |
| Plasma | 121 | Vallee & Gibson (1948) |
| Plasma | 104 | Prasad et al. (1965a) |
| Plasma | 91 | Kapú et al. (1976a) |
| Plasma | 105 | Butler et al. (1978) |
| Serum | 126 | Vikbladh (1950) |
| Serum | 102 | Pekarek et al. (1972) |
| Erythrocytes | 1440 | Vallee & Gibson (1948) |
| Erythrocytes | 1440 | Prasad et al. (1965a) |
| Erythrocytes | 1080 | Mansouri et al. (1970) |
| Erythrocytes | 950 | Butler et al. (1978) |
| Leukocytes | 5 µg/g dry wt. | Mikac-Devic (1970) |
| Milk | 300 - 500 | Underwood (1971) |
| Saliva | 8 | Sullivan (1975) |
| Gastric Juice | 17 - 16 | Sullivan (1975) |
| Bile | 35 | Sullivan (1975) |
| Sweat | 115 | Prasad et al. (1963b) |
| Urine | 633 µg/24 h. | Prasad et al. (1976) |
| Urine | 673 µg/24 h. | Butler et al. (1978) |

Table 1.3. Zinc Content of Certain Foods, Wet Weight.

| Food Item | Zinc µg/g | Reference |
|---|---|---|
| **Seafoods** | | |
| Oysters, fresh | 1487 | Schroeder et al., 1967 |
| Shrimp, frozen | 14.76 | Schroeder et al., 1967 |
| Lobster, frozen | 1.48 | Schroeder et al., 1967 |
| Tuna in oil, canned | 17.40 | Schroeder et al., 1967 |
| Sardines, smoked canned | 29.08 | Schroeder et al., 1967 |
| Meats | | |
| Beef, ground steak | 56.61 | Schroeder et al., 1967 |
| Beef liver | 39.23 | Schroeder et al., 1967 |
| Beef kidney | 19.96 | Schroeder et al., 1967 |
| Lamb chop | 3.61 | Schroeder et al., 1967 |
| Pork liver | 36.17 | Schroeder et al., 1967 |
| Pork kidney | 35.34 | Schroeder et al., 1967 |
| Chicken breast | 11.0 | Halsted et al., 1974 |
| Chicken wing | 23.08 | Schroeder et al., 1967 |
| Chicken leg | 29.05 | Schroeder et al., 1967 |
| Dairy Products | | |
| Cows milk, whole, raw | 4.18 | Schroeder et al., 1967 |
| Cows milk | 3.4 | Halsted et al., 1974 |
| Egg white | 0.27 | Schroeder et al., 1967 |
| Egg yolk | 35.54 | Schroeder et al., 1967 |
| Cereals & Grains | | |
| Maize, whole | 54.0 | Oke, 1965 |
| Sorghum, whole | 4.4 | Oke, 1965 |
| Rice, whole | 26 | Oke, 1965 |
| Wheat, whole | 31.52 | Halsted et al., 1974 |
| Bread, whole wheat | 5.33 | Schroeder et al., 1967 |
| Bread, whole wheat | 1.04 | Osis et al., 1972 |
| Bread, white | 0.6 | Osis et al., 1972 |
| Bread, white | 1.21 | Schroeder et al., 1967 |

Table 1.3. Continued

| Food Item | Zinc µg/g | References |
|---|---|---|
| Vegetables & Fruits | | |
| Beans, string, fresh | 1.25 | Schroeder et al., 1967 |
| Beans, string, fresh | 3.10 | Cresta et al., 1976 |
| Peas, fresh | 6.9 | Halsted et al., 1974 |
| Onions | 0.89 | Halsted et al., 1974 |
| Onions | 1.81 | Cresta et al., 1976 |
| Bananas | 1.50 | Cresta et al., 1976 |
| Starchy tubers | | |
| Cassava | 4.45 | Cresta et al., 1976 |
| Yam | 2.40 | Cresta et al., 1976 |

Table 1.4. Copper content of Human Organs and Tissues*

| Organs & Tissues | µg/g ash | | µg/g dry wt. | | µg/g wet wt. | |
|---|---|---|---|---|---|---|
| | Mean | Range | Mean | Range | Mean | Range |
| Brain | 360 | 240-500 | 21.9 | 14.6-30.5 | 5.40 | 3.60-7.50 |
| Liver | 510 | 320-1300 | 18.8 | 11.8-48.0 | 6.63 | 4.16-16.9 |
| Kidney | 260 | 190-340 | 11.9 | 8.75-15.6 | 2.86 | 2.09-3.74 |
| Heart | 350 | 250-460 | 14.0 | 10.0-18.4 | 3.85 | 2.75-5.06 |
| Muscle | 74 | 50-120 | 3.11 | 2.10-5.05 | 0.89 | 0.60-1.44 |
| Skin | 100 | 80-150 | 1.70 | 1.36-2.55 | 0.70 | 0.56-1.05 |
| Spleen | 89 | 67-120 | 4.45 | 3.35-6.00 | 1.25 | 0.94-1.68 |
| Pancreas | 140 | 92-210 | 4.90 | 3.22-7.35 | 1.68 | 1.12-2.52 |
| Lung | 120 | 92-180 | 5.75 | 4.41-8.65 | 1.32 | 1.02-1.98 |
| Adrenal | 200 | 160-250 | 2.20 | 1.76-2.75 | 0.92 | 0.74-1.15 |
| Testis | 80 | 58-110 | 4.55 | 3.30-6.27 | 1.10 | 0.63-1.54 |
| Prostate | 100 | 57-140 | 4.50 | 2.56-6.30 | 1.10 | 0.63-1.54 |
| Bone | 5.0 | 2.0-5.0 | 1.45 | 0.58-1.45 | 0.75 | 0.30-0.75 |

* from Sass-Kortsak (1965) (Adult figures)

Table 1.5. Copper content of Biological Fluids in Man.

| Fluid | Copper µg/100 ml ± S.D | References |
|---|---|---|
| Blood | | |
| Whole | 98 ± 13 | Wintrobe et al., 1953 |
| Plasma | 109 ± 17 | Wintrobe et al., 1953 |
| Plasma | 108 ± 26.95 | Kapu et al., 1976b |
| Serum | 102 ± 13 | Olatunbosun et al., 1975a |
| Serum | 108 | Olatunbosun et al., 1975b |
| Erythrocytes | 115 ± 22 | Wintrobe et al., 1953 |
| Erythrocytes | 93 ± 6.3 | Butler et al., 1978 |
| Saliva | 31.7 ± 15.1 | De Jorge et al., 1964 |
| Bile | 5.6 - 205.0 | Van Ravensteyn, 1944 |
| Cerebrospinal fluid | 1.6 ± 0.4 | Kjellin, 1963 |
| Urine | 18.0 ± 7.2 µg/24 h. (4-30 ug/24 h.) | Butler and Newman 1956 |
| Urine | 60 µg/24 h. | Schroeder et al., 1966 |

Table 1.6. Copper Metalloenzymes in Mammals*

| Protein or Enzyme | Source |
|---|---|
| Albocuprein I | Human, brain |
| Albocuprein II | Human, brain |
| Caeruloplasmin | Human and others, plasma |
| Cytochrome C oxidase | Meat mammals, heart, liver, etc. |
| 3, 4-Dihydroxyphenylethylamine B-hydroxylase | Cattle, adrenals |
| Dopamine B-hydroxylase | Cattle, adrenals |
| Ferroxidase II | Man, serum |
| Hepatomito chondrocuprein | Man and cattle, liver |
| L-6-D (metallothianein) | Man and cattle, liver |
| Lysyl oxidase | Chicken and rat cartilage |
| Mitochondrial monoamine oxidase | Man, rat and cattle, liver and brain |
| Pink copper protein | Man, Erythrocytes |
| Plasma/serum monoamine oxidase | Man, rabbit and pig, plasma/serum |
| Superoxide dismutase (cytocuprein) | |
| Cerebrocuprein | Man, brain |
| Erythrocuprein | Man, erythrocytes |
| Homocuprein | Man, blood |
| Hepatocuprein | Man, liver |
| Tryptophan - 2, 3 - dioxygenase | Rat, liver |
| Tyrosinase | Man, skin, eye |

*Prepared from data according to Scheinberg (1976).

Table 1.7. Content of Copper in Certain Foods*

| Food Item | Copper µg/g |
|---|---|
| Seafoods** | |
| Clams, raw | 3.33 |
| Oysters | 137.00 |
| Sardines, canned | 1.12 |
| Shrimp, frozen | 3.40 |
| Meat and Meat Products | |
| Beef - flesh | 0.20 |
| Beef - liver | 46.00 |
| Chickens - flesh | 0.15 |
| Meat sauce | 0.60 |
| Mutton - flesh | 0.45 |
| Pork - flesh | 0.23 |
| Pork liver | 5.50 |
| Nuts and Legumes | |
| Beans - stringbeans | 0.40 |
| Beans - pods | 0.75 |
| Groundnut - seed | 3.50 |
| Beans - dry | 2.95 |
| Groundnut | 2.70 - 5.60 |
| Palm nut | 3.00 |
| Starchy roots and tubers | |
| Cassava tuber - raw | 0.50 - 0.80 |
| Cassava tuber - boiled | 1.00 |
| Carrot | 0.70 |
| Potato | 1.21 |
| Onion | 1.12 |
| Radish | 0.54 |
| Yam tuber | 0.90 |
| Yam meal | 3.40 |

Table 1.7. (continued).

| Food Item | Copper µg/g |
|---|---|
| Cereal Products | |
| Barley | 4.07 |
| Maize - grain | 0.80 - 2.40 |
| Maize - whole meal | 1.20 - 1.70 |
| Rice | 1.10 - 1.70 |
| Wheat - whole grain | 1.70 |
| Wheat - flour | 1.19 |
| Fruits and Vegetables | |
| Bananas | 1.10 |
| Pineapple | 0.04 |
| Oranges | 0.04 |
| Cabbage* | 0.40 |
| Cassava leaves | 1.30 |
| Lettuce | 0.37 |
| Okra | 0.40 - 1.80 |
| Tomato | 0.40 |
| Dairy Products | |
| Cheese - American | 1.10 |
| Cheese - Cottage | 0.020 |
| Cheese - Swiss | 0.10 |
| Milk | 0.05 |

*from Cresta et al. (1976).

**from Schroeder et al. (1966).

FIGURE: 1.1. SCHEMATIC REPRESENTATION OF SOME METABOLIC PATHWAYS OF COPPER

The numbers in the boxes refer to milligrams of copper in each pool. The numbers next to the arrows refer to milligrams of copper traversing the pathway each day.

CU-ALB = direct-reacting-fraction

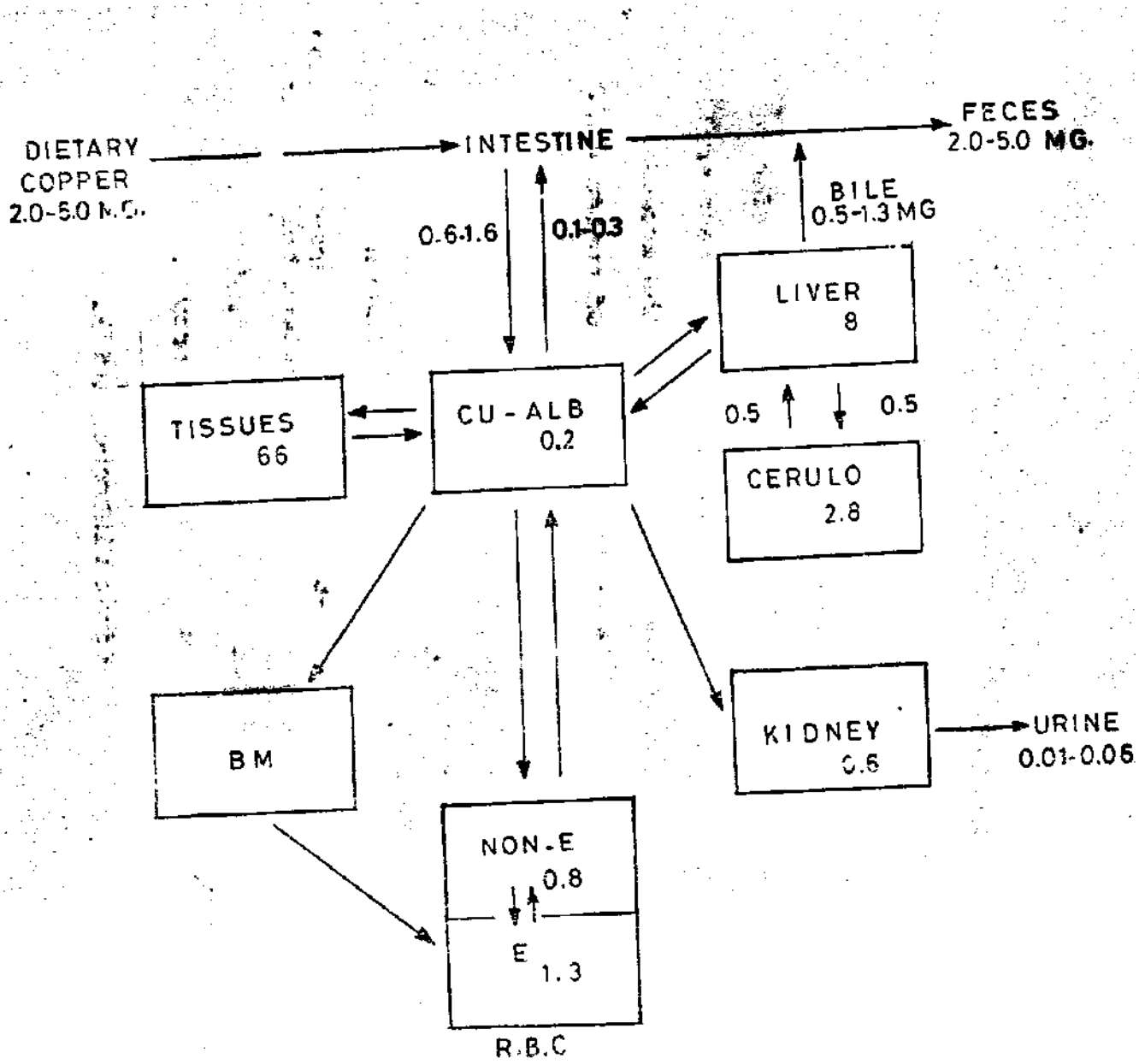
CERULO = caeruloplasmin

NON-E = non-erythrocuprein fraction of red blood cells (RBC)

E = erythrocuprein

BM = bone marrow

(from Wintrobe, 1967).


DIETARY
COPPER
2.0-5.0 MG.
INTESTINE
FECES
2.0-5.0 MG.
BILE
0.5-1.3 MG
0.6-1.6
0.1-03
LIVER
8
TISSUES
66
CU - ALB
0.2
0.5
0.5
CERULO
2.8
BM
KIDNEY
0.6
URINE
0.01-0.06
NON-E
0.8
E
1.3
R.B.C

# CHAPTER 2

## SERUM ZINC AND COPPER IN ELITE AND NON-ELITE NIGERIAN ADULTS

### 2.1. Introduction.

### 2.2. Normal ranges and physiological variation.

A number of studies have established the normal range of plasma or serum zinc in different populations (Halsted, et al., 1974; Kapu et al., 1976a; Underwood, 1977a, Butler et al., 1978; Olatunbosun et al., 1978). Individual variability is great but differences due to age among adults, race or sex are small or non-existent (Underwood, 1977a). However, according to Weismann (1976), the serum zinc level is lower in women than in men, and shows a minor fall with age, probably owing to a falling protein content in the serum. The normal ranges reported lie between approximately 70 and 120 µg/100 ml, mean values being approximately 95 µg/100 ml (Davies et al., 1968; Halsted and Smith, 1970; Kapu et al., 1976a; Olatunbosun et al., 1978). Significant regional differences in the U.S.A. in the plasma zinc levels of normal males have been reported (Underwood, 1977a), probably reflecting dietary intakes. Haemolysis as well as decayed thrombocytes may present considerable source of error in serum zinc determinations.

Plasma or serum copper in healthy Nigerians (Edozien, 1958; Reiff and Schnieder, 1959; Olatunbosun et al., 1973, 1974, 1975a, 1978; Kapu et al., 1976b) have been reported to be in the same range as found in populations of temperate countries (Cartwright, 1947; Cartwright et al., 1948; Wintrobe, et al.1953, Halsted et al., 1968). Plasma copper has been shown to be higher in human females than in males. Normal values of plasma copper reported are 105.5 ± 5.03 µg/100 ml for men and 114.0 ± 4.67 µg/100 ml for women (Underwood, 1977b). Although no explanation has been given for the observed sex differences, it is important to note that plasma copper levels can be significantly increased by the administration of œstradial in humans.

2.2.1. Effect of social class.

In a preliminary study, Kapu et al. (1976a) have shown that non-elite Nigerians had significantly lower mean plasma zinc concentrations (91.2 ± 28.16 µg/100 ml) than elite Nigerians (161.4 ± 52.06 µg/100 ml) and Europeans (134.4 ± 32.75 µg/100 ml) in Nigeria. Their results show that elite Nigerians and Europeans had similar plasma zinc levels. Spears et al. (1976) have confirmed this observation in elite and non-elite Indians in northern India. The socioeconomic differences observed in Nigerians and Indians in their plasma zinc levels suggest differences in dietary habits, occupation and environmental influences such as

intercurrent diseases among the non-elite population.

There seems to be no socioeconomic difference associated with plasma copper levels. Kapu et al. (1976b) reported that the mean plasma copper levels in Nigerian elite (115.1 ± 29.17 µg/100 ml) and non-elite (108 ± 26.95 µg/100 ml) and in Caucasians (110.8 ± 16.17 µg/100 ml) did not differ significantly.

2.2.2. Sickle cell trait.

Plasma or serum zinc concentrations have been reported to be lower in sickle cell trait (Hb.AS) than in normal (Hb.AA) subjects from Jamaica (Seaman et al., 1970) from New York State, U.S.A. (Karayalcin et al., 1974). However, Kapu et al. (1976a) were unable to confirm these results in non-elite Nigerians with normal haemoglobin pattern, sickle cell trait or sickle cell anaemia (91.2 ± 28.16, 86.8 ± 13.95, 93.6 ± 13.96 µg/100 ml, respectively).

Olatunbosun et al. (1975a) have reported equal serum copper concentrations (102 µg/100 ml) in normals (Hb.AA) and individuals with sickle cell trait (Hb.AS) living in the western area of Nigeria. This has been confirmed in Nigerians with normal haemoglobin pattern (Hb.AA) and those with sickle cell trait (Hb.AS) from the savannah areas of northern Nigeria (Kapu et al., 1976b). Kapu et al. (1976b) discovered that the mean plasma copper levels in normal elite Nigerians (115.1 ± 29.17 µg/100 ml), normal non-elite Nigerians (108.2 ± 26.95 µg/100 ml), those with sickle cell trait (117.7 ± 35.84 µg/100 ml), and Caucasians (110.8 ± 16.17 µg/100 ml) did not differ significantly.

2.2.3. Effect of food.

The plasma or serum zinc levels are slightly affected by diurnal variations with circadian rhythm related to the ingestion of food (Underwood, 1977b). It has been shown that in normal subjects the fasting serum value is significantly higher than the value approximately an hour after a meal (Vikbladh, 1951; Davies et al., 1968;

Withers et al., 1968; Walker et al., 1973). McBean and Halsted (1969), however, were unable to demonstrate any statistical difference between fasting plasma zinc and the value one hour after a meal. These changes may indicate that during rapid protein breakdown, plasma zinc levels decline.

Plasma copper does not increase after meals or decrease during fasting and there appears to be no cyclic pattern of variation in man (Underwood, 1977b).

### 2.2.4. Effect of pregnancy

Decreased serum zinc levels have been reported in late pregnancy (Halsted et al., 1968; Halsted and Smith, 1970; Underwood, 1977a). It was not clear from these reports whether these changes reflect a redistribution of zinc, or an emergency call on available circulating zinc loosely-bound to albumin. In a recent study Schechter et al., (1978), attributed the significantly reduced serum zinc levels in late pregnancy to decreased serum albumin and decreased zinc affinity for serum albumin.

Elevated plasma or serum copper levels during human pregnancy have been reported from different parts of the world (Underwood, 1977b). Nielson (Underwood, 1977b) observed an average of 270 µg/100ml in pregnant women compared with

120 µg/100 ml in non-pregnant women. Similar findings have been reported by Halsted et al. (1968) and in Nigeria by Olatunbosun et al.(1974). The significance of the increased serum copper was not clear from these reports.

This investigation was undertaken in order to study further the normal ranges and physiological variations of serum zinc and copper in Nigerians from this environment.

## 2.3. Subjects studied

The subjects for the study of normal ranges included 127 non-elite and 18 elite male Nigerians with Hb.AA or Hb.AS. The elite Nigerians were members of the senior staff of the Ahmadu Bello University, Zaria. The non-elite Nigerians were rural farmers presenting as blood donors from the rural areas around Zaria.

The effect of ingesting food on serum biochemical values was determined in a group of 12 Nigerian undergraduate students living in halls of residence on the main campus of the Ahmadu Bello University, Zaria. 12 pregnant women were Nigerians in the third trimester of pregnancy and were attending the ante-natal clinic of the Ahmadu Bello University Teaching Hospital, Zaria. The control group comprised 10 housewives of similar socioeconomic background, from the Zaria area. They had not received any form of oral contraception.

## Results

Table 2.1. shows no signficant differences in the concentrations of almost all the serum parameters studied between the Hb.AA and Hb.AS of both elite and non-elite groups. The PCV in the elite with Hb.AS was statistically signficantly reduced in comparison with that in the elite with Hb.AA, but this was almost certainly the result of chance. These results were therefore combined as shown in Table 2.2.

Table 2.2. shows signficantly reduced concentrations of serum zinc ($P < 0.001$), albumin ($P < 0.001$) and PCV ($P < 0.01$) in the non-elite than in the elite Nigerians. The distributions of serum zinc and copper concentrations in elite and non-elite Nigerians are shown in Figures 2.1, 2.2., 2.3, and 2.4.

In Figure 2.5, the mean serum zinc measured at 13.00 h. (90.4 µg/100 ml) was significantly less ($P < 0.01$) than that at 0.9.00 h. (140.8 µg/100 ml) with a corresponding mean percentage fall of about 35.8%. However, the mean serum copper (Figure 2.6), serum albumin (Figure 2.7), serum total protein (Figure 2.8) and mean serum alkaline phosphatase (Fig. 2.9) showed only slight variations. The serum zinc values at 0.9.00 h. were negatively correlated with the serum albumin values measured at the same time ($r = 05467$, $P<0.05$) (Table 2.3).

The results of serum zinc and copper in pregnant and non-pregnant Nigerians are shown in Figures 2.10 and 2.11.

The mean serum zinc in pregnant Nigerians (60.8 µg/100 ml) was significantly reduced ($P < 0.001$) while the mean serum copper (223 µg/100 ml) was significantly elevated ($P < 0.001$) compared to the non-pregnant (Figure 2.11). The elevated copper levels in the pregnant Nigerians paralleł the caeruloplasmin levels (49.6 mg/100 ml). In the group of pregnant Nigerians the correlation between serum zinc or serum copper and the carrier proteins is shown in Table: 2.3.

## 2.5. Discussion

The finding of similar mean serum zinc levels between individuals with Hb.AA and those with Hb.AS in the present study does not agree with those of similar studies from Jamaica (Serjeant et al., 1970) and from New York State, U.S.A. (Karayalcin et al., 1974), probably because these workers did not match their subjects with respect to socio-economic factors including environmental differences such as dietary habits, occupation and parasitic infestations. The finding reported in the present study confirms the earlier reports by Kapu et al. (1976a, 1976b) that elite and non-elite Nigerians with Hb.AA or Hb.AS had similar mean serum zinc and copper concentrations. However, the non-elite Nigerians had significantly reduced serum zinc but not serum copper levels in comparison with elite Nigerians, confirming the findings of Kapu et al. (1976a; 1976b) in similar patients in the savannah areas of northern Nigeria, and that by Spears et al. (1976) in Indians living in northern India.

The reasons for these differences were probably socioeconomic, including environmental differences in health and dietary practices.

The mean haemoglobin concentration in the non-elite group was similar to that of the elite group, but the PCV was significantly lower ($P < 0.01$).

The significantly reduced albumin in the non-elite group ($P < 0.001$) may be due to the low animal protein intake, so common among rural African populations. A similar finding has been reported for another rural population in Nigeria (Edozien, 1958). However, the mean total protein concentrations were identical, indicating that the non-elite group had higher globulin status, more likely as a result of increased antibody production.

This investigation has demonstrated that in normal Nigerians the serum zinc at 13.00 h. is significantly reduced compared with the fasting value. It is therefore of importance that for every estimation specimens should be collected with the subject fasting. Davies et al. (1968) and Walker et al. (1973) have made similar suggestions for studies in the temperate country of England. This turnover has been suggested to be an essential requisite of normal metabolic activity instead of a temporary depletion due to loss in the exocrine

pancreatic juice (Davies et al., 1968). However, the serum copper and other serum contents studied showed no such metabolic fluctuations, which agrees with the findings of Davies et al. (1968), for plasma copper and total protein.

A more comprehensive study utilising different physiological loads to monitor serum zinc response in Nigerians might prove valuable in assessing the utilization of zinc in different groups of people in this country.

Zinc absorption is increased in the latter stages of pregnancy possibly due to pH changes in the intestine or possibly related to the elaboration of large amounts of hormones in which zinc metalloenzymes are involved (Davies, 1972). The mean serum zinc levels of pregnant Nigerians in the present study was significantly lower than in non-pregnant Nigerians, and may be secondary to the increased metabolic demand for zinc during mammalian gravidity. This finding is in agreement with the results of other workers in temperate populations (Halsted et al., 1968). The significantly raised serum copper levels in this study confirmed other Nigerian studies (Olatunbosun et al., 1974) and those from other parts of the world (Underwood, 1977b). The raised copper levels in the pregnant women was possibly as a consequence of increased caeruloplasmin activity as a result of

the needed control of iron mobilization by caeruloplasmin, a role that is greatly increased during gravidity.

## 2.6. Summary

Nigerians with normal haemoglobin (Hb.AA) showed trace element levels, protein, enzyme and haematological Parameters equal to those with sickle cell trait. The serum zinc, albumin and haematocrit were much reduced in the non-elite than in the elite Nigerians. Socioeconomic factors accounted for most of the differences observed, and must be considered in selecting normal control groups in a similar study.

The significant fall in serum zinc levels after the ingestion of food has also been confirmed in Nigerians and this physiological fluctuation must be considered in comparing results in different groups of subjects. The negative correlation between serum zinc and serum albumin in pregnant subjects may suggest that the low serum zinc levels may be associated with other carrier factors other than albumin.

Significantly reduced serum zinc and elevated serum copper levels during the third trimester of pregnancy have been confirmed in Nigerians. Increased caeruloplasmin activity accounted for most of the elevated serum copper levels in the pregnant subjects, probably due to increased demand for caeruloplasmin in iron mobilization.

Table: 2.1. Serum Zinc, Copper, Proteins and Haematology of Symptom-free Nigerians according to Haemoglobin Types (Mean ± Standard Error)

| | Elite | | Non-Elite | |
|---|---|---|---|---|
| | Hb.AA | Hb.AS | Hb.AA | Hb.AS |
| n | 12 | 6 | 84 | 43 |
| Zinc (µg/100 ml) | 127.5±6.6 | 131.6±12.6 | 110.9±10.8 | 94.4±3.6 |
| Copper (µg/100 ml) | 115.0 ±4 | 104.0± 4.7 | 123.6±10.6 | 113.0±148 |
| Caeruloplasmin (mg/100 ml) | 24.8±0.9 | 24.0± 0.6 | 36.8±11.5 | 24.0±0.5 |
| Alkaline Phosphatase (K.A.U.) | 9.8±0.7 | 7.4± 1.3 | 18.8±11.8 | 8.0±0.4 |
| Albumin (g/l) | 47.7±1.4 | 48.4± 0.9 | 42.8± 0.4 | 43.0 ±0.6 |
| Total Protein (g/l) | 79.3±3.2 | 78.9± 1.6 | 83.7±11 | 73.0 ±0.9 |
| Haemoglobin (g/dl) | 15.3±0.4 | 14.2± 0.6 | 14.0±0.2 | 14.5±0.3 |
| Packed Cell Volume (%) | 47.5±1.0 | 43.5± 1.0** | 42.0 ±0.6 | 42.9±0.6 |

** = Significant difference between Hb.AS of Elite Nigerians ($P < 0.01$)

Hb.AA = Normal Haemoglobin

Hb.AS = Sickle Cell trait

n = number of subjects in each group

Table: 2.2. Serum Zinc, Copper, Proteins and Haematological Values in Symptom-free Nigerians (Mean ± Standard Error)

| Items | Elite Mean ± S.E. | Non-Elite Mean ± S.E. |
|---|---|---|
| Number of Subjects | 18 | 127 |
| Zinc (µg/100 ml) | 128.9±5.8** | 105.3±7.3 |
| Copper (µg/100 ml) | 111.7±3.5 | 120.2±7.0 |
| Caeruloplasmin (mg/100 ml) | 24.6±0.7 | 32.8±7.6 |
| Albumin (g/l) | 48.0±0.9*** | 43.0±0.3 |
| Total Protein (g/l) | 79.2±1.4 | 80.3±7.3 |
| Alkaline Phosphatase (K.A. Units) | 9.0±0.7 | 15.2±7.8 |
| Haemoglobin (g/dl) | 14.8±0.4 | 14.2±0.2 |
| Packed Cell Volume (%) | 46.2+0.9*** | 42.5+0.5 |

Comparison of means to non-elite

** = $P < 0.01$

*** = $P < 0.001$

Hb.AA + AS = Normal haemoglobin + sickle cell trait values.

Table: 2.3. Pearson's Coefficient of Correlation (r) and Probability Constant (P) in Pregnant Nigerians.

| | Third Trimester of Pregnancy | |
|---|---|---|
| | r | P |
| Serum zinc and serum albumin | -0.7460 | $<0.01$ |
| Serum zinc and serum total protein | -0.5089 | $<0.05$ |
| Serum zinc and serum alkaline phosphatase | +0.2852 | ns |
| Serum zinc and serum copper | +0.7577 | $<0.01$ |
| Serum copper and serum albumin | -0.7385 | $<0.01$ |
| Serum copper and serum caeruloplasmin | -0.2128 | ns |

ns = non significant difference.

FIGURE: 2.2. THE MEAN SERUM COPPER IN 84 NON-ELITE MALE NIGERIANS WITH Hb AA (123.34 ± 10.63 µg/100 ml) DID NOT DIFFER SIGNIFICANTLY FROM THAT IN 43 MALES WITH Hb AS (113.46 ± 2.27 µg/100 ml).

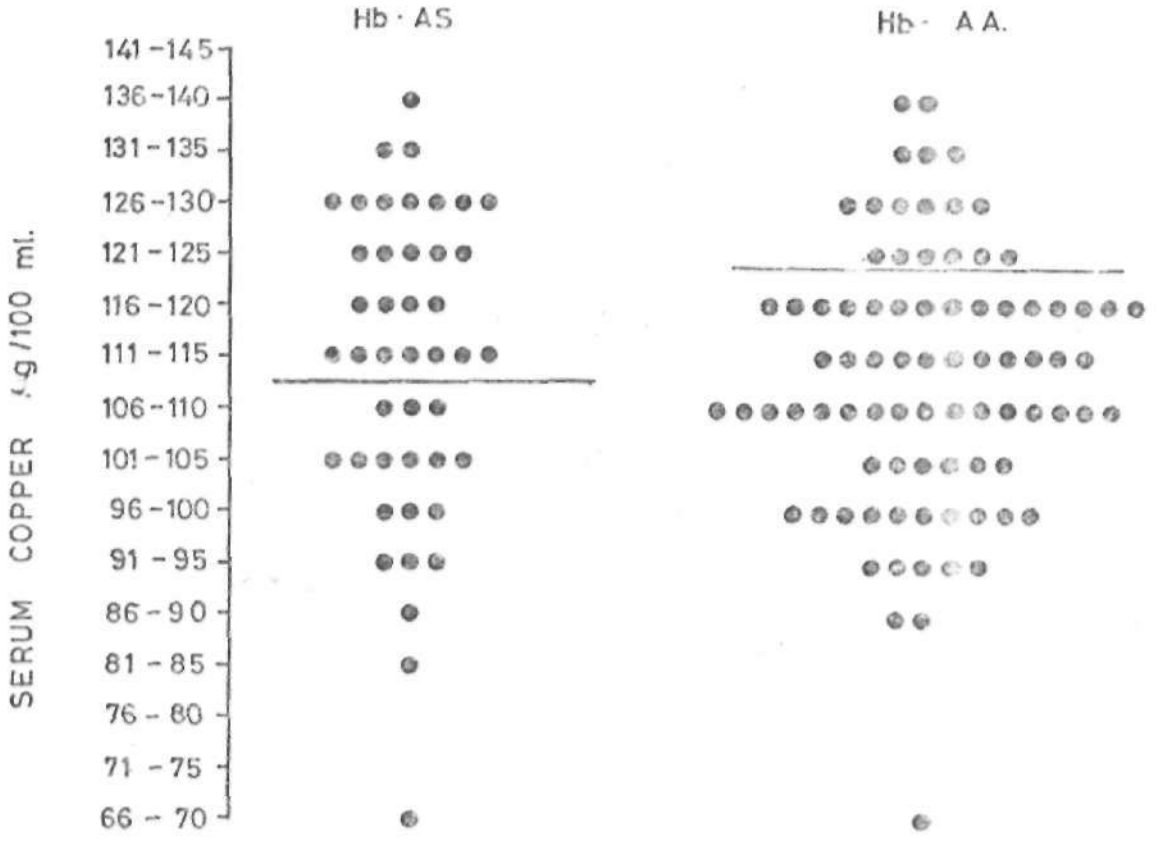

Hb · AS
Hb · A A.
SERUM COPPER µg/100 ml.
141 - 145
136 - 140
131 - 135
126 - 130
121 - 125
116 - 120
111 - 115
106 - 110
101 - 105
96 - 100
91 - 95
86 - 90
81 - 85
76 - 80
71 - 75
66 - 70

FIGURE: 2.3. THE MEAN SERUM ZINC IN 127 NON-ELITE MALE NIGERIANS WITH Hb AA AND Hb AS (105.32 ± 7.3 µg/100 ml) WAS SIGNIFICANTLY LOWER ($P < 0.01$) THAN THAT IN 18 ELITE MALE NIGERIANS WITH Hb AA and Hb.AS (128.89 ± 5.85 µg/100 ml).

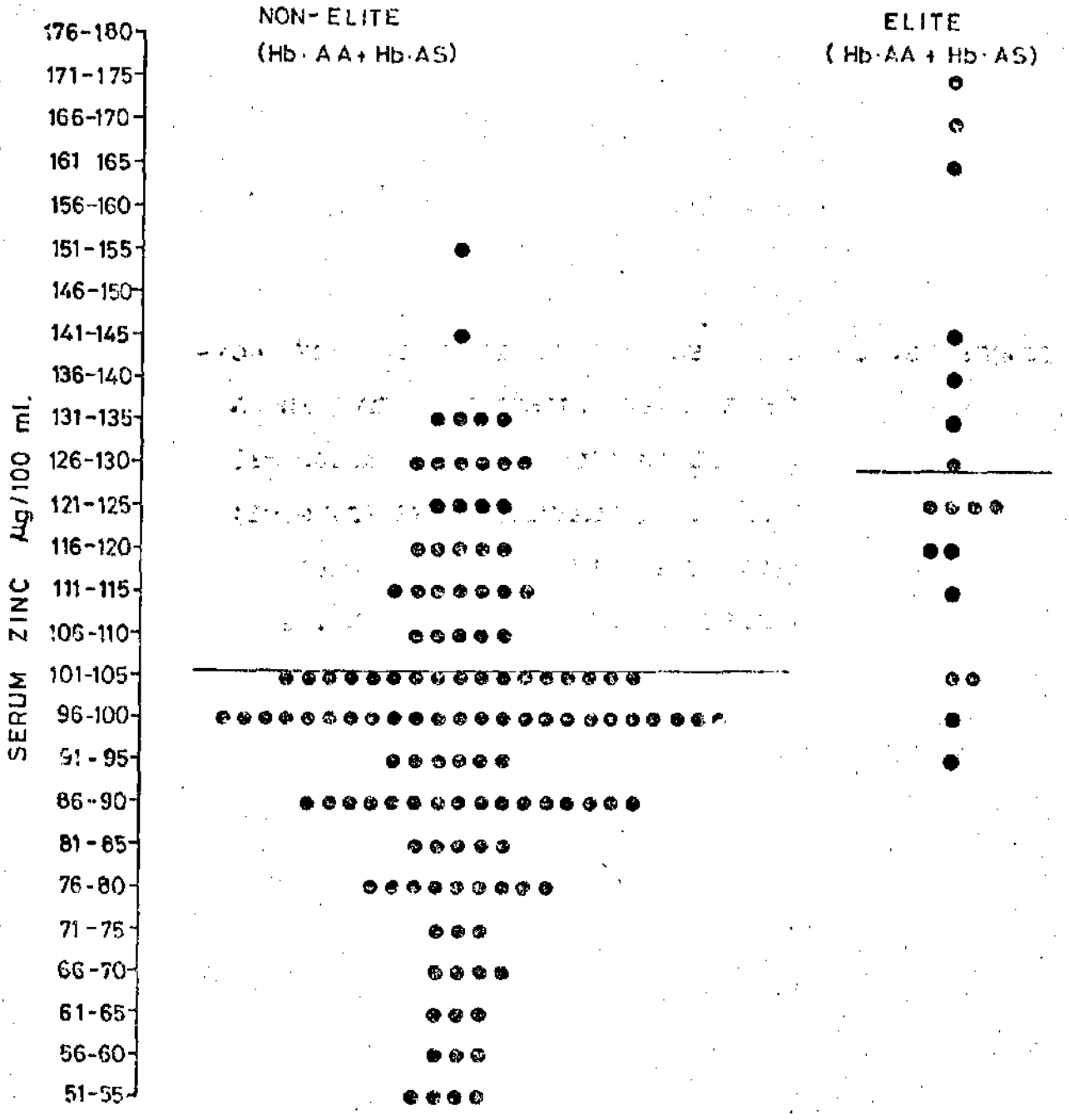

NON- ELITE
(Hb · AA + Hb · AS)
ELITE
( Hb · AA + Hb · AS)
SERUM ZINC µg/100 ml.
176-180
171-175
166-170
161 165
156-160
151-155
146-150
141-145
136-140
131-135
126-130
121-125
116-120
111-115
106-110
101-105
96-100
91 - 95
86 - 90
81 - 85
76 - 80
71 - 75
66 - 70
61 - 65
56-60
51-55

FIGURE: 2.4. THE MEAN SERUM COPPER IN 127 NON-ELITE MALE NIGERIANS WITH Hb.AA AND Hb.AS (120.19 ± 7.07 µg/100 ml) DID NOT DIFFER SIGNIFICANTLY FROM THAT IN 18 ELITE MALE NIGERIANS WITH Hb.AA AND Hb.AS (111.67 ± 3.5 µg/100 ml).

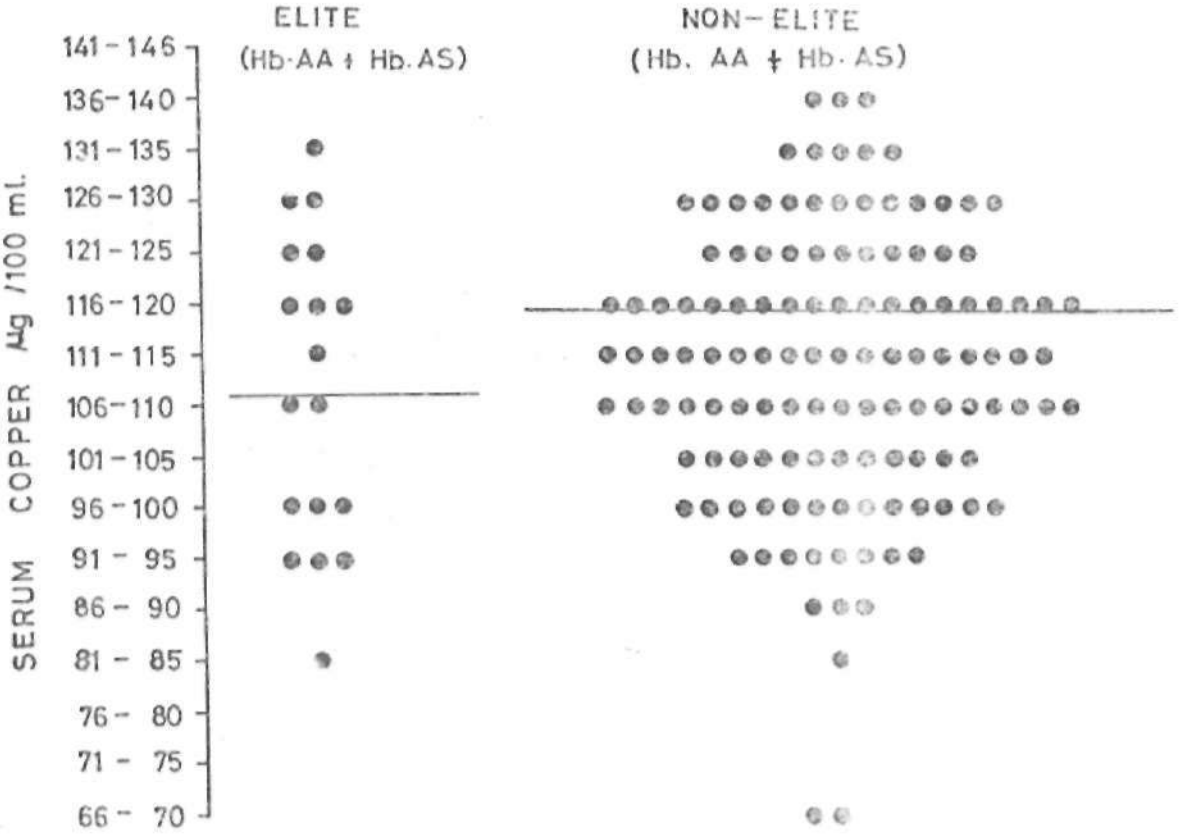

ELITE
(Hb·AA + Hb.AS)
NON-ELITE
(Hb. AA + Hb·AS)
SERUM COPPER Mg /100 ml.
141 - 146
136 - 140
131 - 135
126 - 130
121 - 125
116 - 120
111 - 115
106 - 110
101 - 105
96 - 100
91 - 95
86 - 90
81 - 85
76 - 80
71 - 75
66 - 70

FIGURE: 2.5. THE SERUM ZINC MEASURED FOLLOWING THE MIDDAY MEAL (13.00 h.) IN NORMAL NIGERIAN MALE UNDERGRADUATES (Mean = 90.4 µg/100 ml) WAS SIGNIFICANTLY LESS ($p < 0.01$) THAN THAT AT 09.00 h. AFTER AN OVERNIGHT FAST (Mean = 140.8 µg/ml). THE LINES CONNECT VALUES FROM INDIVIDUAL SUBJECTS.

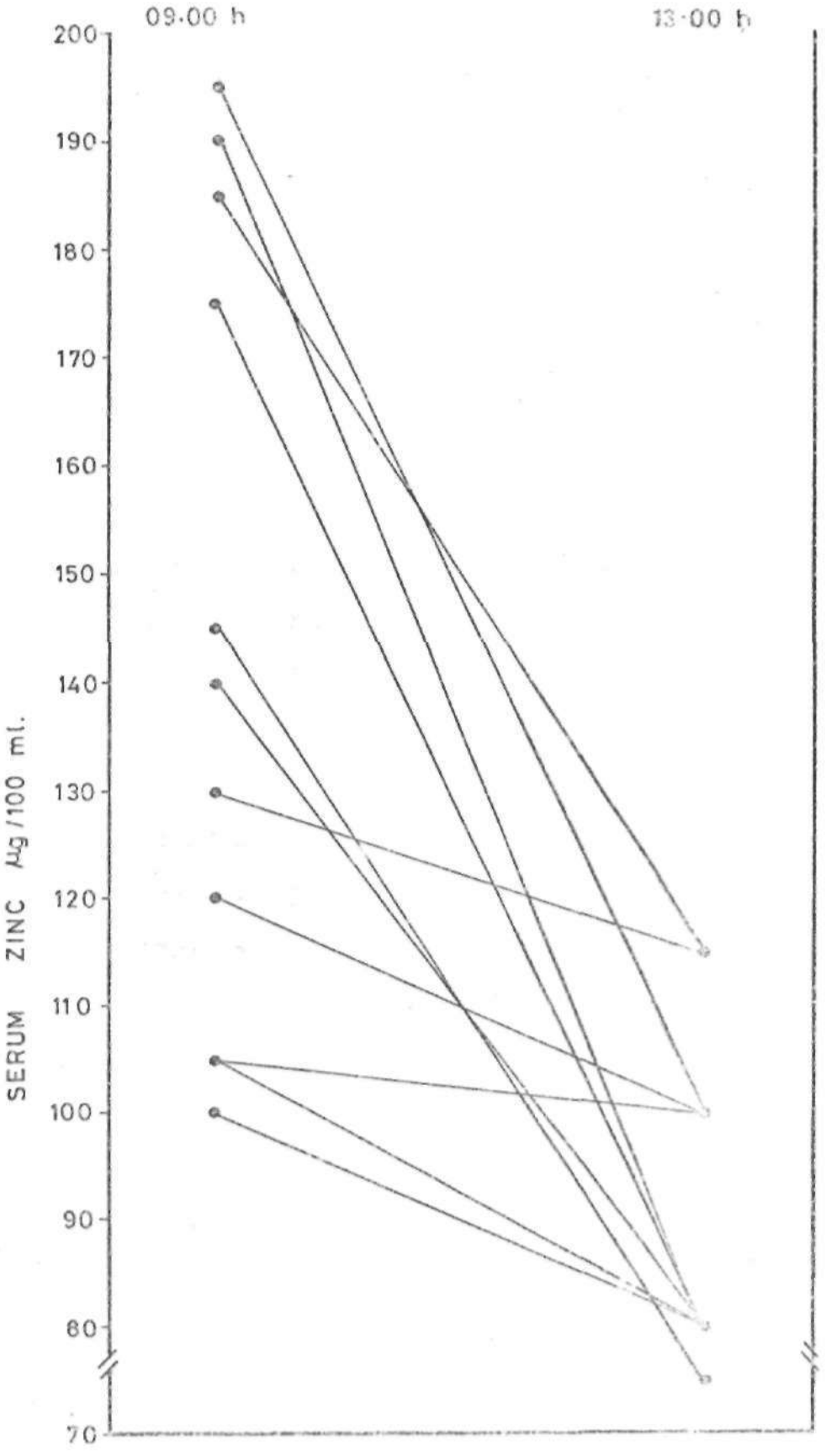

09·00 h
13·00 h
200
190
180
170
160
150
140
130
120
110
100
90
80
70
SERUM ZINC µg/100 ml.

FIGURE: 2.6. THE SERUM COPPER (mean = 106.3 µg/100 ml) MEASURED AFTER AN OVERNIGHT FAST (09.00 h.) DID NOT DIFFER SIGNIFICANTLY FROM THAT (mean = 109.6 µg/100 ml) FOLLOWING THE MIDDAY MEAL (13.00 h.) IN NORMAL NIGERIAN MALE UNDERGRADUATES. THE LINES CONNECT VALUES FROM INDIVIDUAL SUBJECTS.

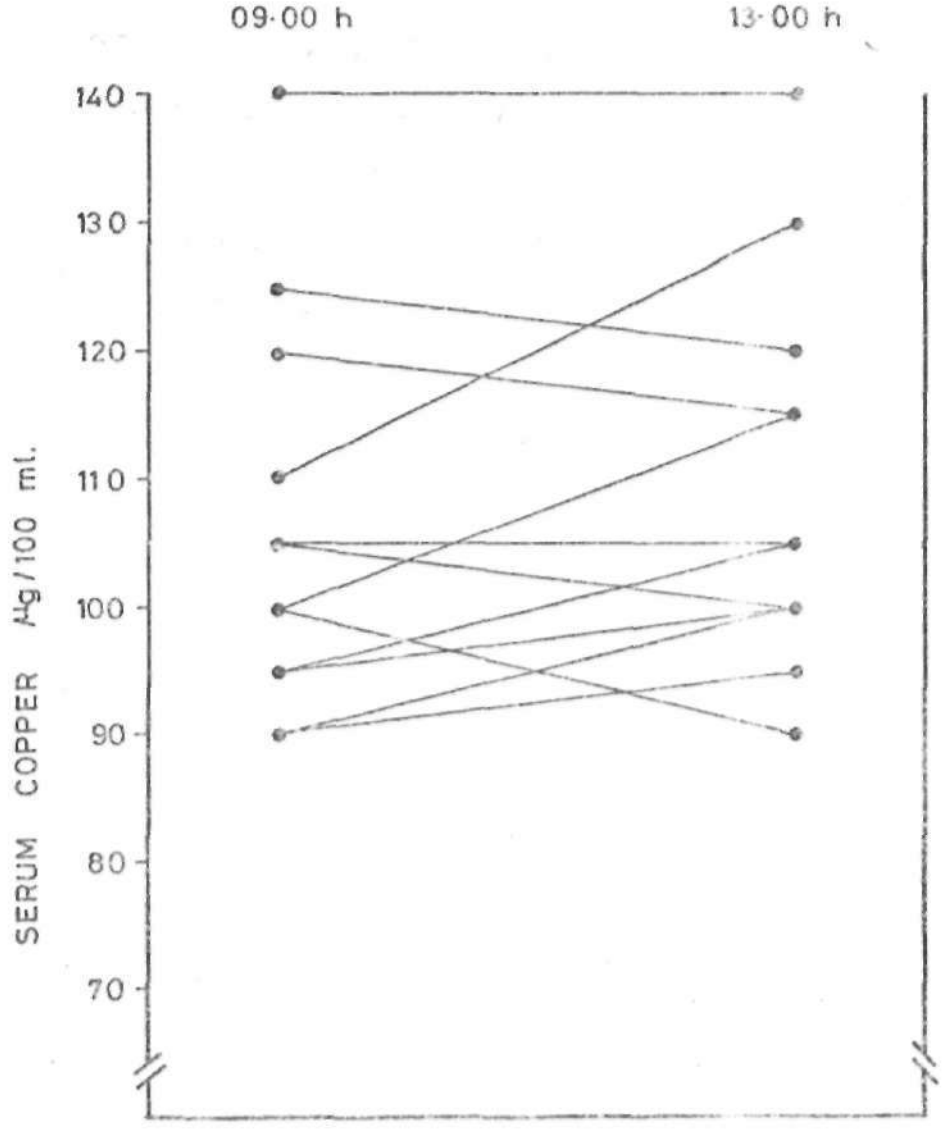

09·00 h
13·00 h
140
130
120
110
100
90
80
70
SERUM COPPER µg/100 ml.

FIGURE: 2.7. THE SERUM ALBUMIN MEASURED AFTER AN OVERNIGHT FAST (09.00 h.) IN A GROUP OF NORMAL NIGERIAN MALE UNDERGRADUATES (mean = 46.96g/l) DID NOT DIFFER SIGNIFICANTLY FROM THAT MEASURED (mean = 46.52g/l) FOLLOWING THE MIDDAY MEAL (13.00 h.). THE LINES CONNECT VALUES FROM INDIVIDUAL SUBJECTS.

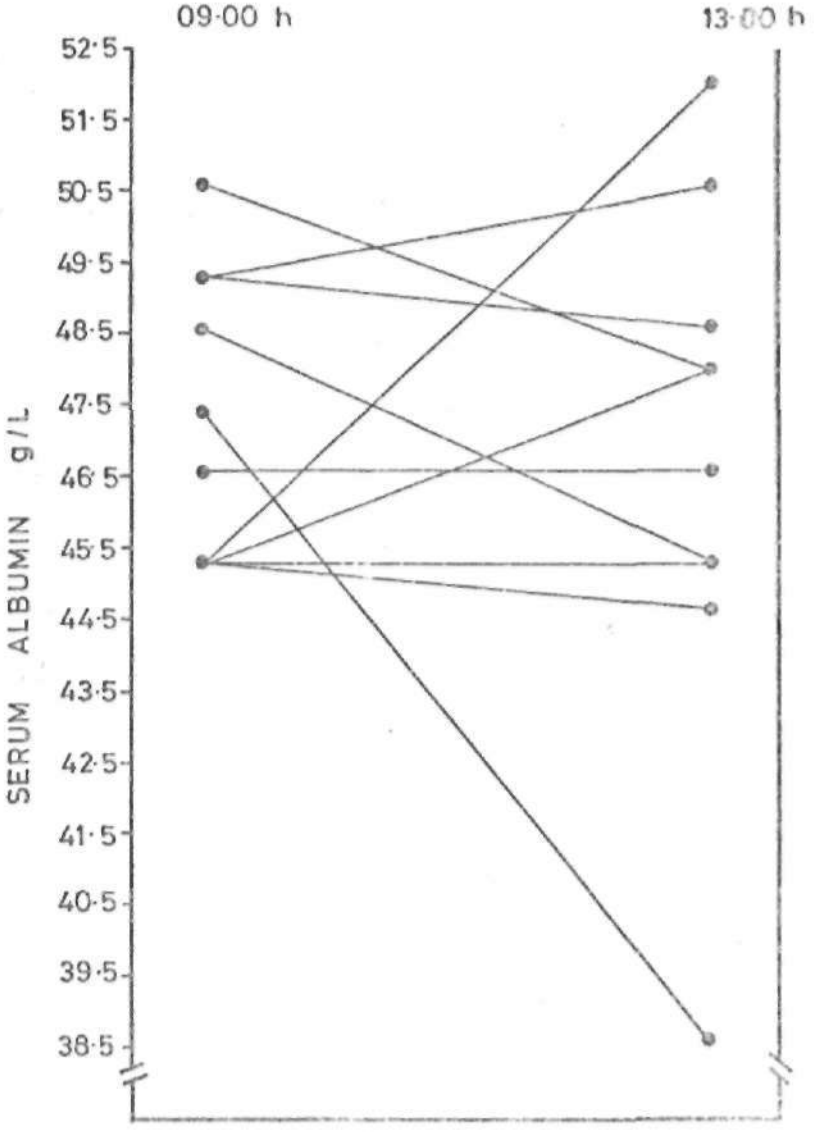

09·00 h
13·00 h
SERUM ALBUMIN g/L
52·5
51·5
50·5
49·5
48·5
47·5
46·5
45·5
44·5
43·5
42·5
41·5
40·5
39·5
38·5

FIGURE: 2.8. THE SERUM TOTAL PROTEIN MEASURED AFTER AN OVERNIGHT FAST (09.00 h.) IN A GROUP OF NORMAL NIGERIAN MALE UNDERGRADUATES (mean = 71.78g/l) DID NOT DIFFER SIGNIFICANTLY FROM THAT MEASURED (mean = 71.02g/l) FOLLOWING THE MIDDAY MEAL (13.00 h.). THE LINES CONNECT VALUES FROM INDIVIDUAL SUBJECTS.

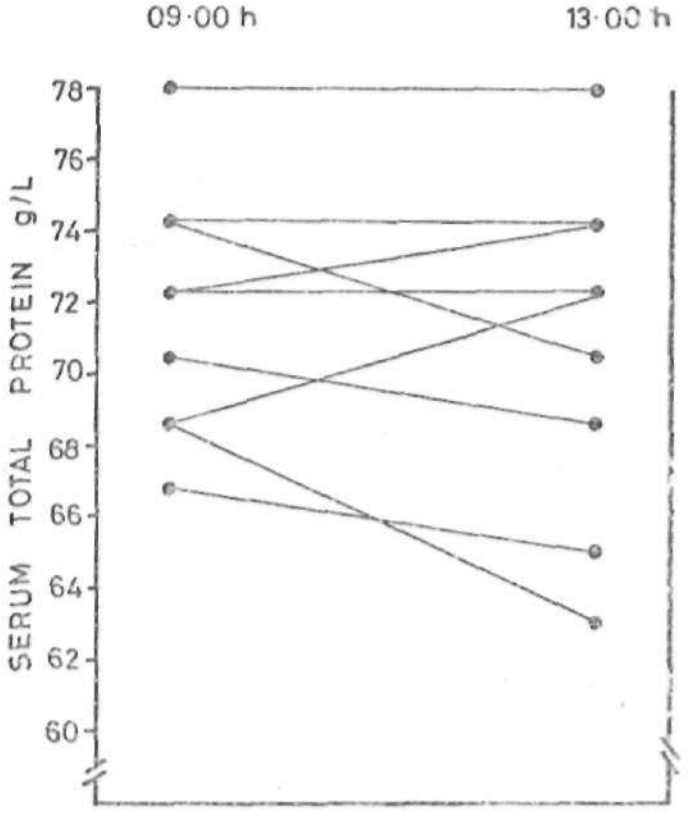

09·00 h
13·00 h
SERUM TOTAL PROTEIN g/L
78
76
74
72
70
68
66
64
62
60

FIGURE: 2.9. THE SERUM ALKALINE PHOSPHATASE MEASURED AFTER AN OVERNIGHT FAST (09.00 h.) IN A GROUP OF NORMAL NIGERIAN MALE UNDERGRADUATES (mean = 4.96 K.A.U) DID NOT DIFFER SIGNIFICANTLY FROM THAT MEASURED (mean = 5.67 K.A.U.) FOLLOWING THE MIDDAY MEAL (13.00 h.). THE LINES CONNECT VALUES FROM INDIVIDUAL SUBJECTS.

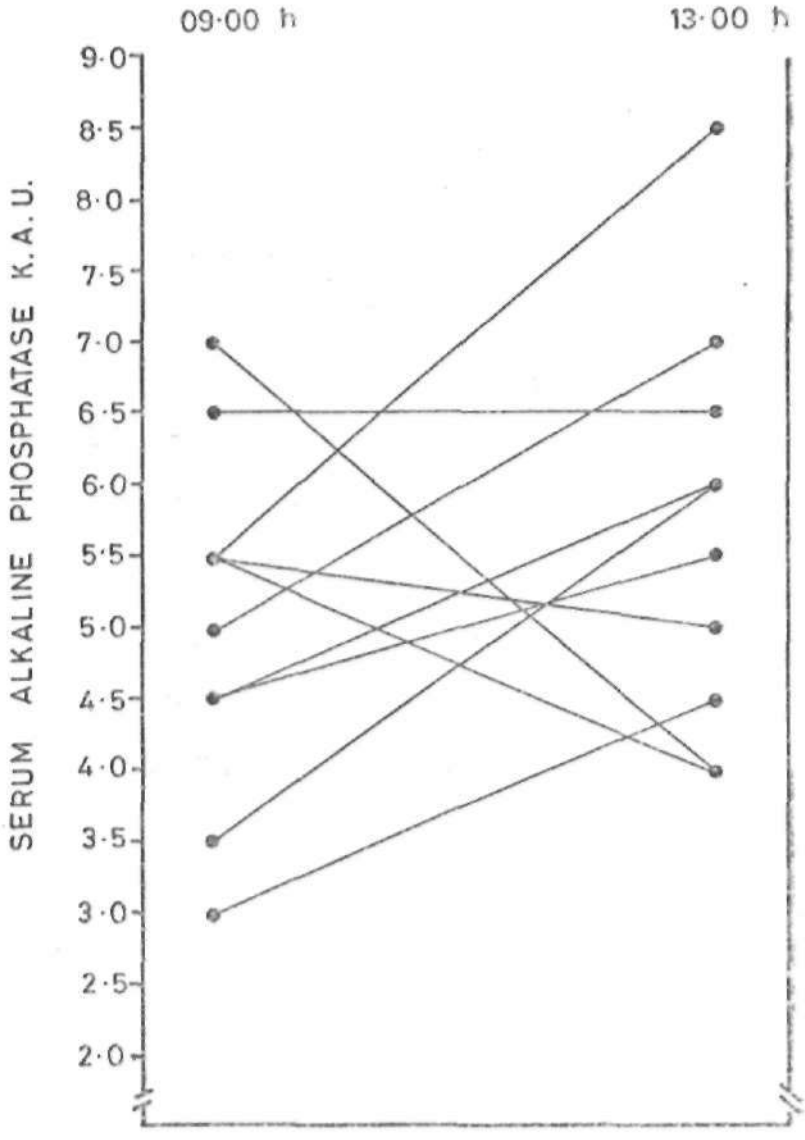

09·00 h
13·00 h
SERUM ALKALINE PHOSPHATASE K.A.U.
9·0
8·5
8·0
7·5
7·0
6·5
6·0
5·5
5·0
4·5
4·0
3·5
3·0
2·5
2·0

FIGURE: 2.10. THE DISTRIBUTION OF SERUM ZINC AND SERUM COPPER LEVELS IN PREGNANT AND NON-PREGNANT FEMALE NIGERIANS. THE MEAN VALUE OF EACH GROUP IS INDICATED BY THE HORIZONTAL LINES.

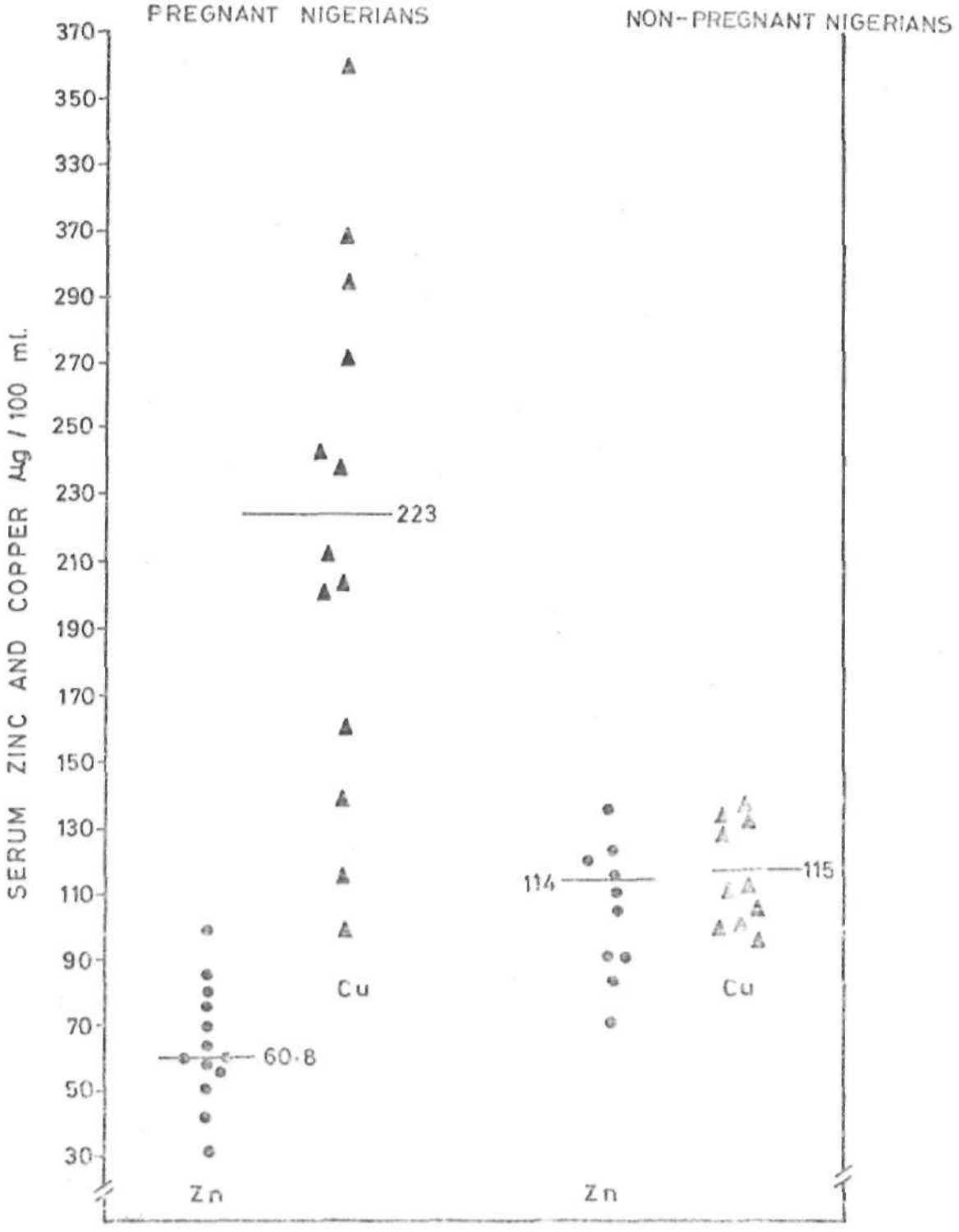
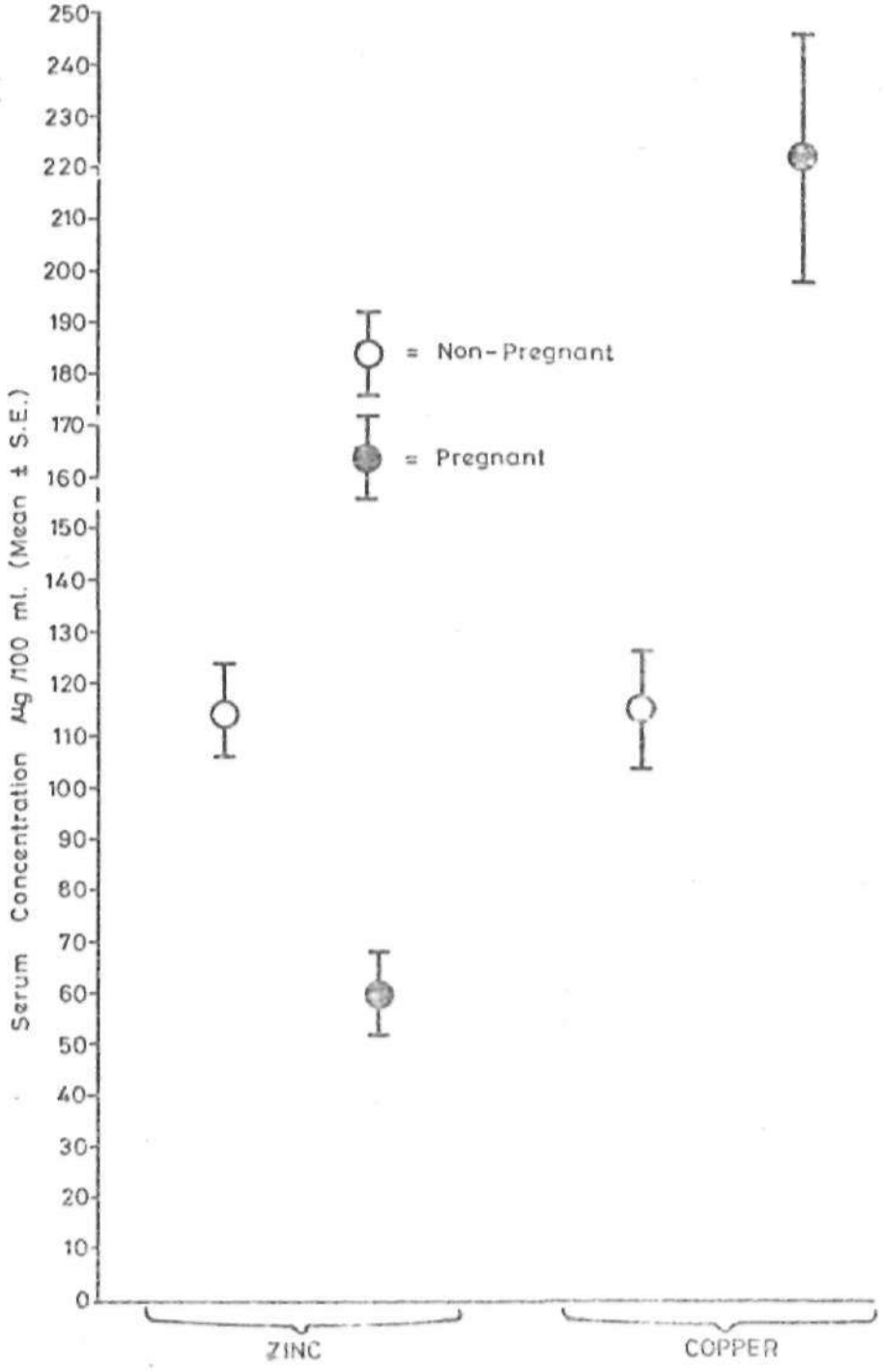

PREGNANT NIGERIANS
NON-PREGNANT NIGERIANS
SERUM ZINC AND COPPER µg / 100 ml.
370
350
330
370
290
270
250
230
210
190
170
150
130
110
90
70
50
30
223
60·8
114
115
Cu
Cu
Zn
Zn

FIGURE: 2.11. WHILE THE MEAN SERUM ZINC IN 12 PREGNANT NIGERIANS (60.8 ± 6.08 µg/100 ml) WAS SIGNIFICANTLY LESS ($P < 0.001$) THAN THAN IN 12 NON-PREGNANT FEMALE NIGERIANS (114 ± 10.00 µg/100 ml), THE SERUM COPPER IN THE 12 PREGNANT NIGERIANS (123.00 ± 24.10 µg/100 ml) WAS SIGNIFICANTLY INCREASED ($P < 0.001$) THAN THAT IN 12 NON-PREGNANT FEMALE NIGERIANS (115.00 ± 11.01 µg/100 ml).


Serum Concentration Ag /100 ml. (Mean ± S.E.)
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
= Non-Pregnant
= Pregnant
ZINC
COPPER

# CHAPTER 3

# ZINC AND COPPER IN SICKLE CELL DISEASE

## 3.1. Introduction.

### 3.1.1. Plasma or serum zinc and copper concentrations.

Plasma or serum zinc concentrations have been reported to be lower on average in sickle cell anaemia (Hb.SS) than in normal (Hb.AA) subjects in Jamaica (Serjeant et al., 1970), New York State (Karayalcin et al., 1974) and in Michigan (Prasad et al., 1976). The plasma ribonuclease activity was significantly higher in the sickle cell patients, decreasing but not significantly during the administration of zinc. During this process also, the plasma zinc increased significantly whereas only slight increases in erythrocyte zinc and carbonic anhydrase activity were observed (Prasad et al., 1976).

Contrary to the above findings, Kapu et al. (1975d) observed no significant difference in mean plasma zinc concentrations between non-elite Nigerians with normal haemoglobin pattern, sickle cell trait or sickle cell anaemia. According to Butler et al. (1978) sickle cell anaemia subjects in the Washington metropolitan area had plasma zinc concentrations comparable to healthy Negro controls.

Sachs et al. (1935) first reported raised serum copper levels in a patient with sickle cell anaemia. This observation has been confirmed in patients from the forest area of Western Nigeria (Olatunbosun et al., 1975a), in similar patients from the savannah areas of northern Nigeria (Kapu et al., 1975b) and in sickle cell patients from the U.S.A. (Prasad et al., 1976; Butler et al., 1978).

Elevated caeruloplasmin accounted for most of the increased plasma copper (Butler et al., 1979), and was likely needed because of the increased need for iron and its fast turnover rate in sickle cell anaemia. The diverse findings in plasma zinc concentrations in sickle cell patients may reflect differences in local dietary habits which might lead to mild zinc deficiency in some cases due to the anorexia in these patients compared with normal controls.

3.1.2. Erythrocyte zinc and copper levels.

In Michigan, sickle cell patients were found to have depressed levels of zinc in erythrocytes accompanied with high urinary zinc excretion (Prasad et al., 1976). Butler et al. (1978) were unable to confirm these findings in sickle cell anaemia patients from the Washington metropolitan area. These workers reported that their patients showed zinc levels equal to the healthy Negro control group in red cell and urinary zinc, although the urinary zinc levels were higher than the caucasian controls. Prasad et al. (1976)

argued on the basis of their findings that the raised urinary zinc in their patients may have been responsible for the depressed plasma and red cell zinc.

From Texas, (Butler et al., 1978) there is a report of high erythrocyte copper with values about double in sickle cell anaemia patients compared to healthy controls. In Michigan and the Washington metropolitan area, sickle cell anaemia patients showed normal erythrocyte copper concentrations (Prasad et al., 1976; Butler et al., 1978). These differences in the local dietary copper intakes, which is about 8.1 mg/day in Texas, 1.09 mg/day in Iowa and 0.58 mg/day in the Chicago area (Butler et al., 1978).

3.1.3. Zinc and copper in hair.

Hair zinc levels have been reported to be significantly lower than normal in sickle cell patients (Prasad et al., 1976). Olatunbosun et al. (1977) were unable to confirm this finding in Nigerian patients with sickle cell anaemia. Butler et al. (1978) have also reported that sickle cell anaemia patients showed zinc levels equal to healthy Negro controls in hair, although values were lower than the caucasian controls.

To the knowledge of the author, however, there are no previous reports on copper in hair from sickle cell patients.

There seems to be very little, if any, available data on zinc and copper metabolism in sickle cell anaemia. Moreover, in view of the seemingly conflicting reports from various areas of the U.S.A. and the world over the zinc and copper metabolism in sickle cell anaemia, it was found necessary to investigate zinc and copper metabolism in sickle cell patients in Nigeria.

## 3.2. Subjects Studied.

Thirty two sickle cell male patients (31 Hb.SS and 1 Hb.SC) were aged 11-25 years. Sex was matched because women are reported to have higher blood copper levels than men (Wintrobe, 1967). The sickle cell patients were probably comparable to elite controls than non-elite as most non-elite children with sickle cell disease die before the age of 2 years (Fleming, 1975).

## 3.3. Results.

Table 3.1. shows significantly reduced concentration of zinc in serum and hair between sickle cell anaemia patients and elite controls. Erythrocyte zinc and total protein levels showed no significant differences. The serum albumin, and alkaline phosphatase levels showed significant differences.

The serum copper and caeruloplasmin values were significantly elevated ($P < 0.001$) in patients with sickle cell

anaemia [illegible] The erythrocyte and hair copper levels did not differ significantly between the sickle cell anaemia and elite control groups.

The haemoglobin and packed cell volume were significantly lower in the sickle cell anaemia patients than elite control groups ($P < 0.001$).

However, the serum zinc (105.3 ± 7.3 µg/100 ml), serum albumin (43.0 ± 0.3 g/l), serum total protein (80.3 ± 7.3 g/l) and serum alkaline phosphatase (15.2 ± 7.8 K.A. Units) in non-elite Nigerians (Tables 2.3. and 4.1.) did not differ significantly from those observed in patients with sickle cell anaemia (Table 3.1.).

## 3.4. Discussion

The significantly reduced serum zinc levels in the sickle cell anaemia patients in comparison with elite controls agrees with the reports from Jamaica (Serjeant et al., 1974) and Michigan (Prasad et al., 1976). The reduced hair zinc in sickle cell anaemia patients in this study compared to elite controls agrees with the observation of Prasad et al. (1976). It must be noted, however, that these reports did not consider socioeconomic and other environmental factors which are known to affect plasma or serum zinc values.

The non-significant difference in the serum zinc between patients with sickle cell anaemia and non-elite Nigerians in

the present study is in agreement with the reports of Kapu et al. (1976a) and those of Butler et al. (1978) who studied sickle cell patients from the same ethnic background as the control groups. The seemingly diverse findings may be due to differences in socioeconomic and other environmental factors which operate in each study area. Although our sickle cell patients were probably comparable to elite Nigerians, it is possible that the anorexia and intercurrent disease factors were probably responsible for the significant difference between these patients and elite controls. These diverse findings point to the importance of using socioeconomic and ethnic matched groups.

The significantly reduced hair zinc levels in the sickle cell anaemia patients does not agree with the observation of Olatunbosun et al. (1977) who studied similar patients. While the present study compared sickle cell anaemia patients to elite Nigerians, Olatunbosun et al. (1977) did not consider socioeconomic factors. Further study would be needed to compare hair zinc in sickle cell anaemia patients with non-elite and elite groups to discover any socioeconomic effects, as it has been shown for plasma or serum zinc (Kapu et al., 1976b; Spears et al., 1976).

The reduced albumin levels in the sickle cell patients may in part be responsible for the reduced plasma zinc levels

Low plasma albumin levels are usually associated with low protein intake. The increased alkaline phosphatase activity in sickle cell anaemia was most probably a reflection of liver function.

It has been said that sickle cell anaemia patients with leg ulcers are zinc deficient, and leg ulcers present in about 75% of patients with sickle cell anaemia have been reported (Serjeant et al., 1970) to respond to oral zinc sulphate. In the present study none of the sickle cell anaemia patients had leg ulcers, but they did have lower zinc levels. It has been reported that patients with leg ulcers from other causes showed lowered zinc levels (Halsted and Smith, 1970). In both conditions the reduced serum zinc may be reflecting an immobilization rather than an absolute deficiency. It has been suggested, however, that Nigerians with sickle cell anaemia may have better zinc status than sickle cell patients in Jamaica or America, and this may protect them from leg ulcers (Kapu et al., 1976a; Olatunbosun et al., 1977), which are rarely seen in active sickle cell anaemia clinics in rural West Africa (Kendall et al., 1973; Kapu et al., 1976a).

The findings of raised serum copper concentrations in sickle cell anaemia patients in the present study agrees with the observations of Olatunbosun et al. (1975a), Kapu et al. (1976b), Prasad et al. (1976) and Butler et al. (1978). The elevation in serum copper in the sickle cell anaemia patients in this study was due mainly to the increase

in caeruloplasmin activity because of the increased need for iron and its fast turnover rate in severe anaemias such as sickle cell anaemia. On the other hand, the accumulation of copper in plasma or serum and red cells in sickle cell anaemia and other haematological disorders could be secondary to disturbed iron metabolism in the mitochondria of normoblasts (Kapu et al., 1976b).

The differences in zinc and copper concentrations observed in HbSS from various parts of the world may reflect socioeconomic and other environmental factors such as dietary differences, anorexia and intercurrent disease factors.

## 3.5. Summary

Nigerian sickle cell anaemia patients had erythrocyte zinc, erythrocyte copper and hair copper levels comparable to elite Nigerians, but had significantly lower serum and hair zinc levels than elite Nigerians. Sickle cell anaemia patients and non-elite Nigerians with normal haemoglobin pattern did not differ significantly in their serum zinc. The serum [illegible] copper with the caeruloplasmin levels were significantly increased in sickle cell anaemia patients in comparison with elite or non-elite Nigerians.

The differences in serum and hair zinc between sickle cell anaemia patients and elite Nigerians may have been due

to the anorexia and intercurrent disease factors associated with sickle cell disease and increased loss of zinc in urine. Caeruloplasmin activity accounted for most of the elevated serum copper probably because of the increased need for iron and its fast turnover rate in this severe anaemia, and/or because of the role copper is known to play in haemosynthesis.

Table: 3.1. Zinc and copper levels in sickle cell anaemia and elite Nigerian groups (Mean ± Standard Error)

| Variable | Sickle Cell Hb.SS | Elite controls | Probability |
|---|---|---|---|
| Number of subjects | 32 | 18 | |
| Zinc | | | |
| Serum Zn, µg/100 ml | 90.80±1.9 | 128.90±5.80 | <0.001 |
| Serum alkaline phosphatase K.A.U. | 13.57±1.19 | 9.91±1.19 | <0.001 |
| Serum albumin, g/l | 44.80±1.20 | 48.00±0.90 | <0.05 |
| Serum total protein g/l | 77.70±1.30 | 79.20±1.40 | NS[a] |
| Erythrocyte Zn, µg/ml | 11.71±0.40[b] | 11.91±0.53 | NS |
| Hair Zn, µg/g | 130.80±14.44 | 155.78±19.52 | <0.01 |
| Copper | | | |
| Serum Cu, µg/100 ml | 175.20±6.30 | 111.70±3.50 | <0.001 |
| Serum caeruloplasmin mg/100 ml | 50.30±2.10 | 24.60±0.70 | <0.001 |
| Erythrocyte Cu, µg/100 ml | 134.07±1.67[b] | 137.98±0.72 | NS |
| Hair Cu, µg/g | 16.97±0.93[b] | 17.18±1.09 | NS |
| Haematology | | | |
| Haemoglobin, g/100 ml | 9.57±0.28 | 14.80±0.40 | <0.001 |
| Packed cell volume, % | 28.60±1.09 | 46.20±0.90 | <0.001 |

[a] NS = non-significant

[b] Number of subjects studied = 12

FIGURE: 3.1. THE SERUM ZINC AND COPPER IN NORMAL NIGERIANS AND THOSE WITH VARIOUS DISORDERS.

EN = symptom-free elite Nigerians (18)

NEN = symptom-free non-elite Nigerians (127)

LU = leg ulcer (27)

SCD = sickle cell disease (32)

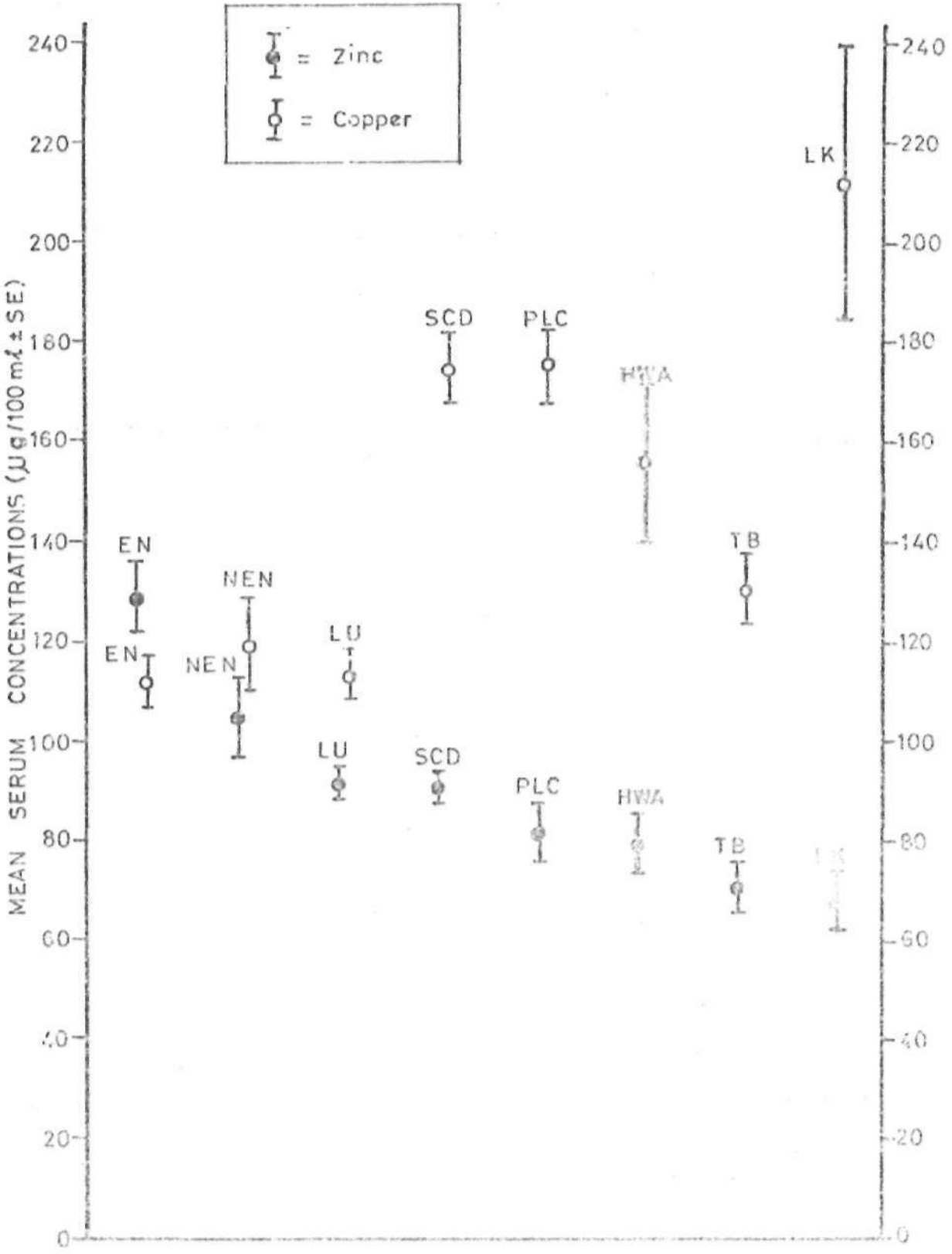
PLC = primary liver cancer (20)

HWA = hookworm anaemia (14)

TB = tuberculosis (22)

LK = leukaemia (12)

The number of subjects in each group is indicated in brackets.


= Zinc
= Copper
MEAN SERUM CONCENTRATIONS (µg/100 ml ± SE)
240
220
200
180
160
140
120
100
80
60
40
20
0
EN
EN
NEN
NEN
LU
LU
SCD
SCD
PLC
PLC
HWA
HWA
TB
TB
LK

# CHAPTER 4

# ZINC AND COPPER IN MISCELLANEOUS DISEASES

## 4.1. Introduction

The role of trace metals in a variety of disease conditions has been the subject of conjecture, and reports of different authors are often conflicting and contradictory. A major reason for these discrepancies is the difficulty of analyzing trace elements and the problems associated with collecting specimens without contamination.

However, the use of highly sophisticated analytical procedures that involve absorption or emission activation have permitted the documentation of even small alterations in the trace element content of biological fluids, giving rise to new concepts concerning the regulatory mechanisms which influence trace element metabolism in both health and disease (Beisel et al., 1973).

### 4.1.1. Zinc

Low extracellular zinc levels are seen in many chronic diseases such as infections, liver and pancreas disorders, various forms of anaemia, neoplastic disorders, and leg ulceration (Halsted and Smith, 1970; Strain et

al., 1978). In most cases, low extracellular zinc must be considered to be due to low serum protein concentration, especially low albumin concentrations. However, this has not been adequately investigated (Weismann, 1976). In Acrodermatitis enteropathica, the low serum zinc levels are most probably due to defective intestinal absorption. Except in diabetes mellitus (Mateo et al., 1975), increased serum values have rarely been reported in pathological conditions.

Elevated serum zinc values reported in 12 patients suffering from primary liver cancer (Karlinsky and Roomere, 1967) were not substantiated in 31 similar patients (Dunn et al., 1969).

The wide range of these conditions suggests that these changes may be non-specific effects of systemic illness (Greaves and Boyde, 1967). Furthermore, other workers have been unable to confirm some of these results (Davies et al., 1968; Withers et al., 1968) apparently due to the problems connected with collecting specimens without contamination.

However, it is generally accepted (Walker et al., 1973) that a more specific abnormality of zinc metabolism occurs in patients with hepatic cirrhosis, resulting in low serum zinc levels and increased urinary zinc; and these values

may be returned to normal by oral zinc supplementation. According to Walker et al. (1973), there is evidence of a correlation between serum zinc level and serum albumin level. It is therefore possible that the reduced plasma levels of zinc found in patients with cirrhosis may be a reflection of the reduced albumin concentration commonly found in such patients.

4.1.2. Copper

There is also general agreement that copper metabolism is altered in hepatolenticular degeneration or Wilson's disease and in Menke's steely hair disease. In Wilson's disease, levels of copper in body fluids are lower than normal, between 40 and 60 µg per 100 ml, while the urine copper is raised to over 100 µg in a 24 hourly sample (Cummings, 1968). One of the most striking features in Wilson's disease is the very great reduction in caeruloplasmin in blood. Serum copper is also depressed in Menke's steely hair disease. The treatment of this disease with a complex of copper with nitiloacetic acid (CUNTA) suggests the defect in the disease may relate to the genetic absence of a suitable ligand by which copper is transported across cell membranes (Henkin and Grover, 1978).

Only a single study reported depressed serum copper values in patients with urogenital tuberculo-

sis; and inconsistent changes were noted in syphilis (Borisenko, 1965; Tumasheva and Borisenko, 1965).

Increased plasma or serum copper concentrations have been reported in acute and chronic liver disease (Sundermann, 1973) and a variety of other lymphomas (Hrgovcic et al., 1973). The elevations in Hodgkin's disease were related to increases in caeruloplasmin. The raised copper levels in Hodgkin's disease are regularly reduced to within normal limits at complete remission (Thorling and Thorling, 1976). Increased serum copper levels have been confirmed in Nigerian patients with epilepsy (Olatunbosun et al., 1978), schizophrenia (Olatunbosun et al., 1975b) and those with kwashiorkor (Reiff and Schnieden, 1959). Increased plasma or serum levels have also been reported in chronic bacterial infections and in viral illnesses in man (Beisel et al., 1973) although depressed serum copper concentrations have been reported in patients with tick-borne encephalitis ( Beisel et al., 1973).

The present investigation was undertaken to document the effect of a variety of diseases on the serum zinc and copper concentrations in Nigerians, and the correlations of these levels to serum albumin, alkaline phosphatase and caeruloplasmin.

## 4.2. Subjects studied

Ninety-five adult male Nigerians (rural farmers from the Zaria area) with proven cases of miscellaneous diseases were studied. These included hookworm anaemia (14), primary liver cancer (20), various leukaemias (12), active pulmonary tuberculosis (22) and venous leg ulceration (27). Diagnosis was reached following appropriate clinical examinations, haematological, histopathological or microbiological investigations.

The controls were symptom-free adult non-elite Nigerian males (127) living in the Zaria area. Sex and socioeconomic background were matched since women have higher serum copper levels than men (Wintrobe, 1967) and plasma zinc has been shown to be higher in the elite than non-elite Nigerians (Kapu et al., 1976a), a finding that has been confirmed in elite and non-elite Indians (Spears et al., 1976).

## 4.3. Results

### 4.3.1. Hookworm anaemia

From Table 4.1. there were significantly decreased serum zinc and albumin ($P < 0.05$), haemoglobin and haematocrit levels ($P < 0.01$) in hookworm patients in comparison with non-elite Nigerians. The mean serum copper level was significantly increased in hookworm patients ($P < 0.05$).

However, the alkaline phosphatase and the caeruloplasmin levels in hookworm patients and non-elite Nigerians did not differ significantly. There was significant correlation between the serum copper and the serum caeruloplasmin ($r = + 0.5877$, $P < 0.05$).

4.3.2. Pulmonary tuberculosis

The serum zinc, albumin and haemoglobin in patients with active tuberculosis were significantly lower ($P < 0.001$) than non-elite controls (table 4.1.). The correlation between the serum zinc and serum alkaline phosphatase was significant ($r = + 0.4967$, $P < 0.01$). The serum copper was significantly correlated with the serum caeruloplasmin ($r = + 0.6467$, $P < 0.001$).

4.3.3. Leukaemia

Patients with leukaemia had significantly lower zinc ($P < 0.01$), albumin ($P < 0.05$), haematocrit and haemoglobin levels ($P < 0.01$), but significantly higher copper and caeruloplasmin values ($P < 0.01$) than normal non-elite controls (table 4.1.). There was a significant correlation between the serum copper and the serum caeruloplasmin ($r = + 0.9331$, $P < 0.01$).

4.3.4. Leg ulceration

Patients with leg ulcers had similar biochemical

values studied with non-elite controls (table 4.1.).

The correlation between the serum zinc and serum alkaline phosphatase was slightly significant ($r = -0.3805$, $P < 0.05$).

4.3.5. Primary liver cancer

Patients with primary liver cancer showed significantly lower zinc albumin, haemoglobin and haematocrit levels, but showed significantly higher copper and caeruloplasmin levels ($P < 0.001$) than non-elite controls (table 4.1.). The alkaline phosphatase and total protein did not differ significantly. There was a slight significant correlation between the serum zinc and serum alkaline phosphatase ($r = -0.3816$, $P < 0.05$). The correlation between the serum albumin was also slightly significant ($r = +0.4613$, $P < 0.05$).

4.4. Discussion

4.4.1. Hookworm anaemia

The significantly reduced serum zinc levels with the serum albumin and alkaline phosphatase values in patients with hookworm in this study may be due to excessive blood loss because blood contains about 10 µg/ml of zinc, meaning that hookworm infestation, like Schistosoma haematobium infestation, can contribute to body zinc depletion (Prasad, 1969; Halsted et al., 1972; Anon, 1973). The

significantly reduced haemoglobin and haematocrit values was due to iron deficiency.

Caeruloplasmin accounted for the elevated serum copper because caeruloplasmin is the major copper carrier protein whose function may be accelerated in response to the need for copper in the utilization of iron during haemosynthesis (Wintrobe, 1967).

The accumulation of copper in red cells and plasma in Hb.SS may have similar mechanism to that in hookworm anaemia, thalassaemia, aplastic anaemia and pernicious anaemia, secondary to disturbed iron metabolism in mitochondria of normoblasts. The significant correlation between serum copper and serum caeruloplasmin might reflect that serum copper is directly related to caeruloplasmin levels not depending solely on lateration in hepatic function.

### 4.4.2. Pulmonary tuberculosis

The reduced plasma zinc noted in the present study in patients with pulmonary tuberculosis is in agreement with the findings of Halsted and Smith (1970). This reduction could be the result of loss through sputum, and altered concentration of albumin and alkaline phosphatase. Another reason that has been advanced to be responsible for a depression of serum zinc in inflammatory conditions, as in iron, is thought to be mediated by Leukocytic

Endogenous Mediator (LEM) after its release from phagocytic cells (Beisel et al., 1973). The significant correlation between the serum zinc and serum alkaline phosphatase confirms that zinc is an essential metal for the function of alkaline phosphatase.

4.4.3. Leukaemia

The reduced serum zinc levels in this study agrees with reported studies by Davies (1972). The same author reported elevated erythrocyte levels in leukaemia. These changes suggest a re-distribution phenomenon with respect to zinc. It is possibly that in view of an increased metabolic activity of the erythrocyte as a result of haemorrhage, the requirement of zinc by the red blood cells might increase, thus causing a shift from the plasma pool to the red blood cells. However, low zinc values in serum cannot be explained by a movement of zinc into areas of tissue injury (Beisel et al., 1973).

The elevated serum copper with the serum caeruloplasmin levels is in agreement with the findings reported by Davies (1972) and Beisel et al. (1973). The highly significant correlation between the serum copper and serum caeruloplasmin in this study indicates that increase serum copper accompanies an increased hepatic secretion of caeruloplasmin in response to the need for copper in haemopoiesis.

### 4.4.4. Leg ulceration

The finding of non-significant reduction in the serum zinc concentration of patients with venous leg ulceration with controls does not agree with the earlier reports of Greaves and Boyde (1967), Withers et al., (1968), Halsted and Smith (1970) and Greaves and Skillen (1970). Myers and Cherry (1970) were also unable to demonstrate any reduction of plasma zinc concentrations in their patients with leg ulceration. The slightly raised alkaline phosphatase in the leg ulcer patients in this study may have originated from the bones, liver or kidney to provide phosphate for growth and tissue repair, or participate in bone metabolism. The slight negative but significant correlation between the serum zinc and serum alkaline phosphatase might mean that the low zinc levels were not influenced by the alkaline phosphatase activity. Raised serum alkaline phosphatase in a similar study was mainly of the intestinal form (Greaves and Skillen, 1970).

### 4.4.5. Primary liver cancer

The significantly reduced serum zinc in patients with primary liver cancer in this study does not agree with the finding of elevated serum zinc concentrations in association with liver cancer reported by Karlinsky and

Romere (1967) in South African Bantus. Dunn et al. (1969) were unable to substantiate any statistically significant differences in serum zinc concentrations between 31 male Bantus. Although it is reported that normal liver tissue contains considerable amounts of zinc and that this is increased in liver cancer (Dunn et al., 1968), Butt and Higginson (1957) have found mean hepatic zinc to be subnormal in Bantu cirrhotics. And decreased serum zinc levels have been found in cirrhosis (Versieck et al., 1974). According to Dunn et al. (1969), local epidemiological studies have established that between 80 and 100% of hepatomas in South African Bantu develop in such cirrhotic livers, and a change of similar magnitude occur in other areas of the tropics where liver-cell carcinoma is common (Edington and Gilles, 1969).

While low plasma zinc is a notable feature of cirrhosis (Versieck et al., 1974) increased urinary zinc is associated with cirrhosis (Vallee et al., 1957; Sullivan, 1965; Kahn et al., 1965; Walker et al., 1973; Schwartz, 1975), with the serum zinc in liver disease correlating particularly with the serum albumin level (Kahn et al., 1965). In the present study, the slight but significant correlation between the serum zinc and serum

albumin levels indicates that the reduced zinc levels found in patients with primary liver cancer may be a reflection of reduced albumin concentrations as commonly found in patients with hepatic cirrhosis (Walker et al., 1973).

The significantly raised serum copper obtained in the present study is similar to that usually found in acute and chronic liver disease (Sunderman, 1973), although a similar finding has not been reported in primary liver cancer. The elevated mean serum copper levels in patients with primary liver cancer in this study was associated with elevated caeruloplasmin levels, probably as a result of disturbance in hepatic copper metabolism. Also, the increased serum copper may be attributed to interference with the excretion of copper in the bile, independent of caeruloplasmin activity (Schwartz, 1975). The latter is the likely explanation since the serum copper and serum caeruloplasmin were found to be uncorrelated in this study.

The findings of elevated serum copper levels with the serum caeruloplasmin levels in this study requires further comprehensive study to determine the difference in serum copper levels in relation to disease activity, as is in Hodgkin's disease (Thorling and Thorling, 1976) and in a variety of other lymphomas (Hrgovcic et al., 1973).

## 4.5. Summary

Serum zinc, copper, caeruloplasmin, albumin, total protein, alkaline phosphatase, haemoglobin and packed cell volume were measured in 95 rural farmers with a variety of diseases from the Zaria area in northern Nigeria. While abnormally low values of zinc, albumin and haemoglobin were obtained in hookworm anaemia, active pulmonary tuberculosis, leukaemia and primary liver cancer, the copper with the caeruloplasmin values were abnormally elevated, except in active pulmonary tuberculosis. In venous leg ulceration there were no sifnificant changes in all biochemical parameters estimated.

These observed changes could be the result of excess loss through sputum, urine and blood; or altered concentrations of albumin, alkaline phosphatase and caeruloplasmin, since signficant correlations occured between the elements and their carrier proteins. In inflammatory conditions a depression of serum zinc, as in iron, is thought to be mediated by leukocytic endogenous mediator factor (LEM) after its release from phagocytic cells. The accumulation of copper in red cells and serum in haematological disorders could be secondary to disturbed iron metabolism in the mitochondria of normoblasts.

Table 4.1. Serum Biochemical Values in Nigerians with Various Pathological Conditions (Mean ± SE)

| Items | Controls (non-elite) | Primary Liver Cancer | Leukaemia | Hookworm Anaemia | Leg Ulcer Patients | Pulmonary Tuberculosis |
|---|---|---|---|---|---|---|
| Number of Subjects | 127 | 20 | 12 | 14 | 27 | 22 |
| Zinc (µg/100 ml) | 105.3±7.3 | 81.80±4.3 | 67.5±4.9 | 80.45±4.88 | 92.2±1.9 | 70.9±4.04 |
| Copper (µg/100 ml) | 120.2±7.0 | 175.4±6.8 | 211.7±25.7 | 156.36±14.35 | 114.1±3.1 | 130.7±5.9 |
| Caeruloplasmin (mg/100 ml) | 32.6±7.6 | 33.1±0.9 | 45.00±3.9 | 29.18±2.01 | 26.9±0.5 | 27.3±0.6 |
| Albumin (g/l) | 43.0±0.3 | 34.2±1.4 | 38.5±1.6 | 35.98±1.6 | 43.0±1.0 | 36.2±1.0 |
| Total Protein (g/l) | 80.3±7.3 | 77.5±1.9 | 74.10±3.8 | 71.28±2.87 | 92.2±1.9 | 74.6±1.6 |
| Alkaline Phosphatase (K.A. Units) | 15.2±7.8 | 9.9±1.10 | 7.6±1.2 | 14.18±1.52 | 16.5±1.3 | 7.1±0.7 |
| Haemoglobin (g/dl) | 14.2±0.2 | 10.6±0.6 | 10.73±0.91 | 10.94±1.02 | 14.1±0.3 | 12.04±0.4 |
| Packed Cell Volume (%) | 46.2±0.9 | 32.1±1.3 | 32.5±2.3 | 34.33±2.8 | 42.0±0.9 | 40.31±1.2 |

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## ADDENDUM

Since the preparation of this thesis, Prasad *et al.* (1978) have reported copper deficiency in an adult patient with sickle cell disease who had received oral zinc 150mg daily in divided doses of 15mg daily for nearly two years. The oral administration of 1mg of copper per day corrected the deficiency.

Zinc affects membranes in several ways (Prasad, 1979). It prevents induced histamine release from mast cells, an effect believed to be due to its action on cell membrane. Zinc controls the activities of many plasma membranes. Adenosine triphosphatase (ATPase) and phospholipase A are known to be inhibited by zinc, and it is believed that this effect may result in immobilization of energy - dependent activity plasma membrane or increase integrity of the membrane structure (Prasad, 1979). Zinc also binds to haemoglobin, and it is possible that the antisickling effect of zinc is not only due to its effects on red cell membrane but in part is related to its binding to the haemoglobin molecule (Prasad, 1979).

Iron absorption is impaired in copper deficient animals and this defect represents impaired transfer of iron from mucosa to plasma, rather than from duodenal lumen to mucosa (Prasad, 1979). It is believed that caeruloplasmin, a copper protein, catalyzes the oxidation of ferrous iron. This step is essential before iron can be bound to transferrin and circulate in the plasma for marrow utilization (Prasad, 1979; Imbembo, 1979).

It is believed (Palmisano, 1979) that all the patients will develop the clinical picture of anaemia, leukopenia and neutropenia if their sole nourishment is copper-free parentenal hyperalimentation given for a long period of time. About six cases of such copper deficiency have now been reported (Palmisano, 1979). During total parenteral nutrition therapy, decreased levels of trace elements can occur by a number of mechanisms:

(1) decreased intake in the solution itself;

(2) increased need secondary to anabolic processes;

(3) increased excretion due to concomitant physical conditions (Imbembo, 1979).

Copper deficiency may be corrected by a daily dosage of 500 µg of copper to restore tissue stores, or by the oral administration of 5mg of copper sulphate in water solution daily.

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# The Natural Forages of Northern Nigeria
# I. Nitrogen and Mineral composition of Grasses and Browse from the Northern Guinea Savanna and Standing Hays from the different Savanna Zones

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SUMMARY

**THE mineral status of nine native grass species and ten browse species from the Northern Guinea Savanna Vegetation was investigated together with the mineral Composition of standing hays from Daura and Kano areas of the Sudan Savanna, Bauchi ranch of the sub-Sudan, Samaru (Shika Fadama) of the Northern Guinea Savanna and Jos area of the Plateau vegetation, in Northern Nigeria. All forage types (grasses, browse and standing hays) which were sampled in April of 1975 were analysed for nitrogen (N), Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg). sodium (Na), zinc (Zn) and copper (Cu). The grass species included:** *Andropogon gayanus, A. ascinodis, Brachiaria jubata, Cynodon dactylon, Hyparthelia dissoluta, Pancium phragmitoides, Setaria anceps, Sporobolus pyramidalis* **and** *Urelytram muricatum,* **while the browse consisted of** *Adenodolichos paniculatus, Anmogeissus leocarpus, Briddelia ferruginea, Crossopteryx febrifug, Dichrostachys cenerea (glometata), Khaya senegalensis, Mimosa pigra, Parinari curatellifolis, Psorospermum guineense,* **and** *Pterocarpus erinaceus.* **Both grass and browse species were leafy materials from among those species considered to be highly consumed by cattle, in general, the average values for Na, P, and Zn in the grasses, browse and standing hays were inadequate for the nutritional needs of cattle one standing hays from all .ocations contained suboptimum amounts of N, Ca, P, K, Mg, Na and The Cu content of all three forage types was adequate for cattle nutrition The grass and browse species contained adequate amounts of N, K, Mg and Cu. The trace elements and Ca and Mg tended to be more concentrated in the browse species than in the grass species The opposite was true of K. Grasses, browse and standing hays contained equal amounts oi Na. Similarly, the grass and browse species contained identical amounts of P. Supplementation of mineral concentrates to include Na, P, K, Ca and Zn was suggested.**

## INTRODUCTION

THE vast majority of Nigerian cattle are found in the Northern States of Nigeria (Oyenuga, 1968) and are mostly kept under traditional systems of management. The major fodder resources include grasses, browse, crop residues and the standing hays (Miller and Blair Rains, 1963a; Miller, Blair Rains and Thorpe, 1963b and 1964; Van Raay and de Leeuw, 1974). However, very little is known of the chemical constituents of these fodder resources (Oyenuga, 1968; Loosli, 1974). Any study that provides information on the mineral composition of the indigenous forage species of Northern Nigeria would contribute significantly to the progress of systematic nutritional work in Nigeria and possibly serve as a basis for appropriate formulation of feeds for livestock. This study was undertaken to find out the levels of nitrogen and some important minerals in the local forage species found in Northern Nigeria.

## EXPERIMENTAL METHODS

### Materials

Nine grass and ten browse species and standing hays were sampled during the month of April of 1975. The grass and browse species were leafy materials native to Northern Nigeria, and were collected from the Samaru area (on the Shika Fadama) of the Northern Guinea Savanna about 11° 11′ north of the equator and 07° 38′ east of the prime meridian at an

altitude of about 685.8m (2250ft) above sea level. The annual rainfall of the area is 111.40cm (43.86in) The standing hays were whole materials and were collected from Daura and Kano areas of the Sudan Savanna, Samaru in the Northern Guinea Savanna, Bauchi ranch of the sub-Sudan and Jos area of the Plateau vegetation Daura is about N13° 02′ and longitude E08° 13′ and Kano is about latitude N12° 03′ and longitude E08° 32′. The altitudes of Daura and Kano are 426.72m (1400ft) and 472.14m (1549ft) above sea level, with annual rainfall of 67.56cm (26.6in) and 88.42cm (34.81in), respectively. Bauchi is about latitude N10° 11′ and longitude E09° 49′ with an altitude of 609.30m (1999ft) above sea level and an annual rainfall of 110.19cm (43.38in). Jos is about Longitude E08o 54′ and latitude N09° 52′ with an altitude of 1284.73m (4215ft) above sea level and an annual rainfall of 141.02cm (55.52in) (Fig. 1. after Clayton, 1957).

A representative sample of each forage type was collected according to the sampling procedures for field hay, pasture or forage grasses (Jones, Large, Pfeidere, and Klosky, 1971). All samples were washed several times in distilled water to avoid any contamination from accumulated dust and soil particles. They were air dried under the shade and later in an oven at about 85°C, cooled and then ground in a stainless Microhammer mill. The samples were then stored in plastic jars after oven drying at about 104°C prior to digestion.

**Analytical Methods**

One gram of the oven-dried sample was wet digested in Kjeldahl flask in duplicate as described by Sandell (1950). The digest was washed with distilled water into 100m volumetric flask and made to mark. The concentrations of K, Ca, Mg, Zn and Cu were determined with a Perkin-Elemer Model 290B atomic absorption spectrophotometer.* P was determined by the Vanadomolybdate colorimetric method (Cavell, 1955). The Standard flame photometric method was used to estimate the concentration of Na, while the micro-Kjeldahl method was used for N determination. All results were expressed on an oven-dry basis.

TABLE 1

***Nitrogen and Mineral Composition of Grasses from the Northern Guinea Zone of Nigeria***

| *Species* | *N* % | *P* % | *K* % | *Ca* % | *Mg* % | *Na* % | *Zn* ppm | *Cu* ppm | *Ca to P ratio* |
|---|---|---|---|---|---|---|---|---|---|
| Andropogon gayanus | 1.78 | 0.11 | 1.43 | 0.30 | 0.14 | 0.013 | 28 | 17 | 2.73 |
| Andropogon ascinodis | 2.31 | 0.19 | 2.49 | 0.32 | 0.25 | 0.052 | 42 | 20 | 1.68 |
| Brachiaria jubata | 2.84 | 0.28 | 3.49 | 0.29 | 0.32 | 0.043 | 40 | 20 | 1.04 |
| Cynodon dactylon | 2.30 | 0.32 | 1.99 | 0.75 | 0.20 | 0.024 | 43 | 10 | 2.34 |
| Hyparthelia dissoluta | 1.50 | 0.13 | 1.62 | 0.28 | 0.16 | 0.011 | 30 | 12 | 2.15 |
| Panicum phragmitoides | 2.87 | 0.28 | 2.53 | 0.24 | 0.45 | 0.024 | 54 | 24 | 0.86 |
| Setaria anceps | 2.64 | 0.21 | 5.54 | 0.26 | 0.15 | 0.020 | 42 | 10 | 1.24 |
| Sporobolus pyramidalis | 3.31 | 0.22 | 2.07 | 0.35 | 0.26 | 0.019 | 77 | 5 | 1.59 |
| Urelytram muricatum | 1.92 | 0.21 | 1.86 | 0.25 | 0.18 | 0.013 | 27 | 12 | 1.19 |
| Average Values | 2.39 | 0.22 | 2.56 | 0.34 | 0.23 | 0.02 | 42.50 | 14.44 | 1.55 |
| Standard Deviations | 0.59 | 0.07 | 1.27 | 0.16 | 0.10 | 0.01 | 15.56 | 6.13 | |

* The Perkin-Elmer Corporation (1971), Norwalk, Connecticut, U.S.A.

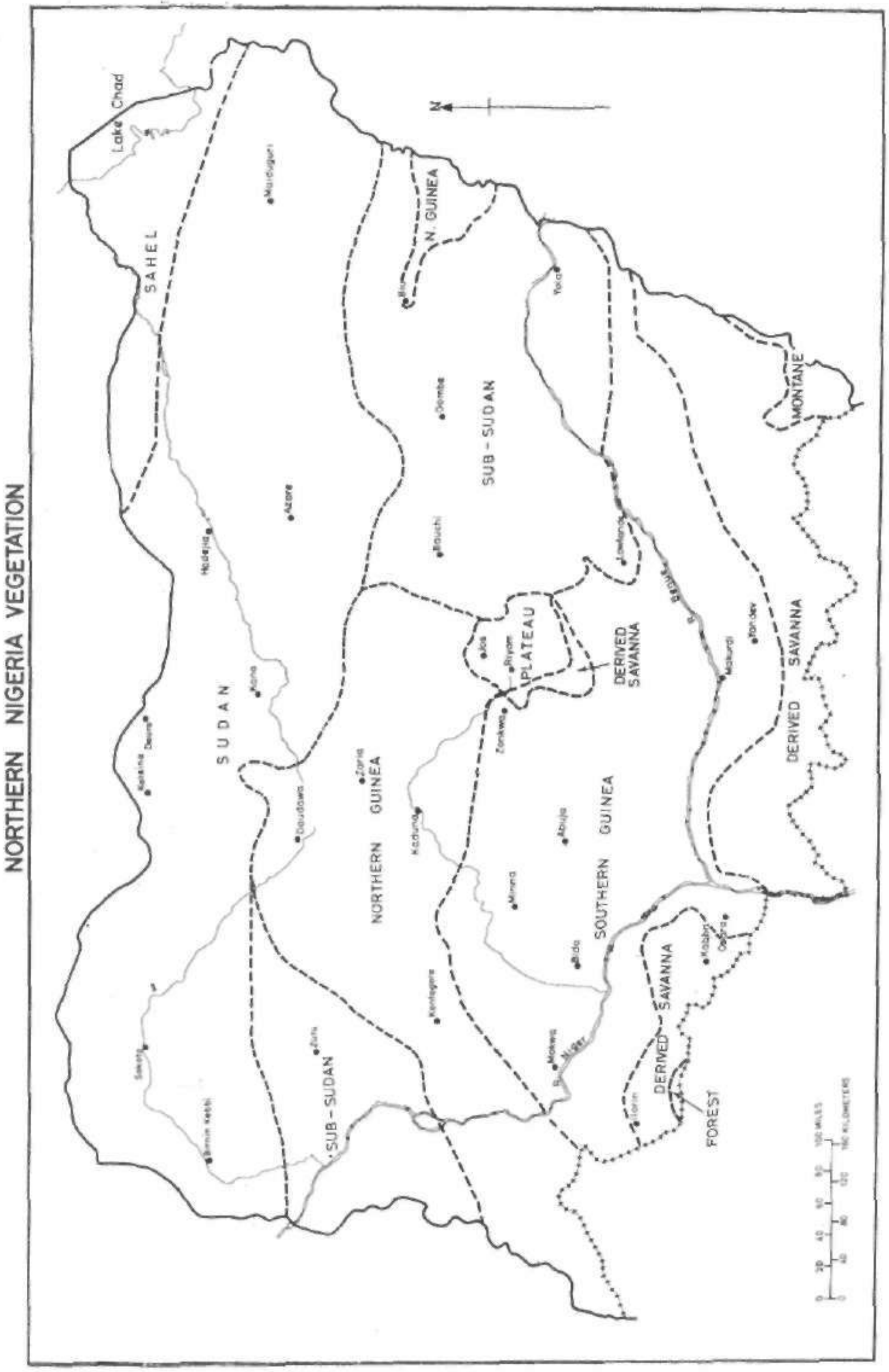

NORTHERN NIGERIA VEGETATION
Lake Chad
SAHEL
SUDAN
SUB-SUDAN
N. GUINEA
NORTHERN GUINEA
SOUTHERN GUINEA
PLATEAU
DERIVED SAVANNA
MONTANE
FOREST
N

Fig. 1

TABLE 2

**Nitrogen and Mineral Composition of Browse from the Northern Guinea Savanna of Nigeria**

| *Species* | *N* % | *P* % | *K* % | *Ca* % | *Mg* % | *Na* % | *Zn* ppm | *Cu* ppm | *Ca to P ratio* |
|---|---|---|---|---|---|---|---|---|---|
| Adenodolichos paniculatus | 3.31 | 0.39 | 2.49 | 0.31 | 0.21 | 0.018 | 16 | 10 | 0.79 |
| Anogeissus leocarpus | 1.86 | 0.20 | 1.09 | 1.34 | 0.31 | 0.023 | 22 | 14 | 6.70 |
| Briddelia ferruginea | 1.64 | 0.12 | 2.33 | 0.70 | 0.41 | 0.028 | 53 | 15 | 5.83 |
| Crossopteryx febrifuga | 1.82 | 0.16 | 1.40 | 0.53 | 0.20 | 0.017 | 48 | 20 | 3.31 |
| Dichrostachys cinerea | 2.61 | 0.19 | 1.30 | 0.79 | 0.39 | 0.24 | 43 | 16 | 4.16 |
| Kyaya Senegalensis | 1.51 | 0.15 | 1.37 | 0.90 | 0.27 | 0.021 | 31 | 11 | 6.00 |
| Mimosa pigra | 2.72 | 0.27 | 1.50 | 0.98 | 0.22 | 0.026 | 70 | 25 | 3.63 |
| Parimari curatellifolia | 1.39 | 0.12 | 1.05 | 0.53 | 0.19 | 0.021 | 19 | 10 | 4.42 |
| Psorospermum guineense | 1.25 | 0.18 | 1.47 | 0.48 | 0.18 | 0.017 | 49 | 10 | 2.67 |
| Pterocarpus erinaceus | 3.56 | 0.30 | 2.58 | 0.43 | 0.45 | 0.019 | 63 | 20 | 1.43 |
| Average Values | 2.17 | 0.21 | 1.66 | 0.70 | 0.28 | 0.021 | 45.90 | 15.10 | 3.33 |
| Standard Deviations | 0.82 | 0.09 | 0.78 | 0.31 | 1.10 | 0.003 | 17.29 | 5.20 | |

TABLE 3

**Nitrogen and Mineral Content of Standing Hays from the Savanna Zones of Northern Nigeria**

| *Vegetation Zone* | *N* % | *P* % | *K* % | *Ca* % | *Mg* % | *Na* % | *Zn* ppm | *Cu* ppm | *Ca to P ratio* |
|---|---|---|---|---|---|---|---|---|---|
| Sudan | | | | | | | | | |
| Daura — Northern Sudan | 0.32 | 0.02 | 0.65 | 0.12 | 0.12 | 0.015 | 42 | 15 | 6.00 |
| Kano — Southern Sudan | 0.45 | 0.05 | 0.17 | 0.31 | 0.11 | 0.029 | 43 | 10 | 56.20 |
| Sub-Sudan | | | | | | | | | |
| Bauchi — ranch | 0.26 | 0.02 | 0.45 | 0.20 | 0.12 | 0.016 | 35 | 11 | 10.00 |
| Northern Guinea | | | | | | | | | |
| Samaru (Shika-Fadama) | 0.29 | 0.03 | 0.36 | 0.23 | 0.10 | 0.020 | 36 | 10 | 7.67 |
| Plateau | | | | | | | | | |
| Jos | 0.22 | 0.01 | 0.20 | 0.22 | 0.07 | 0.027 | 29 | 13 | 22.0 |
| Average Values | 0.31 | 0.03 | 0.37 | 0.22 | 0.11 | 0.02 | 37.00 | 11.80 | 7.33 |
| Standard Deviations | 0.09 | 0.02 | 0.20 | 0.07 | 0.02 | 0.01 | 5.70 | 2.17 | |

## RESULTS AND DISCUSSION

The average values of nitrogen and the seven mineral elements in each forage type are presented in tables 1, 2 and 3. Figures 2, 3, 4, 5 and 6 show the mean protein and mineral values.

In all three forage types, the concentration of N (or protein) was directly proportional to the concentration of Zn ($P < 0.05$), while in the standing hays alone, the K concentration was inversely proportional to the concentrations of Ga and Na ($P < 0.05$). The N concentration in Browse species was directly related to the concentrations of $P < 0.05$) and K ($P < 0.01$). Similarly, the concentration of N in the standing hays was directly related to the concentration of P ($P > 0.05$.)

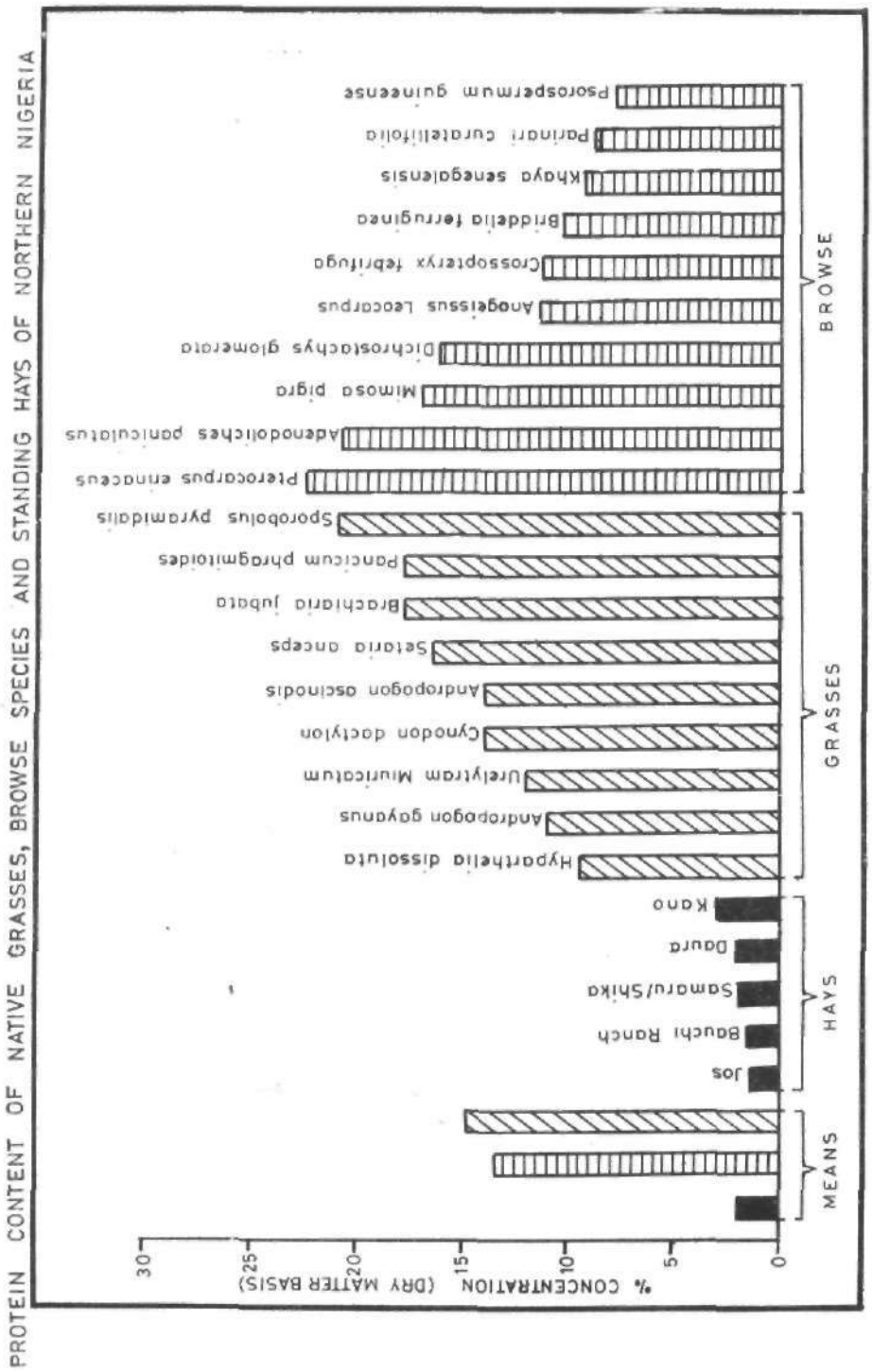

PROTEIN CONTENT OF NATIVE GRASSES, BROWSE SPECIES AND STANDING HAYS OF NORTHERN NIGERIA
% CONCENTRATION (DRY MATTER BASIS)
0
5
10
15
20
25
30
MEANS
HAYS
GRASSES
BROWSE
Jos
Bauchi Ranch
Samaru/Shika
Daura
Kano
Hyparrhenia dissoluta
Andropogon gayanus
Urelytrum Muricatum
Cynodon dactylon
Andropogon oscinodis
Setaria anceps
Brachiaria jubata
Panicum phragmitoides
Sporobolus pyramidalis
Pterocarpus erinaceus
Adenodoliches paniculatus
Mimosa pigra
Dichrostachys glomerata
Anogeissus leiocarpus
Crossopterix febrifuga
Bridelia ferruginea
Khaya senegalensis
Parinari curatellifolia
Psorospermum guineense

Fig. 2

**Major Elements**

The crude protein values for each grass and browse species and the standing hays were calculated from the nitrogen figures (Figs. 2 and 3). According to the A.R.C. (1965) recommendations, 8% crude protein which is equivalent to 1.28% nitrogen was adequate for milk production. On this basis all the grass and browse species provided adequate amounts of nitrogen. The standing hays from all locations contained inadequate amounts of nitrogen. The mean crude protein values of temperate zone pastures (Wienmann, 1955) and Uganda grasses (Long, Thornton and Marshall, 1969a) were 20% and 6.24 respectively, compared with 14.91 $\pm$ 3.66% for grasses, 13.55 $\pm$ 5.15% for browse and 1.93 $\pm$ 0.54% for standing hays obtained in this study. There was no significant difference between the average protein (or nitrogen) contents of the grass and browse species. The average protein content of the standing hays was significantly lower ($P < 0.01$) that those of the grass or browse species.

All forage types sampled contained low concentrations of Na. with equal values of 0.02% (Fig. 4), which were lower than the A.R.C. (1965) recommendations of 0.13% or good United Kingdom pastures which normally contained 0.167% Na (Orr, 1929). Long, Ndyanabo, Marshall and Thornton (1969b) obtained a higher Na value of 0.044% for grasses in Uganda while lower values of 0.015% were obtained by Howard, Burdin and Lampkin (1962) for Kenya highland pastures.

Since the requirements of beef cattle are reported to be lower than dairy cattle, the amounts of Na found in the present study may be barely adequate for beef grazing but definitely deficient for dairy production.

The average K values were 2.56 $\pm$ 1.27, 1.66 $\pm$ 0.78 and 0.37 $\pm$ 0.20% for grasses, browse and the standing hays respectively. K intended to be more concentrated in the grasses than in the browse or the field hays ($P < 0.01$) (Figure 5). K contents of grass and browse species were within the range of 1 to 4% reported for temperate grasses. The field hays from all locations were deficient in K. The differences between forage and types may be due to differences in age at sampling, since K concentration drops with in creasing maturity (Widdowson Penny and Willams '1965).

The average P levels of the grasses and browse were about identical and were significantly higher ($P < 0.01$) than that of the field hays (Fig. 4) In general, the P levels of all forage types were below the ARC (1965) recommendations of 0.36% necessary for dairy cattle. It is interesting to note that the grass species *Cynodon dactylon* contained a very high amount of P (table 1). This is in agreement with the finding of Long *et al* (1969) for the same grass found in Uganda. The browse species *Adenodolichos paniculatus* (table2) contained 0.39% of P which was higher that the ARC (1965) recommended requirements. It is concluded that P is deficient in all forage types.

The Ca level tended to be more concentrated in the browse than in the grass or standing hays ($P < 0.01$) and more in the grass ($P \pm 0.01$) than in the hays Fig. 4). The Ca concentration of most of the browse exceeded the A.R.C. (1965) suggested value of 0.43% for dairy cattle (table2), while the Ca values for all hays and grasses with the notable exception of *Cynodon dactylon* were well below the A.R.C. (1965) stipulations (table 1).

The average Mg values in the grasses, browse and field hays were 0.23 $\pm$ 0.10, 0.28 $\pm$ 0.10 and 0.11 $\pm$ 0.02, respectively The A.R.C. (1965) has recommended a Mg requirement of 0.18%. On this basis,

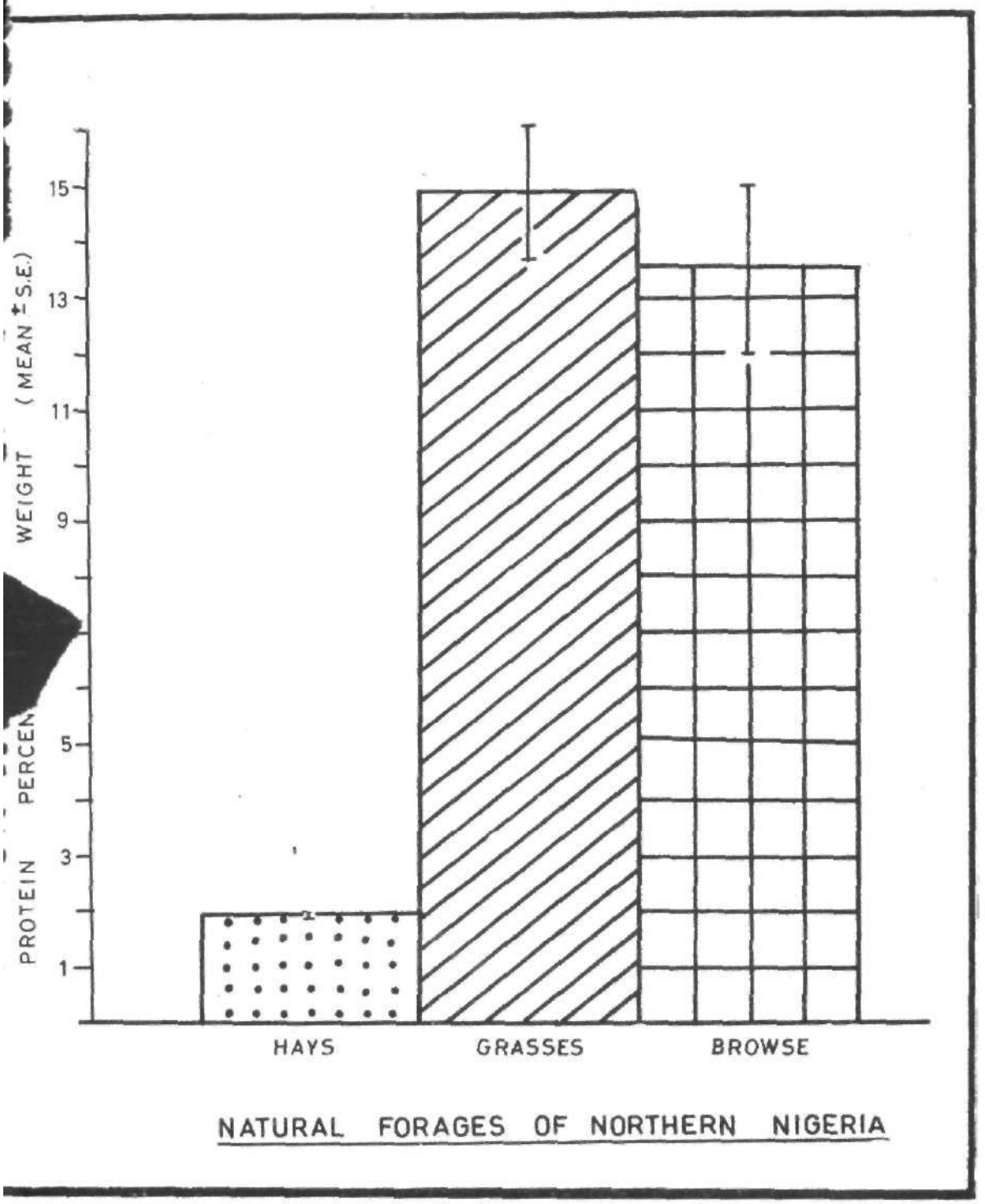

PROTEIN PERCEN WEIGHT (MEAN ± S.E.)
15
13
11
9
5
3
1
HAYS
GRASSES
BROWSE
NATURAL FORAGES OF NORTHERN NIGERIA

Fig. 3

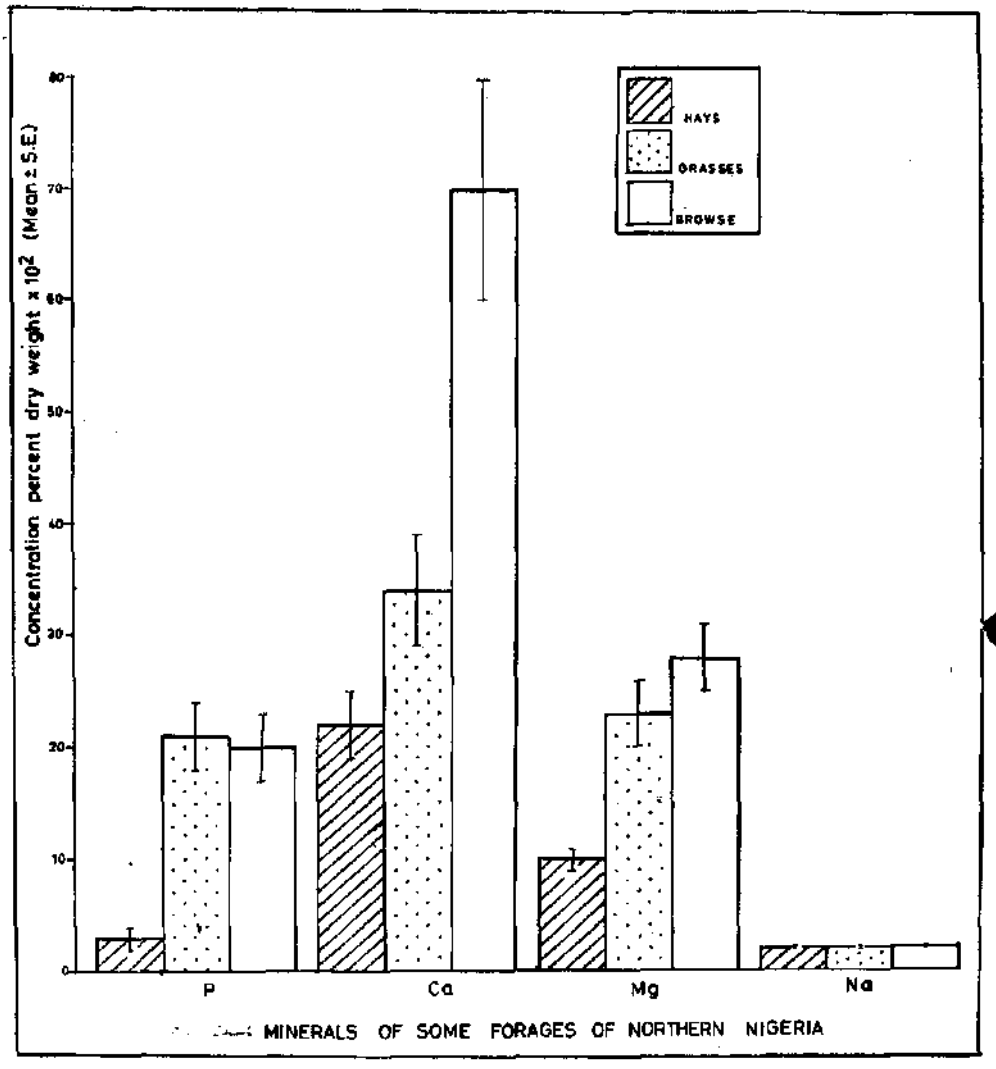

HAYS
GRASSES
BROWSE
Concentration percent dry weight x 10² (Mean ± S.E)
0
10
20
30
40
50
60
70
80
P
Ca
Mg
Na
MINERALS OF SOME FORAGES OF NORTHERN NIGERIA

Fig. 4

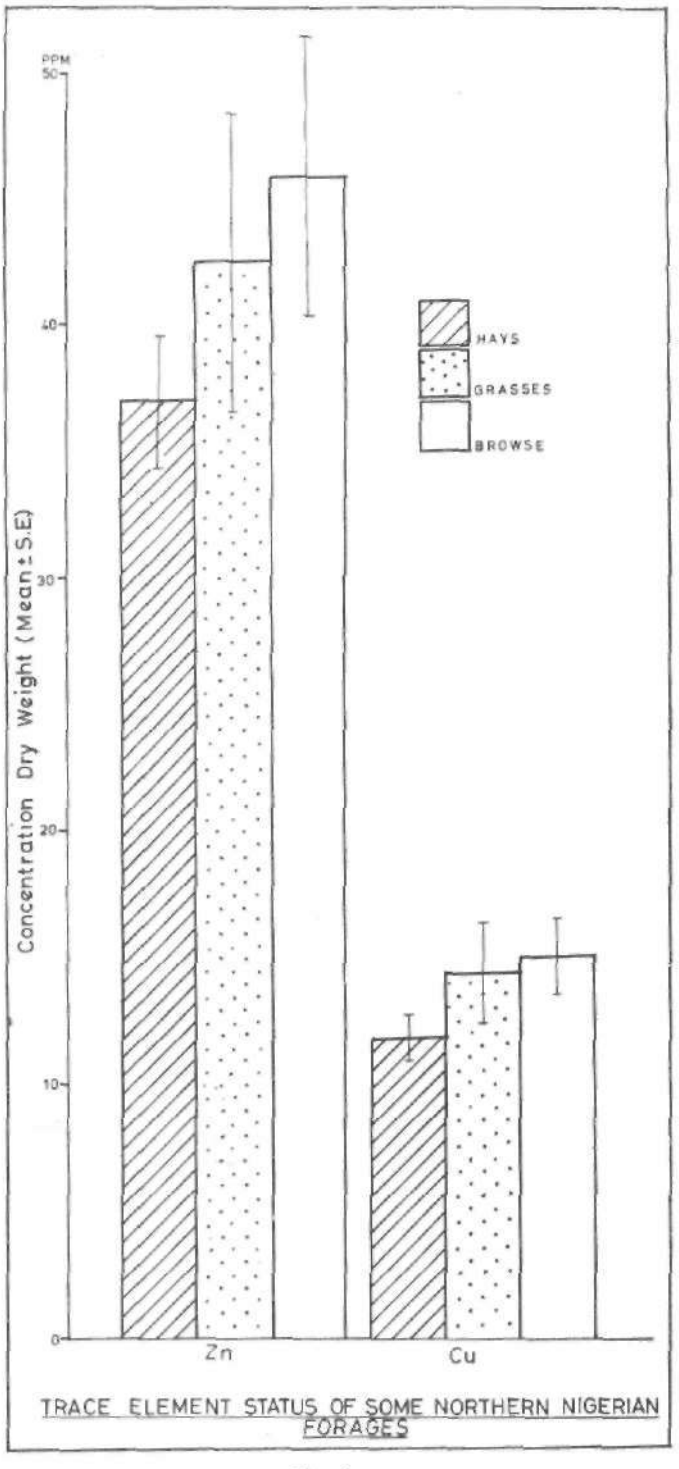

PPM
50
40
30
20
10
0
Concentration Dry Weight (Mean ± S.E)
HAYS
GRASSES
BROWSE
Zn
Cu
TRACE ELEMENT STATUS OF SOME NORTHERN NIGERIAN FORAGES

Fig. 6

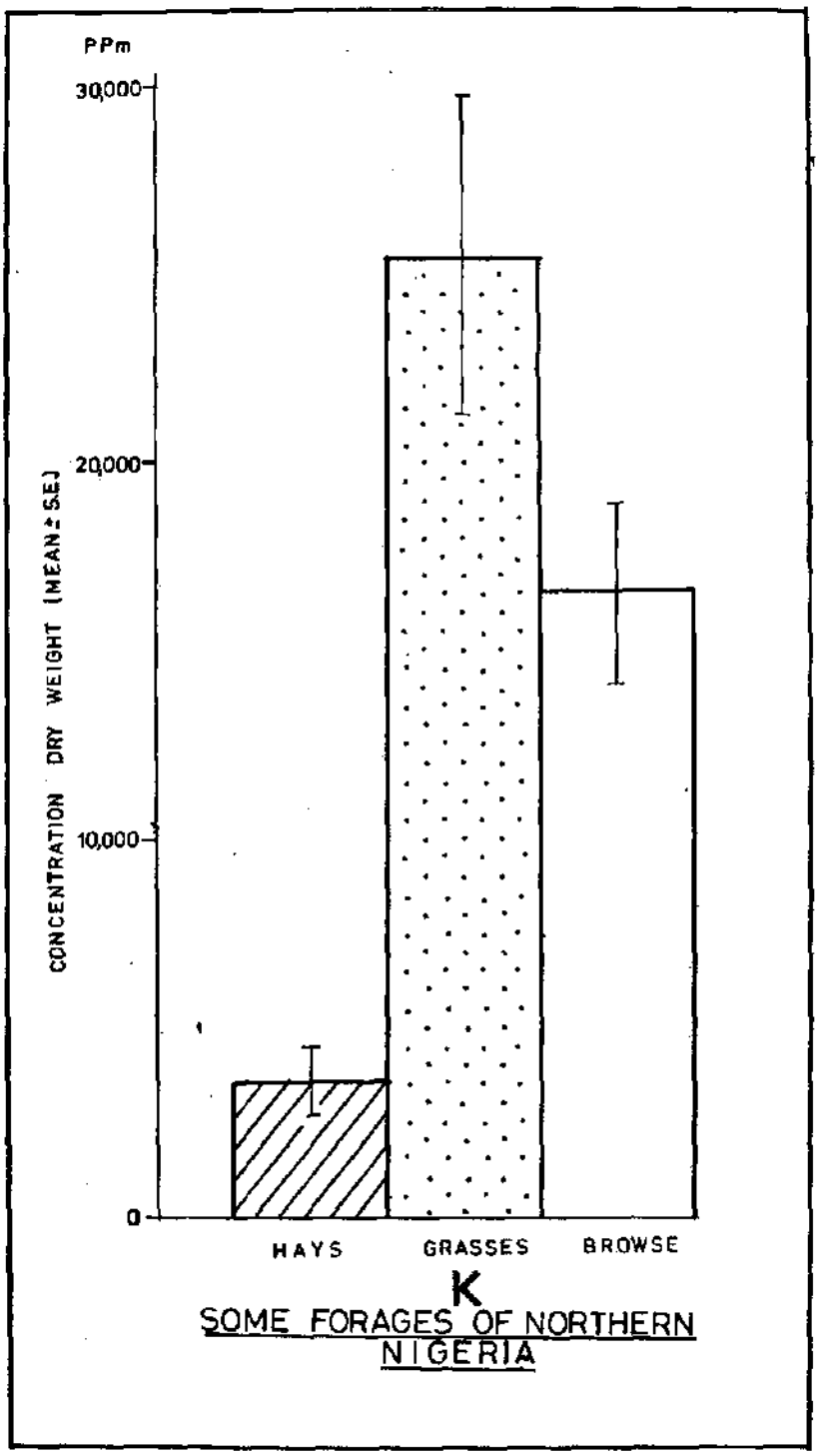

PPm
30,000
20,000
10,000
0
CONCENTRATION DRY WEIGHT (MEAN ± S.E.)
HAYS
GRASSES
BROWSE
K
SOME FORAGES OF NORTHERN NIGERIA

Fig. 5

the grass and browse species contained adequate amounts of Mg while the hays were deficient. There was no significant difference between the average Mg contents of grasses and browse. The average Mg level in the field hays was significantly lower ($P < 0.01$) than in the grasses or the standing hays (Fig. 4). Calcium: Phosphorus ratio.

Hignett and Hignett (1951 and 1953) and Hignett (1959) have suggested that the Ca:P ratio was a factor influencing animal fertility. They proposed an optimal ratio of between 1 and 2.1 which was later disputed by Little John and Lewis (1960). Long *et al* (1969) obtained a ratio between 1:1 and 2:1 for grasses in Uganda. The Ca: P ratios obtained in this study are presented in tables 1, 2 and 3 The Ca: P ratio for grasses varied between 1:1 and 2:1.

**Trace elements**

Cu levels were about within the range of 12 to 18 ppm reported by Oyenuga and Olubajo (1974)for forages grown at Ibadan (tables 1, 2 and 3). The grass species *Sporobolus pyramidalis* had the lowest Cu level (table 1). Lower average Cu levels of $6.0 \pm 3.6\%$ have been reported for Dairy farms in Uganda (Long, Marshall, Ndyanabo and Thornton, 1972). There were no significant differences in average Cu level between the different forage types (Fig. 6). It is suggested that all three natural forage types provided adequate amounts of Cu for cattle production. According to Kapu (1975) Native Northern Nigerian Zebus had normal serum Cu status which were above Cu levels of Zebus in Senegal or temperate breeds of cattle.

The average Zn levels in all three forage types were below the A.R.C. (1965) recommendations of 50 ppm, but were within the range of 36 to 52 ppm reported by Oyenuga and Olubajo (1974) for forages grown at Ibadan (tables 1, 2 and 3). There were no significant differences in Zn levels of the three forage types. Both Cu and Zn tended to be more concentrated in the browse species than in the grasses or hays (Fig. 6).

Mineral supplementation containing Nacl, P, K, Ca and Zn is suggested. Cu is unlikely to be deficient and should not be included.

## ACKNOWLEDGEMENTS

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# Mineral Composition of Serum from and *Dermatophilus* Infected Zebu Cattle Under Grazing Conditions in Nigeria

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SUMMARY

**SERUM zinc (Zn), copper (Cu), calcium (Ca), magnesium (Mg) and potassium (K) were determined by atomic absorption spectrophotometry in Zebu cattle with dermatophilosis and in normal cattle under grazing conditions during the rainy season in Northern Nigeria. The mean serum Zn, Ca, Mg and K levels in cattle with dermatophilosis were found to be significantly lower than in normal cattle. The serum Zn, Ca and Mg values in Nigerian Zebu cattle during the rainy season appear to be lower than values found by most other workers for cattle found elsewhere. The converse is true for Cu and K. The differences in serum mineral values between Nigerian Zebu cattle and cattle in other countries may be due, among other reasons, to differences in the nutrient quality of the natural forages upon which grazing cattle subsist.**

## INTRODUCTION

MINERAL elements found in the body of animals are of importance in a number of physiological activities and may be found in either increased or decrease quantities in association with certain diseases (Coles, 1974). Garner (1950) reported that average serum Ca value in osteophagic Zebu cattle was significantly higher, while the average serum Ca value in similar cattle with trypanosomiasis was significantly lower, in comparison with the normal Zebu cattle. Bonomi (1964) suggested that an incidence of infectious pododermatitis in cattle may be related to a low Zn status of animals and Dermertzis and Mills (1973) found oral Zn therapy as an effective agent in the control of the disease in young bulls.

This paper reports findings on the level and variability of five minerals in serum from Zebu cattle with dermatophilosis and from normal cattle.

## MATERIALS AND METHODS

Blood samples of 98 normal Zebu cattle and 43 Zebu cattle with dermatophilosis were obtained from grazing fields during the months of July, August—September and October of 1974. Venous blood was collected in disposable syringes and transferred to deionized glass test tubes. The blood was allowed to clot at room temperature, centrifuged and serum decanted in deionized plastic vials and stored at 4° C prior to analysis. All elements were determined by a Perkin-Elmer Model 290 B atomic absorption spectrophotometer in accordance with the manufacturer's operating manual.

Diagnosis of the disease was based on lessions and demonstration of the presences of *D. conglolensis* (Kelley and Bida, 1970)

## RESULTS AND DISCUSSION

Serum mineral concentrations are presented in Table 1.

TABLE 1

**Serum Minerals (mg/l) in Normal and Dermatophilus infected Zebu cattle in Nigeria**

| | *Zn* | | *Cu* | | *Ca* | | *Mg* | | *K* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *N* | *D* | *N* | *D* | *N* | *D* | *N* | *D* | *N* | *D* |
| Cattle | | | | | | | | | | |
| Maximum | 1.88 | 1.28 | 1.56 | 1.41 | 1.58 | 117 | 39 | 28 | 7.27 | 298 |
| Minimum | 0.72 | 0.45 | 0.67 | 0.77 | .83 | 72 | 17 | 14 | 182 | 1214 |
| Mean | 1.28 | 0.89 | 1.05 | 1.08 | 97.82 | 89.84 | 23.83 | 20.86 | 290.0 | 221.84 |
| Standard Deviation | 0.28 | 0.18 | 0.16 | 0.16 | 11.24 | 10.61 | 4.23 | 2.68 | 89.36 | 38.18 |
| Significance | ** | | NS | | | ** | | ** | | ** |

NS = Non Significant Difference ($P < 0.01$)
N = Normal cattle
D = Cattle with dermatophilosis

Figs 1 and 2 show the mean serum mineral variability. A comparison of the levels of serum minerals obtained in this study with those reported by other workers in Africa and in temperate countries is presented in Table 2.

There was no significant difference ($P > 0.05$) in serum Cu between *Dermatophilus* infected cattle and normal animals. The average Zn, Ca, Mg and K levels in Zebu cattle with dermatophilosis were significantly lower ($P > 0.01$) than in normal cattle (Table 1, Figs 1 and 2).

From Table 2 it is evident that the figures for Cu and K in normal Zebus in this study are higher than reported for cattle in Western Nigeria (Oduye and Fansami, 1971), but lower than that for Zebu cattle in Senegal (Friot and Calvet, 1973) and Uganda (Jones, 1943) or for temperate cattle (Underwood, 1971). Anderson (1933) reported a mean Ca value of 114 mg/l for Zebu cattle under grazing conditions and receiving concentrates at the Shika Research Station in Northern Nigeria. The higher Ca value obtained by Garner (1950) was from similar cattle from the Vom Livestock Improvement Centre and Laboratory cattle.

The mean Mg level is similar to that for English cattle, but lower than those for similar cattle from the Livestock Improvement Centre and Laboratory cattle at Vom and for similar cattle under grazing conditions in Uganda or in Senegal (Table 2).

The mean Zn level was lower than that for Zebu cattle in Senegal (Friot and Calvet 1973) or for temperate cattle (Underwood, 1971). The importance of reduced serum mineral levels in dermatophilosis is yet to be established.

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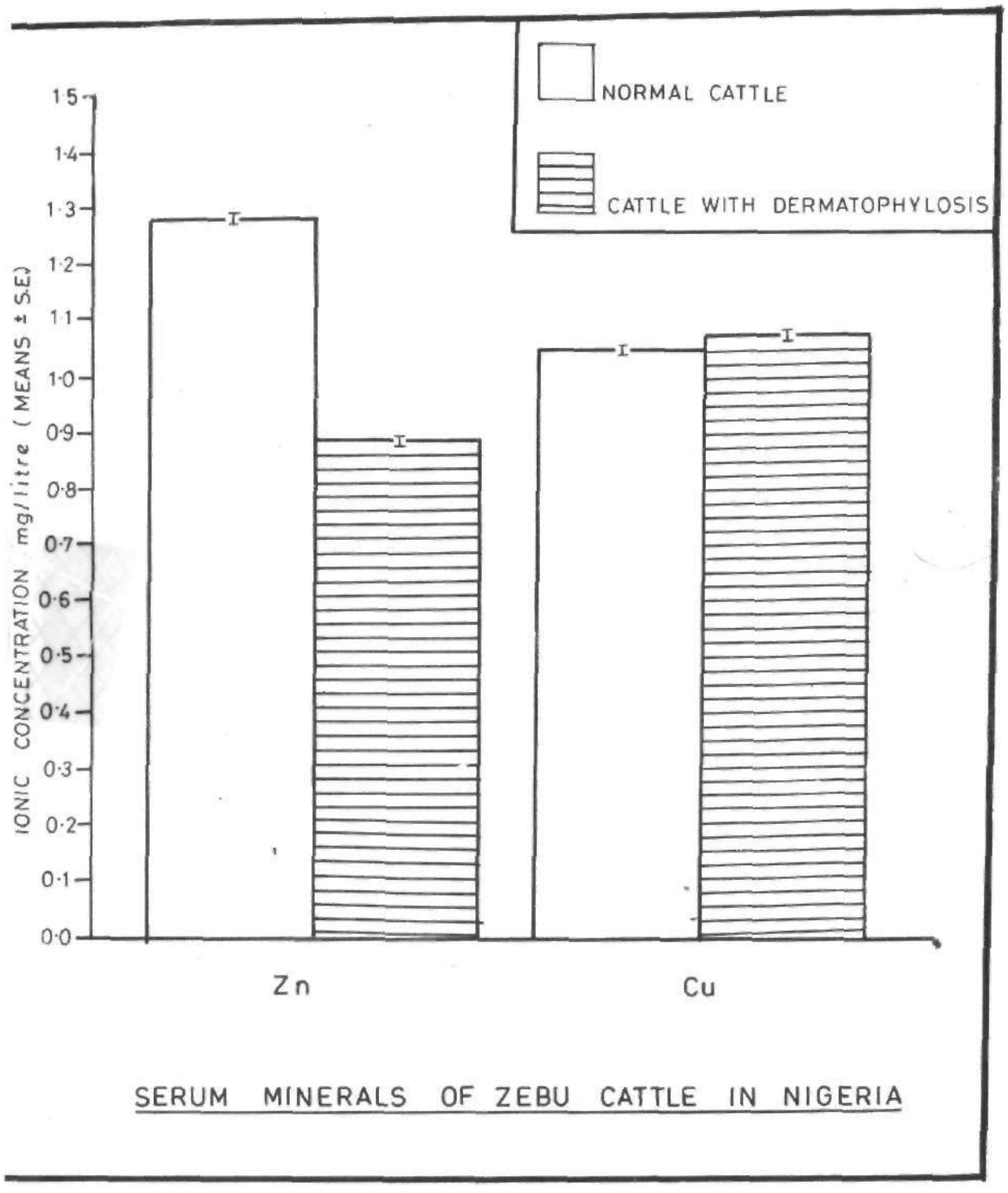

NORMAL CATTLE
CATTLE WITH DERMATOPHYLOSIS
IONIC CONCENTRATION mg/litre ( MEANS ± S.E)
1·5
1·4
1·3
1·2
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0·0
Zn
Cu
SERUM MINERALS OF ZEBU CATTLE IN NIGERIA

Fig. 1

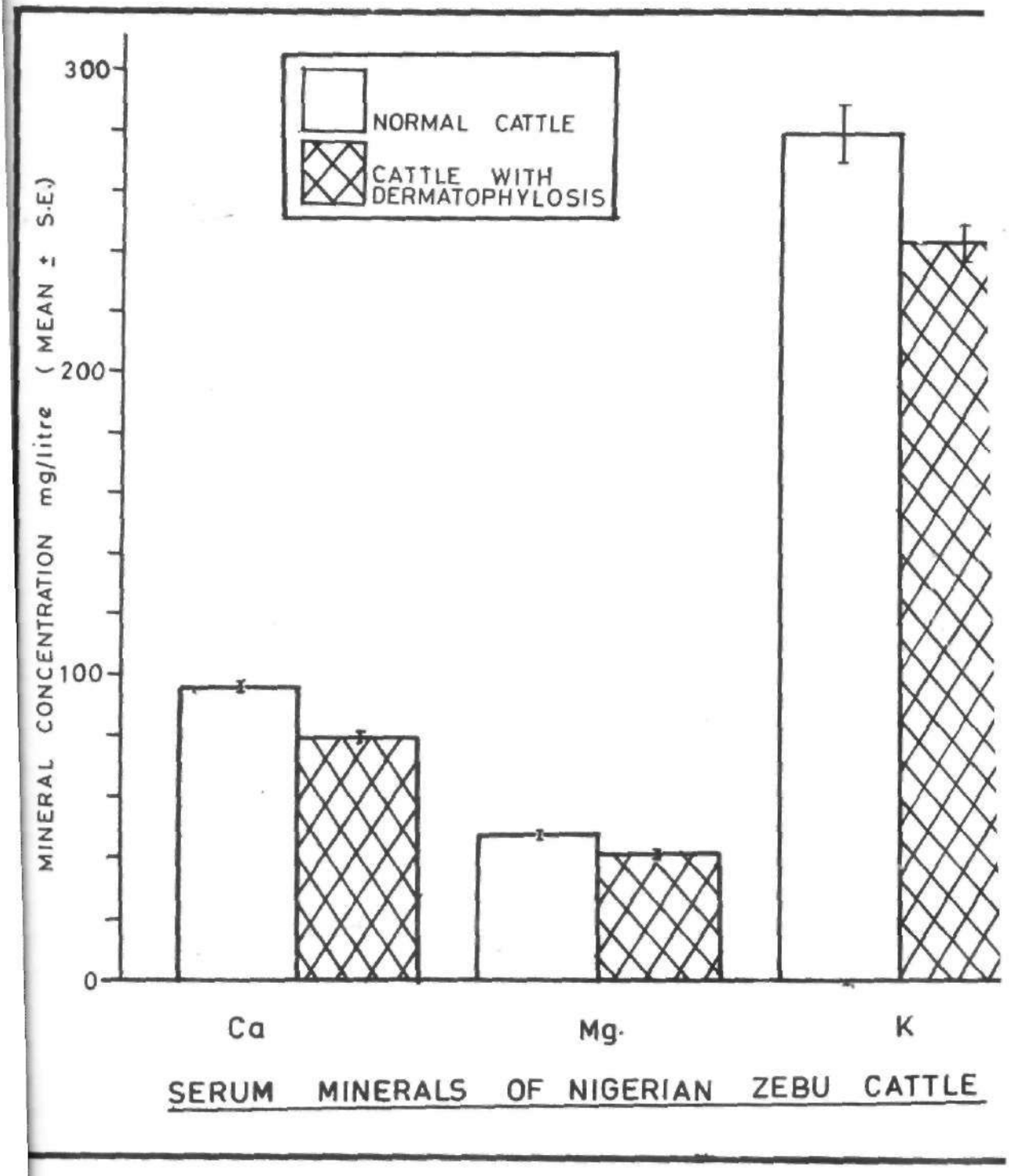

300
200
100
0
MINERAL CONCENTRATION mg/litre ( MEAN ± S.E.)
NORMAL CATTLE
CATTLE WITH DERMATOPHYLOSIS
Ca
Mg.
K
SERUM MINERALS OF NIGERIAN ZEBU CATTLE

Fig. 2

tural Research, Ahmadu Bello University for use of facilities. The helpful advice of D. I. Saror of the Department of Veterinary Pathology, Ahmadu Bello University is sincerely acknowledged.

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TABLE I

Serum Minerals (mg/l) in Normal and Dermatophilus infected Zebu cattle in Nigeria

| Cattle | Zn | | Cu | | Ca | | Mg | | K | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | D | N | D | N | D | N | D | N | D |
| Maximum | 1.88 | 1.28 | 1.56 | 1.41 | 158 | 117 | 39 | 28 | 7.27 | 298 |
| Minimum | 0.72 | 0.45 | 0.67 | 0.77 | 83 | 72 | 17 | 14 | 182 | 121 |
| Mean | 1.28 | 0.89 | 1.05 | 1.08 | 97.82 | 89.84 | 23.83 | 20.86 | 290.0 | 221.84 |
| Standard Deviation | 0.28 | 0.18 | 0.16 | 0.16 | 11.24 | 10.61 | 4.23 | 2.68 | 89.36 | 38.18 |
| Significance | | ** | | NS | | ** | | ** | | ** |

** = Highly significant difference ($P < 0.01$)

NS = Non-significant difference

N = Normal cattle (98 sampled)

D = Cattle with dermatophilosis (43 sampled)

Comparison of Mean Serum Mineral Levels of Zebu cattle in Nigeria, Senegal, Uganda and of temperate cattle.

| Breed of Cattle | No. of Animals | Sex | Age Group | Zinc mg/l | Copper mg/l | Calcium mg/l | Magnesium mg/l | Potassium mg/l | Literature Reference |
|---|---|---|---|---|---|---|---|---|---|
| English Cattle | - | - | - | - | - | 100 | 24 | - | Allcroft (1947) |
| Temperate | - | - | - | 1.50-1.80 | 0.93 | - | - | - | Underwood (1971) |
| Ankole,& Zebu, Uganda | | | | - | - | 115± 1.38 | 28 ± 0.46 | - | Jones (1943) |
| Zebu, Senegal | - | M & F | - | 1.80±0.25 | 0.68±0.02 | 114.1±2.1 | 31.2± 0.6 | 237.6±4.4 | Friot and Calvet (1973) |
| Zebu, Nigeria (Vom) | - | - | - | - | 0.77 | 105±1.07 | 26 ± 0.30 | - | Garner (1950) |
| Zebu, Nigeria (Ibadan) | 147 | M & F | 1 mth - 11 yrs | - | - | 98.1± 15.2 | - | 174.33±33.54 | Oduye and Fasanmi (1971) |
| Zebu, Nigeria (North) | 98 | M & F | Adult | 1.28±0.28 | 1.05±0.16 | 97.86± 11.24 | 23.82± 4.28 | 289.96±89.36 | Table 1 |
| Zebu*, Nigeria (North) | 43 | M & F | Adult | 0.08±0.18 | 1.08±0.16 | 89.84± 10.61 | 20.86± 2.68 | 221.84±38.18 | Table 1 |

* Cattle with dermatophilosis.

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their experiments, if expressed as specific responses and not as *stimulation indices*. I think that the capacity of neonatal lymphocytes to proliferate spontanously as well as in response to allogeneic stimulation has to be considered when their mitosis-inhibiting effect[1 12] is studied, especially when neonatal lymphocytes are cultured together with unrelated cells. It may have an effect on maternal neonatal lymphocyte interactions, too, as the M.L.C. responses of maternal cells to related neonatal cells are in some instances either very weak or absent. Mitosis inhibition may be caused by simple overgrowth or by some kind of crowding effect or factor known to inhibit the proliferation of lymphocytes in vitro[13] and even shown to be produced by crowded lymphocytoblasts.[14] This suggestion does not, of course, exclude the existence of more specific mitosis inhibitor(s) perhaps produced by neonatal lymphocytes.

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## PLASMA-ZINC IN SICKLE-CELL ANÆMIA

SIR,—Plasma or serum zinc concentrations have been reported to be lower on average in sickle-cell trait (Hb AS) and sickle-cell anæmia (Hb SS) than in normal (Hb AA) subjects in Jamaica[15] and New York State.[16] Oral zinc therapy was said to improve the healing of sickle-cell ulcers in Jamaicans with low plasma-zinc.[15]

Rural workers in developing countries may have low plasma-zinc concentrations as the result of poor absorption due to phytates in the diet,[17] excessive loss in sweat,[18] increased excretion in urine associated with acute and chronic diseases including infection,[19] and loss in fæces associated with hookworm or *Schistosoma hæmatobium* infestations.[20-22]

We measured plasma-zinc by atomic-absorption spectrophotometry in 10 élite Nigerians (university senior staff), 38 non-élite Nigerians (farmers who were voluntary blood-donors), 13 adult patients with sickle-cell anæmia (Hb SS), and 10 Europeans (university senior staff) (see accompanying table).

PLASMA-ZINC LEVELS IN NIGERIANS AND EUROPEANS

| | No. of individuals | Plasma-zinc(μg/dl) (mean±S.D. |
|---|---|---|
| Nigerian élite (Hb AA) | 10 | 161·4±52·06 |
| Nigerian non-élite (Hb AA) | 20 | 91.2±28.16 |
| Nigerian non-élite (Hb AS) | 18 | 86.8±13.95 |
| Nigerian sickle-cell (Hb SS) | 13 | 93.6±13.96 |
| Europeans (Hb AA) | 10 | 134.4±32.75 |

Similar plasma-zinc levels were found in Nigerian élite and Europeans, which agrees with the finding that differences due to race are small or non-existent.[23] Non-élite Nigerians had significantly lower mean plasma-zinc concentrations than élite Nigerians and Europeans ($P<0.001$), but none had levels suggesting deficiency; further study would be necessary to ascribe the lower levels to diet, sweat loss, chronic inflammation, or intestinal parasites.[17-22]

No significant difference in mean plasma-zinc was observed between non-élite Nigerians with normal hæmoglobin pattern, sickle-cell trait, or sickle-cell anæmia. However, the only sickle-cell patient with leg ulcers had the lowest observed plasma-zinc value (65 μg/dl).

Nigerians with sickle-cell anæmia may have better zinc status than patients in Jamaica or America, and this may protect them against leg ulcers, which are rarely seen in active sickle-cell clinics in rural West Africa. Alternatively, low plasma-zinc may be the result of chronic ulcers from any cause,[19 20] and not reflect a deficiency. Further study is required of patients with leg ulcers, with or without sickle-cell anæmia.

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## DEVELOPMENTAL MIXOPLOIDY AND TRISOMY-20 SYNDROME

SIR,—Dr Benn and Professor Harnden (March 6, p. 541) responded to our letter (Feb. 21, p. 431) about two patients with trisomy-20 mosaicism by pointing out that cytogenetically abnormal fibroblasts may also be found in early passages of cultures from normal persons. They suggest that such changes may be present in vivo without resulting in phenotypic abnormality, but that they may also arise early in vitro, and that duplicate lines should therefore be studied to guard against attributing the patient's abnormal phenotype to a tissue-culture artefact. This precaution cannot be emphasized too strongly, for we have also observed several chromosomally abnormal lines arising early in fibroblast culture; whenever we observe such a cell line, we routinely repeat the biopsy and culture.

From our two patients, three biopsies with two duplicate cultures each were grown at three different times. This makes it highly unlikely that the repeated finding of F-trisomy mosaicism was a culture-related change. Also, the fact that the two patients have the same rare and highly abnormal phenotype and are the only two who have ever been found to have F-trisomy in this laboratory makes it highly probable that the chromosome abnormality is causally related to their syndrome. Indeed the findings in these patients emphasise the importance of doing chromosome studies on fibroblast cultures in all patients with "idiopathic" multiple congenital-anomaly/mental-retardation syndrome who were previously found to have normal chromosomes in lymphocyte cultures.

The finding of tissue mosaicism in malformed individuals or in apparently normal individuals[1] emphasises that mitotic non-disjunction can occur at any time during prenatal or postnatal life. Although it *may* be insignificant once development is complete, somatic chromosome mutation is suspected of playing an important role in the cause and/or pathogenesis of many cancers (e.g., chronic myelogenous leukæmia). Our two patients with F-trisomy mosaicism again illustrate that developmental mixoploidy is also a well-documented, albeit poorly understood, cause of malformation syndromes. The remarkable distribution of their two cell lines is not unprecedented: we have studied other patients with malformation syndromes who repeatedly demonstrated abnormal chromosomes in one tissue and normal chromosomes in another.[2 3]

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During the second three months patients crossing over from œstrogen to placebo showed a striking increase in flushes to pre-treatment levels. Several patients, including two oöphorectomised women, who had been nearly free of flushes for several years, suddenly developed severe flushes on stopping œstrogen treatment. This would seem to provide some evidence that flushes occur when œstrogen levels are falling, especially if exogenous œstrogen is withdrawn suddenly in patients whose endogenous œstrogen production is lacking. Women left without treatment finally settled at their pre-treatment level of flushes.

Thus the results of this small trial would suggest that œstrogens are effective in the treatment of flushes and that withdrawal of œstrogen causes flushing, but that placebo given de novo is also effective, possibly by stimulating production of endogenous œstrogen. It is difficult to measure the therapeutic value of exogenous œstrogen when the body manufactures its own supply, which varies in response to excitement and sexual stimuli and also shows diurnal changes. I agree with Mulley and Mitchell that œstrogens should not be prescribed routinely for all patients with hot flushes, especially in situations where there are increased risk factors for serious conditions.

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SIR,—The survey of Mulley and Mitchell[1] questioning an œstrogen-gonadotrophin cause for menopausal flushing prompts the following comments. In our clinical experience of about 200 adrenalectomies for the treatment of advanced breast cancer in women post-menopause or post-oophorectomy,[2] hot flushes and sweating identical with the menopausal syndrome occurred in 2-3% of cases. The true figure may have been higher since no specific attempt was made to record the disturbance. None of the women showed overt psychological symptoms assumed to be part of the menopausal complex.

Mulley and Mitchell rightly question the œstrogen-gonadotrophin dogma. Both ovary and adrenal secrete or provide precursors for a number of steroids within the C18 (œstrogen), C19 (androgen), and C21 (progestagen) series. Within each category are many substances without known biological properties of the classical type. Any of these could be related to the odd vasomotor disturbance.

Likewise, the pituitary secretes more hormones than just the gonadotrophins. Prolactin, with vascular effects,[3] is one such compound, and, like the gonadotrophins, it is œstrogen regulated.[4] However, my group has noted that prolactin falls after the menopause and oophorectomy and rises after adrenalectomy.[5] These data confound the idea that prolactin may be a primary mediator for menopausal flushing.

Since deprivation of both ovarian and adrenal steroids can produce the symptoms, one or other of their secretory products or metabolites seems to occupy a primary role, with perhaps secondary effects from pituitary peptides.

It is time for a serious study of the menopause; this, besides giving an insight into breast cancer,[5] could provide information on the relations between the endocrine system and vascular diseases.

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## PLASMA-COPPER IN SICKLE-CELL ANÆMIA

SIR,—Sachs et al.[1] first reported raised serum-copper levels in a patient with sickle-cell anæmia. This observation has been confirmed in patients from the forest area of western Nigeria[2]: the mean serum concentration (115 μg/dl) in those with hæmoglobin (Hb)SC disease was intermediate between the mean in HbSS patients (136 μg/dl) and normals (HbAA or HbAS subjects) (102 μg/dl). Schaeffer et al.[3] described copper-containing granules in sickled erythrocytes which were not present in normal cells.

Raised serum-copper concentrations have been reported in other hæmatological disorders, including thalassæmia, aplastic anæmia, pernicious anæmia, leukæmia, and lymphomas.[4-6]

We have measured plasma-copper by atomic absorption spectrophotometry in patients with sickle-cell anæmia (HbSS), in élite Nigerians (university senior staff), in non-élite Nigerians (Hausa farmers presenting as blood-donors) who were either normal Hb(AA) or had sickle-cell trait (HbAS), and in Caucasians (university senior staff) living in northern Nigeria. The mean plasma-copper concentrations in patients with sickle-cell anæmia were significantly higher than in other groups ($P<0.001$) (see accompanying table). The mean plasma-copper levels in Nigerian élite and non-élite, with or without sickle-cell trait, and in Caucasians did not differ significantly.

PLASMA-COPPER CONCENTRATIONS IN NIGERIANS AND CAUCASIANS

| Subjects studied | | No. of subjects | Plasma-copper (μg/dl) Mean ± S.D. |
|---|---|---|---|
| Nigerian élite | HbAA | 13 | 115·1 ± 29·17 |
| Nigerian non-élite | HbAA | 20 | 108·2 ± 26·95 |
| Nigerian non-élite | HbAS | 18 | 117·7 ± 35·84 |
| Sickle-cell anæmia | HbSS | 17 | 164·4 ± 33·40 |
| Caucasians | HbAA | 10 | 110·8 ± 16·17 |

Our finding of raised plasma-copper concentrations in sickle-cell anæmia confirms the observations of Olatunbosun et al.[2] They suggested that copper, with zinc, calcium, sodium, and potassium, were involved in the maintenance of the structural integrity of the red-cell membrane, and that changes in the serum levels of these metals were an index of irreversible sickling. This explanation does not account for the similarly raised plasma-copper in thalassæmia, aplastic anæmia, and pernicious anæmia, nor does it relate these findings to the essential role which copper is known to play in erythropoiesis.[7]

Copper deficiency in animals leads to changes in many systems of the body, including the development of hypochromic microcytic anæmia. Copper-containing enzymes or co-factors seem to be necessary for the uptake of iron by the intestinal mucosa, the transfer of iron into the blood, and its uptake by normoblasts and other tissue cells; and copper is also required in iron utilisation during hæmosynthesis.[7] The accumulation of copper in red cells and plasma in sickle-cell anæmia could be secondary to disturbed iron metabolism in the mitochondria or normoblasts. The hypercupræmia of other hæmatological disorders is likely to have a similar mechanism.

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