DISEASE: ABNORMALITIES OF COPPER, ZINC AND MAGNESIUM IN BLOOD

Pages with reference to book, From 325 To 333

William W.T. Manser (Department of Biochemistry, the Aga Khan University Medical College, Karachi.)

INTRODUCTION

More than 2000 years ago, the Greeks noted the medicinal properties of elements such as iodine, iron and arsenic. Since the industrial revolution and with this the increasing cxposure of man to metals, there has been increased interest in the biological aspects of metals and the recognition of their toxic states. Since the advent of atomic absorption spectrophotometry and many improvements in the technique over recent years, it has been possible to estimate more than 60 elements in various materials and at least 25 present in body fluids and tissues are known to be essential for health. Fourteen of these are termed trace elements as their concentration is less than 50 mg/kg body weight:

1. Iron
2. Iodine
3. Copper
4. Maganese
5. Zinc
6. Cobalt
7. Molybdenum
8. Selenium
9. Chromium
10. Tin
11. Vanadium
12. Flurine
13. Silicon
14. Nickel

Trace elements take part in bolic pathways, competing with each other for active metabolic sites, thus a change in concentration of one element is expressed by a new balance worked out by the other competing trace elements. Evidence is accumulating that even the toxic element arsenic may have an essential role to play and may therefore be an essential element but no roles have yet been found for lead and aluminium which may therefore still be regarded as truly toxic elements. Amongst other elements, it is now known that even seemingly inert metals such as tungsten and tantalum when inspired as fine alloy dusts, appear in the blood and are ultimately excreted, and are therefore metabolized[1]. Studies have shown that an altered concentration of a trace element can affect different systems. For example, copper deficiency has been associated with some forms of anaemia[2], chromium deficiency with impaired glucose tolerance[3], zinc deficiency with retarded growth[4], and selenium deficiency with the cardiomyopathy, Keshan disease[5], to mention but a few. The possibility that a trace element abnormality can produce psychiatric illness has been considered in several studies[6]. The first real breakthrough providing evidence for the therapeutic potential of trace elements in neuropsychiatry came in 1948 with the use of lithium in the treatment of manic depressive psychosis[7]. This review is centered around the essential elements, copper, zinc and magnesium only, as the medical aspects of these metals have not been reviewed in any Pakistani journal.

COPPER

Copper levels in tissues and body fluids depend on diet, state of health, sex and age. It is a component of at least 16 mammalian metalloproteins, many of which are central to haematopoiesis, bone and connective tissue physiology, and parts of the nervous system. Metallothioneins normally prevent excessive copper absorption in C .I.T., but that which is absorbed, is complexed with albumin for transportation to the liver, where it is incorporated into ceruloplasmin and enters into the plasma pool. Excretion is mainly in the bile and storage is in the liver, complexed with metallothioneins. In serum, about 93% is bound to ceruloplasmin, and the rest to albumin and aminoacids, the main one being histidine[8]. Ceruloplasmin is also an acute phase protein and hence factors which affect ceruloplasmin levels often alter copper levels[9]. There is no correlation between copper intake and either plasma, copper or ceruloplasmin levels, the correlation coefficient between copper balance and plasma copper being very poor[10], at r = -0.26. Hence plasma copper levels do not necessarily reflect the copper status of the subject. Intestinal absorption of copper is antagonised by zinc[11] and symptoms of copper deficiency (hypocbromatic — microcytic anaemia, leukopenia, and neutropenia) have been induced by a high zinc intake[12]. However, we obtained the following correlation coefficients for normal adults, males r = -0.247, females r = -0.095[13], reflecting a poor correlation for Zn V Cu for normal Pakistani males and none for females.

Toxicity and Excess

Copper toxicity is rare, resulting in nausea, vomiting, haemolysis, hepatic necrosis, oliguria, azotaemia, convulsions and coma. Raised copper levels in RBC's and whole blood have resulted from haemodialysis together with the following manifestations: pancreatitis (with raised serum amylase levels), myoglobinaemia, G.I. problems, leukocytosis, metabolic acidosis and haemolysis[14], Elevated serum copper levels have been found in some malignancies like ovarian carcinoma. Levels fell on successful treatment but rose again on relapse[15]. Wilson's disease[16], is probably the best known copper related disorder. Laboratory findings include reduced serum ceruloplasmin, an increase in non-ceruloplasmin copper levels, reduced total serum copper and increased urinary copper excretion. Copper deposits in various organs and the main presenting features include chronic liver disease, osteoarthropathy and possible renal calculi, and Kayser-Fleiseher rings in the iris[17]. Neurological symptoms include a deterioration in co-ordination of voluntary movements, involuntary movements, disorders of tone and posture, a loss of intellect, disturbed behaviour, progressive brain degeneration, multiple sclerosis and Parkinsonism , [17] as well as a schizophrenia-like picture with delusions, hallucinations and flattening of effect[6]. Penicillamine has been used for many years in the treatment of this disease[18]. Copper is removed from the organs by complex formation and then excreted. Zinc therapy, to reduce the absorption of copper from the diet, has been reported with great success[19]. Hepatie copper accumulation occurs in Indian childhood cirrhosis which is endemic in India and usually fatal. It resembles Wilson's disease except that it occurs much earlier in life[20]. Whether the cause is dietary or metabolic, it is not yet known. Causes of fatal copper poisoning from copper contaminated drinking water, with similar symptoms, have recently been reported from Germany[21]. Hepatic copper accumulation together with raised serum levels have been found in cholestatic liver disorders, primary biliary cirrhosis, in sclerosing cholangitis[22] and in alcoholic liver cirrhosis[23]. However , other researchers have found hypereupruria and hypocupraemia in the last of these[24]. Pyridoxal is a cofactor for the enzymatic decarboxylation of aminoacids to neurotransmitter amines. Impairment in the decarboxylation of glutamate (exeitory) to GABA (inhibitory) might be expected to lead to an excess of excitory response to sensory stimuli. Actually, raised levels of pyridoxal phosphate, together with

copper and zinc in the brain lead to hyperexcitability probably because other neurotransmitter systems are involved. Copper is also a cofaetor for hydroxylases involved in the biosynthesis of the neurotransmitters dopamine and noradrenaline and for amine oxidases involved in the catabolism of neurotransmitters[25] Copper accumulation has been found in sonic chizophrenics[6].

Deficiency

Classical symptoms of copper deficiency are neutropenia, leukopenia, bone demineralisation and impaired immune functions and neurologically may be associated with multiple sclerosis, refractory depression, demyelinatión and some schizophrenias[7,25] There are also many other associations as outlined below. Klevay hypothesised in 1975 that coronary heart disease (C.H.D.) is predominantly a disease of zinc and copper metabolic imbalance[26] and that is such disease, the ratio of the levels of zinc to copper in serum or plasma should be greater than normal. This was derived in part from his earlier investigations that the average diet in the West had a higher Zn: Cu ratio and that there was a higher incidence of C.H.D. than in developing countries. Klevay et al[27], found a correlation (r = 0.854) between plasma cholesterol levels and copper depletion. Other researchers have found that a diet low in copper gave a decrease in HDL- cholesterol and an increase in LDL-eholesterol levels in plasma which were reversed on copper supplementation[28]. Others have found no relationship between levels of copper or zinc in plasma and lipids or lipoproteins in serum[29], and nothing conclusive for humans between impaired cardiac functions and low copper and/or excess zinc eonsumptions, although it was so for rats[30]. In fact, after acute myocardial infarction raised serum copper levels have been found during the first four which then returned to normal[31] but increased plasma zinc: copper is known in cerebral artherosclerosis[32], Menkes disease[32], is related to copper deficiency. Clinical symptoms[33] include abnormal hair, hypopigmentation[34], bone changes and arterial changes, together with progressive cerebral degeneration and death between the ages of 6 months and 3 years. Hypercholesterolaemia does not occur[35]. Impaired glucose tolerance has been found in rats due to low copper and/or excess zinc consumption[29]. Conversely, humans given fructose as 20% of their diet developed copper deficiency and cardiac disrhythmias[36]. Copper deficiency may impair the normal formation of elastin in the arterial wall as cross linking and maturation of connective tissue is dependent on lysyl oxidase which requires the metal cofactor[37]. In infants, failure to thrive, altered iron metabolism, anaemias, defective clastin and collagcn, skeletal changes, scurvy, cerebral and myocardial defects, altered carbohydrate or lipid metabolism and pancreatic function, defective synthesis of polypeptide hormones or of coagulation factor V, may be due to copper deficiency. These are hazards particularly for preterm low birth weight babies as symptoms do not become apparent until after about three months of age when maternally derived hepatic reserves of copper arc depleted[38]. Care must be taken not to cause copper toxicity in such babies receiving intravenous nutrition[39]. Cases of suspected child abuse have arisen which were actually cases of copper deficiency resulting in bone fractures. The syndrome of copper deficiency in infants includes psychomotor retardation, hypotonia, hypopigmentation, prominent scalp veins in palpable pcriosteal depressions, osteoporosis, bone fractures, abnormal bone formations, pallor, sideroblastic anaemia resistant to iron therapy, hepatosplenomegaly, neutropenia and low copper and ceruloplasmin levels[40] Superoxide dismutase has copper and zinc cofactors and is important in preventing oxygen toxicity. This toxicity has been implicated in bronchopulmonary dysplasia in premature infants possibly resulting from copper deficiency[41].

ZINC

Zinc is part of over 80 metalloenzymes[42] found in all metabolic pathways. Zinc levels in body fluids and tissues vary with age, sex and nutritional states.f It is required for DNA, RNA and protein synthesis, cell mediated immunity, reproduction, and development of the epidermis and CNS.There is no store as such in the body, all zinc being locked up in proteins and in the skeleton, much of it being unusable although some can be released on catabolism of these parts. Sixty five percent of the zinc is transported by albumin and the rest by a2-macroglobulin and transferrin. In the liver, kidney and intestine it is bound to metallothioneins. Malabsorption occurs in G.I.T. problems and in pancreatic insufficiency and stress; inflammation and infection lower plasma levels owing to increased cell demand. Most of the blood zinc is present in erythrocytes and that in plasma represents about 1% of body zinc. Zinc deficiency is often accompanied by normal serum levels hence a serum level is not a reliable index of zinc status. Also it varies with time of day and often falls after a meal[43], but the situation may be improved by sampling at approximately the same time of day. Levels in erythrocytes, leucoeytes, hair, nails and tissues have been used to assess zinc status more accurately but are more difficult to do. To add to the validity of serum level estimations, suggestions have been made to supplement the estimation with those of zinc dependent enzymes such as serum alkaline phosphatase or red cell carbonic anhydrase[44], the zinc taste test[45] or the copper-zinc ratio[26]. The only reliable method of detecting zinc deficiency is to see if the symptoms are alleviated after zinc supplementation. Correlation coefficients between zinc and copper have already been mentioned and that between zinc and calcium in plasma or serum has been found by various authors to be 0.167[46], 054[47] and also 0.334 for males and 0.187 for females[48]. A correlation coefficient of about 0.2 has been found between zinc and albumin[48].

Deficiency

Zinc deficiency has been associated with dementia [possibly linked to the importance of zinc in RNA synthesis and the link of the later with memory[7]], depression thought disorders and schizophrenia . It has also been linked with senile dementia[49] but this has been disputed[50]. In senile dementia, there is evidence for a primary degeneration of the noradrenergic system and loss of nerve cells in the locus ceruleus6. Clinical symptoms of zinc deficiency are similar to those of acrodermatitis enteropathica, itself due to a congenital often fatal inability to absorb zinc from the diet: watery diarrhoea, seborrheié skin lesions acrally and around body orifices, alopecia, immunodefieiency and impaired healing. Other manifestations and associations are dealt with below. Zinc deficiency and the response to zinc supplementation was investigated in Egypt[51] and then in Iran[52], in causing growth retardation, absent sexual development, iron deficiency anaemia, geophagia, rough skin, hyperpigmentation, span nails and open epiphyses. The deficiency was due to phytate in cereals, which in a diet lacking in animal protein, forms as insoluble salt with zinc. Infectious diseases (due to the flux of zinc from the plasma to the liver), chronic sepsis, chronic active liver disease, uraemia, pregnancy, malabsorption and nephrotic syndrome were found to be associated with zinc deficiency[53], and with parasitic infestation such as hookworm[54], and schistosomiasis, hookworm and excessive sweating[55]. Complexes of zinc and insulin arc stored in the pancreatic B- cells[56] and variations of the normal zinc: insulin ratio alter the antigenic properties of the latter[57]. But in an investigation, it wasfound that[58] out of 20 Type II diabetics hadzinc deficiency due to zinc malabsorption and excessive zinc excretion, as well as due to the byperglycaemia itselP. Diabetes produces altered immune function typical of zinc deficiency[59]. Levels of zinc in serum are low and urinary excretion high in liver cirrhosis[24,60] In alcoholism, serum zinc levels are also low and alcohol appears tobe responsible for the lowserum zinc levels in the cirrhosis itself[23] possibly due to associated undernutrition[6]. The zinc deficiency would partly inactivate superoxidc dismutase, for which it and copper are cofactors, thereby opening the hepatocytes to oxidative -damage[61]. When acute, the Sc deficiency manifests itself as anorexia, dysfunction of smell

and taste[60], and neuropsychiatric complications such as Korsakoff's syndrome and alcohol dementia[6], and when chronic, as growth retardation, anaemia, testicular atrophy and impaired wound healing[61]. Low serum zinc levels have been found in various types of heart and pulmonary disease, as well as in maliganancy hypertension, arthritis, ulcers and psychoses . in myocardial infarction, serum zinc levels were found to be low for the first two days after infarction, but were normal after about two weeks[31]. No correlation was found between plasma cholesterol and zinc levels in normals[10] althous zinc does lower HDLcholesterol levels[6] One result of zinc deficiency is the diminution of the senses of smell and tasteM. Zinc sulphate solution is used to semi- quantify the latter[45]. Taste acuity towards sodium chloride and sucrose decreases with age as does zinc tolerance[65]. Anorexia nervosa patients tend to have low serum zinc levels as was found in 7 of 14 patients in one study[66]. As in liver cirrhosis, loss of zinc in nephrotic syndrome and renal insufficiency can lead to the manifestations of chronic zinc deficiency[60]. Some reports[67] state that plasma levels increase after haemodialysis but decrease on haemofiltration in chronic renal failure, but the same authors earlier[68] stated that plasma zinc levels decrease and copper levels increase on dialysis. Nickel and zinc supplements are required where necessary to control anaemia and improve nutrition. Hormones influence trace element metabolism including excretion and transport[69]. For example, byperthyroidism results in a high excretion of zinc resulting in low erythrocyte levels, with high plasma and RBC copper levels. Hypothyroidism results in high RBC zinc levels[70]. These authors[70] found no difference in plasma zinc levels or in urinary copper excretions but others[71] found normal plasma zinc levels in byper-, but lower plasma Sc and excretion of zinc for hypo-thyroidism. Serum zinc levels in pregnancy are lower[72] than in non-pregnancy falling after 35 weeks[73], the cord tissue level rising at this time and then remaining constant[74]. There is a positive correlation between this and birth weight[74] and cord blood levels are 25% higher than maternal[72] ($r = 0.45$). Iron therapy in pregnancy tends to decrease plasma zinc levels[75] and older pregnant smokers have lower birth weight babies. This is because of cadmium accumulation which crosses the placenta to the exclusion of some of the zinc[76]. Mothers of low birth weight babies have lower serum zinc levels. Low maternal plasma zinc levels are associated with pregnancy complications and abnormal labour[77] for which zinc supplements are of no help[78]. Anencephaly and other CNS disorders can be caused and neuronal development can be permanently disturbed byvarious factors including maternal malnutrition and zinc deficiency during pregnancy[25,79]. Infant malnutrition, especially zinc deficiency can have irreversible effects on behaviour . It was found that dyslexic children, had, in sweat, 67% of the normal zinc level, and high levels in sweat and hair of lead, cadmium and copper. The parents had low zinc levels. It was concluded that zinc should be supplemented in parents diets before conception and through pregnancy and lactation, especially if vegetarian[80]. It is interesting to note that conditions associated with zinc deficiency such as pregnancy, postoperative states, alcoholism, liver failure, porphyria and steroid medication are all potential situations where a schizophrenic-like manifestation can occur[6].

Zinc Excess

Zinc toxicity is rare and may produce fever and GLT. distress. As discussed under copper toxicity and excess, in many hyperexcited states high levels of copper, zinc and pyridoxaiphosphate are found in the brain[25].

MAGNESIUM

Magnesium is a cofactor for about 300 cellular enzymes and has an important role in energy metabolism, participating in phosphate-transfer reactions involving ATP and nucleotide triphosphatases

and for the functional integrity of the nervous system. About 60% of body magnesium is in the bone, of which that in the bone surface is the main reservoir, 20% is in skeletal muscle and 20% in the organs. Less than 1% is in blood of which about one-third is in plasma. In plasma 25% is bound to albumin, 8% to the globulins, 12% is complexed to anions and 55% is free. Although the physiological role is primarily intracellular, most experimental data is from cxtracellular sources, mainly blood, which gives a poor guide only to the magnesium status of the subject. Estimations in leukocytes, erythrocytes or in muscle, although providing useful information are not without their drawbacks[81]. A series of ten tests has been devised to assess magnesium status[82] but the simplest procedure is to estimate the serum level and the excretion rate. If the excretion rate is normal, the magnesium status is normal; if the excretion is low and the serum level is normal, the Magnesium Load Test should be applied to see if there is magnesium deficiency. If both are low there is deficiency but a low serum level with a high excretory rate, signifies renal magnesium loss[83]. A correlation exists between serum levels of magnesium and calcium, and a coefficient of 0.40 has been reported[84].

Magnesium deficiency

Magnesium deficiency is very common: far more common than most people realise. It can even be life-threatening. In a survey of 37,000 people in U.s., 75% had a magnesium intake of less than the recommended daily allowance[85]. This is often due to food processing: oriental diets are rich in magnesium[86]. Clinical symptoms are memory and concentration loss, apathy, confusion, hallucinations, paranoia, neuromuscular problems (e.g tetany and infantile seizures), personality changes, and overt psychosis[7,87]. Other associations are discussed below. Although hypermagnesaemia has been found to cause decreased glucose tolerance[88], magnesium deficiency has been associated with insulin resistance[89], insulin dependence in diabetic children. [90] and a case of focal seizures in diabetes[91]. Correlation coefficients between serum magnesium levels and glycosylated haemoglobin levels or 24 hr. glycosuria were -0.358 and -0.296 respectively. Better control over the diabetes led to improved magnesium levels[90]. Low serum magnesium levels have been found in hypertension[92]. Hypertension improved on giving magnesium supplements to those on long term diuretics, according to one research group[93], but another group found the changes not significant[94]. The results of stress are made worse in cases of magnesium deficiency but supplements aid recovery and even help those who are not magnesium deficient[95]. Magnesium deficiency blocks the response of the parathyroid to hypocalcaemia96 and causes resistance to parathormone[96,97] and to vitamin D[97]. Magnesium is required for the hepatic 25-hydroxylation of vitamin D and other steps in calcium homeostasis[98]. Hypomagnesaemia often accompanies hypocalcaemia[98]. Dietary treatment of phenylketonuria has been known to cause vitamin D resistant rickets due to magnesium deficiency[99]. Magnesium inhibits the uptake of calcium by smooth muscle cells[100] and the formation of calcium oxalate stones[101]. It has been reported that hyperthyroidism in humans is accompanied by low plasma and erythrocyte levels of magnesium. A lower excretion of magnesium in hypothyroidism than in hyperthyroidism[71] occurs but magnesium deficiencyis responsible for low serum levels of T4 but not T3 in rats[102]. As in normals, a correlation exists between serum calcium and magnesium levels in postoperative thyroidectomy cases but not preoperative[84]. Low serum magnesium levels have been found in liver cirrhosis . Chronic alcoholism often results in magnesium depletion, which can have serious consequences in the withdrawal reactions and often leads to death in delerium tremens[103] In rats magnesium deficiency has been associated with the coronary factors: raised serum levels of triglycerides, cholesterol VLDL-cholesterol, LDL-cholesterol and low levels of cholesterol esters and HDL-cholesterol[104]. In humans low serum magnesium levels have been found to be associated with acute myocardial infarction and with ventricular arrhythmias[105]. However, others have found an association with myocardial infarction

but not with arrhythmias[106] or have found no difference in magnesium levels with myocardial infarction[107]. In another group, only 6% of myocardial infarct cases had a low serum magnesium but of 13 patients with a low serum magnesium and who had a myocardial infarction, 10 had ventricular arrhythmias and 8 had low serum potassium levels also[108]. Reductions in the intracellular magnesium content of infarcted areas as well as of non-infarcted areas have been found[109] and these reductions, as well as of serum magnesium, were secondary to changed hormonal secretions, e.g. catecholamines, after infarction or after cardiac surgery also[110]. It has been reported that low serum magnesium levels in an otherwise healthy individual predisposes him/her to myocardial infarction[111]. The incidence of ischemic heart disease and myocardial infarction is inversely correlated with the hardness of the drinking water, its magnesium content, and to the magnesium content of the soil. This has been found, for example, indifferent areas of Finland. Possibly, a high magnesium intake counteracts the detrimental effect of a high salt and calcium intake and it has been suggested that magnesium supplements should be given, where necessary, to prevent cardiovascular disease[112].

Magnesium Excess

Magnesium toxicity is uncommon, renal insufficiency being the most usual cause, and may result in hypercalcaemia, general depression of the nervous system, retardation of the cardiac conduction system, loss of deep tendon reflexes, respiratory paralysis, general anaesthaesia, cardiac arrest or death. Hypermagnaesaemia may cause decreased glucose tolerance88. Magnesium sulphate enemas, which are often given to patients in hepatic coma, have been known to cause hypercalcaemia and fatal magnesium toxicity through bowel absorption where there has been the tendency towards renal failure[113]. Hypermagnesaemia has been found in unspecified schizophrenias[114-115] and in drug treated chronic schizophrenia[115] but low levels were found in acute schizophrenia[116]. However, other researchers could find no difference between normals and schizophrenics[117].

REFERENCES

1. Sabbioni, B. Biomedical trace metal research at the Joint Research Centre, Ispra, Italy. Metabolism of minerals and trace elements in human diseases, international symposium: Aligarh/New Delhi/Srinagar, 1987.
2. Dunlop, W.M., James, G.W. and Flume, D.M. Anemia and neutropenia caused by copper deficiency. Ann. Intern. Med., 1974; 80 :470.
3. Mertz,W. Chromium occurrence and function in biological systems. Physlol. Rev., 1969; 49: 163.
4. Hambridge, K.M., Chavez, M.N., Brown, R.M. and Walravens, P.A. Zinc nutritional status of young middle-income children and effects of consuming zinc-fortified breakfast cereals. Am. J. Clin. Nutr., 1979; 32: 2532.
5. Chen, X., Yang, G., Chen, J., et al. Studies on the relations of selenium and Keshan disease. Biol. Trace Elenient Res., 1980; 2 : 91.
6. Srinivasan, D.P. Trace elements in psychiatric illness. Br. J. Hosp. Med., 1984; 32: 77.
7. Linter, C.M. Neuropsychiatriic aspects of trace elements. Br. J. Hosp. Med., 1985; 34 : 361.
8. Neumann, N.Z. and Sass-Kortsak, A. The state of copper in human serum; evidence for an amino acid-bound fraction. J. Clin. Invest, 1967; 46 : 646.
9. Klevay, L.M., Rech, S.J., Jacob, R.A., Logan, G.M. Jr., Munoz, J.M. and Sandstead, H.H. The human require.ment for copper, I. Healthy men fed conventional, American diets. Ani. J. Clin. Nutr., 1980; 33:45.
10. Festa, M.D., Anderson, H.L., Dowdy, R.P. and Eller sieck, MR. Effect of zinc intake on copper excretion and retention in men. Am. J. Clin. Nutr., 1985; 41 : 285.
11. Hoffman, H.N., Phyliky, RL. and Fleming, C.R. Zinc-induced copper deficiency. Gastroenterology,

1988; 94:508.
12. Manser, W.T., and M. Altaf Khan. Trace element studies on Karachi populations, Part I. Normal ranges for blood copper, zinc and magnesium for adults. JPMA., 1989;39:43.
13. Klein, WJ. Jr., Metz, E.N. and Price, A.R. Acute copper intoxication; a hazard of hemodialysis. Arch. Intern. Med., 1972; 129: 578.
14. Margalioth, EJ., Udassin, R., Maor, J. and Schenker, J.G. Serum copper levels in ovarian carcinoma. Cancer, 1985; 56: 856-859.
15. Wilson, S.A.K. Progressive lenticular degeneration: a familial nervous system disease associated with cirrhosis of the liver. Brain, 1912; 34 : 295.
16. Danks, D.M. Inborn errors of trace element metabolism. Clin. Endocrinol. Metab., 1985; 14 : 591.
17. Walshe, J.M. Current therapeutics 192. Penicillamine Practitioner, 1963; 191 : 789.
18. Hoogenraad, T.U. and van den Hamer, C.J.A. 3 years of continuous oral zinc therapy in 4 patients with Wilson's disease. Acta Neurol. Scand., 1983; 67: 356.
19. Mehrotra, R., Pandey, P.K. and Nath, P. Hepatic copper in Indian childhood cirrhosis. Histopathology, 1981; 5:659.
20. Muller-Hocker,J., Meyer, U., Wiebecke, B., Hubner, G., Eife, R., Keliner, M. and Schramcl, P. Copper storage disease of the liver and chronic dietaiy copper intoxication in two further German infants mimicking Indian childhood cirrhosis. Pathol. Res. Pract., 1988; 183: 39.
21. Ritland, S., Steinnes, E. and Skrede, S. Hepatic copper content, urinary copper excretion and serum ceruloplas min in liver disease. Scand. J. Gastroenterol., 1977; 12:81.
22. Zarski, J.P., Arnaud, J., Dumolard, L., Favier, A. and Rachsul, M. Oligo-elements (Zn, Cu, Mn) dans la cir rhose alcoolique: influence de I' alcoolisme chronique. Gas*roenterol. Clin. BioL, 1985; 9: 664.
23. Rodriguez, M.C., Henriquez, MS., Turon, A.F., de M. Novoa, F.J., Diaz, J.G. and Leon, P.B. Trace elements in chronic alcoholism. Trace Elements In Medicine, 1986;3:164-167.
24. Bryce-Smith, D., John Jayes Lecture: Environmental Chemical influences on behaviour and mentation. Chern. Soc. Rev., 1986; 15 : 93.
25. Klevay, L.M. Coronary heart disease; the zinc copper hypothesis. Am. J. Clin. Nutr., 1975; 28: 764.
26. Klevay, L.M. Association between the amount of fat and the ratio of zinc to copper in 71 foods; inferences about the epidemiology of coronary heart disease. Nutr. Rep. Int., 1974; 9: 393.
27. Klevay, L.M., Inman. L., Johnson, L.K. et al. Effects of a diet low in copper on a healthy man. Ctin. Res., 1980; 28 758 A.
28. Tiber, A.M., Sakhaii, M., Joffe, C.D. and Ratnaparkhi, M.V. Relative value of plasma copper, zinc, lipids and lipoproteins as markers for coronary heart disease. Atherosclerosis, 1986; 62.: 105.
29. Madeinos, D.M. The copper; zinc hypothesis and cardiovascular disease. Biochem. Arch., 1985; 1: 67-73 and references contained therein.
30. Dumolard, L., Arnaud, J., Veyrat, B. and Favier, A. Oligoelements serques et infarctus du myocarde. Ann. Biol. Clin. (Paris), 1986; 44: 217.
31. Bustamente, 3., Martin Mateo, M.C., Fernandez, J., de Quiroz, B. and Manchado, 0.0. Zinc, copper and ceruloplasmin in arteriosclerosis. Biomedicine, 1976; 25:244.
32. Menkez, 3.11., Alter, M., Steigleder, O.K., Weakley, D.R. and Sung, J.H. A sex-linked recessive disorder with retardation of growth, peculiar hair, and focal cerebral and cerebellar degeneration. Paediatrics, 1962; 29: 764.
33. Danks, D.M., Campbell, P.E., Stevens, Di., Mayne, V. and Cartwright, B. Menke's kinky hair syndrome; an inherited defect in copper absorption with widespread effects. Paediatrics, 1972; 50:188.
34. Blackett, P.R., Lee, L).M., Donaldson, D.L., Fesmire, J.D., Chan, W.Y., Ilolcombe, J.Ji. and Rennert, 0.M. Studies of lipids, lipoproteins, and apolipoproteins in Menke's disease. Petliatr. Res., 1984; 18: 864.
35. Reiser, S., Smith, J.C. Jr., Mertz, W., Holbrook, J.T., Scholfield, Di., Powell, A.S., Conficld,W.K.

and Canary, J.J. Indices of copper status in humans consuming a typical American diet containing either fructose or starch. Am. J. Clin. Nutr., 1985; 42: 242.
36. Allen, K.G.D. and Klevay, L.M. Hyperlipoproteinaemia in rats due to copper deficiency. Nntr. Rep. Int., 1980; 22 :295.
37. Copper and the infant. Lancet, 1987; 1: 900.
38. Soo, T.L., Simmer, K., Carlson, L. and McDonald, L. Copper and very low birthweight babies. Arch. Dis. Child., 1988; 63: 79.
39. Shaw, J.C. Copper deficiency and non-accidental injury. Arch. Dis. Child., 1988; 63 : 448.
40. Autor, A.P., Frank, L. and Roberts, R.J. Developmental characteristics of pulmonary superoxide dismutase; relationship to idiopathic respiratory distress syndrome. Pediatr. Res., 1976; 10: 154.
41. Golden, M.H. Trace elements in human nutrition. hum. Nutr. Clin. Nntr., 1982; 36: 185.
42. Solomons, N.W. On the assessment of zinc and copper nutriture in man. Am. J. Clin. Nutr., 1979; 32 : 856.
43. Danks, D.M. Diagnosis of trace metal deficiency with emphasis on copper and zinc. Am. J. Clin. Nutr., 1981; 34 : 278.
44. Bryce-Smith, D. and Simpsom, R.I.D. Anorexia, depression, and zinc deficiency. Lancet., 1984; 2: 1162.
45. Markowitz, M.E., Roscn,J.F. and Mizruchi, M. Circadian variations in serum zinc (Zn) concentrations; Correlation with blood ionised calcium, serum total calcium and phosphate in humans. Am. J. Clin. Nutr., 1985; 41: 689.
46. Spcich, M. Correlation between calcium and zinc in plasma. Clin. Chem., 1986; 32: 1427.
47. Abu-Farsakh, F.A.,Thajeel, AU., Al-Khalily, AS., Itani, S.M. and Al-Awqati, M.A. Sex-related correlation between zinc and calcium in serum. Clin. Chem., 1988; 34:467.
48. Hullin, R.P. Serum zinc in psychiatric patients, in zinc deficiency in human subjects: progress in clinical biology. cal research. New York, Liss, 1983, p.129.
49. Kcnn, C. and Gibb, E. The role of zinc in senile dementia. Br. J. Psychiatry, 1986: 149 : 221.
50. Sandstead, H.H., Prasad, AS., Schultcr, A.R., Farid, Z., Miale, A. Jr., Bassilly, S. and Darby, W.J. Human zinc deficiency, endocrine manifestations and response to treatment. Am. J. Clin. Nutr., 1967; 20 : 422.
51. Halstead, J.A., Ronaghy, F.A., Abadi, P., Hughshcnass, M.,Anishakemi, G.H., Barakat, R.M. and Reinhold,J.G. Zinc deficiency in man; the Shiraz Experiment. Am. J. Med., 1972; 53 : 277.
52. McCance, R.A. and Widdowson, E.M. The absorption and excretion of zinc. Biochem. J., 1942; 36: 692.
53. Lemann, I. A study of the type of infantilism in hookworm disease. Arch. Intern. Med. (Chicago), 1910; 6:139.
54. Prasad, A.S., Schulert, A.R., Sandstead, H.H., Miale, A. Jr. and Farid, Z. Zinc, iron, and nitrogen content of sweat in normal and deficient subjects. J. Lab. Clin. Met, 1963; 62:84.
55. Scott, D.A., Fisher, AM. The insulin and zinc content of normal and diabetic pancreas. 3. Clin. Invest., 1938; 17:725.
56. Arquila, E., Thieve, P., Bugman, T., Ruess, W. and Suuijama, R. Effects of zinc on the conformation of antigic determinants of insulin. Biochem. 3., 1978; 175:289.
57. Kinlaw, W.B., Levine, A.S., Morclcy,J.E., Silvis, S.E. and MeClain, C.J. Abnormal zinc metabolism in Type II diabetes mellitus. Am. J. Med., 1983; 75 : 273.
58. Fovenyi, J., Totpal, K., Thaisz, E. and Garam, T. Nonspecific cellular immunity in type I and type II diabetes. Exp. Clin. Endocrinol., 1984; 83: 203.
59. Linderman, R.D., Baxter, Di., Yunice, A.A. and Kraikitpanitch, S. Serum concentrations and urinary excretions of zinc in cirrhosis ncphrotic syndrome and renal insufficiency. Am. J. Med. Sei., 1978; 275: 17.
60. Ribarov, S.R. and Boehev, P.O. A ehcmiluminescent method for measurement of activated oxygen

forms in biological fluids and homogenates. 3. Biochem. Blophys. Methods, 1983; 8: 205.
61. Sullivan, J.E., Blotcky, A.J., Jetton, M.M., Hahn, H.K.J. and Bureh, R.E. Serum levels of selenium, calcium, copper, magnesium, manganese and zinc in various human diseases. J. Nutr., 1979; 109: 1432.
62. Hooper, P.L., Viscanti, L., Garry, Pi. and Johnson, G.E. Zinc lowers high-density lipoprotien cholesterol levels. JAMA., 1980; 244: 1960.
63. Henkin, R.L., Schechter, P.J., Friedewald, W.T., de Mets, D.L. and Raff, M.S. A double blind study of the effects of zinc sulphate on taste and smell dysfunction. Am. 3. Med. Sci., 1976; 272: 285.
64. Bales, C.W., Steinman, L.C., Freeland-Graves, J.H., Stone, J.M., and Young, R.K. The effects of age on plasma zinc uptake and taste acuity. Am. 3. Clin. Nutr., 1986; 44: 664.
65. Ainley, C.C., Cason, J., Carlsson, L., Thompson, R.P., Slavin, B.M. and Norton, K.R. Zinc state in anorexia nervosa. Br. Med. 3. Clin., 1986; 293 : 992.
66. Marumo, F., Tsukamoto, Y., Iwanami, S., Kishimoto, T. and Yamagami, S. Trace element concentrations in hair, fingernails and plasma of patients with chronic renal failure on haemodialysis and haemofiltration. Nephron, 1984; 38: 267.
67. Tsukamoto, Y., Iwanami, S. and Marumo, F. Disturbances of trace element concentrations in plasma of patients with chronic renal failure. Nephron, 1980; 26: 174.
68. flenkin, R.I. Trace elements in endocrinology, Med. Clin. North Am., 1976; 60: 779.
69. Aihara, K., Nishi, Y., Hatano, S., Kihara, M., Yoshhnitsu, Nutr., 1986; 5 : 69. K., i'akcichi, N., Ito, T., Ezaki, H. and Usui, I. Zinc, copper, manganese and selenium metabolism in thyroid disease. Am. J. Clin. Nutr., 1984 ; 40: 26..
70. Dolev, E., Deuster, P.A., Solomon, B., Trostmann, U.H., Wartofsky, L. and Burman, K.D. Alterations in magnesium and zinc metabolism in thyroid disease. Metabolism, 1988; 37: 61.
71. Marsal, K., Furgyih, S. Zinc concentrations in maternal blood during pregnancy and post-partum, in cord blood and amniotic fluid. Ada ObsteL Gynecol. Scand., 1987;66: 653.
72. Hambidge, K.M. and Droegemueller, W. Changes in plasma and hair concentrations of zinc, copper, chromium, and manganese during pregnancy. Obstet. CynaecoL, 1974; 44 : 666.
73. Arumanayagam, M., Wong, F.W.I., Chang, A.M. and Swaminathan, R. Zinc concentration in umbilical cord tissue and cord plasma in appropriate-for-gestatinnalage babies. Eur.J. Obstet. Gynaecol. Reprod. Biol., 1986; 23:121.
74. Hambidge, K.M., Krebs, N.F., Sibley, L. and English, J. Acute effects of iron therapy on zinc status during pregnancy. Obstet. Gynaecol., 1987; 70 : 593.
75. Kuhnert, B.R, Kuhnert, P.M. and Zarlingo, T.J. Associations between placental cadmium and zinc and age and parity in pregnant women who smoke. ObsieL Gynaecol., 1988; 71 : 67.
76. Hambidge, K.M., Neldner, K.H. and Walravens, P.A. Zinc, acrodermatitis enteropathica and congenital malformations. Lancet, 1975; 2: 577.
77. Lazebnik, N., Kuhnert, B.R., Kuhnert, P.M. and Thompson, K.L. Zinc status, pregnancy complications and labour abnormalities. Am. J. Clin. NuIr., 1987; 46:763.
78. Soltan, M.H. and Jenkins, D.M. Maternal and fetal plasma zinc concentration and abnormalities. Br. J. Obstet. Gynaecol.,, 1982; 89 : 56.
79. Anon. Zinc deficiency linked to dyslexia. New ScientisL 17th March 1988, p.38.
80. Edmondson, R.P., Thomas, R.D., Patrick, J., Hilton, PJ. and Jones, N.F. Leukocyte electrolytes in cardiac and non-cardiac patients receiving diuretics. Lancet, 1974; 1:12.
81. Elm, R.J. Assessment of magnesium status. Clin. ChenL, 1987; 33: 1965.
82. Berkelhammer, C. and Bear, R.A. A clinical approach to common electrolyte problems. Hypomagnesemia. Can. Med. Assoc. j., 1985; 132 : 360.
83. Chueca, P., Serrano, S., Encarnacion Carrasco, M.I. and Chueca, C. Correlation between calcium and magnesium in plasma or serum? Clin. CheTn., 1987; 33: 1304.
84. Pao, E.M. and Mickle, Si. Problem nutrients in U.S. Food Technol., 1981; 35 : 58.
85. Seelig, M.S. The requirement of magnesium by the normal adult. Summaiy and analyses of

published data. Am. J. Clin. Nutr., 1964; 14: 242.
86. Wester, P.O. Magnesium. Am.J. Clin. NuIr., 1987; 45 (5 Suppl): 1305.
87. Zofkova,1., Nedvidkova,J., Zamrazil,V. and Simeckova, A. Influence of magnesium on glucose tolerance. Acute hypermagnesemia reduces the glucose tolerance independently on hormonal indicators. Ilorm. Metab. Res., 1987; 19: 228.
88. Moles, K.W. and McMullen, J.K. Insulin resistence and hypomagnesaemia; case report. Br. Med. J. (Clin. Res.), 1982; 285 : 262.
89. Fort, P. and Lifshitz, F. Magnesium status in children with insulin dependent diabetes mellitus. J. Am. Coll.
90. Matthey, F., Gelder, C.M. and Schon, F.E. Isolated hypomagnesaemia presenting as focal seizures in diabetes mellitus. Br. Med. .J. (Clin. Res.), 1986; 293:1409.
91. McCarron, D.A. Calcium and magnesium nutrition in human hypertension. Ann. Intern. Med., 1983; 98 (Spt.2) 800.
92. Dyckner, T. and Wester, P.O. Effect of magnesium on blood pressure. Br. Med. 3., 1983; 286: 1847.
93. Henderson, D.G., Schierup, 3. and Schodt, T. Effect of magnesium supplementation on blood pressure and electrolyte concentrations in hypertensive patients receiving long-term diuretic treatment. Br. Med. J. (Clin. Res.), 1986; 293: 664.
94. Classen, H.G. Systemic stress, magnesium status and cardiovascular damage. Magnesium, 1986; 5: 105.
95. Rude, R.K, Oldham, S.B. and Singer, F.R Functional parathyroidism and parathyroid hormone end-organ resistance in human magnesium deficiency. Clin. Endocrinol., 1976; 5 : 209.
96. Masscy, S.G. and Coburn, J.W. Comments on the mechanisms of disordered divalent ion metabolism in renal failure. A possible role of magnesium depletion. Am. J. Clin. Nutr., 1970; 23: 1005.
97. Levine, B.S. and Coburn, J.W. Magnesium, the mimic/antagonistof calcium. N. Engl. 3. Med., 1984; 310: 1253.
98. Rotloh, A., Riva, E., Zecchini, 0., Magro, F., Fiocchi, A., Longhi, Rand Giovanini, M. Magnesium deficient rickets in a phenyl-ketonuric patient on dietaty treatment. J. Inherited Metab. This., 1986; 9 (suppl.2) : 215.
99. Altura, B.M. and Altura, B.T. Magnesium ions and contraction of vascular smooth muscles; relationship to some vascular diseases. Fed. Proc., 1981; 40: 2672.
100. Bataille, P. and Fournier, A. Role du magnesium dans la lithiase calcique. Presse Med., 1988; 17: 301.
101. Hsu, J.M., Root, A.W., Duckett, G.E., Smith, J.C. Jr., Yunice, A.A. and Kepford, G. The effects of magnesium depletion on thyroid function in rats. 3. NuIr., 1984; 114 1510.
102. Wolfe, S.M. and Victor, M. The relationship between hypo- magnesaemia and alkalosis to alcohol withdrawal symptoms. Ann. N.Y. Acnd. ScL, 1969; 162 : 973.
103. Rayssiguier, Y., Gueux, B. and Weiser, D. Effect of magnesium deficiency on lipid metabolism in rats fed on a high carbohydrate diet..). Nutr., 1981; 111: 1876.
104. Dyckner, T. Serum magnesium in acute myocardial infarction. Relation to arrhythmias. Ada Med. Scant, 1980; 207: 59.
105. Abraham, A.S., Eylath, U., Weinstein, M. et al. Serum. magnesium levels in patients with acute myocardial infarction. N. Eng. 3. Med., 1977; 2%: 862.
106. Boyd, J.C., Sugg, N.}C, Bruns, D.E. et al. Relationship of serum potassium and magnesium to cardiac arrhythmias in coronary care unit patients, abstracted. Ann. Clin. Lab. Sd., 1983; 13 : 334.
107. Kafka, H., Langevin, L and Armstrong, P.W. Serum magnesium and potassium in acute myocardial infarction. Influence on ventricular arrhythmias. Arch. Intern. Med., 1987; 147: 465.
108. Ebel, H. and Gunther, T. Role of magnesium in cardiac disease..). Clin. Chem. Clin. Blochem., 1983; 21 : 249.
109. Lindsay, R Changes in plasma and urine magnesium following subcutaneous insulin. Curr. Med.

Res. Opin., 1976; 4 : 296.
110. Crampton. R.S. and Clark, C.W. Varying extracellular Mf alters ischemic and reperfusion ventricular tacky arrhythmias, abstracted. Circulation, 1983; 68(supl. 3) 146.
111. Karppanen, H. Epidemiological aspects of magnesium deficiency in cardiovascular disease. Magnesium BulL, 1986;8: 199
112. Collinson, P.O. and Burroughs, A.K. Severe hypermagnesaemia due to magnesium sulphate enemas in patients with hepatic coma. Br. Med. J. (Clin. Res.), 1986; 293:1013.
113. Cade, J.F. A significant elevation of plasma magnesium level in schizophrenia and depressive states. Med. J. Aust., 1964; 1: 195.
114. Chugh, T.D., Dhingra, R.K., Galali, R.G. and Bathia, J.C. Magnesium in schizophrenia. Ind. J. Med. Sd., 1973; 61 :998.
115. Chhatre, S.M., Ganeriwal, S.K. and Reddy, B.V. Serum magnesium levels in schizophrenia. Indian J. Med. Sci., 1983; 39: 259.
116. Paul, E.A., Daniel, P., Vankomen,W.E. and Bunney,J.R. Serum calcium and magnesium in schizophrenia: Relationship to clinical phenomena and neuroleptic treatment. Br. J. Psychiatry, 1978; 133: 143.