Introduction
1. INTRODUCTION

What is termed as trace element includes certain metals, like Zinc, Copper, Aluminium, Manganese and Cobalt which are found in very minute quantity in the environment. Environmental protection in the form of safe disposal of industrial waste material is one of the most important task of our present generation. If we change the balance of nature by disturbing the balance of these elements in nature it will not be very far to see the fruitful plants of our present time will turn to poisonous plants for our future generations. The determination of zinc bioavailability from various food is an important consideration in human nutrition.

Trace elements also enjoyed historical recognition in an unidentified way-by the ancient Greeks and Romans, whose folklore medicine unknowingly prescribed supplementation of various minerals.

Animal studies permit research which cannot be performed in humans and 'in vitro'. The importance of zinc deficiency during embryonic development, and congenital malformation in the fetuses is well known. Anencephally and hydrocephally occur following 'in utero' zinc deficiency in the rat (Dreosti 1983). In this study most, attention was focussed on the requirement of the zinc during fetal
development from gestation day 12 to term completion, for the normal formation of the brain, especially the cerebellum.

The involvement of neurological signs, both teratological and behavioral, with trace element deficiencies and heavy metal toxicity, served to highlight the importance of trace elements in a neurological context. Probably best known historically would be the early studies on copper related enzootic ataxia in sheep and the neurological cretinism accompanying iodine deficiency in man.

Of the 15 trace elements currently deemed to be essential, zinc has received most attention in recent times. The association of the metal with some 50–100 enzymes (Chelebowski et al 1976) has ensured the interest of biochemists, while the rapid onset of a physiological deficiency (Dreosti et al 1968), coupled with the comparatively low level of zinc in the average human diet (Sandstead 1973) commanded the attention of nutritionists and health professionals. However, central to this growing concern was the discovery by Hurley et al (1966) linking 'in utero' zinc deficiency in rats to abnormal fetal development, especially of the central nervous system, and proposal that a similar situation might obtain in humans (Hurley 1968).

Several indirect lines of evidence suggest that divalent transition-metal ions, such as Ca\(^{++}\), Co\(^{++}\), Cu\(^{++}\),
Fe\(^{++}\) or (Fe\(^{+++}\)), Mn\(^{++}\) and Zn\(^{++}\) may play an important role in the regulation of neuronal excitability.

The involvement of metal ions in complex processes of transmission within the central nervous system and their potential role in the enzymatic processes accompanying synthesis, storage and release of neurohormones is currently receiving increasing scrutiny by neuro-biologist.

Zinc and Vitamin A are known to play a role in cell membrane stabilization zinc is also required for the mobilization of Vitamin A from the liver. Dietary manipulation of both nutrients leads to tetratogenic effects in animals and subsequent impairment of neural tube development.

1.1 Availability

The importance of zinc for human health was first documented in 1963. During the past 28 years, deficiency of zinc in humans due to nutritional factors and several disease states have now been recognized.

The high phytate content of cereal proteins is known to decrease the availability of zinc, thus the prevalence of zinc deficiency is likely to be high in population consuming large quantities of cereal proteins. Alcoholism, malabsorption, sickle-cell anemia, chronic renal disease, and chronically debilitating diseases are now known to be
predisposing factors for zinc deficiency. A spectrum of clinical manifestations ranging from mild to severe degree have now been recognized in human zinc-deficiency states. Zinc is required for many biological functions, including DNA synthesis, cell division, and gene expression. It is also required for the activity of many enzymes in biological systems. Recent studies indicate that zinc is needed for cell-mediated immunity (Prasad 1991).

Everyone likes to eat the foods and drink the beverages that taste good, or that make us feel good, at least in the short term. These are the foods high in sugar, fat and alcohol. Unfortunately, these highly processed foods are frequently deficient in the essential nutrients, and are low in or devoid of fibre. Dietary fibre is that portion of a food carbohydrate which can not be digested by enzymes secreted by the host normally present in the gastrointestinal tract. Vegetarians and persons consuming special diets must also pay strict attention to their dietary intakes to receive the proper balance of nutrients, energy and dietary fibre (Harland et al 1988).

Unrefined cereal grains have been shown to be the best single food source of both dietary fibre and minerals. The refining of grains, essential for edibility, and desirable for palatability, reduces both the mineral and dietary fibre content of the grains. However, these grain products contain
anti-nutrients as well: oxalate, tannins (polyphenols) and phytates -- all of which may adversely affect mineral bioavailability. Increased dietary fibre is recommended for improved general health. Foods or diets high in dietary fibre may alter mineral metabolism, and phytate (phytic acid) normally present in fibrous foods is the primary cause of alteration of mineral metabolism, rather than the fibre itself. Increased dietary fibre can be achieved by the introduction of foods higher in phytate – fruits, vegetables, brown bread.

Oxalates are organic acids found in cauliflower, spinach, rhubarb and chocolate. Tannins, also known as polyphenols, are responsible for the astringent quality of tea and are found in certain grains such as sorghum. There is some evidence that if milk is added to tea before it is drunk the milk protein casein will bind to tannin and lessen the binding of minerals of tannins. Phytate, like oxalates and tannins is an organic compound (myo-inositol hexaphosphate) which occurs in all plants. Phytate is potent chelator of minerals and thus, it's presence in a food will strongly dictate the outcome of mineral associated with this molecules. Nutritional deficiency, due to marginal zinc intake may be quite common even in countries such as United States (Sandstead 1973). In rural areas of Egypt and Iran, where human zinc deficiency was first identified, dietary
intake of zinc appeared initially to be quite adequate (Sarram et al 1969). In these rural areas unleavened (flat) bread, made without yeast to raise the dough, is a principal part of the diet. In larger towns where 'yeast leaving' is used, the incidence of zinc deficiency is much lower suggesting an explanation for this observation.

As with Fe, Zn absorption may be improved by increasing the consumption of animal protein in the meal (Sandstrom, 1987). Unlike Fe, Zn status in humans is difficult to assess. There is no reliable biochemical marker. Due to a normally large endogeneous intestinal excretion of zinc, at times greater than or equal to that absorbed, the technique of apparent balance, in which the intake of zinc is compared with urinary and fecal excretion of Zn, poorly represents the Zn status. It is likely that a number of 'at risk' population groups may be exhibiting borderline deficiencies which impair such normal functions as tissue growth, wound healing and cause immune deficiencies. The endogenous (fecal) zinc excretion is important for the zinc hemeostasis at low zinc intake, whereas at high intake the hemeostasis is regulated via absorption from the digestive tract (Heddie 1990).

Zinc is provided to the organism mainly via the diet. Dietary zinc intake may vary over a wide range, and thus hemeostatic adjustments in absorption and retention to maintain a balanced supply for tissue and organs.
1.2 Body content

With the exception of the magnesium, calcium, and iron, the element zinc is the most abundant divalent metal ion in the brain, being present in concentration of about 12ug/g brain fresh weight (Wallork et al, 1983).

The bone content of zinc in a 240-g rat that consumes adequate zinc is approximately 4,200 ug and muscle content is 6,300 ug (Giugliano et al 1984).

The total zinc content of a normal 70 kg man is approximately 1.4 to 2.3 g (Widdowson et al, 1951). The metal is present throughout all body fluids and tissues (Tipton, et al 1963) and is homogeneously distributed in most organs. They contain about 20 to 30 ug zinc/g wt tissue. However, hair, the prostate, retina, and choroid of eye have relatively high zinc concentrations (Prasad 1979).

1.3 Requirement

Zinc requirement for optimum weight gain in the rat is about 18 ppm when the basal dietary zinc is derived from isolated soyabean protein. However if egg white, as the protein source, was used zinc required by a young rat approximates 12 ppm of the diet (Forbes et al 1960). Zinc (3 ppm) available in diet is the approximate level necessary for the maintenance of pregnancy in rats without available zinc stores (Apgar 1969).
Inspite of large number of investigations which have been carried out in the field of zinc nutrition and metabolism during the past two decades, the requirement of zinc in man is, not yet, known. The National Research Council (1980) has recommended 15 mg zin/day for the adult human consuming American diet adequate in animal protein. However, due to the above-mentioned factors that limit the bioavailability of zinc from plant sources, it is expected that the human requirement for zinc would be higher if plants rather than animals were to be used as major source of protein.

1.4 Clinical manifestation

Geophagy, or the deliberate consumption of earth substances, is a complex and perplexing human behaviour. It has been generally classified as a form of pica (Danford 1982). The most prevalent explanation for human geophagy is that it is a response to alleviate nutrient deficiency (Danford 1982 and Danford et al 1982).

Clay is seen as one of several substances (and foods), the consumption of which offers positive nutritional or other health benefits. The concepts of craving and specific appetite are of secondary or no relevance in the explanation of geophagy. Analysis of clays consumed by people in Africa and central America indicated that in certain instances the
clay was an important source of supplementary calcium, copper, iron, magnesium, or zinc (Hunter 1973 and Vermer 1966).

Timothy et al (1991) believe that geophagy plays a useful role in its proper context and should be appreciated as a normal human behaviour. In traditional societies, it is usually a non-pathologic activity that in appropriate circumstances can contribute to human health. Like any behaviour, it can be inappropriate when engaged in, out of context or to excess.


Hypogonadal dwarfism is rare. It is reported in Egyptian and Iranian children who mature on a diet poor in animal protein and high in phytate, a combination that, presumably, maintained chronic zinc deficits (Prasad et al 1961). In addition to hypogonadism and dwarfism, the children have hepatosplenomegaly, iron deficient anemia, and often exhibit geophagia.

Adults who have become zinc deficient over periods of week to months typically develop skin rashes. The rashes are similar to those seen in acrodermatitis and are described as
popular erythematous eruptions, appearing initially on ala nasi and around the lips. Next these lesions extend to the scrotum and the perianal regions, and later to the joints of the hands and feet and the scalp, usually with some loss of hair (Okada et al 1976).

Zinc deficiency may also lead to disturbances of smell, taste and vision. A new syndrome of idiopathic hypogeusia with dysgeusia and dysosmia, (Henkin 1971). Regarding vision, recent work has demonstrated that an important relationship exists between zinc and vitamin A. Zinc may be required for the proper functioning of the rods, cones and optic nerve (Russell et al 1983).

Zinc deficiency has also been implicated as a cause of poor wound healing after surgery (Burch et al 1976; Henkin 1974).

Neurological symptoms associated with deficiency or toxic overdose of these ions frequently include convulsion (Prasad 1978).

1.5 Toxicity

Three forms of toxic reactions to zinc have been reported in human beings. First metal-fume-fever characterized by pulmonary manifestation, has been reported to occur in industrial workers exposed to fumes. The second type of toxicity was observed in a 16-year-old Iranian boy
who ingested 12 g zinc sulfate over a period of 2 days. The third type of acute toxicity involved a patient with renal failure after hemodialysis (the water for hemodialysis was stored in galvanized tank). The patient suffered from nausea, vomiting, fever and severe anemia. Vomiting, a protective phenomenon occurs after ingestion of large quantities of zinc. In fact 2 g zinc sulfate has been recommended as an emetic.

The signs and symptoms of human zinc toxicity include dehydration, electrolyte imbalance, abdominal pain, incoordination. Acute renal failure, caused by zinc chloride poisoning, has also been reported. The symptoms occurred within hours of ingesting large quantities of zinc. Death is reported to have occurred after ingestion of 45 g of zinc sulfate.

High zinc diets fed to rats produced various effects on tissue zinc levels (Huber et al. 1970). Some tissues, such as liver, kidney, bone and pancreas accumulate zinc in excessive amounts, while muscle, skin, and spleen maintain their normal zinc concentration.

Large amount of zinc interferes with the assembly of microtubuli and causes the formation of supramolecular structures, both 'in vitro' and 'in vivo' (Kress et al., 1981).
Intracerebroventricular injection of zinc led to epileptic seizures in experimental animals, possibly caused by inhibition of the Na\(^+\), K\(^+\) - ATPase (Donaldson et al., 1971).

1.6 Zinc deficiency and development

Impaired hemostasis is one of the many signs of zinc deficiency in rat. Pregnant rats deprived of zinc during the gestation period exhibit dystocia and excessive blood loss occurs at parturition (Apgar 1968), along with prolonged and difficult parturition.

Zinc deficient rats exhibit impaired hemostasis, a pathological sign related to defective platelet function (Michelle et al 1990).

When female rats were fed a diet severely deficient in zinc from the beginning of pregnancy, the concentration of zinc in the plasma fell rapidly, after only 1 day of the deficient regimen, plasma zinc fell by approximately 38% (from 96 to 60 \(\mu g/100\) ml) and a plateau was reached at about 30 \(\mu g/100\) ml after 14 days (Dreosti et al 1968). Hurley et al, (1971a) have shown that there is insufficient maternal zinc mobilization to support normal development of embryos. Zinc deprivation, during pregnancy decreased the concentration of bone zinc slightly, but had no effect on liver concentration, although the weight of liver decreased.
Hurley et al (1971b), have shown that, when a zinc-deficient diet was fed to female rats throughout gestation (days 0 to 21), surviving full-term fetuses had less than half the normal body weight and nearly all showed gross congenital malformations affecting every organ system. Short-term deficiencies of dietary zinc during embryonic development were also teratogenic. For example, with a maternal dietary deficiency from day 6 to 14 of gestation (only 8 days) nearly half the young were malformed at term when zinc intake was insufficient (Dreosti et al 1968). The maternal organism is apparently unable to mobilize body stores of zinc to meet the needs of developing embryos (Hurley et al 1971a and 1972). These experiments exemplify a situation in which pregnant mammals fail to protect their embryos from a nutritional deficiency although they have stores of nutrients in their body. This behaviour differs from many other situations in which the embryos draw from the maternal tissue stores in case of dietary deprivation. Apgar (1969) has shown that, after 4 weeks on a low zinc diet (<1 ppm), the number of female rats that would mate was greatly reduced. Addition of 2.3 ppm zinc to the drinking water resulted in mating of all rats. If zinc was removed on the day sperm was found, only half of the females were pregnant on day 8, and all of them had completely resorbed their fetuses by day 21. If the deficient females were given progesterone and estrogen from day 4 to 21, all of them were
pregnant on day 8, and half of them were able to maintain pregnancy to term. If the females were maintained on 3 PPM zinc throughout gestation, pregnancy was maintained and the zinc content of the fetuses was as high as that in fetuses of females receiving 50 ppm, zinc. An unexpected result of the administration of progesterone - estrogen to the females receiving 3 ppm zinc was the increased fetal mortality, where there had been no fetal mortality between day 8 and 21 in the females receiving 3 ppm zinc and no hormone therapy. Since females receiving 50 ppm zinc plus hormone therapy had a greater fetal survival (almost 70%) than did the females receiving the hormones and only 3 ppm zinc, zinc may be required for the metabolism of excess hormone. High levels of progesterone have been reported to cause fetal deaths in rats (Moore 1961).

Zinc deficiency is associated with growth retardation and hypogonadism (Sanstead et al. 1967; Mahajan et al. 1982; Prasad 1982 a,b), reduced secretion of growth hormone (GH) and testosterone but increased of luteinizing hormone (LH) and follicle-stimulating hormone (Millar et al. 1960; Lei et al. 1976 Root et al (1979). The thyroid axis of zinc deficient animals is also altered (Morley et al. 1980). These effects of zinc may be manifested at one or more endocrinological regulatory sites i.e. testes, hypothalamus or pituitary. Evidence exists that zinc may directly inhibit
basal prolactin (Prl) secretion from the anterior pituitary gland (Bella La et al. 1973) and directly decrease Prl and GH release from pituitary secretory granular (Lorenson et al. 1983 a, b).

Zinc could inhibit Prl release by altering membrane stabilization through direct binding to cell membrane proteins. Alternatively, zinc may directly affect secretory granules since it inhibited Prl and GH release from a preparation of isolated pituitary secretory granules (Lorenson et al. 1983 a,b). This effect may be mediated by an action on specific granular membrane protein or by inhibiting the intragranular conversion of the large oligomeric hormone molecules to the monomeric form in which they are released.

It is of interest that CNS zinc concentration fluctuates during the estrous cycle and menstruation (Merriam et al 1979 Kishi et al. 1982), but whether these changes are related to anterior pituitary function, remain unclear.

In humans made severely zinc deficient by administration of large amounts of dietary histidine, a variety of neuropsychological abnormalities occurred (Henkin et al 1975).

During the last several years, the effects of folic acid and zinc deficiency on the outcome of pregnancies has
aroused much interest, sometimes with conflicting results. Some workers have demonstrated a positive connection between folic acid deficiency and obstetric complications (Hibbard, 1964; Streiff et al 1967) while others seem to doubt it (Pritchard et al 1969 and Rothman 1970; Speidel 1973). Some doubts also exist as to the effects of zinc on the outcome of pregnancy. Thus while some authors have shown positive connection (Hunt et al., 1984; Simmer et al 1985), others question this relationship (Swanson et al 1987).

1.7 Zinc and Brain Development

It has been suggested that zinc plays a key role in the structure and function of biomembranes (Bettger et al 1981). The requirement for zinc in fetal rat brain development was first reported by Hurley et al (1966). Congenital malformation associated with an induced zinc deficiency included hydrocephaly and gross brain defects.

Zinc restriction during early neurogenesis appears to affect the development of many derivatives of the primitive neural tube and defects include agenesis and dysmorphogenesis of the brain, spinal cord, eyes and of the olfactory tract (Hurley, et al 1972).

In studying the effects of zinc deficiency on growth and development, attention must be given to the effect of secondary inanition. Pair-feeding or paired weight gain experiments are of value in distinguishing between primary
effects of zinc deficiency and secondary effects of reduced food intake. There is considerable evidence that malnutrition during infancy and early childhood inhibits maximum mental development of an individual. To understand better the influence of nutrition on mental processes, investigation of the changes in chemical composition and metabolism of brain provides useful information. Poor nutrition of rodents during the prenatal or neonatal periods (or both) at the time of most rapid brain differentiation, and growth, changed the chemical composition of the brain (Zamenhof et al. 1968). Even during the post-weaning period, nutritional deprivation caused reductions of ribonucleic acid (RNA), protein and lipids, indicating aberrant metabolism of these components of brain (Guthrie et al. 1968). Although the concentration of total zinc in the brain was normal in zinc deficient rats, the high uptake of $^{65}$Zn by these brains may reflect that a functional deficiency of this element increased uptake of zinc in a tissue of a deficient animal even though total zinc content of that tissue was normal (Robert et al. 1969).

In humans, unlike in animals, a direct causal relationship has not yet been established between zinc deficiency and brain dysmorphogenesis. Nevertheless, all current evidences suggest that the developing human fetus is no less vulnerable to zinc depletion than are the offspring from other species, and tentative associations have been
drawn between various brain terata and zinc deficiency by several workers in a number of countries.

Damyanor (1971) and Severetal (1973) have reported anencephalus in middle east, Cavdar et al (1980) in Turkey Stewart et at (1981) in USA and Soltan et al (1982) in Britain. Myelomeningocoele was reported by Jameson (1976) in Sweden. Spina bifida (Bergmann et al 1980), exencephalus and microcephalus (Hurd et al 1983) have also been reported.

Disturbed zinc status in man has been associated with several established disorders of the central nervous system. Reduced zinc level is associated with dementia (Burnet 1981), depression (McLardy 1975), mental retardation (Pihl RO et al 1977), schizophrenia (Pfeiffer 1972), fifth-day fits (Goldberg 1982). While increased zinc status is associated with, epilepsy (Barbeau et al 1974) and Pick's disease (Constantinidis 1981).

Joseph et al (1974) have shown that in maternal zinc deficiency from day 18 of gestation and/or throughout lactation, resulted in reductions in pup body, brain, weights at birth. Zinc deficiency and throughout the suckling period when compared with pup from dams fed a diet adequate in zinc yet restricted in the amount consumed also caused reduction in brain weight. The concentration of glutamic dehydrogenase (GDH) was unchanged, and no differences in the concentration of brain zinc were detected. A significant reduction in
2',3'-cyclic Nucleotide 3'-phosphohydrolase (CNP) was found, which implied a delay in myelination.

When a zinc-deficient diet was given throughout gestation to female rats fed a marginally zinc-deficient diet from weaning to mating, 54% of the implantation sites were resorbed and 99% of the total sites were affected. Of the young living at term, 98% exhibited gross congenital malformations involving all organ systems (Hurley et al 1966). Transmission electron microscopic study of zinc deficient 11-day-old rat embryos revealed severe neuroepithelial cell death, and disrupted cell and mitochondria membranes in some living cells. The supporting mesenchymal tissue was less affected. On the other hand, hypervitaminosis A exerted its teratogenic assault mainly on mesenchyme, with a lesser insult on neuroepithelium abutting the lumen. Cell death was common and severe swelling and breakdown of mitochondrial membranes was observed in many living cells. (Joschko et al, 1988). Deficiencies of both zinc and vitamin E are teratogenic in animals, including defects in the development of neural tube. Both substances function in cellular antioxidant defence mechanisms and in membrane stabilization. Transmission electron microscopy of the 11 day old vitamin E deficient embryo revealed cell death, primarily in the mesenchyme but also in the neural tube. (Harding et al., 1986). During foetal and early
neonatal life, mammals have elevated concentrations of copper and zinc in their liver (Mason et al. 1981). This zinc and copper could be stored to provide these essential elements to the developing animals or the metals may be sequestered in a non-toxic form until the liver has matured sufficiently to dispose of the surplus. Much of the copper and zinc is found associated with the low molecular weight cysteine-rich proteins, metallothioneins. Zinc deficiency has profound effects on brain function of both humans and experimental animals (Pfeiffer et al., 1982). Severe zinc deficiency, during the first third of gestation, produces congenital malformations affecting many systems, including the central nervous system in rat offspring (Hurley et al. 1971 and 1972). Severe zinc deficiency during the latter third of pregnancy or from birth to weaning causes subsequent behavioral abnormalities in nutritionally rehabilitated offspring (Caldwell et al. 1973 and Peter 1978).

Overall, the pattern of early brain malformations appears to be consistent with impaired mitosis during embryonic development, and the involvement of zinc in cell division offers an acceptable mechanism to account for the wide range of abnormalities seen in the brains of zinc-deficient fetuses. Record, et al (1979) have shown that the activity of thymidine kinase fell more in the brain (53%) than in liver (34%) of zinc deficient 20-day-old fetuses, when compared with restricted-fed controls.
Zinc is essential for DNA, RNA and protein synthesis (Sandstead et al. 1969 and Fosmire et al. 1977). These functions influence neuron proliferation and maturation. Prenatal zinc deficiency is associated with impaired thymidine kinase activity and depressed incorporation of thymidine into the neural crest (Dreosti et al. 1975). Zinc deficiency during the later part of pregnancy results in a decreased in brain DNA (McKenzie et al. 1975) which is a reflection of a decrease in cell number (Dvergsten 1981).

Axonal transport 'in vitro' has been shown to be increased by the addition of zinc (Edstrom et al. 1975). Zinc also appears necessary for neural microtubule and tubulin synthesis and assembly (Gaskin et al. 1977 and Kress et al. 1981). Cerebellar morphology is adversely affected by severe postnatal zinc deficiency (Dvergsten 1981). The number and migration of granule cells are also depressed. Purkinje, basket and stellate cell dendritic arborizations are deformed and suppressed and parallel fibres are decreased (Dvergsten 1981).

1.8 Zinc and Calcium

Hurley et al. (1972) have shown that a combined deficiency of zinc and calcium resulted in larger litters, fewer malformed fetuses and fewer resorption than in a group of rats that were fed a diet deficient only in zinc. They suggest that the greater breakdown of bone caused by calcium
insufficiency also increased the availability of skeletal zinc. Similarly, by restricting the intake of pregnant rats fed a zinc deficient diet, resorption and malformed fetuses were fewer than when the zinc-deficient, diet was fed ad libitum, suggesting that tissue catabolism was induced early in pregnancy, thus providing zinc released during this process to developing fetuses (Masters 1985).

1.9 Zinc and cadmium

Keun et al (1988) have shown that short term cadmium administration did not influence level of zinc and copper or zinc bound to putative metallothionein fraction in rat liver. However, longer term administration of cadmium eventually resulted in an increase in the amount of zinc associated with metallothionein.

1.10 Zinc and copper antagonism

Among the biological interaction seen with excessive dietary zinc is the antagonism and reduced uptake of copper (Van Campen, 1966). High zinc concentration can, therefore, exaggerate or induce copper deficiency. Anemia, dermatosis, depressed growth rate, neutropenia and lymphopenia are common clinical signs of a copper deficiency in many species (Maynard, et al 1956). Zinc in excess of the minimum daily requirement may lead to depletion of copper stores and even clinical copper deficiency (Prasad et al 1978). So it has
been recommended that daily doses should not exceed 45 mg (Sandstead 1978). Zinc is also known to compete with iron, cadmium and lead (Brewer 1983, Brewer et al 1987).

1.11 Protective action of zinc on lead toxicity

Dilts et al (1979) have shown that lead toxicity is associated with failure in human reproduction. High levels of ingested lead during pregnancy resulted in reduced maternal food intake and maternal fetal weight gain and increased fetal wasting. Dietary zinc provided protection for the fetus but not the dam.

1.12 Zinc status and Immunity

Zinc appears to play an important role in immune function. Animals with severe zinc deficiency may exhibit atrophy of the thymus and lymph nodes (Allen, et al 1981). Similarly, thymic atrophy has been noted in malnourished and zinc deficient children. This has been reversed with zinc repletion (Golden et al 1977).

The immunocompetence of animal species has also been shown to be intimately connected to zinc status (Luecke et al 1979). While a zinc deficiency generally results in an immunologically impaired organism, few studies have been reported concerning the effects of exposure to elevated dietary concentration of zinc (Michael et al 1983).
1.13 Stressors and zinc

Stressors such as infection, inflammation and trauma induce biochemical and endocrine changes within the body that have been collectively called an acute phase response (Dinarello 1984 and Pepys et al 1983). Plasma trace mineral concentrations are altered during an acute-phase reaction. Plasma zinc and iron concentrations increase. Serum ferritin and ceruloplasmin concentrations also increase in response to stress; these changes may constitute an adaptive mechanism wherein tissue repair, immune reactivity, and resistance to infection are promoted (Cannon et al 1986 and Liesen et al 1977). Prolonged physical and psychological stress produce changes characteristic of an acute-phase responses. Plasma zinc, iron and selenium decline (Anita et al 1991).

1.14 Zinc and Cell division

The trace metal zinc (Zn) performs an essential role in normal cellular homeostasis, both 'in vivo' and 'in vitro' (Chvapil, et al 1977). Zinc likewise occupies an important position in the regulation of cell growth and division (Taylor, 1982). Biochemically, the teratogenicity of zinc deficiency is widely ascribed to impaired nucleic acid synthesis during embryonic development (Dreosti et al 1972). Considerable evidence suggests that diminished activity of the zinc-dependent enzyme, thymidine kinase, may be an important factor responsible for reduced mitotic activity.
(Duncan et al 1975), since this enzyme is widely recognized to represent a rate-limiting step in DNA biosynthesis.

The growth rate of the rapidly 'Ehrlich ascities cell-line' in mice is directly correlated with host zinc content. The quantity of zinc in the bathing ascities fluid determines the rate of uptake and incorporation of thymidine into the replicating DNA required for cell proliferation. Tumor cells were capable of withdrawing zinc from the liver. (Minkel et al 1979). Availability of zinc in the ascities fluid was closely correlated with the DNA replication process. Both $[^3H]$ thymidine uptake and its incorporation into DNA was inhibited by a deficiency of zinc (Saryan et al 1979).

1.15 Zinc and Alcohol

Alcohol has been reported to enhance iron absorption, and there appears to be a good correlation between hepatic Fe levels and consumption of alcoholic beverages. Conversely, it has been demonstrated that alcohol increased urinary zinc excretion, which has led to the suggestion that a high intake of alcohol might lead to Zn deficiency (Carey et al 1971) and patients with alcoholic cirrhosis (Kahn et al 1985; Mills et al. 1983). Testes were the first tissue to show a reduction in Zn concentration in rats fed on a Zn-deficient liquid diet containing ethanol, (Ahmed et al 1982). Alcohol-induced
hepatic damage is a major determinant of urinary zinc excretion, since hepatic disease is accompanied by increases in urinary Zn excretion (Helwig et al. 1966). Alcoholic beverages and ethanol alone can effect Zn and Fe metabolism. (Susan et al 1988).

The extent and nature of possible reversibility of alcoholic brain damage is of considerable importance. Available data suggest that abstinent chronic alcoholics may recover significantly from both cerebral atrophy and cognitive deficits (Artmann, et al 1981; Ron, 1982). The hippocampal formation is vulnerable to the effects of chronic ethanol exposure. Previous research has provided quantitative evidence that chronic ethanol treatment (CET) produces 10-30% loss of both CA1 pyramidal and dentate gyrus granule cells in the rodent hippocampus (Irle, et al 1983, Lescaudron, et al 1985, Walker, et al 1981) CET is likely to produce a complex sequence of changes, including cell loss, differentiation and synaptic reorganization. The surviving neurons will likely obtain new targets for their projections and or receive additional afferents to replace those which were lost. Thus, it is likely that destructive and compensatory changes occur simultaneously during CET and that furhter reorganizational or regenerative changes may occur during ethanol abstinence (Michael et al 1988).
1.16 Zinc deficiency in fetal alcohol syndrome (FAS)

The mechanisms underlying the teratogenic effects of ethanol is unknown. One hypothesis is that ethanol functions as a teratogen by inducing a state of maternal/fetal nutritional deficiencies as a result of primary and/or secondary malnutrition (Lieber, 1982).

One class of nutrients that can be affected by ethanol consumption is essential trace elements. In recent years, abuse of ethanol has been reported to produce deficiencies of zinc, magnesium, phosphate and iron (Hurley, 1977 and Altura, 1986). Zinc deficiency has been reported to be one manifestation of alcoholic liver disease (McClain, et al 1983). In addition, ethanol administration unrelated liver disease has been shown to decrease zinc absorption in experimental animals (Antonson, et al 1983). Flynn and Co-workers (1989) have reported that there is inverse relationship between maternal plasma zinc concentrations and the expression of FAS in humans. Assadi et al (1986) have reported low plasma zinc levels and hyperzincuria in FAS infants.

Studies of zinc deficiency in experimental animals have shown a strong positive correlation with abnormal fetal development and epidemiological studies have indicated that maternal zinc deficiency may result in abnormal development in humans (Keen, et al 1987 and Hurley, 1981). Zinc
deficiency does not have to be severe to result in developmental defects. Even a mild deficiency of zinc in rats and monkeys is teratogenic, resulting in fetal growth retardation and skeletal defects (Leek, et al 1988). The molecular explanation for the abnormalities seen as a result of prenatal zinc deficiency is unknown, however, zinc has been reported to be required for fetal DNA and protein synthesis. Brain microtubule polymerization and membrane stabilization and protection of fetal membranes from free radical damage (Keen, et al 1987 and Hurley, 1981, and Dreosti, 1987) Therefore, it is likely that the structural lesions associated with prenatal zinc deficiency arise through a variety of mechanisms.

FAS is associated with a wide spectrum of abnormalities in the infant, including growth retardation, anomalies of face, heart and genitalia and mental deficiencies (Lemoine, et al 1968 and Iosub, et al 1985). In addition defects in the cardiovascular, skeletal, urogenital and immune system are frequently observed in FAS (Rosett, et al 1983).

Children with foetal alcohol syndrome (FAS) can be identified by three characteristic features mental retardation, craniofacial malformations and growth retardation marked by microcephally (Lemoine et al 1968, Apgar et al, 1973). In fact prenatal exposure to alcohol is
a principal cause of mental retardation in the United States of America (Abel and Sokol, 1986).

Sheri, et al (1988) have show that ethanol-induced fetal zinc deficiency does not seem to have a role in the production of gross structural malformations associated with fetal alcohol syndrome when adequate zinc is provided in the diet.

1.17 Zinc binding proteins

The symptoms of zinc deficiency or zinc toxicity have not been causally related to alterations in any of zinc metalloenzymes. Zinc levels of most tissues are maintained during the feeding of zinc deficient diets so that they are inadequate criteria for the assessment of zinc nutrition (Macapinlac et al 1966 Huber et al 1970). The suggestion has been made that under conditions of insufficient dietary zinc intake, growth is limited to preserve tissue zinc levels (Oberleas et al 1969). Exceptions to this are bone (Huber, et al. 1970 and Williams et al 1970) intestinal mucosa (Reinhold et al 1970) and serum (Wilkins et al 1972) where significantly decreased zinc concentration have been observed during zinc deficiency. Proteins that bind zinc may be classified into at least three major groups consisting of

a) Metalloenzymes
b) Metallothioneins
c) Metalloproteins
As an essential substance, zinc is involved in the function and/or structure of at least 90 metalloenzymes.

Mammalian metalloenzymes include carbonic anhydrase, carboxypeptidases, aminopeptidases, alkaline phosphatase alcohol, retinol, malate, lactate, glutamate and glyceraldehyde-3-phosphate dehydrogenases (Aggett, et al 1979). These and other zinc metalloenzymes participate in numerous metabolic processes. Including the synthesis and the degradation of carbohydrates, lipids, proteins and nucleic acid (Riordan 1976).

Metallothioneins are low molecular weight (6000), cystein-rich (25-30%) metal-binding proteins, which are involved in zinc and copper homeostasis and in cadmium and mercury detoxification. Peripherally administered zinc became bound rapidly to albumin, α-macroglobulin, transferrin, ceruloplasmin, haptoglobin, γ-globulins, and probably other proteins (Prasad 1979) and hence are unavailable to be transferred to the CNS (Itah et al 1982).

Interestingly the intraperitoneal administration of zinc sulfate stimulated the metallothionein in the liver but not in the brain (Itah et al 1983), and the zinc deficiency state altered neither the concentration of zinc (Wallwork, et al 1983) nor that of the zinc-binding protein in the brain (Ebadi, et al 1983). There is no reason to believe the
mechanisms involved in the homeostasis of zinc in the peripheral organs and in the central nervous system are identical. In the absence of any known function Ebadi et al (1984), postulated that in the CNS, zinc binding proteins not only do function as physiological donors of zinc to zinc apometalloenzymes, but also by their inducibility many play a decisive role in avoiding CNS toxicity by preventing the rise of free zinc ions shown to be so deleterious.

Metallothionein (MT) is a copper-and zinc-binding protein present in most, if not all, tissues of higher eukaryotic species of animals. The two most widely proposed functions for MT are a role in detoxification of excess level of copper and zinc and a role in copper and zinc homeostasis. (Bremner, 1987a and Kagi et al 1988). Acute or chronic exposure to high levels of either of these two metals leads to the accumulation of copper-and zinc-MT in various tissues. A cessation of exposure to excess levels of copper and zinc results in concomitant loss of tissue metal and MT-bound metal (Bremner, 1987b and Whanger, 1982, and Bremner, 1987a). Thus, when copper and zinc levels exceed a certain threshold they are retained by MT in what is presumed to be a non-toxic form until such time as these metals can be cleared from the tissue (Bremner, 1987b, Hamer, 1986).

An alternative function for MT in copper and zinc homeostasis has been proposed that encompasses a role in the
regulation of such processes as hepatic storage, intestinal absorption and renal excretion of copper and zinc (Cousins, R.J. 1985 and Dunn, et al 1987). Organs such as liver, kidney, intestine and pancreas, which play important roles in general nutrient homeostasis, actively synthesize and accumulate zinc and copper-thioneins. The levels of MT, as well as the amount of bound copper and zinc, change dramatically in fetal and neonatal liver during the course of development (Webb, 1987). It has been proposed that elevated levels of MT might have a function in providing a temporary storage site for copper and zinc required later in development for the synthesis of new tissue mass (Webb, 1987). Alternatively, the accumulation of copper and zinc bound to MT in fetal hepatic tissue may result from a delay in the development of normal export pathways for these metals from the liver, such as via biliary excretion or the synthesis and secretion of plasma metalloproteins (Webb, 1987).

The key of establishing a direct role to MT in zinc and copper homeostasis involves the demonstration that the metal bound to MT can be utilized for normal cellular functions such as the formation of active metalloenzymes. To date, this evidence has been accumulated exclusively in vitro (Kagi, et al 1979 and Hamer, 1986 Kagi, et al 1988). The newly synthesized thionein binds with the excess metals, reducing the level of free metal ions and effectively reducing the
stimulus to additional thionein synthesis. In this way, the amount of intracellular free zinc or copper is maintained at levels compatible with normal cellular requirements. Zinc bound to MT is released after degradation of the protein in the lysosomes, while copper accumulates in the form of insoluble polymerized copper-rich MT. Alternatively, copper and zinc bound to MT could be available for direct ligand exchange with metalloenzymes or other metal-requiring proteins. There is some evidence to suggest that MT is secreted intact, along with its metal, from the cell (Bremner, 1987b).

Chandra (1984) have shown that zinc intake of 300 mg/day in healthy person resulted in decreased maximal response to phytohemagglutinin, increased low density lipoprotein and decreased high density lipoproteins.

Milos, et al (1974) have shown that, the dietary zinc controls lipid peroxidation only in tissue such as liver and the red blood cells. This may be related to the capacity of the tissue to retain zinc in proportional to dietary zinc intake.

1.18 Zinc dependent enzymes

Zinc is necessary for the action of about 120 enzymes (Jeejeebhoy, 1984).
1.19.1 Alkaline phosphatase

The relationship of alkaline phosphatase to dietary zinc intake has been most commonly studied. Zinc deficiency results in lowered activities in bone (Prasad, 1971) and intestinal mucosa (Williams, 1972). However, serum alkaline phosphatase findings are controversial (Prasad, et al 1971). Iqbal (1971) has shown that the food consumption of the mild zinc deficient group was fairly high and alkaline phosphatase activities in its paired-fed control were nearly like those in animals on standard diets.

Feeding of high zinc diets depressed carbonic anhydrase activity and increased alkaline phosphatase in some tissue when compared to ad libitum-fed rats. Mitochondrial glutamic dehydrogenase was not affected by low or high dietary zinc intakes in any tissue analyzed (Agnes, et al 1973). Myeline, formed by the spiral infolding about the axon of a membrane-bound process, is an assembly of interacting biological membranes. Effects of zinc on myelin structure may be attributable to zinc-basic protein interactions.

Enzymes associated with barrier tissues may modulate exchange between blood and parenchyma (Mrsulja et al 1979). Cerebral capillaries are rich in alkaline phosphatase (AP) gammaglutamyl transpeptidase, and Na⁺, K⁺-ATPase (Betj et al, 80 Lidinsky et al 1983). Although no definitive functions for these enzymes have, as yet, been demonstrated in
endothelial cells, their distribution suggests that they are involved in transport (Spatz et al 1982; Inomata et al. 1984). However, AP is considered an important constituent of the enzymatic composition of the cerebral vessels and consequently has been used as a marker for vessels in the adult brain (Bell et al. 1984).

1.18.2 Carbonic Anhydrase

Carbonic anhydrase appears in oligodendrocytes and astrocytes only 6–8 days after birth. Before this time, it is present only in the choroid plexus. (Roussel, et al, 1979).

Its general function is to catalyze the hydration of carbon dioxide, resulting in the formation of a proton and a bicarbonate ion (i.e. the ionized form of carbonic acid). Consequently, it plays an important role in such diverse physiological phenomena, such as acid secretion by the kidney and stomach, removal of carbon dioxide produced by glycolysis, and transport of carbon dioxide across erythrocyte membranes. The enzyme would appear to be important in a variety of physiological processes involved in the regulation of ion exchanges and acid-base balance in the brain. During cerebrospinal fluid (CSF) formation, carbonic anhydrase in the choroid plexus provides H⁺ ion to exchange with Na⁺ ion from blood into the CSF, and it is apparently for this reason that inhibition of carbonic anhydrase reduces CSF pressure (Tschirgi et al 1954, Fishman, 1981). Other
functions attributed to glial carbonic anhydrase activity include the hydration of carbon dioxide produced by metabolic activity of adjacent neurons (Giacobini 1961). Carbonic anhydrase has been reported to be lowered in brain of zinc-deficient rats (Iqbal, 1971).

Many neurochemical and metabolic processes are dependent on, or affected by, zinc. The synthesis of the excitatory neurotransmitter glutamate is dependent on the activity of glutamate dehydrogenase—a zinc-containing enzyme (Vallee, 1960). Also, the activity of a number of enzymes involved in the synthesis and breakdown of -amino butyric acid are dependent on zinc (De Boer et al, 1979; Ebadi et al, 1981).

Zinc may be involved in the storage of biogenic amines in synaptic vesicles (Colburn et al, 1965) and in the storage of nerve growth factor-like substances in the hippocampus (Stewart et al., 1984).

Zinc in low concentration stimulates the rate of rapid axonal transport (Edstrom et al, 1975). But the excess zinc adversely affects neuronal metabolism and function. The activities of glutamate dehydrogenase are inhibited by high level of zinc (Ebadi et al., 1981).

Neurotransmitter synthesis and/ or metabolism are affected by zinc deficiency. In severely zinc-deficient
weanling rats, total brain and hippocampal norepinephrine is increased (Wallwork, et al 1982). Similarly, high levels of striatal catecholamines were reported to be associated with behavioural changes in zinc deficient rats (Bradford, et al 1981). Hypothalamic catecholamine levels were abnormal in zinc-deficient rats (Reeves, et al 1981).

Other aspects of hippocampal metabolism are affected by zinc nutrition/malnutrition. It has been reported that zinc deficiency causes increased taurine and subsequently decreased γ-aminobutyric acid levels in the hippocampus (Barbeau et al 1974) activity of -glutamic acid dehydrogenase (zinc metalloenzyme) was found to be diminished in hippocampus from 18-and 20-day-old zinc deficient rat pups that were deprived of zinc from day 19 of gestation to the time of assay (Dreosti et al 1981).

Recently, zinc-deficient rats were shown to have increased binding of synaptic ligands to muscarinic and dopamine receptors in the cerebellar and striatal membranes, respectively (Geiger et al 1982).

1.18.3. Superoxide dismutase (SOD)

Superoxide dismutase (SOD, superoxide oxidoreductase, is an enzyme which scavenger the superoxide radical, a toxic species generated by metabolic reduction of oxygen in respiring cells (Grankvist, 1979). This enzyme has been shown
to inhibit lipid oxidation and protect against pathologic changes resulting from DNA damage and protein sulfhydryl oxidation by the superoxide radical (Fridovich I, 1975).


Neurons exhibit the most intense staining in the brain and have shown to be vulnerable to oxygen toxicity in mice, rats and dogs (Balentine 1982). Cu-Zn SOD is possibly maintained in the cells of this sensitive system to protect them against superoxide from varying oxygen tension in the blood as well as free radicals generated by normal cellular processes.

The roles that cofactors play in influencing the expression, metabolism and net accumulation of their related proteins and enzymes is an important area of nutritional investigation (Tinker, et al 1985 and Rucker, et al 1986), SOD is often induced under conditions in which free radicals are generated Stevens, et al (1977) and Slater, (1984), one function of metallothionein is thought to be as an antioxidant (Thornalley, et al 1985 and Thomas, et al 1986). Conditions that lead to peroxidative damage often result in increased expression (SOD) (Fields et al 1984, Hamermueller et al 1987).
The failure of Swenerton and Hurley (1968) to notice any effect of zinc deficiency on zinc metalloenzymes in liver is of great interest. It is remarkable, however, that Hsu et al, (1966) observed reductions in liver and brain. But Iqbal (1971) has shown that reduction in kidney and intestinal zinc metalloenzymes, may well be related, among other factors, to differences in cell turnover rates in these organs.

1.19 Zinc and hippocampus

Many questions concerning the accretion of zinc by the hippocampus remain unknown. For example, little is known regarding the form in which zinc is stored in the mossy fibers, or indeed in other areas of the hippocampus, or of respective functions of these metal complexes.

Mclardy (1975) has reported that zinc levels are much reduced in hippocampi from alcoholic patients and that the granular cell layer of the dentate gyrus is abnormally thin, suggesting zinc deficiency may be involved pathologically in alcohol related mental deterioration. Of particular relevance in this connection is the report by Walker et al (1980) that in adult rats, chronic ethanol consumption led to loss of hippocampal pyramidal cells and granule cells in the dentate gyrus. Also pertinent is the observation by West et al (1981) that the organization of mossy fibers in the hippocampus was significantly altered in rats exposed to alcohol prenatally,
due to the appearance of an aberrant band of intrapyramidal fibers in hippocampal subfield CA3a.

In the rat, most development of the hippocampus occurs during first 3 weeks postnatally (Altman, et al 1965). Functionally, the hippocampus is involved in the processing of memory and in the control of emotion (Sahgal 1980). However, little is known concerning the kinetics of zinc uptake by the hippocampus, especially in relation to the period of development when zinc accumulation is most active.

The co-occurrence of unusual amounts of both zinc and an apparent NGF-like factor in the mossy fibres suggests that the two substances may be physiochemically related.

The mechanisms by which divalent metal ions exert their influence on neuronal function, although the subject of wide speculation, is, as yet, unknown.

1.19.1 Zinc and mossy fibres

Zinc ions may be important regulators of opioid peptide action in brain region where they are co-localized (Stengaard, 1982). Opioid peptides and zinc in the mossy fibres may interact in physiologic regulation of hippocampal excitability.

The mere co-localization of zinc and opioid peptides in mossy fibres has suggested to some investigator that there
may be functional interaction between them (Stengaard et al 1981).

Severe zinc deficiency in rats markedly affects the electrophysiological response of the hippocampus mossy fibres to stimuli (Hesse, 1979).

1.20 Zinc and Blood Brain Barrier

Kasarskis, (1984) has shown that intraperitoneal pulse of $^{65}$Zn penetrate very slowly into the rat brain. Intracerebroventricular injections of zinc lead to epileptic seizures in experimental animals possibly caused by inhibition of Na$^+$, K$^+$ - ATPase (Donaldson et al., 1971). The concentrations of zinc in 14 regions of rat brain, including the hippocampus, were often higher than the administered doses producing convulsive seizures (Ebadi Itah, et al 1981).

The mechanisms operating at the blood-brain barrier level on zinc uptake into the brain are yet to be determined. Due to low lipid solubility of ionized substances, zinc would be expected to cross the cerebral capillaries very slowly under normal physiological condition, unless facilitated by transport system. The finding that zinc concentration in the mossy fiber region is about 20 times higher than in plasma and about 100 times higher than in cerebrospinal fluid suggests the possibility of a selective, perhaps high affinity uptake system. However, one of the major problems in
the understanding of the effects of zinc 'in vivo' is represented by our scarce knowledge of the ratio between free and bound zinc in the brain, as well as of the regulation of its homestasis.

The blood-brain barrier presents technical problems for 'in vivo' studies. However, there is good evidence that materials injected intracerebrally are distributed throughout the brain a short time after injection. Opening of BBB may prove useful for the delivery of therapeutic agents into the brain. There are other potential uses eg., trace elements, or agents that may modify appetitive behaviours, such as hunger, Na appetite and thirst (Denton 1982). Blair et al (1990) have shown that (I) CSF zinc concentration in sheep is approximately one tenth of the total concentration of zinc in plasma. (II) Zinc administered intravenously (i.v.) was almost completely excluded from the CSF. (III) Increased cranial blood osmolarity or extracellular space or (ECS) increased CSF Zn in sheep. (IV) CSF Zn subsequently fell towards the low values of zinc in normal CSF. (V) The animal suffered no evident ill effect. It is also possible that the treatments also opened the BBB to protein and that some of zinc appearing in CSF entered with this protein.

The reduction of CSF Zn after the peak value as well as indicating reduced rate of entry as the BBB closed, suggests
clearance of zinc by uptake into brain tissue. Possibly, transport out of the CSF compartment by the choroid plexus occurs (Kasarskis, 1984).

Wensin et al (1987) have shown that the rate of uptake of $^{65}\text{Zn}$ into synaptosomes is dependent on $[\text{Zn}]$ free. In presence of the strong zinc binder EDTA, $[\text{Zn}]$ free will be almost zero and no $^{65}\text{Zn}$ uptake is observed. Regarding the 'in vivo' situation, zinc is transported from the plasma into the brain across the capillary endothelial cell and/or across the choroid plexus. At present, no data are available on this mechanism of zinc transport. Once inside the brain, zinc must be distributed over the brain cells via the brain interstitial fluid, the composition of which is generally believed to be identical to that of the CSF.

Release of substantial amount of zinc into the brain interstitial fluid has been observed from the zinc-rich hippocampal mossy fibres after neuronal stimulation, both 'in vivo' (charton et al; 1985) and in hippocampal slices.

The brain's requirement of zinc (Zn) and other essential trace cofactors differs both quantitatively and qualitatively from its need for metabolic substrates. Substrates must be immediately available to the entire brain in order to respond rapidly to changing metabolic demands (Duffy, 1982; Pappius, 1981). In order to achieve this, transport of these compounds is regulated by specific
systems, localized to the cerebral capillary network, which are saturable, stereospecific, and bidirectional (Pardrige, 1977). In contrast, micronutrient co-factors must be available in minute, but stable, amounts for enzyme activation and other functions. Moreover, their transport must be relatively independent of cerebral metabolic activity and dietary intake because of potential disruption of the membrane ionic milieu (Kawa, 1979).

Zinc inhibits the binding of glutamate, aspartate neurotransmitters and opioid peptides to their respective receptors (Stengaard-Pederson, 1982).

Experiments by Kasarskis (1984) and Wensink et al. (1987) have suggested, however, that zinc homeostasis in the brain is more likely to be affected by a mechanism where-by the rate of efflux of zinc from the brain interstitial fluid back into plasma is the determining factor. Moreover it was demonstrated that, once inside the brain interstitial fluid zinc is rapidly distributed over the different brain subcellular fraction (Wnsink et al., 1987).

1.21 Histochemical consideration

Both histochemical and combined histochemical electron microscopic studies indicate that zinc in the Ammon’s horn is highly concentrated within the giant boutons of mossy fibres
that terminate at the apical dendrites of the pyramidal cells (Hauq, 1967).

Histochemical staining for metals in the normal mammalian brain reveals their presence in a number of neural pathways (Haug, 1973). In the normal rat hippocampus, transitional metals (e.g. zinc, copper, lead) are present (Danscher et al 1976) in a striking series of laminae. The very dense staining of one particular bundle of axons, the mossy fiber pathway formed by the axons of the dentate granule cells, is attributed to the high concentration of zinc that is present in the mossy fibre synaptic boutons (Haug, 1967, Hassler et al 1968). Hippocampal mossy fibres are among the most densely stained with sulfide histochemical methods for demonstrating heavy metals (Maske 1955).

1.21.1 The Timm's Sulfide Method

Timm's method detects several metals that may be precipitated with sulfide, but the different metals cannot be discriminated by this method. The staining of the mossy fibre layer is confined to boutons. The Timm's stainable material in the mossy fibre boutons is generally thought to be zinc, whereas there is hardly any evidence, so far, on the nature of the Timm-stainable material associated with other fibre systems.
1.21.2 The Dithizone Method

The dithizone method stains the whole brain. In the hippocampus the hilus fascia dentata and CA3 mossy fibre zone stain deep red. Maske (1955) showed that dithizone could react with many metals, e.g. manganese, iron, cobalt, nickel, copper, cadmium and lead.

At high magnification dithizone stains tissues in the form of dense "granules" or "grains" which are distributed unevenly throughout the tissue. These dithizonate granules have been variously interpreted in the past as, for examples, concentration of zinc in secretory granules (Okamoto, et al 1966) or concentration of zinc in mitochondria (Fleishhauer 1957). However, careful work by Logothetopulous et al (1961) suggests that the granules are artifacts.

1.21.3 Zinc-zincon complex

The zinc-zincon method stains the whole brain in the form of uniform reaction-product. The nuclei are light brown in colour, while the cytoplasm and the nerve fiber stain very light blue. This difference in colour is due to difference in physiological state of zinc in the nucleus and cytoplasm (Hasan 1977).

1.22 Zinc and gene expression

The importance of zinc-binding finger-loop domains in DNA-binding proteins as regulators of gene expression has
been recognized. The first finger protein to be recognized was transcription factor-IIIA of *xenopus laevis*, which contains tandem repeats of segments with thirty amino acid residues, including pairs of cysteines and histidines (Miller et al 1985 and Klug et al 1987). The presence of zinc in these proteins is essential for site-specific binding to DNA and gene expression. The zinc ion apparently serves as a strut that stabilizes folding of the domain into a finger loop, which is then capable of site-specific binding to double-strand DNA. The zinc finger-loop proteins provide one of the fundamental mechanisms for regulating gene expression of many proteins. In humans, the steroid hormones (and related compounds such as thyroid hormones (cholecalficiferol and retinoic acid) enter cell by facilitated diffusion and combine with respective receptors which contain the DNA binding domain of the zinc finger either before or after entering the nucleus. Complexation of a hormone by its specific receptor evidently initiates a conformational change that exposes the zinc finger loops, so that they bind to high-affinity on DNA and regulate gene expression (Hollenberg et al 1985 and Hughes et al 1988).

The clinical significance of zinc fingers in hormone receptors was demonstrated by Hughes et al (1988), who analyzed the amino acid exchange at the tip of the CI finger loop, and in the other family there was a single amino acid
exchange at the top of the CII finger loop. Evidently, both finger loops are essential for correct binding of the vitamin D receptor to its hormone response element.

1.23 Types of cells in the central nervous system (CNS)

The central nervous system (CNS) has a large population of cells in addition to neurons and macroglia, which are or have the potential to become, phagocytic. This group of cells is heterogeneous and consists of subpopulations of several cell types (Jordan et al 1988, Oehmichen et al 1978) each with its own topographic location, although some intermixing is possible. The cells have many functions in common but also may have some specific antigens and functions, and may even have different embryonic origins (Tsuchihashi et al 1981, Wood et al 1979).

The CNS has at least five major subpopulations of macrophages. Microglia are scattered throughout the CNS and most likely represent the resident macrophages. Supraependymal macrophages are found on the ventricular side of the ciliated ependymal cells, epiplexun cells are associated with choroid plexuses, pericytes are distributed along the cerebral blood vessels and-derived monocytes/macrophages; enter the CNS primarily at the site of injury, but may possibly also be present in small number in normal non-traumatized CNS. Additionally, astrocytes,
oligodendrocytes and ependymal cell may, in specific situations, become phagocytic.

Furthermore, each of the cell types in the phagocytic cell subpopulations described above can appear in different states, resting or functional, which are associated with remarkable changes in the cell surface antigens and receptors for cytokines (Freik, et al 1988).

Cultures of astroglia when subjected to nutritional deprivation, form a large number of macrophage-like cells. This supports the hypothesis that the macrophage-like cells are of neuroectodermal origin and probably correspond to resident microglia of the CNS. (Hao, et al 1991).

1.24 Hydrocephaly:

When there is an excess volume of cerebrospinal fluid, the condition is known as hydrocephalus, of which there are several types. External hydrocephalus, in which the excess fluid is mainly in the subarachnoid space, is found in senile atrophy of the brain. Internal hydrocephalus refers to dilation of the ventricles. The term communicating hydrocephalus refers to a combination of internal hydrocephalus and subtentorial external hydrocephalus. In this instance, the flow of cerebrospinal fluid through the incisura of the tentorium around the mid-brain is obstructed.
1.24.1 Ultrastructural changes in hydrocephaly

Moderately increased cerebrospinal fluid (CSF) pressure over a period of years can lead to extreme ventricular enlargement and thinning of the cortex, and yet, even in the absence of treatment, this need not necessarily lead to neurological and intellectual handicap (Lorber, 1983). Hasan et al (1990) have shown that hydrocephalic oedema in the brain tissue is in the form of swollen astrocytic processes and enlarged extracellular spaces filled with fluid.

1.24.1.1 The Neurons

In hydrocephalic infants, the neurones continue maturing (Hasan et al, 1989a). Furthermore, the increased instance of growth cones and a few maturing neurones apparently stuffed with ribosomes clearly demonstrate that the ultrastructural machinery for protein synthesis and neuritic growth is not irreversibly damaged by the hydrocephalic oedema.

A number of dark neurones exhibiting abundant ribosomes and a network of rough endoplasmic reticulum but with well-preserved nucleus and perikaryal profiles occur occasionally. Some dark neurones exhibiting degenerating mitochondria and a number of electron-dense bodies, lysomes or lipid were also detected. However, many neurons were having near normal organelles and rough endoplasmic reticulum (Hasan et al, 1989a).
1.24.1.2 The capillaries

In the microvasculature of infants with hydrocephalic condition there is marked quantitative increased in pinocytotic vesicles on the contraluminal aspect, mainly in pericytic processes (Hasan et al 1989b).

Presence of numerous pleomorphic vesicles in the endothelium of vessels and swollen pericyte in hydrocephaly suggests a flow direction toward the capillary lumen in hydrocephalic human. These numerous pinocytotic vesicles and long endothelial processes protruding into the lumen (Glees, et al 1989) suggest that the hydro dynamic conditions in hydrocephalus promote an excessive fluid transport toward the cerebral capillary lumen. The normal brain capillaries are characterized by the occurrence of very few pinocytotic vesicles (Wolff 1963). A quantitative estimate of micropinocytotic vesicles in the pericyte processes recently revealed that at times more than 10-fold increase in their number per square micrometre occurs in hydrocephalic oedema (Hasan and Glees, 1989b).

The cerebral capillaries provide the blood-brain interface regulating tissue exchange. Any alteration in the morphology of the vessels may well influence or control cerebral metabolism. Vascular changes also may influence the volume and pressure of the cerebro-spinal fluid (CSF). Nakagawa et al (1984) have shown widening of the
interendothelial cleft between the tight junction and formation of interendothelial blisters. The astrocytic processes control transport of substances to and from the nervous tissue (Wolff 1967). The three basic components of the capillary wall are: 1) endothelial cell 2) basal lamina 3) Pericytes and the controversial perivascular space. The perivascular space is said to be wide and irregular in the human embryo and early foetus (Xiang et al 1988) Glees et al (1967) have reported a 150-200 A wide perivascular space in the 10 day-old mouse embryo. Glees, (1990a) have shown that in hydrocephalic human infants the endothelial nuclei as well as surrounding perikarya are flattened or concave towards the lumen but occasionally the nucleus indents the latter. On the abluminal aspect an electron dense basal lamina is ordinarily visualized which shows clefts to enclose pericytes or processes of pericytes lying flat around the capillary wall. The basal lamina forms the boundary between the capillary and the surrounding perivascular tissues. The micro pinocytotic vesicles were mostly lying free in the endothelial cytoplasm. The vesicles were present almost in the entire capillary wall, from without inward. Also, the pericytes enclosed by thickened basal lamina, exhibited large vacuoles besides micro-pinocytotic vesicles, pleomorphic mitochondria and electron dense cytoplasm containing many microfilaments.
Bruns et al (1968) have demonstrated that vesicles were capable of transporting macromolecules when injected into the circulation. The vesicles are thus considered as the morphological equivalent of the large pore system.

The mean transit time taken by vesicles for transendothelial passage has been estimated by various methods to be 15 to 24 seconds (Casley-Smith 1977). Pinocytosis is a bidirectional phenomenon (Lorber, 1983). Flynn et al (1989) have reported that hydrocephalic "interstitial" oedema may result as the obstruction of ventricular system forces CSF into the periventricular areas. Glees (1990b) are of the opinion that with the passage of time, this interstitial oedema is, to some extent, resolved by the transendothelial transport of excess fluid.

1.24.1.3 Pericytes

The pericytes are ordinary enclosed within a common basal lamina with the endothelial cells of small blood vesccels (Rouget 1873). Pericytal phagocytes have also been reported in the cerebral microvasculature (Torack 1961 Hasan et al 1974). The presence of numerous lysosomes in some pericytes suggested that the cells are phagocytic even in normal circumstances (Lafarga, et al, 1975, Glees, 1987). Pericytes apparently represent an intermediary stage in the differentiation of microglia. They can migrate into the brain tissue and aquire the function of microglial elements.
(Baldwin et al 1969, Baron et al 1972). Pericytes appear in
the brain at the time of vascularization (Peters et al
1970).

Pericytes are also called extravascular or
juxtavascular mitotic cells, which give rise to microglial
cells. Furthermore, Jeynes (1985) showed that a
subpopulation of pericytes, the so-called granular pericytes
function primarily as phagocytes and increase significantly
after ischemic insult. Farrell et al (1987) claimed that a
great majority of pericytes are of granular type and if true
granular pericytes do exist in human cerebral
microvasculature. They must constitute less than 5% of the
population. The uptake of debris by microglia would assign a
definite function for microglia in the normal brain, a
function which is not limited to the removal of dying nerve
cells or their processes. The perivascular microglial cells
are bone-marrow derived (Cammermeyer 1970, Hickey et al
1988). These cells would not have to wait for the occurrence
of infection or trauma to the nervous tissue but would
already normally carry out such removal duties. The
specialized neuron on its own is incapable of coping with
waste material (Glees 1985). Besides phagocytosis, other
functions assigned to pericytes include: their involvement in
the synthesis of basal lamina (Rhodin 1968), offering
mechanical support and stability to vessel walls and forming
a protective layer between endothelium and interstitium (Cancilla et al. 1972). Majno (1965) emphasized the morphologic similarities between endothelial cells, smooth muscle cells and pericytes and suggested that in times of functional demand pericytes may differentiate into smooth muscle cells. Their possible role in the defence reacting and antibody formation was stressed by (Movat et al. 1964).

The morphological relationship of the pericyte to the capillary plays an important role in blood-brain barrier (Dodson et al. 1975). In human oedema, pericytic micropinocytotic, transport may be regarded as loss of their barrier function (Hasan, et al 1989b) by formation of transpericytal channel by process of membrane fusion (Casteyon 1982) in human edema aggregation of pinocytotic vesicles oedema on the contraluminal aspect of pericytes, suggests that the fluid is transported by these cells from the extracellular spaces towards the capillary endothelium (Hasan et al. 1989b). This is contradictory to the results of Brightman et al. (1970) who claimed that the passage of horse-radish peroxidase was blocked regardless of the direction from which the tracer reached the endothelium.

An important argument for the vesicular transport is the electron microscope (EM) appearance of tracer molecules in the pericytal vesicles when injected into the lateral ventricle of the brain (Wagner et al. 1974).
It is possible that besides their role in hydrocephalic edema resolution, these phagocytic cell may normally function as scavengers removing the debris of cell death normally occurring during perinatal development (Hasan et al 1989b).

1.24.1.4 The Neuroglia

Neuroglia (nerve glue) was first described by Virchow (1846) as an independent cellular and fibrous element of the central nervous system which is different from normal connective tissue. Neuroglia surround the blood vessels in the CNS (Glees, 1955). Oligodendroglia include's the numerous "interfascicular glia" of the white matter and a larger part of the perineuronal satellites of the gray matter. Microglia, morphologically, biologically and developmentally differ from neuroglia. (Brierley J.B. 1982) while microglia (mesoglia) was proposed as the true "third element" microglia were described as small cell with dark nuclei. The phagocytic role of microglia was emphasized from the very beginning by (Rio Hortega 1919). Still microglia cells were not difficult to identify with electron microscope that they were described ambiguously by many authors (Bodian 1964 and Herdorn 1964 Robertson 1900). A number of investigators doubted the existence of microglia in normal nervous tissue (Maxwell, 1965). For a long time, microglia were considered to be a type of neuroglia (Mori et al Robain 1970) or even a simple form of oligodendroglia without relation to phagocytic elements present in pathological processes, in that "a
subdivision" of glia which includes the "microglia" involved in repair of brain injury remains open to question (Maxwell et al 1966).

Peter et al (1991) have reported that in visual cortex of young monkey, the microglia have very similar nuclei to the oligodendrocytes, but their cytoplasm is usually paler. In addition, the microglia cell bodies seem to mould themselves to the elements in the surrounding neuropil, so that they have irregular shapes, and their long cisternae of rough endoplasmic reticulum, which are studded with distinct ribosomes, have contents similar in density to the cytoplasm. Consequently, microglial cells can be readily distinguished from oligodendrocytes in thin sections, but it is often difficult to distinguish between them in light microscopic preparations.

In young monkeys microglial cells frequently contain a few inclusions in their perikaryal cytoplasm. But in the older animals nearly all of the microglial cells contain significant amounts of membrane-bound inclusions. Some of the inclusion bodies exhibit closely packed, dark granules of slightly varying density, but in other inclusion the granular contents are dispersed in clump and it is difficult to be sure that they are isolated from the cytoplasm by a membrane. Yet other inclusions are vacuoles containing a few lamellae, and some have a fruity appearance (Peter et al
Indeed, there is a variety of inclusions within microglial cells and they can be so large that they almost entirely fill the cell body, pushing the nucleus to one side. Because of this it is not unusual to encounter profiles of microglial cells that are little more than thin bags of cytoplasm surrounding inclusions that can be 5 to 10 um in diameter.

It is apparent, then, that of the neuroglial cells, the microglia contain the most heterogeneous inclusions and that their inclusions are more numerous and frequently larger than those within either the astrocytes or oligodendrocytes, since it is generally accepted that microglial cells are the prime phagocytes of the brain (Blakemore, 1972, Torvik, 1972, Vaughan et al., 1974; Dolman, 1985). These cells are presumably responsible for removing some of the degenerating myelinated axons, dendrites and axon terminals that are encountered in the visual cortex (Vincent et al. 1989) and in other cortices in aging animals (Rees, 1975. Mervis, 1981. Peters and Vaughan 1981).

Attempts to provide diagnostic features for differentiation between glia cell types relied on the studies from light microscopy of such criteria of the size and general configuration of the cell, shape and density of the nucleus, the amount and density of the perinuclear cytoplasm, the number and nature of processes and positional
characteristics, such as the relationship to blood vessels, neuronal perikarya, and nerve fibres. Maxwell et al (1966) differentiating the macroglia cells into "astrocytic" and "non-astrocytic" types astrocytes would be identified with reasonable certainty by the unique presence of glycogen granules and bundles of microfilaments in their cytoplasm. The "non-astrocytic" macroglia was observed to display wide range of nuclear and cytoplasmic densities. Hasan et al., (1972) highlighted the positive features for the ultrastructural identification of oligodendrocytes: (1) Large dilatation of nuclear cleft; (2) highest density of inhomogenously distributed chromatin near the nuclear membrane and irregular light patches interrupting the dense chromatin at the nuclear membrane, usually identified as the sites of nuclear pores.

In general, the oligodendrocytes are known to exhibit densely packed perinuclear circumferential organization of the organelles, uniformly dark condensed cytosol, and abundant free ribosomes and ribosomal rosettes (Bunge 1968). The shape of the nucleus varies markedly, possibly depending upon the plane of the section (Hasan M, et al 1977). Microgliacytes, on the other hand, commonly have an elongated nucleus, which is seen both cross sectionally and longitudinally (Moris, et al 1969) chromatin masses are ordinary dense in contrast to the light nucleoplasm between
them, and for the most part line the nucleus envelope. The cytoplasm is usually minimal with few mitochondria and little endoplasmic reticulum, although occasional microglia show a large cytoplasm with many electron dense bodies. Although in the classical literature microglia are recognized as phagocytes in the central nervous system (Glees 1955), fat-stained or phagocytosed material was long ago identified also in reactive oligodendrocytes (Ferraro 1928).

Hasan et al (1972) reported that in the deafferented lateral geniculate body of the monkey, reactive oligodendrocytes containing electron dense debris. Spoerri et al (1979) have demonstrated oligodendrocytes containing heterogeneous electron dense structure in the area postrema of the rat and in the human cerebral cortex distant to brain tumors. It is of interest to note the presence of lipid vacuoles in the cytoplasm of cells which correspond to the classical geniculate nucleus of blinded monkey (Hasan et al 1974). It is apparent that the exact identification of different type of glial cells has been a vexing problem ever since the discovery of neuroglia. The existence of transitional (or intermediate) forms between astrocytes and oligodendroglia on the basis of their ultrastructural features were reported by (Farquhar et al 1957). Glia cells were found possessing "some feature ordinarily ascribed to astrocytes and some feature ordinarily ascribed to oligodendrocytes (Mugnaini et al 1964).
Glees et al (1990b) reported that in human brain and particularly in the wake of hydrocephalic edema, the cytological features of macrogliacytes and microgliacytes are likely to differ from the classical description of mature cells in the normal human brain. The astrocytic processes in the hydrocephalic condition were swollen and oedematus but characteristic astrocytic nuclei with perikarya exhibiting distinct micro filaments were only occasionally present. While classical interfascicular glia and perineuronal satellite, oligodendrocytes were more in number. At times, two or more oligodendrocytes were in close contact. The cytoplasm of these oligodendrocytes was found to be confluent in the periphery while very thin electron dense line separated them. In some cases, up to three oligodendrocytes nuclear profiles were found close together not separated by anu electron dense line. These were the oligodendrocytes in the process of division. Some oligodendrocytes showed presence of lysosomes in the vicinity of nucleus. Oligodendrocytes with irregular nuclear configuration contained electron-dense cytoplasm rich in ribosomes and vacuoles. A number of growth cones were also seen in close proximity. Some oligodendrocyte nuclei showed marked invagination and in some, a tangential section of the nucleus showed sequestered intranuclear cytoplasmic inclusion. Microgliacytes with rod-shaped nucleus were also present.
Some microglia showed irregular nuclei with a number of vacuoles and lysosomes in the cytoplasm. In some cases microfilament were also present. Some globular microgliacytes were full of vacuoles and contained bundles of microfilaments. Glees et al (1990b) concluded that the maturing glial cells differ markedly from the classical adult type. Many transitional forms were, apparent Microtubules were not observed in the astrocytic cytoplasm at any stage of development; however, the characteristic microfilaments were commonly observed. The function of abundant microfilaments are not well understood. They may provide rigidity to the astrocytic processes or endow them with contractile properties. Whereas mature oligodendrocytes are known to exhibit fewer visible processes, the maturing oligodendrocyte may possess a number of processes and abundant rough endoplasmic reticulum (Phillips 1973, de Vaughn 1969). Ludwin (1984) reported even in the mature oligodendrocytes, proliferation freely occurs after trauma to the central nervous system. Previously, it was thought that mature oligodendrocytes in the adult mammalian brain were post-mitotic and unable to proliferate even in respect to injury. This failure of oligodendrocytes to undergo mitosis was cited to explain the failure of the human CNS to undergo remyelination after demyelinating disease. But Ludwin (1984) has convincingly showed that mature oligodendrocytes in adult animals, as well as astrocytes and microglia are able to respond to damage in
the CNS following trauma by incorporation of tritiated thymidine into their nuclei. Although the nature of stimulus to proliferate is unclear, it is possible that these cells are responding to axonal reactions following neuronal death at a molecular or chemical level. Recently, Glees et al (1988) have shown neuronal degeneration due to hydrocephalus in the maturing human frontal cortex. Maxwell and Kruger (1965) considered the dense bodies of oligodendrocytes to be the products of "normal" degenerative processes such as those related to aging. The occurrence of similar dense bodies in the maturing oligodendrocytes can well be explained on the basis of neuronal death and consequent axonal alteration known to occur during development which have been further influenced by hydrocephalic oedema (Glees et al 1990a). The unambiguous differentiation between oligodendrocytes and microglia is, at times, difficult. In a great majority of cases, the occurrence of inclusion bodies and vacuolar profilers tilts the balance in favour of microglia. A variety of cell types, endogeneous as well as exogeneous, have been regarded as sources of phagocytes when neurones of the CNS have been destroyed. (Hasan et al 1989b). According to Brierley et al (1982) source of phagocytes will be determined by the nature of injury (toxin, hypoxia-ischemia, infection trauma, etc.) and whether damage is restricted to neurones or involves glial and mesodermal elements upto the level of information (with or without hemorrhage). Some authors stress
the migratory activity as disclosed by Smart et al (1961) by autoradiographic means. They consider microgliacytes as only temporary guests in brain tissue. However, in normal and pathological studies of the CNS it seems advantageous to take their presence for granted and to retain the term microglia well without replacing it by the term macrophage or histiocytes (Glees 1972). When attempting to prepare (on the basis of richness in ultrastructural organelles) a graded scale of metabolically active neuroglia, one is led to conclude that the most metabolically active neuroglia cell is the perineuronal satellite oligodendrocytes. Then follow the astrocytes, and last, the microglia (Glees et al 1990b). The maximum number of oligodendrocytes were found in layer VI of the motor cortex and layer V of the lateral geniculate body. The presence of many types of vacuoles, lipid bodies and array of microfilaments in the maturing and hydrocephalic subjects was remarkable. Vacuoles and intracellular debris indicate their phagocytic role and the richness in microfilament is suggestive of mobility of these cells, (Glees, 1972).

The oligodendrocytes and particularly microgliacytes play a supporting role in oedema resolution (Glees et al 1989). Many amoeboidal microgliacytes were detected in the midst of oedematous astrocytic processes. Hasan et al (1989b) have shown these proliferating oligodendrocytes and microgliacytes in hydrocephalic human brain.
1.25 Nuclei of hypothalamus

Most regions of the hypothalamus contain population of neurons which have been divided on the basis of phylogenetic, developmental, cytoarchitectonic, neuronal circulatory histochemical studies into a number of nuclear aggregations, which have given specific names, but some are much more clearly defined than others.

In mammals, the hypothalamic suprachiasmatic nucleus (SCN) is involved in the generation and entrainment of a number of circadian rhythms. This nucleus is considered to receive information of the environment dark-light cycle via a direct neuronal input, called retino-geniculo-suprachiasmatic pathway. The efferents of SCN reach several hypothalamic and non-hypoathalamic areas. Their output is considered of importance in transformation of rhythms to other central structures. Transmitters identified in the efferents of SCN neurons are, therefore, likely to be involved in circadian rhythmicity. In recent years, the projections of suprachiasmatic neurons containing vasoactive intestinal peptide (VIP) and vasopressin have been determined (Mikkelsen 1988 Sofronier et al 1987).

Gastrin releasing peptide might play a role in the regulation of circadian rhythms in hypothalamic nuclei generated by the suprachiasmatic nucleus. (Mikkelsen et al 1991).
Growth hormone releasing hormone (GHRH) immunoreactivity was identified in neuronal perikarya in the hypothalamic arcuate and suprachiasmatic nuclei as well as in cortical and subcortical telencephalon. Fibers were most evident in the median eminence, paraventricular and periventricular nuclei and molecular layer of the cerebral cortex. Fine fibers were also accumulated in the bed nucleus of the stria terminalis and the amygdala (Edythe et al 1991).

1.26 Functions of the hypothalamus

Clinically, it has long been recognized that lesions in the hypothalamus often lead to widespread and bizarre combinations of symptoms and signs, which stem from endocrine, metabolic, visceral and behavioural disturbances. The hypothalamic action depends upon different information channels, both nervous and vascular, and that it is interlocked, both structurally and functionally with higher regions of the nervous system. The latter include the complex of structures which includes the limbic lobe or system and the pre-frontal regions of the cerebral cortex. The suprachiasmatic region in some, but not all, mammals synthesises either oxytocin or ADH as part of a larger precursor molecule. In the rostral part of the supraoptic nucleus, oxytocin and ADH neurons are equally distributed, but in the caudal part only oxytocin neurons are found.
In mammalia the frontal cortex, limbic system, hypothalamus, and lower regions of the brain stem and spinal cord are conveniently regarded as forming a hierarchy of controls particularly directed towards those homeostatic cycles, which are mediated by the autonomic nervous system, the endocrine system, and the locomotor patterns associated with them.

1.27 Nucleus ceruleus

It is a bluish-grey area in the floor of fourth verticle, which does its color to a group of pigmented nerve cells as seen through overlying tissue. Dahlstrom et al (1969) and Fuxe et al (1970) demonstrated that all the centrally placed neurons of the nucleus are noradrenergic. Stereotactic lesions of both nuclei coerulei (in rats) showed that they were the main and probably sole source of fibres constituting the dorsal noradrenergic bundle, part of the tegmental fasciculus through which they approach some of their many destinations (Ungerstedt 1971) Ramon-Moliner et al (1974), however, have demonstrated in the feline nucleus ceruleus high concentrations of acetylcholinesterase. Locus ceruleus cells are characterized by thin processes that resemble axons and arise directly from the perikaryon (somatic gemmules).

Moreover, the locus ceruleus seems to be active in physiologic process like regulation of respiration micturition and the sleep-wake-rhythm.
1.28 Zinc and Free radicals:

Zinc may also intervene in nonenzymatic free radical reactions (Anonymous 1978 cited by Lancet). In particular, zinc is known to protect against iron-catalyzed free radical damage. It has bee known that the free-radical oxidation (autooxidation) of polyunsaturated lipids is most effectively induced by the interaction of inorganic iron, oxygen and various redox couples. This interaction may be responsible for the pathological changes and clinical manifestations of iron toxicity. Iron-catalyzed free radical oxidation is known to be inhibited by zinc, ceruloplasmin, metalloenzymes (catalase peroxidases, and zinc-and copper-dependent superoxide dismutase), and free radical-scavenging antioxidant vitamin E.

Bettger and O'Dell (1981) hypothesized that zinc plays a biochemical role analogous to that of vitamin E by stabilizing membrane structure and thus reducing peroxidative damage to the cell. Carbon tetrachloride-induced liver injury is another model for studying free radical injury to tissues. Animals maintained on a high zinc regimen are resistant to this type of biochemical injury, thus suggesting that zinc may be protective against free radical injury. Zinc also inhibits the analogous metna amidazole-dependent free radical sequences.
Free radicals being very transient species merely set in motion a destructive chain reaction. Where lipids react with free radicals they undergo a series of molecular rearrangement termed peroxidation and form a number of oxidation derivatives including lipid peroxides, lipid hydroperoxides and aldehydes. Cell damage may be caused by active oxygen metabolites such as hydroxyl peroxidation/superoxidation, anion and hydroxyl radical.

1.29 The morphology of various types of cell death in prenatal tissues:

Large number of necrotic cells are seen when studying embryonic tissues. Morphologically, they occur in nearly all parts of the embryo, and may range from isolated instances to being so numerous in certain areas that whole organs or tissue regions are thus broken down (Nussbaum 1901, Emrnst 1962, Kallius 1931) Physiological cell death is an important component of normal morphogenesis, yet little is known about its trigger and course (Saunders, 1966).

It is obvious that endogeneous (i.e., genetic) factors are important because necrosis always appear in the same place and at the same stage of development in various species. But it is not yet known whether the determinants are situated in the cell types. (Hamburger 1952, Jurand 1965, Ballard et al 1968, Fallon et al 1968).
It is also necessary, when carrying out teratological and embryotoxic experiments to identify the physiological necrosis and to compare them with those due to toxic substances. It would be a mistake to use physiological necroses as indicators of teratogenic or toxic effects, and if morphological differences between physiological and experimentally induced necrosis could be found, it would be possible to avoid this error.

The development of the nervous system involves a complex, precisely timed series of events that includes cell proliferation, migration extension of processes, Synaptogenesis, and cell death. One molecule that has been implicated in some of these events is the growth associated phosphoprotein (GAP-43). Neurons growing either 'in vivo' or 'in vitro' show high levels of GAP-43 expression coincident with the beginnings of outgrowth of processes (Costello et al. 1990, Federoff 1988) with the protein being conveyed in the rapid phase of transport down axonal, but not dendritic, processes (Gosling et al. 1988; Skene et al 1981).

It is quite likely that GAP-43, through its regulation by protein kinase C, participates directly in the process essential for the growth of axons and the formation of synaptic relationships (Dani et al 1991). Moreover, as has been suggested, the persistence of the protein in certain regions of the neuraxis may reflect synapses in which dynamic
remodeling may remain possible throughout life, perhaps in relation to functional plasticity (Dani et al 1991).

1.30 Prenatal development in neocortex of rat

Most of what is known about mammalian brain development has been derived from studies of altricial (born in an immature state) species, presumably because they allow easy access to early developmental stages (Brunjes 1988). Many species, however, are born relatively mature and thus may exhibit different rates and patterns of early brain growth. The laboratory rats are born after 22 day gestation period lacking fur and with only rudimentary motoric and sensory capabilities. The terminology used here is based on the description of cortical development by Astrom (1967), Derer (1974), Marin et al (1982), Luskin et al (1985) and Rakic (1988).

1.30.1 Postconception day (PC) 14

At this stage the developing neocortex contains a deep germinal and superficial marginal layer. The marginal zone contains occasional cells, the first neurons to be found in the region. The layer will subsequently be split by the formation of the cortical plate (Luskin et al 1985 Marin et al 1982).

1.30.2 Postconception day 18

Since PC 14 the rat has undergone substantial changes including the formation of several distinct layer within the
neocortex. Closest to the ventricle is a prominent germinal zone. A broad intermediate zone, the future white matter of the cortex, lies above it, and is divided into (a) a deep or subependymal layer containing occasional mitotic figures, (b) an intermediate zone with decreased cell density and tangentially oriented cells and fibers, and (c) a superficial plexiform layer which contains occasional neurons and which forms the deep border of the cortical plate. The plate is approximately 7 cell bodies thick in the dorsal regions of the pallium, and becomes thicker more laterally. Differentiated neurons are seen at the border (Sub-plate) between the cortical plate and the superficial aspect of the intermediate zone. The most superficial layer is the marginal zone, which will become layer 1 of the neocortex. The cortical plate gradually thins medially and ventrally, where it will form the developing pyramidal cell zones of Ammon's horn. A few cells cap the medial end of the cortical plate representing the beginning of the dentate gyrus. More medial still is a cell free zone representing the developing fimbria.

1.30.3 Post conception day 20

The developing cortical region contains all the layers seen at previous age. The cortical plate has reached its maximum thickness. The region deep to the plate contains a broad strip of radially oriented neurons representing the
deep layers of the neocortex. This subplate region of differentiated neurons is as thick as that of the plate. The plate shows few signs of stratification. The developing dentate gyrus is now easily seen as a large cloud of cells bounding the medial end of the cortical plate.

1.30.4 Postconception day 22

Cortical plate has become much more loosely packed and contains only the rudiments of layer 2 and 3. The plate is no longer continuous with the developing pyramidal zone of the hippocampus. The layers which have emerged from the deeper portion of the plate (subplate) are now 3-4 times deeper than the plate. The dentate gyrus is formed, with the suprapyramidal blade being much more distinct than the infrapyramidal. The hilus contains a relatively high density of cells.

1.31 Control of cell number in the developing neocortex

In a variety of developing vertebrate neural system, cells, dendrites, axons and synapses are initially overproduced and subsequently reduced by a variety of regulating processes (Cowan et al 1973, Hamburger et al 1982, Oppenheim, 1981, Purves, et al 1980). Cell death has been shown in several systems to serve in correction of early errors of connectivity, the matching of cell number in interconnecting populations, and in the sculpting of in homogeneity in the density of cell distribution.
(Oppenheim, 1981 Purves et al 1980, Sengelaub et al 1986). In the development of neocortex neuronal loss occurs but its function have not yet been elucidated.

This normal neuronal loss in the neocortex, variable by cortical layer and area has been demonstrated in a variety of ways, including cell count in early development (Heumann et al 1977, Heumann, et al 1978), comparison of early and late cell numbers in thymidine-labelled material (Kane, 1984) and observation of pyknosis (Pearlman, 1985). All counts indicate relatively large amount of neuronal loss in the last-generated, external cortical layer (11-IV) and lesser amounts of loss in the earliest generated, internal layer (V and VI). Cortical areas vary widely in the incidence of pyknotic cells.

Population matching is a second possiblity for the role of cell death in the cortex (Cowan 1973).

1.32 Aim and objective of present study

In view of the demonstrated need for zinc during embryonic development, this study is mainly focussed on the, histological, ultrastructural and quantitative biochemical changes in the fetal rat brain development caused by zinc deficiencies from gestation day 12 to the completion of pregnancy (Day 22).