PART I

CHAPTER I

INTRODUCTION

INTRODUCTION

The role of nutrition in brain function although a neglected field till a decade or two ago is now eliciting interest. The possibility that nutritional factors can affect peripheral or central nervous function was dramatically brought out by the profound CNS symptoms brought about by vitamin deficiencies.

Thiamine, the first member of the B-complex vitamins can be considered as the first nutrient that has been reported to influence brain structure and function. Chromatolysis of the nerve cells, damage of basal nuclei and lesions in the brain stem and diencephalon are found in cats, monkeys, foxes and pigeons subjected to deficiency (Goldsmith, 1964). Behavioural changes have also been reported in rats (Maurer and Tsai, 1931; Poe and Muenzinger, 1939), dogs (Gantt and Wintrobe, 1945), cats (Everett,1944) and monkeys (Waisman and McCall, 1944). Aphonia is a striking feature associated with thiamine deficiency in human infants (Goldsmith, 1964). Adults suffering from thiamine deficiency show symptoms such as anxiety, depression, irritability and increased sensitivity to noise and pain (Brozek 1957; Eiduson, Geller, Yuwiler and Eiduson, 1964).

According to Bennett, Jones and Nelson (1966) convulsions associated with thiamine deficiency are due to a decreased ability of the brain tissue to oxidise pyruvate. It has been shown that the cocarboxylase content of the brain was decreased and the rate of respiration was reduced in thiamine deficient animals (Peters, 1940; Von Muralt, 1962). Increase in the pyruvate and 2-oxoglutarate levels and a decrease in the activities of pyruvate dehydrogenase, transketolase and 2-oxoglutarate dehydrogenase were also observed (Holowach, Kauffman, Ikossi, Thomas and McDougal, 1968). The thiamine content of the different regions of the brain was found to be reduced and specific sites of histological lesions were identified (Collins, Kirkpatrick and McDougal, 1970; Dreyfus and Victor, 1961). The concentration of 6-phosphogluconate, pyruvate and 2-oxoglutarate were also found to be elevated in several regions including cerebellum (Collins, Kirkpatrick and McDougal, 1970).

According to Dreyfus and Hauser (1965) transketolase is more susceptible to thiamine deprivation than pyruvate decarboxylase and a decrease in transketolase activity may be responsible for initiating the clinical and histopathological events. However, Koeppe, O'Neal and

Hahn (1964) have reported that during thiamine deficiency the ability of brain tissue to decarboxylate pyruvate is not decreased.

Thiamine is believed to have an inhibitory action on choline esterase as the concentration of acetyl choline was reported to be reduced in the brains of thiamine deficient animals (Mann and Quastel, 1940). A decrease in brain glutamate has also been reported in thiamine deficient pigeons (Peters, 1962).

The effects of pyridoxine deficiency on the central nervous system have been identified in experimental animals (Coursin, 1968; Tower, 1958a). Maternal pyridoxine deficiency in rats during gestation and lactation was found to result in decreased weight gains during the first sixteen days of life as well as lowered levels of aromatic L-amino acid decarboxylase in both brain and liver (Eberle and Eiduson, 1968). Convulsions caused by pyridoxine deficiency are shown to be associated with a decreased activity of glutamate decarboxylase and a lowered concentration of Gamma-amino butyric acid (GABA) (Minard, 1967; Roberts, Younger and Frankel, 1951; Tower, 1963). Decreases in the levels of GABA, serine and alanine

and increases in cystathionine and methionine were noted in the brains of pyridoxine deficient rats (Hope, 1964; Tews and Lovell, 1967). A fall in the activity of cystathionase was also reported (Hope, 1964). Convulsions are known to occur in human infants with pyridoxine deficiency (Maloney and Parmelee, 1954; Hunt, Stokes, McCrory and Stroud, 1954) with abnormal electroencephalographic pattern and reduced respiration of brain tissue slices (Roberts, Wein and Simonson, 1964).

Pellagra, a nutritional deficiency primarily attributed to a deficiency of niacin and amino acids is characterized by CNS disturbances such as loss of recent memory, loss of clarity, general lassitude, depression and other clinical manifestations (Goldsmith, 1956; Eiduson et al., 1964). The brain lesions found in pellagra include tigrolysis of brain stem and ganglia, nerve cell atrophy, vasodilation and chromatolysis (Denton, 1928). In experimental animals brain concentrations of NAD, NADH and NADP are found to be significantly reduced with a smaller nonsignificant decrease in NADPH (Garcia-Bunuel, McDougal, Burch, Jones and Touhill, 1962).

Riboflavine deficiency produces impaired motor coordination and learning performance in rats, though

not to same extent as thiamine (Poe and Muenzinger,1939). Behavioural abnormalities were also observed in dogs (Street, Cowgill and Zimmerman, 1941). A degeneration of neurons (Eiduson et al., 1964) and of myelin in the peripheral nerves and the posterior columns of the spinal cord has been found in dogs, mice and swine with a deficiency of riboflavine (Zimmerman, 1943).

Deficiencies of other vitamins of the B-Complex series are also shown to affect the central nervous system. Anemia caused by a deficiency of vitamin B_{12} is found to be associated with symptoms such as mental confusion (Wokes, Badenoch and Sinclair, 1955) and changes in the spinal cord and EEG patterns (Baker, 1967; Wilson and Langman, 1966). Mental symptoms have been found even in the absence of anemia (Vilter et al., 1950). A deficiency of folic acid in early life is found to result in delayed maturation of EEG. Patients with inborn errors of folic acid metabolism are found to be mentally retarded (Arakawa, Fujii and Hayashi, 1967). Deficiencies of pantothenic acid and biotin have been associated with neurological symptoms in man and with impaired learning in experimental animals (Coursin, 1968; Thornton, Bean and Hodges, 1955).

Pantothenate deficiency causes malfunctioning of central nervous system involving paralysis, coma or convulsions in a number of animal species (Gantt, 1957).

The effect of vitamin A deficiency on the functioning of the retina is well known. Apart from its function in vision vitamin A plays an indispensable role in general metabolism.

It appears that the general role that vitamin A fulfills in metabolism outside the visual cycle is that of a "membrane active" compound, which may well regulate the transport of metabolites across the membrane of the cell itself and influence the stability of the membrane of subcellular particles, thereby governing the release of enzymes from subcellular particles into the cytoplasm and the extracellular fluid (Roels, 1967). The stability of liver lysosome is greatly impaired in vitamin A deficiency (Roels, Trout and Guha, 1964). According to DeLuca, Manatt, Madsen and Olson (1963), increased oxidation rates of pyruvate, citrate, 2-oxoglutarate, glutamate, succinate and fumarate in the liver of vitamin A deficient rat are related to changes in the mitochondrial structure.

The level of liver glycogen is depressed in vitamin A deficiency. It appears that vitamin A deficiency causes a block in the incorporation of acetate-C^{14} into glycogen (Wolf, Lane and Johnson, 1957). The same authors have confirmed this by using labelled lactate-C^{14} and glycerol-C^{14}. The incorporation of these into liver glycogen was found to be reduced in vitamin A deficiency. Mucopolysaccharide synthesis in the rat colon is affected by vitamin A deficiency (Wolf and Varandani, 1960).

Disordered ossification resulting in mechanical interference with nervous tissue leading to blindness, deafness and paralysis has been reported in vitamin A deficiency (Spillane, 1955; Dam and Sondergaard, 1964). Malformation of the bones of the skull in vitamin A deficiency often leads to lesions in the nervous system because of the compression caused by close twisting of poorly developed skull bone (Mellanby, 1944). The cranial cavity and spinal canal fail to enlarge sufficiently in vitamin A deficient rats to accommodate the growing brain, resulting in dislocation of the brain towards the foramen magnum and multiple herniations of the cerebrum and cerebellum into the venous sinuses of the dura at sites of arachnoidal villi (Wolbach, 1954).

Lamming, Woollam and Millen (1954) observed hydrocephalus at an incidence of up to 75% in young rabbits whose mothers were deprived of vitamin A. They explained this as due to excessive production of cerebrospinal fluid by the choroid plexuses followed by a secondary distortion of the aqueduct. The former may be a direct effect while the latter may be due to the compression by abnormal bone growth.

Mellanby (1931) observed that vitamin A deficiency produced two types of lesions in the animals, epithelial lesions such as xerophthalmia and nerve injuries. Vitamin A deficiency produces injuries to the optic nerves which may lead to permanent blindness (Moore, 1967). Vitamin A deficiency caused a degeneration of myelin mainly in the sheaths of nerve axons (Mellanby, 1934). Roels (1967) also found a degeneration of myelin in the nerves of rabbits suffering from severe xerophthalmia. Degenerative changes of nerves were found to occur independently of malformation of bone (Dam and Sondergaard, 1964). Blindness, incoordination and spasms have been found in pigs fed the deficient diet for 6-10 months. Degeneration of the nerve in the optic thalamus, the optic femoral and sciatic nerves and spinal cord was found in

these animals (Hughes, Lienhardt and Aubel, 1929). The same authors reported that a similar degeneration has been found in other species. Electroretinograms are found to be affected in vitamin A deficiency (Coursin, 1968).

In rats on the other hand paralysis of the hind limbs was the first symptom of the deficiency. Lesions in the spinal cord consisted of degeneration of the medullary sheaths of the sensory tract. Severe deficiency led to degeneration of the anterior and posterior columns. Supply of cod liver oil removed symptoms of xerophthalmia but the spinal lesions were still present (Aberle, 1934).

Although vitamin E deficiency is rare in man, it is associated with conditions such as congenital biliary atresia and cystic fibrosis of the pancreas. In both these conditions neuraxonal dystrophy characterized by a loss of nerve cells in the nerve nuclei in the medulla has been found. Such changes were also found in experimental animals subjected to deficiency (Gordon and Nitowsky, 1968).

A deficiency of several minerals is also found to affect CNS function and is associated with neurological disorders. This may be presumed from their role as

cofactors. Demyelination in the brain is found to be associated with copper deficiency in sheep (Innes and Shearer, 1940) and guinea pigs (Everson, Sharader and Wang, 1968). Congenital abnormalities of the brain have been noted with a deficiency of zinc in the maternal diet (Li and Vallee, 1968). Extreme retardation of growth, immature hair or alopecia and dermal lesions were found during zinc deficiency in rats (Swenerton and Hurly, 1968). A deficiency of manganese in the maternal diet is found to affect neuromotor development in the progeny in rats (Shils and McCollum, 1943; Follis, 1958). Deficiencies of sodium and potassium can be expected to have serious effects because of their role in the permeability of cell membranes. It is to be noted that kwashiorkor is associated with a deficiency caused by either depletion or defective utilization of potassium (Viteri, Behar, Arroyave and Scrimshaw, 1964).

A deficiency of iodine during the prenatal period has been implicated in the etiology of cretinism, the basic components of which are irreversible changes in mental development, abnormalities in hearing and speech, neuromuscular disorders, impairment of somatic development

and hypothyroidism (Stanbury and Ramalingaswami, 1964). In experimental animals the effects of iodine deficiency are aggravated by neurological stress (Milcu, 1960). Thyroid deficiency in early life is also found to result in severe, and frequently irreversible, behavioural changes associated with structural changes in the cortical neurons, hypoplasia of the neuropil (Eayrs, 1968), retardation of myelination (Balazs, Brooksbank, Davison, Eayrs and Wilson, 1969; Walravens and Chase, 1969) and a retardation of the maturation of the energy metabolism of the brain (Cocks, Balazs, Johnson and Eayrs, 1970). Hypothyoridism induced by iodine deficiency has been found to affect glutamate decarboxylase in the brain synaptosomal fraction (Balazs, Kovacs, Teichgraber, Cocks and Eayrs, 1968).

The early studies on starvation and protein deprivation in adult animals showed no change in the composition of the brain in either condition (Folch,1947; Fulton, 1949; Lehr and Gayet, 1963). The age of the animal as well as the short term nature of the studies lead to the erroneous conclusion about the susceptibility of the brain to the effects of undernutrition and protein deficiency. The brain was looked upon as a static organ

undergoing no change after maturity. This misconception regarding the metabolic activity of the brain arose partly because of the observation that the mature central nervous system shows no sign of renewal of neurons by cell division (Leblond and Walker, 1956) and the young child has all the neurons it will ever have. This notion was reinforced by the observation that the intravenous administration of labelled amino acids was not followed by the rapid incorporation in the brain (Friedberg and Greenberg,1947). It is now known that this is because of the blood-brain barrier and subsequent studies have shown that incorporation of amino acids in brain proteins is quite rapid when they are administered intracisternally (Gaitonde and Richter, 1953, 1955, 1956).

The blood-brain barrier is a label for the differential extent and rate of accessibility of the brain to different substances present in the blood. It is interesting to note that the development of the blood brain barrier takes place during the period of rapid maturation of the brain. This might account for the greater metabolic rates at birth of the young brain. This also accounts for the differential behaviour of the young and adult brains with regard to utilization of

substrates for its metabolic activity. Unlike other capillary or cell membranes the blood-brain barrier is extremely impermeable to proteins including antibodies and bacterial toxins. The brain has to manufacture most of its complex constituents, some of which are uniquely present in the same, absorbing only relatively simple substances from the blood stream. It is reported recently that there are separate systems for the transport of neutral and basic amino acids across the blood-brain barrier (Richter and Wainer, 1971).

Cerebral metabolism accounts for 20-25% of the basal metabolism in man (Kety, 1957) although the weight of the brain is only about 2.5-3% of total body weight and the cerebral blood flow is also considerably high (Kety, 1957).

Several studies suggest that nutrients are made preferentially available to brain tissue even in the face of deficiency or starvation. According to Rajalakshmi and Ramakrishnan (1972) this would be consistent with biological adaptation as small changes in the brain may result in gross disturbances in metabolic regulation and behaviour. With prolonged and severe deficiency, however,

the brain has also to share ultimately the shortage with other organs. In this connection it is of interest to note that during starvation brain oxidizes less glucose and more ketone bodies than it generally utilizes for energy purposes (Cahill, Owen and Morgan, 1968).

As early as 1925 Jackson reported that nerve cells of animals subjected to inanition, especially the motor cells of the spinal cord, undergo atrophic degeneration, chromatolysis, cytoplasmic vacuolation and disorganization of neurofibrils. Degeneration of Nissl substance in the anterior horn cells, chromatolysis, tigrolysis, foaming of the cytoplasm, swollen appearance of the myelin sheath, increase in oligodendroglial cells, neuronal loss and fibrous gliosis have been reported in dogs and pigs (Platt, Heard and Stewart, 1964; Platt and Stewart, 1971).

Undernutrition in new born rats has been reported to reduce brain weight by as much as 46% and cause a reduction in the growth of neuropil so that the normal pattern of the dendritic process is not achieved (Eayrs and Horn, 1955; Horn, 1955). Other investigators have found smaller decreases in brain weight generally of the order of 8-21% (Widdowson and McCance, 1960; 1962; Williamson and Coniglio, 1971).

Guthrie (1968) and Chase, Lindsley, and O'Brien (1969) found no change in DNA concentration of rats undernourished from birth. The DNA content of the whole brain has been found to be affected by undernutrition in early life although its concentration is not appreciably affected (Dobbing, 1968). In these studies on pigs even a reduction in body weight by 96% was not found to affect DNA concentration in the brain. The effects might also vary with the brain region studied according to Chase, Lindsley and O'Brien (1969) and Howard and Granoff (1968) who found a decreased DNA concentration in the cerebellum but not in the cerebrum. A decrease in RNA content of the cytoplasm in purkinje cells has been reported by Novakova, Koldovsky, Hahn and Krecek,(1967). Generally a reduction in protein content has been observed in terms of whole brain values but not when considered in terms of values per gram of fresh weight (Winick and Noble, 1966; Guthrie, 1968; Rajalakshmi, Ali and Ramakrishnan, 1967). This might depend on the brain region studied and a somewhat reduced concentration in the cerebellum has been reported by Chase, Lindsley and O'Brien (1969).

A number of investigators have found that myelination, and cholesterol and phospholipid contents of the brain are affected in undernourished animals (Dobbing, 1964; Dobbing and Widdowson, 1965; Benton, Moser, Dodge and Carr, 1966; Cully, Yuan and Mertz, 1966; Winick and Noble, 1966).

Maturation of evoked cortical response to a visual or auditory stimulus has been found to be delayed in rats subjected to neonatal undernutrition (Mourek, Himwich, Myslivecek and Callison, 1967). Similar results were obtained with regard to a number of other parameters such as oxygen consumption per square meter of body surface, motor co-ordination, brain tissue oxygen consumption and conditioning (Myslivecek et al., 1968). In the case of all these parameters any difference found at younger age levels were largely found to disappear in the older animals so that the effects do not seem to be permanent.

A few studies have suggested the differential effects of protein and calorie deficiencies. In these studies protein deficiency during the postweaning period was found to have adverse effects on behavioural measures although calorie deficiency had no effect (Barnes, Moore, Reid and Pond, 1968). Post-weaning undernutrition so severe as to prevent any increase in body weight preceeded

by neonatal undernutrition has been found to impair psychological performance (Frankova and Barnes, 1968). The impairment was found to be greater with a protein deficient diet. Baird, Widdowson and Cowley (1971) also reported that a malnourished animal performs less well than a well nourished one in the behavioural studies and a low protein diet is rather more harmful in this respect than a low calorie one.

It has long been known that a disease syndrome known as kwashiorkor occurs in children who are fed on diets severely lacking in protein. Clinical observations of the association of this condition with extreme apathy led to the suggestion that the central nervous system is also affected in protein deficiency (Platt, 1961). This suggestion received support from the observations of Platt, Heard and Stewart (1964) that in both children and experimental animals protein malnutrition is associated with changes in the electrical activity of the brain and degeneration of Nissl granules in the anterior horn cells of the spinal cord.

The brain contains 2% of soluble organic constituents of which a major protion is amino acid. 20% of the total

nitrogen in the brain is made up of amino nitrogen 25% of which is contributed by glutamic acid and glutamine (Ansel and Richter, 1954; Waelsch, 1952; 1955; Weil-Malherbe, 1952). While the brain compares with plasma and liver in its concentrations of essential amino acids it has a much higher concentration of non-essential amino acids (Waelsch, 1957).

Glutamic acid, glutamine and Gamma amino butyric acid account for a substantial portion of the non-essential amino acids. Most of the brain glutamic acid is locally synthesized (Strecker, 1957) and the rate of incorporation of labelled glucose in brain glutamic acid is high as compared to that in the liver as can be seen from the following table:

	Percent activity of total amino acids in	
	brain	liver
Glutamate	37.0	5.3
GABA	4.0	-
Glutamine	9.0	5.2
Aspartate	9.0	2.6
Alanine	2.0	3.0
Total	61.0	16.1

Data taken from Gaitonde, Dahl and Elliott (1965).

The formation of glutamic acid is catalysed by the enzyme glutamate dehydrogenase. The importance of this reaction is that it occurs spontaneously i.e. without the supply of energy as soon as a sufficient concentration of ammonia is available. The operation of the blood-brain barrier against this amino acid suggests its synthesis in the brain. Dewan (1938) and Von Euler, Adler, Gunther and Das (1938) established the presence of glutamate dehydrogenase in mammalian tissues including brain. The activity of glutamate dehydrogenase in the brain has not been found to be as high as that in the liver or kidney (Krebs, Eggleston and Hems, 1948) a fact which contrasts with the higher concentration of glutamic acid in the brain. This is explained by the fact that in the brain the equilibrium constant of the enzyme favours reductive amination and the enzyme does not normally operate in the direction of oxidation (Olson and Anfinsen, 1953; Strecker, 1953; Weil-Malherbe, 1957).

The role of glutamic acid in brain metabolism has elicited much speculation and interest since the observation of Thunberg (1923) that the brain can oxidize glutamic acid <u>in vitro</u>. Many subsequent studies suggested

that the mammalian brain in vitro can utilize glutamic acid in place of glucose (Quastel and Wheatley, 1932; Krebs, 1935; Weil-Malherbe, 1936). Extensive studies carried out by Weil-Malherbe (1936) on brain glutamic acid showed that during the oxidation of the same no ammonia is liberated. It is now known that it can be oxidised via the GABA shunt by the successive conversion of glutamic acid to GABA, succinic-semialdehyde and succinate.

Glutamic acid has a high turnover rate in brain slices and is rapidly metabolised to glutamine, GABA and aspartic acid (Waelsch, 1957; Tower, 1959). Enzymes involved in the metabolism of glutamic acid namely glutamine synthetase, glutamate decarboxylase, aspartate amino transferase and alanine amino transferase have been identified in the brain (Cohen and Hekhuis, 1941; Speck, 1949; Elliott, 1951; Krebs, 1935; Lajtha, Mela and Waelsch, 1953; Roberts and Frankel, 1951; 1951a; Roberts, Harman and Frankel, 1951). It is of interest that glutamine synthetase is invariably present in the brains of all animals (Krebs, 1935a) whereas it is variably present or absent in other tissues (Wu, 1963). This enzyme provides a local machinery for the removal of ammonia which is known

to be toxic to the brain (Sapirstein, 1943; Strecker, 1957). In this connection the blood-brain barrier acts against glutamic acid but not against glutamine.

Considerable excitement regarding the role of this amino acid was aroused by the earlier reports on the favourable effects of glutamic acid supplementation on the intelligence of mentally retarded children and learning performance in rats (Waelsch, 1951; Strecker, 1957). In this connection it is of interest to note that the decrease in the activities of glutamate dehydrogenase and glutamate decarboxylase observed in animals fed low protein diets disappeared when the diet was supplemented with glutamic acid (Rajalakshmi, Pillai and Ramakrishnan, 1969). Glutamic acid administration to patients in insulin coma restores consciousness, a phenomenon which may also be due to the release of glucose by adrenaline after administration of this amino acid (Strecker, 1957).

Further, it has been shown that the brain contains a protein which has a high content of glutamic acid (Moore, 1965). In other studies, a similar protein has been found to show a rapid turnover rate (Minard and Richter, 1968).

The distribution of glutamic acid and glutamine in cellular components and in different layers of the brain suggests a functional significance for both. Thus glutamic acid is distributed more in the grey matter (Tower, 1959) which is mainly concerned with nervous activity whereas glutamine is distributed equally in the grey and white matter (Krebs, Eggleston and Hems, 1949; Waelsch, 1952). The distribution of these two amino acids in the cellular particulate shows some variation. Glutamic acid is primarily found in the mitochondrial fraction whereas glutamine is found in the mitochondrial as well as nuclear fractions (Tower, 1959; Weil-Malherbe, 1957).

The concentrations of glutamic acid, glutamine and GABA are found to increase during fetal and postnatal development and reach ceiling values with myelination and maturation of the neurons (Krebs, Eggleston and Hems 1949; Baxter, Schade and Roberts, 1960; Agrawal, Davis and Himwich, 1968). In the rat the attainment of the maximum levels of glutamic acid coincides with active protein synthesis associated with myelination and neuron maturation, and with the shift to adult patterns of metabolism (Rudnick and Waelsch, 1955; 1955a; Tower, 1959).

In this connection it should be noted that the glutamic acid compartmentation takes place during the development of dendritic processes and nerve terminals (Patel and Balazs, 1970).

It has been suggested that the compartmentation of glutamic acid can be used as an index of cerebral maturation which can be used to study the effects of different influences on the development of brain during the early postnatal period (Patel and Balazs, 1970). Patel and Balazs (1971) reported that thyroid deficiency resulted in a marked retardation in the development of metabolic compartmentation of glutamic acid and GABA. Recently Tewari and Baxter (1969) have shown that GABA stimulated the incorporation of amino acids into protein by a ribosomal system from the immature rat brain but not from the immature rat liver.

GABA is uniquely present in the brain and is derived from brain glutamic acid as the enzyme involved in its formation, namely glutamate decarboxylase is present in the CNS. Further, the blood-brain barrier operates against this amino acid (Van Gelder and Elliott, 1958). It has also been shown that the amount

of GABA present in any region of the brain varies with the activity of glutamate decarboxylase (Sisken, Roberts and Baxter, 1960). Hence, it is reasonable to expect that the relative activities of both glutamate decarboxylase and GABA transaminase would determine the level of GABA in any particular region (Roberts, 1960; Tower, 1958).

Both glutamate decarboxylase (Salvador and Albers, 1959) and GABA transaminase (Albers and Brady, 1959) are found to be more in the grey matter of the central nervous system. Various regions of the same exhibit widely differing activities. Miller and Pitts (1967) showed that in the human brain the activity of succinate semialdehyde dehydrogenase is high in the basal ganglia, thalamus, hypothalamus and relatively low in white matter.

Reference has been made to the utilization of glutamic acid in the absence of glucose. This must proceed either through transamination with oxaloacetate or through the GABA shunt. The former mechanism seems more likely on the basis of several studies (Krebs and Bellamy, 1960; Haslam and Krebs, 1963; Balazs, 1965). However it has been shown that a substantial portion

of glucose is oxidised via the GABA shunt and estimates of the proportion so oxidised vary from 10%-40% (Mckhann and Tower, 1959; 1961; Elliott, 1965). A more recent estimate obtained by following the oxidation of glucose in brain slices gave the flux through the GABA bypass as 8% of the flux through the tricarboxylic acid cycle (Patel, Balazs and Richter, 1970). However, no net change in the GABA concentration is found when this is used as substrate (Elliott, 1965) although a negligible net consumption is found in the presence of glucose (Elliott and Van Gelder, 1958). This could be because of the continued synthesis of GABA from glutamate formed during transaminations and the reductive deamination of 2:oxoglutarate.

Apart from the metabolic functions of amino acids, they are believed to exert some electrophysiological action on the brain. They may be considered as transmitter substances causing excitatory and inhibitory effects in central nervous system. Among these amino acids both glutamic acid and GABA are of considerable interest as the concentrations of these amino acids are high in the brain and the enzyme systems necessary for their metabolism are also present in the brain. Acidic amino acids

structurally related to glutamic acid depolarize neurons and are considered as excitatory substances (Curtis,1962; 1965; Hebb, 1970; Krnjevic, 1964) and the neutral amino acids structurally related to GABA which depress the firing of neurons are considered as inhibitory substances.

Thus glutamic acid is found to have an excitatory action and GABA an inhibitory action. It may be considered that these amino acids have mutually complementary roles in the central nervous system. The elementary criteria of a transmitter substance are that it must be produced in the relevant tissue at the appropriate time, stored, released to exert an appropriate action, and be removed (Elliott, 1965). All the criteria except that of release are met by both (Elliott, 1965). Even this criterion appears to be met by GABA as in studies made on crustacean nervous system GABA leaks from the punctured cortical surface (Otsuka, Iversen, Hall and Kravitz, 1966). The rate of release of GABA was increased by about 50% when the electroencephalographic pattern indicated arousal (Jasper, Khan and Elliott, 1965).

The powerful depressant action of GABA when applied on cortical neurons and its presence in large quantities

in the brain have led to the suggestion that GABA may be a postsynaptic inhibitory transmitter substance (Krnjevic, 1964). A stereospecificity of cortical receptors for GABA has also been shown on the basis that amino acidic groups are less potent in blocking cortical neuron activity (Krnjevic, 1964). However, some investigators rule out such a role on the ground that GABA does not act on the same site as strychnine but believe that it may be involved in pre-synaptic inhibition (e.g. Curtis, 1963). However, there is a consensus in favour of the view that GABA does have an important electrophysiological role.

Indirect evidence regarding the role of GABA as an inhibitory transmitter is to be found from the lowered concentrations of this amino acid in epileptic seizures and in seizures induced by convulsive agents (Roberts and Baxter, 1959).

Intravenous administration of GABA to man causes transitory paraesthesias, and produces a fall in blood pressure and respiratory rate in man, dog and rabbits (Elliott and Hobbiger, 1959; Tower, 1960). This is believed to be due to the peripheral action of GABA as it does not cross the blood-brain barrier (Van Gelder and Elliott, 1958).

In contrast to GABA, glutamic acid is believed to have an excitatory effect. There is no strong evidence against the possibility that L-glutamic acid may be the principal excitatory transmitter in the central nervous system (Krnjevic, 1965). Recent studies have shown a similarity between the action of glutamate on the crustacean neuromuscular junction and that of acetyl choline on the vertebrate end-plate (Takeuchi and Takeuchi, 1964). Studies of Krnjevic and Schwartz (1967) in cat pericruciate cortex showed that glutamic acid is more selective in its action than acetylcholine. They were also able to show by means of intracellular recording that the depolarizing action of glutamate on cortical nerve cells was accompanied by a fall in membrane resistance; the mechanism of its depolarizing actions is therefore more in accordance with the expected behaviour of an excitatory transmitter than is the case for acetylcholine.

Thus the concentrations of GABA and glutamate in the central nervous system are probably very critical as any disturbance in them may change the electro-physiological status, resulting in abnormal function of the brain. The rapid flux of glucose carbon to

amino acids is found only at about 10-15 days after birth, when the cerebral cortex becomes functionally mature and is not found in the fetal or neonatal brain (Gaitonde and Richter, 1966). The supply of glucose as a precursor for amino acids becomes therefore a critical factor from 10 days onwards. Therefore any condition in which the glucose metabolism is hampered might be expected to affect the concentration of glutamic acid in the brain. Thus insulin induced hypoglycemia causes a reduction in glutamic acid and GABA in the central nervous system (Cravioto, Massieu and Izquierdo, 1951; Dawson, 1950, 1953).

The learning process is believed to involve the repeated firing of groups of neurons or neuronal assemblies (Hebb, 1949). Such firing cannot be efficient if the background electrical activity is too low, in which case it may not take place, or too high in which case the sequential firing of particular groups of neurons may become disorganised. We may therefore presume that an optimum level of dendritic activity is crucial for efficient CNS function. This may involve the maintenance of critical levels of glutamic acid and GABA.

The above considerations led to the choice of the enzymes of glutamate metabolism as the metabolic parameters in the studies carried out on the effects of nutritional deficiency in this laboratory.

In previous studies in this laboratory, protein deficiency was found to result in decreased activities of brain glutamate dehydrogenase and decarboxylase whereas GABA-transaminase was unaffected (Rajalakshmi, Pillai and Ramakrishnan, 1969). As the food intake differs in animals fed low and high protein diets ad libitum, a question also arises as to whether the effects of a low protein diet are not at least in part due to a reduction in calorie intake. Although preliminary studies carried out in this regard comparing low protein animals with high protein animals pair fed with the former suggested this not to be the case in these studies, both the low and high protein pair fed animals were growth retarded because of environmental conditions.

Further the enzyme techniques used in these studies suffered from some limitations as the homogenates were not treated with Triton X-100. Also in these experiments

no simultaneous study was made of high protein groups fed ad libitum or pair fed with low protein animals. It seemed therefore necessary to repeat and extend these studies. Preliminary studies suggested that the brain enzyme deficits produced by low protein diet are also produced by a diet based on kodri (Paspalum scorbiculatum L.), a millet very poor in protein quality and that these deficits can be restored by improving the protein quality of the diet without increasing the nitrogen content (Rajalakshmi, Pillai and Ramakrishnan, 1969). It seemed necessary to confirm these observations and investigate whether other food grains commonly consumed in this country such as wheat (Triticum aestivum) and maize (Zea mays) produce similar effects.

Low and high protein diets using casein differ not only with regard to protein content but also with regard to the content of amino acids such as glutamic acid which is abundantly present in casein (FAO, 1970). As pointed out earlier, glutamic acid has been found to influence CNS function in both children and experimental animals (Waelsch, 1951; Strecker, 1957) although normally glutamate is not known to cross the blood brain barrier (Waelsch, 1955). Because of the reported effects of

glutamic acid, preliminary studies were carried out on the effects of glutamic acid supplementation to the low protein diet so as to raise the level of glutamic acid in the same to that in the high protein diet. In these studies glutamic acid supplementation to a low protein was found to restore brain enzyme activities to normal levels. It seemed necessary to identify whether such effects are found with smaller doses of glutamic acid supplementation and whether they depend on the level of protein in the diet. These aspects were further investigated in the present studies.

As stated earlier a deficiency of vitamin A in young animals results in malformation of the skull and resultant changes in the central nervous system (Mellanby, 1944). Some CNS changes such as degeneration of nerves are also found independently of skeletal changes. It is well known that vitamin A deficiency may cause irreversible changes in the eye. Vitamin A deficiency is wide spread in this country and in many areas of the world and often coexists with protein deficiency. In children the effects of a vitamin A deficient diet are aggravated by a lack of protein in the diet as the conversion of carotene to vitamin A and absorption,

storage and transport of vitamin A depend on protein status. India has the highest incidence of blindness in the world and most of this blindness occurs in early childhood. A deficiency of vitamin as well as protein is believed to be the major etiological factor. It seemed worthwhile in the above context to carry out studies on the effects of vitamin A deficiency on the brain enzymes studied and the interaction of the same with protein deficiency.

The present investigations were concerned with an extension of the studies on the relation between diet and brain enzymes and were concerned with the following aspects:-

I. the comparative effects of calorie restriction and protein deficiency

II. the effects of differences in protein quality

III. the effects of glutamate supplementation to diets varying in protein content and of different doses of such supplementation

IV. the effects of vitamin A deficiency in relation to the protein content of the diet.

Albino rats were used for these studies and brain glutamate dehydrogenase (GDH) (L-glutamate:NAD oxido-reductase, E.C.1.4.1.2), glutamate decarboxylase (GAD) (L-glutamate 1-carboxy-lyase, E.C.4.1.1.15) and GABA transaminase (GABA-T) (4-amino butyrate:2-oxoglutarate amino transferase E.C.2.6.1.19) were the main parameters investigated. Additional studies were carried out on the partial purification and characterization of GDH in brain and liver. These studies are incorporated in this thesis.