CHAPTER I

INTRODUCTION

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The vital role of the central nervous system in the regulation of the growth, metabolism and function of the organism is well known. It is not surprising that its development precedes that of many other organs (Winick, Brasel and Rosso, 1972). The development of the brain starts with the formation of the neural tube in the embryonic stage, the anterior portion of which develops subsequently into the forebrain, midbrain and hindbrain. The central lumen enclosed by the walls of the neural tube forms the small apparently functionless central canal of the adult spinal cord and the much more important ventricular system of the brain. The neural tube is a collection of primitive embryonic dividing cells from which are formed the spongioblasts which give rise to glia and the neuroblasts which are precursors of neurons (Altman, 1969).

The development of the brain is characterised in the first phase by organogenesis and neural multiplication followed by the growth spurt characterised by axonal and dendritic growth, glial multiplication and growth in size. Briefly, cell division is the first event (hyperplasia) in the process followed by cell division + cell enlaggement (Hyperplasia + Hypertrophy). Finally cell division ceases and only cell growth (Hypertrophy) continues till maturity

(Winick and Noble, 1966). Since the DNA concentration per diploid nuclei tends to be constant in all cells including those of brain, the proliferative rate and number of cells at different stages of development in the brain is generally estimated by the same whereas cell size is indicated by the ratio of protein : DNA and RNA : DNA (Davison and Dobbing, 1968). The maximum cell number is attained in about 3 weeks of age in the rat (Winick, 1968) and one year in man (Altman, 1969).

The brain is composed of anatomically and morphologically distinct regions and the pattern of development varies to some extent from region to region. DNA synthesis in rats continues until about 21 days of age in the cerebrum, 17 days in cerebellum and 14 days in the stem (Fish and Winick, 1969). The hippocampus shows a unique cellular growth characterised by a discrete rise in DNA content between the 14th and 17th days of life. This increase corresponds to the migration of neurons (Altman, 1969; Fish and Winick, 1969). Thereafter an enormous increase is found in the size of the cells.

The sequence of events is not so clearly defined in the human brain. Neuronal multiplication is achieved by 30 weeks of gestation according to Robinson and Tizard (1966) and by 18 weeks according to Dobbing and Sands (1973). Similarly total cell number (neuronal + glial) is reported to be

achieved by 12 months after birth by Mandel, Rein, Hearth-Edle and Mardell (1964) and 8 or 12 months by Winick (1968). Moreover, Dobbing and Sands (1970) have shown that two peaks of DNA synthesis may occur normally in the human brain. The first peak is reached at about 18 weeks of gestation and the second around birth. They have interpreted these periods to correspond to the peak rate of neuronal division and the peak rate of glial division. According to Dobbing and Sands (1973) cell division in the cerebellum, even though it begins to grow later, stops earlier than in the rest of the brain. Adult cell number is achieved in the cerebellum around the age of 18 postnatal months, at a time when the forebrain and stem have only 60% of the same. The studies of Winick, Rosso and Waterlow (1970) show that in the cerebrum and cerebellum cell division levels off between 12 and 15 months of age. In the brain stem DNA synthesis continues at a slow but rather steady rate until atleast up to 1 year of age.

The increase in neuronal size is associated with the growth of axons and dendrites and a tremendous increase in the complexity of the latter. Synaptic contacts with asymmetrical membrane thickenings increase in number during the postnatal maturation of the neocortex in the rat and these represent a "mature" form. After 10 days the synapses found are indistinguishable from those found in adult cortex

and at the same time the dendritic cytoplasm is well-developed containing mitochondria, the endoplasmic reticulum, ribosomes and a dense background matrix (Caley and Maxwell, 1971). In the human cerebral cortex dendritic growth is maximal from the late fetal period to about one year of age and continues at a slower pace for several years thereafter (Schade and Groenigen, 1961).

Axonal and dendritic growth is accompanied by an explosive multiplication of oligodendroglial cells, following which the glial cells surround the nerve axon in spiral fashion and a progressive deposition of lipids takes place which represents about 75% of the dry weight of myelin. The largest lipid component is cholesterol but significant amounts of ethanolamine phosphatide and galactolipids (cerebrosides and cerebroside sulfates) are present. The characteristic composition of myelin lipids show a molar ratio of cholesterol : phospholipid : cerebroside of 4:3:2. In the rat brain, the rates of increase in the myelin lipids are maximum during the second and third postnatal week. (Davison and Dobbing, 1968; Norton and Poduslo, 1973), but some increase in concentration occurs till adult age. In man myelination proceeds at a very fast rate from 7 months of gestation onwards and continues till 4-5 years of age according to Kokrady, Mammen and Bachhawat (1971) and Dobbing

and Sands (1973) although in earlier studies this was believed to take place till a much later age of 10 years (Brante, 1949; Tingey, 1956; Cummings, Goodwin, Woodward and Curzon, 1958) or over adolescence (Davison and Dobbing, 1966).

During the period of myelination recognisable patterns of electrical activity are also found to emerge. The evoked cortical response has proved a powerful measure of the degree of maturation in the central nervous system. In general the evoked response of immature cortical tissue is characterised by an initial negative deflection, which changes during maturation into an initial positive component. In rats the most important changes occur at 14 days of age i.e. decreased latencies and the appearance of all components of the evoked response (Rosen, 1971).

These structural and functional changes are associated with several changes in chemical composition and metabolism as might be expected. The main energy source of the brain is glucose. But it would appear that the immature brain can utilize ketone bodies such as β-hydroxybutyrate and aceto-acetate (Smith, Satterthwaite and Sokoloff, 1969; Toth and Quastel, 1970; Hawkins, Williamson and Krebs, 1971). Moreover, anaerobic glycolysis is predominant in the immature

brain, but as development proceeds oxygen consumption increases and the oxidative pathway predominates. These changes are reflected in the enzyme activities of the brain during development. Throughout the postnatal life of the rat the activities of glycolytic enzymes do not change appreciably (Laatsch, 1962), but those of enzymes of the tricarboxylic acid cycle increase considerably between 10 and 40 days (Robins and Lowe, 1961). During the most rapid phase of lipid synthesis glucose is metabolised in much greater proportions through the hexosemonophosphate shunt and thereafter a small proportion passes through this pathway which favours both nucleic acid and lipid synthesis. For the former it supplies ribose nucleus and for the latter it generates NADPH (Winick, 1970).

It is well known that the brain has a high concentration of aminoacids such as glutamic acid, aspartic acid and glutamine. In the adult animal a significant proportion of the same is derived from glucose and this accounts for a considerable part of the glucose utilization of the brain. In the fetal and neonatal brain the rapid flux of glucose carbon to aminoacids, a characteristic of the adult brain, is not yet present. In the rat this appears about 10 to 21 days after birth when the cerebral cortex becomes functionally mature (Gaitonde and Richter, 1966) and adult levels of

glutamic acid and glutamine are attained at 21 and 14 days of age respectively. GABA and aspartic acid reach adult levels at about 30 days (Agrawal, Davis and Himwich, 1966; Bayer and McMurry, 1967).

Increases in the dicarboxylic acid content of the developing brain may be related to the concomitant increase of glutamic acid decarboxylase activity (Bayer and McMurry, 1967; Pitts and Quick, 1967; Sims and Pitts, 1970). Moreover, the activities of aspartate aminotransferase and glutamate dehydrogenase show a reasonable correlation with glutamate levels (Bayer and McMurry, 1967). A dramatic increase occurs in the sodium-potassium activated ATPase activity of the rat brain between 10 and 20 days of age and this may be related to the increase of dendritic processes and the multiplication of nerve endings (Samson and Quinn, 1967). In this connection, ATP is concerned with the control of such transmitter substances such as glutamate, 5-hydroxy tryptamine, γ-amino-butyrate and noradrenaline (Himwich, Pscheidt and Schweigerdt, 1967).

In the adult mammalian brain, glutamate is divided into 3 metabolic compartmental pools (Balazs, Patel and Richter, 1973). Studies on cats and rats (Berl, 1965; Patel and Balázs, 1970) show that metabolic compartmentation and the

conversion of glucose to aminoacids become apparent at about the same time.

The maturation of the nervous system is associated with the development of more complex reflex activity. Spasmodic twiching and general reflexes such as breathing, crawling, suckling, righting, negative geotaxis, cliff avoidance etc. are found soon after birth. During the second week, behaviour takes on more complex forms and at weaning, the rat becomes self dependent (Schapiro, 1971). Some of the reflexes which are established during this period include righting of the body during a fall, seeking physical support when the vibrisae are touched, climbing a rope etc.

Thus the development of the central nervous system can be viewed as a progression of biochemical, morphological, and functional events which occur in orderly sequence. This sequence may be either interrupted or delayed by nutritional deficiencies as well as toxic factors and such interruption may prove to be irreversible.

Because of recognition that adequate nutrition is necessary for the structural development of the brain in early life, extensive studies have been carried out with experimental animals on the effects of protein deficiency or undernutrition during this period. A few attempts have also been made to study the effects of vitamin deficiencies.

Degenerative changes in the CNS have been found with a deficiency of vitamin A (Dam and Sondergaard, 1964), vitamin E (Gordon and Nitowsky, 1968), thiamine (Collins, Kirkapatrick and McDougal, 1970), and riboflavine (Zimmerman, 1943). Biochemical changes have been reported in deficiencies of thiamine (Holowach, Kauffman, Ikossi, Thomas and McDougal, 1968; Collins et al, 1970), niacin (Garcia-Bunuel, McDougal, Burch, Jones and Touhill, 1962) and pyridoxine (Eiduson, Geller, Yuwiler and Eiduson, 1964). These studies have been reviewed elsewhere (Coursin, 1968; Rajalakshmi and Ramakrishnan, 1972). The most extensively investigated parameters are DNA, RNA and protein which reflect structural development. The various lipids, particularly cholesterol and phospholipids have also been investigated because of their role in myelination.

Only a few studies have been done on the effects of manipulating the plane of nutrition during the prenatal period. Pups born of rats fed a protein deficient diet during gestation had low birth weights and brain weights (Zamanhof, Marthens and Margolis, 1968; Shrader and Zeman, 1969; Zeman and Stanbrough, 1969; Envonwu and Glover, 1973; Siassi and Siassi, 1973). Brain DNA content was reduced but concentration was not affected suggesting a reduction in cell number (Zeman and Stanbrough, 1969; Zamanhof, Marthens and Graud, 1971; Envonwu and Glover, 1973; Siassi and Siassi, 1973). In

contrast, pups born of mothers fed a quantitatively restricted diet had significantly lower body weights but brain weight was not affected (Kumar and Sanger, 1970; Balázs and Patel, 1973).

Many techniques have been used to produce undernutrition in rats during the neonatal period. The most common technique is to manipulate litter size and consequently the supply of milk to the pups (Kennedy, 1957; Widdowson and McCance, 1960). Another method consists of restricting the food intake of the mother during lactation (Chow and Lee, 1964) or feeding the mother a low protein diet (Barnes, Cunnold, Zimmerman, Simmons, Mcleod and Krook, 1966). Restricting the access of the pups to the mother has been used by Eayrs and Horn (1955). Some investigators have manipulated maternal diet both during gestation and lactation (Simonson, Sherwin, Anilane, Yu and Chow, 1968). A combination of both approaches (increase in litter size and maternal protein restriction) has also been used to produce a more severe degree of undernutrition (Guthrie and Brown, 1968). In all these cases the effect is a decreased supply of milk but the protein content of milk is not affected. Consequently, these methods do not enable us to manipulate the protein concentration of the diet.

A more rigidly controlled technique for modifying the diet of the suckling rat was reported by Miller and Dymsza

(1963). In this method newborn pups were fed using a specially designed needle. Czajka and Miller (1968) have achieved various degrees of undernutrition by hand-feeding the pups from birth with a milk formula the protein content of which was varied. They found weight gain during the neonatal period to depend on the protein content of the liquid formula as might be expected. However, this technique has not yet been extensively used for studies on the brain.

Undernutrition during the neonatal period has been associated with a reduction in brain weight (Dobbing and Widdowson, 1965; Winick and Noble, 1966; Chase, Lindsley and O'Brien, 1969; Swaiman, Daleiden and Wolfe, 1971; Sobotka, Cook and Brodie, 1974). A decrease was found in the content of DNA (Winick and Noble, 1966; Dobbing, 1968; Guthrie and Brown, 1968; Swaiman et al, 1970; Envonwu and Glover, 1973), RNA (Winick and Noble, 1966; Swaiman et al, 1971; Envonwu and Glover, 1973), cholesterol (Dobbing, 1964; Benton, Moser, Dodge and Carr, 1966; Geison, 1967; Dobbing, 1968; Dickerson and Jarvis, 1970) and protein (Swaiman et al, 1971; Envonwu and Glover, 1973; Gambetti, Autoillio-Gambetti, Gontas, Shaffer and Steiber, 1972) but the concentrations are not affected (Guthrie and Brown, 1968; Chase et al, 1969; Swaiman et al, 1971; Envonwu and Glover, 1973; Sobotka, Cook and Brodie, 1974). However, a reduction in the concentration of DNA, RNA and protein has been reported by Winick and Noble (1966).

A reduction with neonatal undernutrition has been reported in the concentration of brain lipids including cholesterol and phospholipids (Dobbing, 1968; Geison and Waisman, 1970), gangliosides (Dickerson and Jarvis, 1970; Geison and Waisman, 1970), cerebrosides (Culley and Mertz, 1964; Benton et al, 1966; Geison, 1967; Geison and Waisman, 1970), proteolipids, sulfatides and plasmalogens (Geison, 1967).

The effects of dietary deprivation have been reported to vary from region to region, depending on their maturation period. Amongst the regions extensively studied are the cerebrum, cerebellum and stem. The cerebellum has been reported to be most affected in rats by Culley and Lineberger (1968), Cheek, Graystone and Rowe (1969), Fish and Winick (1969) and Chase et al (1969), and in mice, by Howard and Granoff (1968). The brain stem has been reported to be the least affected (Fish and Winick, 1969). The studies of Patel, Balázs and Johnson (1973) emphasize the timing of undernutrition imposed in relation to the maturity of the regions studied. Moreover, studies by these investigators suggested a compensatory mechanism in undernourished rats namely, a depression of mitotic activity and a reduction in the normal rate of cell loss. All these studies suggested a decrease in DNA and protein synthesis and reduction in RNA content. The

reduction in protein synthesis in undernutrition is believed to be brought about by the effects of the same on RNA metabolism as the same is found to be associated with an increase in alkaline ribonuclease and a decrease in DNA polymerase (Winick, Rosso and Brasel, 1972).

Dendritic arborization and synaptic contacts are responsible for the maintenance of the background electrical activity and the EEG pattern is determined for assessing the functional activity of the brain. Undernutrition is found to affect the proper arborization of dendrites and also reduces the number of synapses (Eayrs and Horn, 1955; Eayrs and Goodhead, 1959; Bass, Netsky and Young, 1970; Cragg, 1972; Shoemaker, Coyle and Bloom, 1974). But Gambetti, Gambetti, Rizzuto, Shafer and Pfaff (1974) failed to find any effect on synaptogenesis. The electrophysiological maturation was found to be affected by Mourek, Himwich, Myslivecek and Gallison (1967). The biochemical maturation of the brain as judged by the ability to incorporate glucose into aminoacids particularly glutamate, glutamine and GABA is found to be delayed by undernutrition in early life. At 21 days of age, the undernourished animals showed only 18% of the radioactivity in brain aminoacids as compared to 37% in controls. Appreciably decreased radioactivity was found in glutamate, glutamine and GABA, but the maturation of the GABA nerve compartment was found to be normal in the undernourished rats

(Balázs and Patel, 1973). Moreover the concentrations of these aminoacids were not found to be affected (Balázs and Patel, 1973; Roach, Corbin and Pennington, 1974). However, a decrease in the concentration of free aminoacids including glutamate, glutamine and GABA in neonatally undernourished rats has been reported in several studies (Reddy, Pleasants and Worstmann, 1971; Envonwu and Glover, 1973) and in the monkey (Envonwu and Worthington, 1973).

Undernutrition during the neonatal period has also been found to result in decreased activities of several brain enzymes, namely, succinic dehydrogenase, aldolase and acetylcholinesterase (Adlard and Dobbing, 1971). Similar observations were made with regard to acetylcholinesterase by Sereni, Principi, Perletti and Sereni (1966) but Gambetti, Steibber and Gontas (1972) found an increase in the activity of this enzyme. Cerebroside sulfotransferase is found to be affected in undernutrition (Chase, Dorsy and McKhann, 1967).

The deficits in brain weight and brain composition due to undernutrition during the prenatal and neonatal period are not found to be fully reversed by subsequent rehabilitation in later life (Dobbing, 1968; Guthrie and Brown, 1968; Geison and Waisman, 1970; Rajalakshmi, Nakhasi and Ramakrishnan, 1974). Normal concentrations of various lipid fractions are achieved with rehabilitation (Howard and Granoff, 1968;

Guthrie and Brown, 1968) but whole brain values were less because of decreased brain weights. However, Culley and Lineberger (1968) reported persistent deficits in the concentrations of cholesterol,phospholipids and cerebrosides even after rehabilitation. Similar results have been reported in rats (Rajalakshmi, Nakhasi and Ramakrishnan, 1974) and in pigs (Dickerson, Dobbing and McCance, 1967) but in the later studies on pigs, the degree of undernutrition was extremely severe and prolonged, the body weight of the undernourished animals being only 3.5% of expected weights. Even so the deficit in the concentration of cholesterol was only 6% and that in brain weight 34% as against a body weight deficit of 96.5%. The relatively smaller deficits in brain weight and brain composition resulting from postweaning undernutrition are found to be completely restored (Dobbing, 1968).

A number of studies have been made on the effects of undernutrition in early life on neuromotor development and the same is found to be delayed in pups of mothers subjected to either undernutrition or protein deficiency during lactation or during both gestation and lactation (Chow and Lee, 1964; Smart and Dobbing, 1971a; Altman, Sudershan, Das, McCornic and Barnes, 1971). Pups born of mothers undernourished during gestation and nursed by normally nourished mothers did not show a similar retardation suggesting that

nutrition during the suckling period is more important for neuromotor development (Smart and Dobbing, 1971b). However, Simonson et al (1968) found some developmental retardation in pups born of undernourished mothers and some behavioural effects to persist even after weaning.

The maturation of evoked cortical response to visual or auditory stimuli were found to be delayed in neonatally undernourished rats. These differences, however, disappeared at 45 days of age (Mourek, et al, 1967).

The degree of maternal and environmental stimulation may also influence neuromotor development in early life. Maternal behaviour has been found to be altered with malnutrition (Frankova, 1972, 1974). Similarly the interaction between pups varies when they are reared in small or large litters. Pups in large litters are found to be more independent and more active (Frankova, 1972).

A slow development of spontaneous exploratory activity (Frankova and Barnes, 1968a) and lower intensity of exploratory behaviour (Lat, Widdowson and McCance, 1961) have been found in rats subjected to neonatal undernutrition.

Although developmental retardation has been noted in early life in several studies, no permanent effects on complex learning were observed in undernourished and rehabilitated

rats (D'Amato, 1960; Guthrie, 1968; Barnes et al, 1966; Frankova and Barnes, 1969b). However, some persisting effects such as hoarding behaviour, increased emotionality and decreased exploratory activity have been found (Seitz, 1954; Novakova, 1966; Frankova and Barnes, 1968a; Chow, Blackwell and Sherwin, 1968).

Severe undernutrition or protein deficiency during the postweaning period preceded by neonatal undernutrition has been found to impair psychological performance (Frankova and Barnes, 1968a). In this case the impairment was much greater with a protein deficient diet. Protein deficiency in the postweaning period has been found to impair performance on visual discrimination and reversal learning and water maze performance in rats. (Rajalakshmi, Govindarajan and Ramakrishnan, 1965; Rajalakshmi and Ramakrishnan, 1969a). In pigs it is found to show impaired performance on tasks such as electric shock avoidance (Barnes et al, [illegible], 1968). Dogs subjected to protein deficiency showed lack of interest in the surroundings and were less playful and more "aggressive" even after rehabilitation (Platt and Stewart, 1968).

It would be reasonable to expect from the foregoing that nutritional deficiency during the prenatal, neonatal and post-weaning period may retard to some extent behavioral development as well. The extrapolation of the results of such studies to

man is a problem because of differences in the ontogenic development of the brain at different stages in different species. In the case of man the peak period of neuronal multiplication is around 18 weeks of gestation whereas in rats it is around birth (Altman, 1969). The glial multiplication begins at about birth in the rat and about mid-gestation in man (Dobbing and Sands, 1973). In the rat the phase of rapid myelination ~~is~~ ends by about 25 postnatal days, a stage reached by the human brain by about 3 to 4 postnatal years.

It can be seen that growth during the neonatal period is much more rapid in the rat than in man. This also means that the neonatal brain may be more vulnerable to malnutrition in this species than in man. Another point is that the stress of reproduction is also much more severe in the rat than in man so that nutritional deficiencies in the mother affect the offspring to a greater extent in the former. However, if the effects of malnutrition on the growth and establishment of synaptic connections are more important than the marginal effects on neuronal number (and this seems likely) man is not perhaps more protected than the rat, since in both species this phase of brain growth is predominantly postnatal. In the rat it occupies the first 25 postnatal days. In man it runs from midpregnancy into the second postnatal year or even well beyond (Dobbing and Sands, 1973).

It is well-known that a substantial section of the world's population is either undernourished or malnourished or both. Children in the postweaning period are the group most vulnerable to malnutrition as the diets provided for them are often more deficient in quantity and quality than adult diets while their nutritional requirements are greater in relation to body weight and calorie intake. Severe protein calorie malnutrition in children has been found to be associated with changes such as decrease in brain weight (Udani, 1962; Brown, 1965; Winick and Rosso, 1969a), DNA (Rosso, Hormazabal and Winick, 1970; Winick, Rosso and Waterlow, 1970; Ganguli, Dutta and Mukherjee, 1972), RNA, protein and total lipids (Winick and Rosso, 1969b; Ganguli et al, 1972; Subbarao and Janardana Sarma, 1972), cholesterol and phospholipids (Winick and Rosso, 1969b; Winick, Rosso and Waterlow, 1970; Subbarao and Janardana Sarma, 1972), cerebrosides and sulfatides (Fishman, Prensky and Dodge, 1969; Mokashi, Mukherjee, Ganguli and Bachhawat, 1972), and gangliosides (Mokashi et al, 1972). Bachhawat (1972) reported deficits in the concentration of glycolipids (cerebroside + sulfatides) and mucopolysaccharides in the whole brain and of gangliosides in the white matter in malnourished children.

Histological changes similar to those found in malnourished pigs and dogs (Platt, Heard and Stewart, 1964) have been found

in the brain of malnourished children (Udani, 1962). Davison (1967) found the myelin in the brains of malnourished children to resemble the immature myelin of malnourished animals.

Abnormalities of the EEG pattern have been found in malnourished children (Engel, 1956; Nelson, 1959; Nelson and Dean, 1959). Normal patterns evolve with nutritional rehabilitation and restoration of body weight to near normal levels (Valenzuela Hernandez and Macias, 1959). In some studies the abnormalities were found to persist (Engel, 1956).

The psychological changes in the malnourished child were recognized long before the other changes, and more than two decades ago, Carothers (1953) stressed the mental arrest of malnourished children in his report to the World Health Organization. Extreme apathy is a common feature in children suffering from kwashiorkor (Platt, 1961) and for all purposes they seem to have lost contact with environment (Rajalakshmi and Ramakrishnan, 1972). They 'stay put' because of edema and are extremely irritable when disturbed. In contrast to this marasmic children are more irritable and tend to be cry babies. They are not as apathetic as the kwashiorkor children and show a fair interest in surroundings.

A number of studies have suggested the poor psychological status of the malnourished child (Stoch and Smythe, 1963; Cabak and Najadanvic, 1965; Stoch and Smythe, 1967; Monckeberg, 1968; Chase and Martin, 1970). Previously established reflexes are depressed or abolished and the elaboration of new conditioned reflexes are affected in protein deficient children (Brozek, 1962).

Persistent effects of early malnutrition on psychological performance are found in some studies (Cravioto, 1966; Hertzig, Birch, Stephen, Richardson and Tizard, 1972). The studies of Cravioto and Robles (1965) and Stoch and Smythe (1963, 1967) suggested a correlation between the age at which the children were hospitalized and intellectual impairment but such correlation was not found in the apparently more systematic studies of Hertzig et al (1972).

Apart from nutritional status factors such as paucity of the cultural environment, social and economic disadvantage, poor housing, excessively frequent and shortspaced reproduction, maternal and child illhealth etc. are also capable of influencing intellectual development. The role of environmental factors has been discussed by Richardson (1968).

In this connection studies carried out in Baroda show rural poor children to have a Cattell I.Q. of about 80 as

compared to 100 in the urban poor, although the two groups have a similar poor nutritional status as judged by height, weight and composition of blood and serum (Rajalakshmi and Ramakrishnan, 1969b; Rajalakshmi and associates, unpublished). Also, more attendance at a play centre in a poor village improved I.Q. scores as much as nutritional improvement. In the studies on urban children, growth retardation so that the body weight deficits were about 25-30% before 2-3 years of age was not associated with deterioration in psychological performance. The children had adequate protein status and were not lacking in affective care by parents. Mclaren (1971) found an improvement in the psychological performance of malnourished children with environmental stimulation.

Most of the studies on the effects of nutritional deprivation are on the composition of the brain with regard to protein, lipids and nucleic acid. However, the aminoacid make up of the brain and the metabolism of the same should be of special interest in view of the special features with regard to both.

The brain contains 2% of soluble organic constituents of which a major portion is aminoacid. Twenty per cent of the total nitrogen in the brain is made up of amino nitrogen 25% of which is contributed by glutamic acid and glutamine (Waelsch, 1952; 1955; Weil-Malherbe, 1952; Ansell and

Richter, 1954). While the brain compares with plasma and liver in its concentrations of essential aminoacids it has a much higher concentration of non-essential aminoacids (Waelsch, 1957).

Glutamic acid, glutamine and GABA account for a substantial portion of the non-essential aminoacids. When U-C^{14} glucose was subcutaneously injected in the rat a greater proportion of labelling was found in the brain than in the liver in glutamate, glutamine and aspartate (Gaitonde and Richter, 1966; Gaitonde, Dahl and Elliot, 1965).

The role of glutamic acid in brain metabolism received increased attention due to the observation of Thunberg (1923) that the brain can oxidize glutamic acid *in vitro*. Extensive studies carried out by Weil-Malherbe (1936) showed that during the oxidation of glutamic acid no ammonia was evolved. It is now known that it can be oxidized via the GABA shunt by the successive conversion of glutamic acid to GABA, succinate semialdehyde and succinate.

In situ, glutamate can be formed by transaminase, namely, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and GABA transaminase and glutamate dehydrogenase. Although brain glutamic acid could be theoretically derived from either the amination of or transamination with 2-oxo-glutarate, the major pathway for its formation appears to be

the direct amination of 2-oxoglutarate 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. (Krebs, Eggleston and Hems, 1948). The brain has a higher concentration of glutamate than the liver but a lower activity of glutamate dehydrogenase. This is explained by the fact that in the brain the equilibrium constant of the enzyme favours reductive amination and that it does not normally operate in the direction of oxidation (Weil-Malherbe, 1957; Olson and Anfinson, 1953). This helps in the removal of ammonia which is toxic to the nervous system (Krebs, Eggleston and Hems, 1948).

Glutamic acid has a high turn-over rate in brain slices and is rapidly metabolized to glutamine, GABA and aspartic acid. Glutamine synthetase is invariably present in the brains of all animals (Krebs, 1935) whereas it is variably present or absent in other tissues (Wu, 1963). This enzyme also provides a local machinery for the removal of ammonia (Sapirstein, 1943). In this connection, the blood brain barrier acts against glutamic acid but not against glutamine (Kamin and Handler, 1951).

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 to a greater extent in the grey matter (Tower, 1959) which is mainly concerned with nervous activity whereas glutamine is distributed equally in grey and white matter (Krebs, Eggleston and Hems, 1949; Waelsch, 1952). The distribution of these two aminoacids in the cellular particulate also shows some variation. Glutamic acid is primarily found in the mitochondrial fraction whereas glutamine is found in the mitochondrial as well as nuclear fractions (Weil-Malherbe, 1957; Tower, 1959).

GABA is uniquely present in the brain and it has been found in all parts of the CNS, but is consistently more in grey matter than in white matter (Berl and Waelsch, 1958; Elliot, 1958; Albers, 1960; Baxter, 1970). The tissue content of GABA is a function of the activities of GAD and GABA transaminase, but it has been shown that the amount of GABA present in any region of the brain varies with the activity of glutamate decarboxylase (Roberts, 1960; Sisken, Roberts and Baxter, 1960; Collins, 1972). Such correlation is not seen with GABA and its degrading enzyme GABA transaminase (Collins, 1972). Certain nerve fibres and terminals such as Purkinje axon terminals in the cerebellar nuclei and the dorsal part of the lateral vestibular nucleus contain relatively high concentrations of GABA and glutamate decarboxylase (Kravitz, Molinoff and Hall, 1965; Fonnum,1973).

Evidence is accumulating that in the hippocampus the basket cells in the area dentata contain GAD which is also concentrated in the molecular layers of the hippocampal regions. Very high concentrations of GAD and GABA are found in nerve terminals in the substantia nigra. These terminals are probably derived from cells in the globus pallidus or striatum (Fonnum, 1973).

The subcellular distribution of the enzymes concerned with glutamate metabolism has been studied by several workers (Vandenberg and Van Kempen and Veldstra, 1965; Balázs, Dahl and Hardwood, 1966; Salgonicoff and DeRobertis, 1965). Glutamate dehydrogenase is localized in the mitochondria whereas aspartate aminotransferase and alanine aminotransferase are having bimodal localization. They are found in the mitochondria as well as the cytoplasm. Glutamine synthetase is concentrated in the microsomal fraction (Sellinger, Verster and Baloein (1962) whereas GABA-T is localized in the mitochondria (Salgonicoff and DeRobertis, 1965; Weinstein, Roberts and Rakefuda, 1971; Fonnum, 1973). GAD is localized in the synaptosomes and is particularly concentrated in the fraction of nerve endings poor in acetylcholine. Salgonicoff and DeRobertis (1965) speculate that in the living condition GAD is fixed to the outer surface of the vesicle by Ca^{2+} bonds.

A substantial portion of glucose is found to be oxidized via the GABA shunt although estimates of the same vary from 10-40%. (McKhann and Tower, 1959; 1961; Elliot, 1965). A more recent estimate obtained by following the oxidation of glucose in brain slices suggest the flux through the GABA bypass to be 8% of the total flux through the tricarboxylic acid cycle (Patel, Balázs and Richter, 1970). However no net change in GABA concentration is found when this is used as substrate (Elliot, 1965) although a negligible net consumption is found in the presence of glucose (Elliot and Van Gelder, 1958). This could be because of the continued synthesis of GABA from glutamate formed during transamination and reductive amination of α-ketoglutarate.

Studies reviewed by Spadoni and Gaetani (1972) suggest that the formation of polysomes and protein synthetic activity in the liver depend on the free aminoacid pool. The increase in the free aminoacid pool in many tissues including the nervous tissue has been found to correlate with protein synthetic activity during development (Miller, 1969).

In this connection GABA is found to have an accelerating effect on protein synthesis. In the brain GABA interacts with aminoacyl t. RNA synthetase whose activity increases as a consequence (Baxter, Tewari and Raeburn, 1972).

As mentioned earlier, the concentrations of glutamic acid, glutamine and GABA are found to increase during fetal and postnatal development and reach adult values with the myelination and maturation of neurons (Baxter, Schade and Roberts, 1960; Agrawal, Davis and Himwich, 1966). The postnatal increase in the cerebral glutamate levels coincides also with the rapid rate of conversion of glucose carbon into aminoacids (Gaitonde and Richter, 1966). In rats these changes parallel the development of compartmentation of glutamate metabolism (Patel and Balázs, 1970) and occur between 14-21 days of age. The enzymes of the GABA shunt in the brain increase during the postnatal period (Vandenberg, Van Kempen and Veldstra, 1965; Bayer and McMurry, 1967; Sims and Pitts, 1970). Changes in GAD activity with age in different areas of the brain are correlated highly with the rapid growth of the surface area of the dendrites while changes in GABA levels are correlated with the increase in the volume of dendrites (Schade and Baxter, 1960; Sisken et al, 1960; Himwich, 1962; Roberts and Kuriyama, 1968). It is found that the appearance of adult EEG patterns and the maturation of the cell body nucleus coincides with the attainment of adult concentrations of GABA (Schade and Baxter, 1960).

Apart from the metabolic importance of glutamate in respiration as well as detoxification of ammonia, glutamic acid and GABA are believed to exert some electrophysiological function in the brain, Glutamic acid is found to have an excitatory effect and GABA, an inhibitory one (Curtis, 1962, 1965; Hebb, 1970; Johnson, 1972).

Extracellular application of glutamate is found to increase the rate of firing which varies with the amount of glutamate released (Galindo, Krnjevic and Schwartz, 1967). Curtis, Phills and Watkins (1960) showed by intracellular recording that the increase in membrane excitability caused by glutamate is associated with a clear depolarizing effect with a fall in membrane resistance. Although the evidence for a significant release of glutamate is hardly conclusive, the release of glutamate was found by electrical stimulation of cortical slices (Hammerstad and Cuttler, 1972) or by stimulating suspensions of cortical synaptosomes (Bradford, 1970). It is also found that the release of glutamate from the surface of the cerebral cortex can be correlated with cortical activity (Jasper and Koyama, 1969).

There is some evidence that the mechanism of excitation is initiated by a mobilization of membrane bound Ca^{2+}, (Krnjevic, 1974).

The powerful inhibitory action of GABA on cortical neurons was first described by Krnjevic and Phills (1963). Extracellular application of GABA showed a hyperpolarizing effect associated with marked increase in membrane conductance which is similar to that of natural inhibition evoked by epicortical stimulation (Krnjevic, Randic and Straughan, 1966a and b; Li and Chou, 1962). These effects were reversed by injection of chloride ions to neurons (Diamond, 1968). These observations provide strong evidence that the action of GABA and that of the natural inhibitory transmitters in the cortex are identical. Some cells of the dieter's nucleus situated in the medulla receive a direct inhibitory innervation from the cerebellar cortex. Studies on these neurons gave convincing evidence of GABA as an inhibitory transmitter (Obata, Otuska and Tanaka, 1970). The inhibitory action of GABA on central neurons appears to be due to a pronounced increase in chloride permeability (Krnjevic, 1965).

If GABA is indeed an inhibitory transmitter it should be released from inhibitory nerve endings. Studies with brain slices and on isolated nerve endings (Machiyama, Balázs, Hammond, Julian and Richter, 1970) show that if they are stimulated electrically or with 40 mM K^+, a large amount of GABA is released in the medium. In vivo studies show that GABA is liberated when the brain is stimulated (Obata and Takeda, 1969; Iverson, Mitchell and Srinivasan, 1971).

Nearly half of the labelled GABA taken up by cortical slices is found in nerve endings which also contain the GAD activity in the tissue (Bloom and Iverson, 1971). These terminals in the cerebral cortex would constitute 4% of the total volume.

Balázs, Machiyama and Patel (1972) were able to demonstrate a small compartment present in the nerve endings which has a small glutamate pool, rapidly in equilibrium with GABA. The concentration of GABA in this compartment is estimated to be 25 to 30% of the total GABA. Thus in 1 g of brain tissue the GABA pool is about 0.5 µmole.

The amount of glutamate and GABA in the synaptosomes is very small but may be functionally most important. Krnjevic and Whittekar (1965) obtained excitatory effects with glutamate and depressant effects with GABA with the rates of release corresponding to 700 and 350 synaptosomes per second. This is important since synaptic activation at these rates is considered to be well within the possible physiological limit (Whittekar and Sheridan, 1965).

The exact mechanism of action of GABA is not understood. On the basis of the studies on the localization of enzymes of glutamate and GABA metabolism (Salgonicoff and DeRobertis, 1965), GABA may be acting in either of the two ways described

below (Himwich and Agrawal, 1969). One possibility is a transynaptic diffusion of GABA to regulate the excitability of adjacent neurons. It has also been suggested that the GABA bound to the synaptic vesicles is released when an impulse arrives in a neuron, and as a result, it crosses the synaptic cleft to exert its effects on the postsynaptic membrane.

According to Roberts (1966) when an excitatory transmitter affects a postsynaptic membrane and depolarization results, an instantaneous postsynaptic release of GABA from bound or stored form into the extraneuronal synaptic environment occurs and this acts as a synaptic feedback transmitter. Such an inhibitory transmitter could be bound to both presynaptic and postsynaptic membrane on the sides facing the synaptic cleft and thus accelerate the rate of return to the resting potential.

Lowered concentrations of GABA in epileptic seizures and in seizures induced by convulsive agents give indirect evidence of the function of GABA as inhibitory transmitter (Roberts and Baxter, 1959).

Nervous transmission is via both the axons and dendrites (Hebb, 1960). The axons either respond fully or not at all to stimulation (all or none response)whereas the response of the

dendrites varies with the strength of the impulse. The latter results in diffused transmission which does not reflect the original nature of this nerve impulse but is very necessary for maintaining background electrical activity in the brain (Magoun, 1963). Dendritic activity is evident even in sleep and is believed to influence the level of arousal. The learning process is believed to involve the repeated firing of groups of neurons or neuronal assemblies. 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 (Hebb, 1949). An optimum level of dendritic activity is critical for efficient CNS function and the relative proportions of glutamic acid and GABA may conceivably determine the level of background electrical activity (Rajalakshmi, Govindarajan and Ramakrishnan, 1965; Hebb, 1970).

Some definite correlations have been observed between the ratio of GABA to glutamic acid in the brain and some behavioural characteristics of the rats. Emotionally stable strain of rats had slightly higher levels of GABA in the brain while a more reactive strain of rats and generally in a higher state of arousal had relatively low ratios of GABA to glutamate (Ricks, Huggins and Kerkut, 1967). In another series of experiments a definite correlation was

seen between cholinesterase activity and GABA production in the cerebral cortex in 5 different strains of rats (Ricks, Morris and Kerkut, 1968).

The high turn-over of glutamate in the brain and the importance of the glutamate system in brain metabolism stimulated studies in this laboratory on the effects of nutritional deficiencies on enzymes involved in glutamate metabolism in the rat brain. In previous studies in this laboratory a low protein diet in the immediate postweaning period was found to result in deficits in brain glutamate dehydrogenase and decarboxylase. This suggests the possibility that similar deficits may be found with deficiency during the neonatal period when the brain can be expected to be even more vulnerable to the effects of nutritional deficiency.

The present studies were carried out in rats on the effects of neonatal undernutrition on brain glutamate dehydrogenase and decarboxylase with special reference to the possible dependence of such effects on the severity and duration of undernutrition and on the brain region studied. Studies were also made of the effects of such undernutrition on the vulnerability of the animals to subsequent nutritional deficiency. The details of these studies are incorporated in this thesis.