C H A P T E R I

Introduction

CHAPTER I

INTRODUCTION

The brain has been looked upon as a more or less static organ undergoing no chemical changes after reaching full development in early infancy. This belief persisted partly because the composition of the brain with regard to protein, fat and other major constituents does not change much and partly because nerve tissue does not regenerate (Leblond and Walker, 1956).

The early work on brain was mostly concerned with chemical analysis of lipid constituents, the credit for which goes to the father of brain biochemistry, Thudichum (Thudichum, 1884). The interest in brain lipids was mainly due to its role in myelination, which is unique to the central nervous system.

The chemical composition of the brain is given in Table 1. It is clear from the table that 50% of the dry weight of the brain is lipid. The myelin sheath which is necessary for its structural maintenance contains a high proportion of lipids. The second largest fraction is protein. The proteins of the brain are highly complex in nature and the lipoproteins are mainly responsible for brain structure. The brain has also a unique type of protein which can be extracted with chloroform-methanol mixture (McIlwain, 1959). Recently a soluble protein having a

Table 1

Approximate composition of brain*

Component	Gram per 100 g. of brain tissue
Water	77 - 78
Inorganic salts	1
Soluble organic substance	2
Carbohydrate	1
Protein	8
Total lipids	11

* McIlwain (1959).

molecular weight of about 30,000 was found in the nervous tissue, but not in other organs (Moore, 1965).

As the brain develops from the embryonic to the adult stage there is a gross change in chemical composition as well as the metabolic activity of the brain. The protein, lipid, and water contents change considerably. The chemical composition of the brain at different ages is given in Table 2.

Because of the rapid growth of the brain during the early period, a rapid synthesis of new proteins is necessary. Each stage of growth requires specific proteins (enzymes

Table 2

Composition of mouse brain* at different ages

Age (days)	Gram per 100 g. of fresh tissue			
	Water	Solids	Protein	Lipids
1	86.8	13.3	8.70	2.36
4	87.5	12.5	7.45	3.18
7	86.4	13.6	7.92	3.57
10	84.4	15.6	9.00	4.60
16	81.5	18.5	10.40	5.68
19	80.7	19.3	10.90	6.30
25	78.7	21.3	11.40	7.55
30	78.5	21.5	11.40	7.50
75	77.2	22.8	12.00	8.04
90	76.8	23.2	12.10	8.42
100	76.2	23.8	12.10	8.84

* Folch Pi,1955.

and structural proteins). Thus the mean protein content of the brain of rat increases from 6.5 per cent at birth to 11.1 per cent at the adult stage (Richter, 1962). It varies in different areas of the brain because of differences in cell distribution and in the maturation of the cells (Randall, 1938; Clouet and Gaitonde, 1956; Richter, 1962). The protein content increases first in the phylogenetically older areas of the brain such as medulla and hypothalamus which are first to become functionally active (Kelly, 1956; Richter, 1959a, 1962). At about the time of the "critical period" (Peters and Flexner, 1950) during which the neurons of the cortex develop their dendrites and become functionally active (Clouet and Gaitonde, 1956),the brain protein changes rapidly in its content and nature (Block, 1937; Clouet and Gaitonde, 1956; Lajtha, 1958,1959; Moore, 1965). The development of the blood brain barrier also starts at this period. The presence of different types of protein at different stages of functional and metabolic maturity suggest the specificity of proteins contributing to different functional properties.

As might be expected, changes in brain composition are associated with changes in enzymes at different stages of development. Thus an increase in the cytochrome-cytochrome oxidase activity of the cerebral cortex takes place

at the period is critical for morphological change (Flexner, Flexner and Straus, 1941). Succinic dehydrogenase and ATPase activities in rat brain are found to be at low levels up to the 6th day of postnatal life and to increase rapidly thereafter (Potter, Schneider and Liebb, 1945). Similarly a number of enzymes such as phosphatases, choline esterase (Cohn and Richter, 1956; Flexner, 1952; 1955) and glutamosynthetase (Rudnick and Waelsch, 1955; 1955a) increase with the progress of myelination. Lactate dehydrogenase activity in the adult brain was found to be twice as much as in the 10 day old rat as in the new born rat (Kuhlman and Lowry, 1956). A similar observation was made with regard to glutamate decarboxylase (Roberts, Harman and Frankel, 1951)

Though it is found that most of the enzymes show an increase during development there are some exceptions. In the guinea pig, acid phosphatase does not change to any extent during gestation and it remains at a high concentration (Flexner and Flexner, 1948). Similarly glucose-6-phosphate dehydrgenase does not increase with age (Kuhlman and Lowry, 1956).

Species variations also exist in the case of enzymes such as acetyl choline esterase which increased sharply even during the gestation period in the guinea pig (Peters and Flexner, 1950; Hamburgh and Flexner, 1956) whereas in rats the maximal value was reached in 10 to 15 days of post-

natal life (Cohn and Richter, 1956).

The activities of a number of different enzymes during fetal development were found to be correlated with morphological and functional changes occuring during maturation of the brain (Peters and Flexner, 1950; Hamburgh and Flexner, 1956). Thus certain enzymes concerned with the synthesis of cholesterol were found to be absent at maturity (Srere, Chaikoff, Treitman and Burstein, 1950; Korey and Stein, 1961). Changes in the amino acid distribution of the brain protein with age has been reported (Clouet and Gaitonde, 1956).

It appears from the available data that the proteins of the immature brain have a higher average turn over rate than those of the mature one (Gaitonde and Richter, 1956; Lajtha, Frust, Grestein and Waelsch, 1957; Lajtha, 1959).

Cerebral metabolism accounts for 20-25% of the basal metabolism in man (Kety, 1957) and cerebral blood flow is also considerably high (Kety, 1957) although the weight of the brain is only about 2% of the total body weight.

The metabolic activity of the brain has generally been thought of mainly in terms of carbohydrate metabolism as the respiratory quotient is one. But during the last few years it has become to be recognised that the proteins in the brain particularly the nerve cells are metabolically highly active (Gaitonde and Richter, 1953, 1955, 1956;

Cohn, Gaitonde and Richter, 1954;Clouet and Richter, 1959; Lajtha, 1958, 1964; Richter, 1962; Richter,Gaitonde and Cohn, 1960; Waelsch and Lajtha, 1961).

Previous misconceptions 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 acid was not followed by rapid incorporation in the brain(Friedberg and Greenberg, 1947). It is now known that this is because of the blood brain barrier. Subsequent studies have shown that incorporation in brain proteins is quite rapid when the amino acids are administered intracisternally (Friedberg, Tarver and Greenberg, 1948; Gaitonde and Richter, 1953,1955, 1956). The reasons for the high rates of protein turn over in the brain are not known (Waelsch and Lajtha, 1961; Lajtha, 1964). The same is consistent with the high rate of cerebral metabolism and the fact that the brain manufactures many of its metabolites such as glutamic acid.

It is well known that in spite of the relatively stable net chemical composition of the brain, factors such as prolonged cold exposure (Andjus, Knopfelmacher, Russel and Smith, 1955; Schneider, 1957; Subbarao and Gupta, 1965),

insulin shock (Cravioto, Massieu and Izquiero, 1951; Dawson, 1953; McIlwain, 1959), hypoxia (Himwich,1951), psychotropic drugs (Quastel and Wheatley, 1932; Himwich and Rinaldi, 1957; Quastel, 1965), and electro-convulsive shock (Holmberg, 1963) severely affect brain functions.

The role of nutritional deficiency in brain function has been largely ignored in spite of the dramatic effects found in central nervous functions with vitamin deficiencies (Coursin, 1965, 1967). This was primarily due to the fact that calorie restriction for a short term was not found to result in changes in brain composition (Folch,1947; Fulton, 1949). However, in view of the high metabolic activity of the brain and its requirement of cofactors and enzymes for the synthesis of brain metabolites, it is unlikely that the brain can continue to function normally in the face of local deficiency. The lack of severe effects in starvation is due to the possibility that the tissue stores are preferentially transferred to the brain. The effects of insulin hypoglyceamia and hypoxia are dramatic instances of the susceptibility of the brain to a lack of glucose and oxygen.

As mentioned earlier, deficiencies of vitamins such as ascorbic acid, thiamine, niacin, and pyridoxine have been

found to be associated with severe behavioural symptoms as well as nervous system disorders (Bourne, 1953;Goldsmith, 1953, 1964; Eudison, Geller, Yuwiler and Eudison, 1964). Pellagra, a nutritional deficiency disease (Goldsmith,1953) primarily attributed to niacin and amino acids, is characterised by psychic disturbances such as loss of recent memory, loss of clarity, general lassitude,depression, and other clinical manifestations (Leigh, 1952; Goldsmith, 1956). The deficiency of thiamine is also associated with mental depression and other behavioural changes apart from neurological changes (Lowry, 1952; Brozek, 1957). Neurological symptoms seem to depend on the lowering of the rates of oxidative decarboxylation and cocarboxylase, and the thiamine level of the brain. They are severe if all three levels are low and occur less frequently if the thiamine level is still high (von Muralt, 1962).

The effect of deficiency of vitamin A on the functioning of the retina is well known. An increasing inefficiency of dark light adaptation , night blindness, and finally total blindness are found to result (Dam and Sondergaard, 1964). Disordered ossification follows the vitamin A deficiency and cause irregularities in skull growth (Spillane, 1955). If the development of the brain continues normally the resulting disproportion leads to mechanical interferance with nervous

tissue. The changes so produced lead to blindness, deafness and paralysis in certain animals (Mellanby, 1941; Wolbach and Bessey, 1942).

More recently effect of a deficiency of pyridoxine (Tower, 1958a; Coursin, 1960), riboflavin (Street, Cowgil and Zimmerman, 1941), pantothenic acid (Bean and Hodges, 1954) and vitamin B_{12} (Newberne and O'Dell, 1959) on central nervous system have been identified in experimental animals and also in man (McIlwain, 1959). Behavioural abnormalities were observed with riboflavin deficiency in dogs (Street, Cowgil and Zimmerman, 1941). Pantothenate deficiency caused in a number of animal species malfunctioning of central nervous system involving paralysis , coma or convulsions (Gantt, 1957). Mental confusion was found to be associated with pernicious anemia, and patients suffering from the same showed an abnormal electroencephalographic pattern (McIlwain, 1959). Convulsions are known to occur in experimental animals (Patton, Karn and Longenecker, 1944) and human infants (Maloney and Parmelee, 1954; Hunt, Stokes, McMory and Stroud, 1954) during pyridoxine deficiency which also produces abnormal electroencephalographic pattern and reduced respiration of brain tissue slices (Roberts, Wein and Simonsen, 1964). The degeneration of neurons was observed with a deficiency of thiamine (Peters, 1962) as well as riboflavin(Eudison, Geller, Yuwiler and Eudison, 1964).

Protein deficiency has been observed to be related to

mental impairment by clinicians (Platt, 1961). The segment of population most severly damaged by malnutrition in nearly all of the developing countries is the age range from weaning to five years. In most areas up to 50% of the children fail to survive to school age directly or indirectly as result of poor nutrition. Among those that do survive, permanent stunting is equivalent to two or three years of their most rapid growth, and the central nervous system also may be irreversibly damaged to a degree comparable to the suppression of this growth (NAS and NRC report, 1963).

Others such as Cravioto, Delicardie and Birch,(1965) have remarked that the mental arrest caused by protein deficiency is almost irreversible. As pointed out by Platt (1961), "clinically mental apathy is so constant a feature of protein malnutrition that it is often left out of description of the disease".

Although the mental changes, misery, apathy, irritability and drowsiness are such constant findings in children suffering from protein-calorie deficiency, there appear to be few studies on either behavioural aspects or central nervous system changes. In contrast, extensive studies have been carried out on the effects of protein-calorie malnutrition on the structure and function of the liver

(Ramalingaswami, Sriramachari and Patwardhan,1954, 1958; Heard, Platt and Stewart, 1958; Platt,Heard and Stewart, 1964), body composition (Widdowson, Dickerson and McCance, 1960; Widdowson, 1966) and vitamin A status (McLaren, 1960,1966; McLaren, Shirajian, Tchalian and Khoury, 1965; Oomen, McLaren and Escapini, 1964)

The effect of inanition on the central nervous system has been reviewed by Jackson (1925), who reports that the nerve cells of animals, and especially the motor cells of the spinal cord, undergo atrophic degeneration, chromatolysis, cytoplasmic vacuolation and disorganization of neurofibrils and in severe cases actual loss of some cells. Secondary diffuse changes in the myelinated fibers are also said to occur. An increase in the neuroglial cells in the spinal cord of mice subjected to starvation from the 65th day of age has been reported (Andrew, 1941). Inanition in new born rats can reduce brain weight by as much as 46 per cent and cause a reduction in the growth of neurophil so that the normal patterning of the dendritic process is not achived (Eayrs and Horn, 1955; Horn, 1955). Widdowson and McCance (1960) induced inanition by increasing litter size and found an 8% decrease in the brain weight at 23 days of age and 21% at 14 days of age. A similar weight reduction of the brain of undernourished pigs was also observed (McCance, 1960; Widdowson and McCance, 1962). Dobbing (1964) has

reported delayed myelination in rats subjected to early inanition. He was able to show a lower cholesterol content in the neurons of the undernourished animals.

Lowrey, Pond, Barnes, Krook and Loosli (1962) have shown that protein deficiency caused by feeding grossly imbalanced diets produces anatomic alterations in the brain, including a reduction in the number of neurons in all grey matter, and swelling of the neurons with poorly developed axons and dendrites.

Platt (1962) found Nissel substances in the anterior horn cells to be deficient in amount and different in appearance in piglets subjected to protein-calorie deficiency. He further observed that animals subjected to protein malnutrition frequently become semicomatose for a short time before death. He found a similar phenomenon in Gambian infants suffering from protein malnutrition and observes that the death in both the cases is a central nervous death apparently meaning thereby that the activity of the brain stops first leading to a cessation of activity of the heart and other organs resulting in death (Platt, 1961)

There is some evidence from animal experiments to show that moderate protein deficiency of the mother may give rise to neurological and behavioural abnormality in young. Thus progressive retardation in development and intelligence with successive generations of rats fed low

protein diet was observed (Cowley and Griesel, 1959, 1963). Barnes and his associates have provided evidence that nutritional deprivation in early life can result in long lasting or permanent retardation in the development of learning behaviour. This could be partly reversed in rats rehabilitated (Barnes, Cunnold, Zimmermann, Simmons, MacLeod and Krook, 1966; Barnes, 1967). Lat, Widdowson and McCance,(1960) observed altered behavioural pattern in adult rats that had been underfed prior to weaning. Similarly Platt (1962); Platt, Heard and Stewart(1964) observed tremor and clumsiness in puppies born to bitches subjected to protein deficiency.

Stoch and Smythe (1963) observed that severe under-nutrition in children during the first two years of life is associated with low head circumference and low intelligence quotients. Abnormal electroencephalographic patterns were obtained in African infants suffering from protein malnutrition (Nelson and Dean, 1959).

Not many studies have been carried out on specific biochemical changes in the brain with protein deficiency. One of the problems in studying such changes is the choice of the biochemical parameters to be studied. Because of the high percentage of lipids in the brain the few studies carried out have attempted to measure changes in brain lipids (Page, 1937).

In this laboratory studies have been carried out in rats on the effects of undernutrition and protein deficiency on psychological performance and glutamic acid metabolism in the brain (Rajalakshmi, Govindarajan and Ramakrishnan, 1965; Rajalakshmi, Ali and Ramakrishnan, 1967). In these studies protein deficiency was found to affect both whereas neither was affected by calorie restriction.

The choice of the glutamic acid system in the above studies was guided by the consideration that the brain has a high concentration of glutamic acid which is metabolised through Gamma-aminobutyric acid to succinate by a unique shunt pathway in the brain (Roberts, 1960).

As mentioned previously the brain contains 2 per cent of soluble organic constituents, of which a major portion is amino acid. Twenty per cent of total nitrogen in the brain is made up of free amino nitrogen and 25 per cent of this contributed by glutamic acid and glutamine (Ansel and Richter, 1954; Waelsch, 1952, 1955;Weil-Malherbe,1952). An outstanding feature of the central nervous system is the existence of exceptionally high concentrations of non-essential amino acids in an easily extractable form. It will be seen from table 3 that whereas the brain compares with the liver and plasma with regard to the concentration of essential amino acids, it has a particularly high concentration of non-essential amino acids, mainly glutamic

Table 3

Concentrations of free amino acids in brain, liver and plasma of rat and cat*

Amino acid	µmoles per gram of fresh tissue					
	Rat			Cat		
	Brain	Liver	Plasma	Brain	Liver	Plasma
Leu	0.20	0.60	0.20	0.14	0.28	0.21
Phe	0.06	0.30	0.06	0.07	0.105	0.049
Try	0.03	0.09	-	-	-	-
Val	0.10	0.50	0.20	0.18	0.36	0.126
His	0.08	0.32	0.04	0.06	0.60	0.09
Lys	0.02	0.05	0.026	0.14	0.252	0.21
Ileu	-	-	-	0.09	0.135	0.063
Tyr	0.10	0.34	0.08	0.06	0.12	0.042
Met	0.08	1.24	0.104	0.10	-	0.03
Thr	1.00	1.30	-	0.22	0.264	0.11
Arg	0.20	0.06	-	0.08	0.011	0.08
Asp	-	-	-	2.20	0.66	0.066
Asp(NH_2)	-	-	-	0.10	0.20	0.07
Glu	10.00	4.00	-	8.70	4.30	0.012
Glu(NH_2)	4.00	4.00	-	3.40	3.40	0.34
Gly	1.30	-	-	1.30	1.30	0.26
Ala	0.60	-	-	1.00	2.00	2.80
GABA	2.00	-	-	2.30	0.092	0.0002
Pro	-	-	-	0.14	0.24	0.21
Cys	-	-	-	0.04	-	0.02
Ser	1.10	-	-	0.72	0.36	0.216
Acetyl Asp.	5.00	-	-	6.00	0.18	-

* Data taken from Waelsch (1957).

Values for liver and plasma recalculated by multiplying with the factors given in the paper.

acid. Further, amino acids such as Gamma amino butyric acid, acetyl aspartic acid (Tallan, Moore and Stein, 1956), cystathionine (Tallan, Moore and Stein, 1958) and homocarnosine (Pisano, Wilson, Cohen, Abraham and Udenfriend, 1961) are uniquely present in the brain.

The role of glutamic acid in brain metabolism has elicited much speculation and interest since the observation of Thunberg (1920, 1923) that the brain can oxidize glutamic acid in vitro. The subsequent studies of Quastel and Wheatley,(1932), Krebs (1935) and Weil-Malherbe (1936) suggested that the mammalian brain can utilize glutamic acid in place of glucose. Thus Krebs stated l (+) glutamic acid to be the only amino acid found capable of maintaining the respiration of brain or retina (Krebs, 1935). Extensive studies carried out by Weil-Malherbe(1936) on glutamic acid showed that during the oxidation of glutamic acid no ammonia was evolved.

Considerable exitement regarding the role of this amino acid was also caused by the earlier reports regarding the effect of glutamic acid supplementation on the intelligence of mentally retarded children and the psychological performance of animals(Waelsch, 1951; Strecker, 1957).

The distribution of glutamic acid and glutamine in cellular components and in different layers of the brain

suggest 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 Gamma-aminobutyric acid are found to increase during fetal and post-natal development and reach ceiling values with myelination and maturation of the neurons (Krebs,Eggleston and Hems, 1949; Roberts, Harman and Frankel, 1951; Waelsch, 1952 Baxter, Schade and Roberts, 1960). In the rat the attainment of 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 behind the blood brain barrier (Rudnick and Waelsch, 1955, Waelsch, 1955a; Tower, 1959).

Glutamic acid has a high turnover rate in brain slices and is rapidly metabolized to glutamine, Gamma-aminobutyric acid and aspartic acid (Waelsch, 1957; Tower, 1959).

Enzymes involved in the metabolism of glutamic acid, namely, glutamine synthetase, glutamate decarboxylase, aspartate aminotransferase and alanine aminotransferase, have been identified in the brain (Cohen and Hekhuis,1941; Speck, 1949; Elliot, 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, 1935) whereas it is variably present or absent in the kidney, liver, heart etc. 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.

The glutamine of brain has remarkable stability (Weil-Malherbe, 1957) though the brain has the enzyme glutamine aminohydrolase (Krebs, 1935a). Cerebral glutamine is in a dynamic state with a rapid turn over rate both in vitro and in vivo (Tower, 1958, 1959). It is used for peptide synthesis (Waelsch, 1952). Both glutamine synthetase and L-glutamyl transferase are present in the microsomal fraction of the brain(Waelsch, 1957) which is involved in protein synthesis.

The brain has also been reported to possess the enzyme machinery for the transamination of glutamic acid to alanine

and aspartic acid (Cohen and Hekhuis, 1941).

As already pointed out Gamma-aminobutyric acid is formed by the decarboxylation of glutamic acid (Roberts, Frankel and Harman, 1950; Awapara, Landua, Fuerst and Seale,1950). It transaminates with one molecule of 2-oxoglutaric acid to yield succinic semialdehyde which is further oxidized to succinate, an intermediate of the Kreb's cycle. The enzyme system which catalyses these reactions, namely, 4-aminobutyrate aminotransferase and succinic semialdehyde dehydrogenase are present in the brain (Bessman, Rossen and Layne, 1953; Roberts and Bregoff, 1953; Roberts, 1960; Albers, 1960; Albers and Salvador,1958). The metabolism of glutamic acid to Gamma-aminobutyric acid and succinate provides a shunt pathway from 2-oxoglutarate to succinate in the brain.

Unlike glutamate decarboxylase, 4-aminobutyrate aminotransferase is present in the liver and kidney (Roberts and Bregoff, 1953). The significance of its presence in the liver has not been commented on but might well be the need to oxidize Gamma-aminobutyric acid present in foods of some vegetable origin as well as animal brain. Both glutamate decarboxylase and 4-aminobutyrate aminotransferase require pyridoxal phosphate as cofactor for their activity

(Roberts and Baxter, 1959). Glutamate decarboxylase is loosely bound with its prosthetic group whereas 4-aminobutyrate aminotransferase is firmly bound (Roberts, Harman and Frankel, 1951; Killam and Bain, 1957; Tower, 1957). The apparent differences in the affinity of these apoenzymes to their prosthetic group is consistent with the differential effects of pyridoxine deficiency on these two enzymes. During pyridoxine deficiency both Gamma-aminobutyric acid transaminase and glutamate decarboxylase will compete for pyridoxine and since the affinity of pyridoxine towards Gamma-aminobutyric acid transaminase is greater, glutamate decarboxylase is affected to a greater extent (Roberts and Baxter, 1959; Killam, 1957). This observation is consistent with the decreased level of Gamma-aminobutyric acid in this condition (Roberts and Frankel, 1951a).

The decarboxylation reaction is essentially irreversible (Roberts and Bregoff, 1953; Albers, 1960). A linear relationship between the amount of Gamma-aminobutyric acid present and glutamate decarboxylase activity in different areas of chick brain was observed (Sisken, Roberts and Baxter, 1960) which would suggest that Gamma-aminobutyric acid is mainly formed by the decarboxylation of glutamic acid by glutamate decarboxylase. The fact that the line can be extrapolated to zero supports the hypothesis that glutamate

decarboxylase is the enzyme responsible for the formation of Gamma-aminobutyric acid in the central nervous system (Sisken, Roberts, and Baxter, 1960). It has been suggested that the relative rate of decarboxylation and the subsequent transamination reaction in a particular cerebral area might determine the Gamma-aminobutyric acid level in that area (Roberts, 1960; Tower, 1958).

The brain derives its energy by the aerobic oxidation of glucose (Kety, 1957). The respiratory quotient is found to be one which is consistent with the observation that the brain is almost entirely dependent on carbohydrate as energy source (Kety, 1957; Waelsch, 1957). However, the perfusion studies of Geiger (1957,1958) have shown that, under extreme conditions, glutamic acid would appear to be used as substrate for oxidation purposes.

Rat brain slices incubated with specific inhibitors of glucose showed decreased oxygen uptake with a lowered respiratory quotient of 0.85, a figure corresponding to the oxidation of non-carbohydrate substances. This was associated with a decrease in glutamic acid suggesting its possible utilization as energy source during such conditions (Tower, 1959, 1963; Waelsch, 1959). During hypoxia when cerebral slices are incubated at low oxygen tension they show significant decreases in glutamate as well as glutamine concentrations (Waelsch, 1959).

The finding that ammonia is formed during nerve activity (Richter and Dawson, 1948; Richter, 1959;Waelsch and Lajtha, 1961; Hyden, 1955) suggests that glutamine may be utilised for energy purposes after deamination to glutamic acid. In the absence of glucose, glutamic acid formed from glutamine or from other amino acids by transamination reaction could support oxidation only for a limited period because of the release of ammonia. As a matter of fact ammonia toxicity has been found with prolonged deprivation of glucose (Geiger, 1957).

These findings suggest that the glutamic acid-glutamine system may serve as a metabolic buffer in the central nervous system. Moreover, as mentioned previously, the presence, distribution and compartmentalization of glutamine and glutamic acid suggest the multiple role of glutamine and functional role of glutamic acid in the central nervous system.

Lack of glucose is found to favour the removal of glutamic acid whereas a high concentration of glucose prevents this (Waelsch, 1949; Cravioto, Massieu and Izquiero, 1951; Takagaki, Hirano and Tuskada, 1957). The utilization of glutamic acid in the absence of glucose must proceed either through transamination with oxaloacetate or through the Gamma-aminobutyric acid shunt. The possibility

of the former mechanism seems to be more likely as the observations of Krebs and Bellamy (1960) and Haslam and Krebs (1963) have shown that a large fraction of the glutamate utilized (80-90%) is metabolised though the former reaction. The findings of many others (Haslam and Krebs, 1963; Balazs, 1965) also suggest this possibility. On the other hand, McKhann, Albers, Sokoloff, Mickelsen and Tower,(1960) and McKhann and Tower (1959, 1961) suggest that, in cat cerebral cortex, over 40 per cent of total glucose is metabolized via the Gamma-aminobutyric acid shunt and any intermediate in this shunt can maintain oxidative phosphorylation. Elliot (1965) has suggested that 10% of total metabolism may proceed through the Gamma-aminobutyric acid shunt. However, no net change in Gamma-aminobutyric acid 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 Gamma-aminobutyric acid from glutamic acid formed during the transamination and the reductive deamination of 2-oxoglutarate.

Although brain glutamic acid could be theoretically derived from the amination and transamination reaction with 2-oxoglutarate or by deamination of glutamine, the major

pathway for its formation appears to be by the direct amination of 2-oxoglutarate (Krebs, Eggleston and Hems, 1948; Waelsch, 1949; Stern, Eggleston, Hems and Krebs, 1949; Terner, Eggleston and Krebs, 1950). The administration of labelled glucose or pyruvate is found to be rapidly followed by the formation of glutamic acid (Roberts, Flexner, and Flexner, 1959; Busch, Goldberg and Anderson, 1956; Busch, Fujiwara and Keer, 1960; Chain, Cantanzaro, Chain, Masi and Pocchiari, 1955). Under these conditions Gamma-aminobutyric acid also showed labeling. Similar results were obtained from in vitro studies (Tower, 1958). When radio-active glucose was administered to rat or cat 65-70% of total radio activity in the brain after 20 minutes was in amino acids as compared to 2-16% in other tissues such as liver, spleen, lung, kidney and blood (Waelsch and Lajtha, 1961; Gaitonde, Marchi and Richter, 1964; Gaitonde, Dahl and Elliot, 1965; Gaitonde, 1965). In the brain the activity was found to be more in glutamic and aspartic acids and to a smaller extent in glutamine, Gamma-aminobutyric acid and alanine (Waelsch and Lajtha, 1961; Gaitonde, 1965).

Apart from the metabolic functions of glutamic acid and Gamma-aminobutyric acid, they are also believed to exert some electrophysiological action on the brain. The former is found to have an excitatory action and the latter an inhibitory one. The inhibitory nature of an alcoholic extract

of the brain was reported by Florey (1956). For this factor they gave the name Florey's Factor 1. Later, many workers have reported that much of the Factor 1 activity could be accounted for Gamma-aminobutyric acid (Bazemore, Elliot and Florey, 1956; 1957; Levin, Lovell and Elliot, 1961; Lovell and Elliot, 1963). Tropical application of Gamma-aminobutyric acid on the cat cerebral cortex caused marked reversible changes in electrical activity (Iwama and Jasper, 1957; Purpura, Girada and Grundfest, 1957). The surface negative dendritic response to direct electrical stimulation is rapidly replaced by surface positive responses. Intravenous injection of Gamma-aminobutyric acid does not show any effect as it does not cross the blood brain barrier (Elliot and Hobbiger, 1959) although it may do so following local breakdown of the barrier by chemical means (Edwards and Kuffler, 1959). Studies on the stretch receptor neurons of cray fish led to the conclusion that in the crustacean, Gamma-aminobutyric acid might be a transmitter of inhibitory neurons. Purpura, Girada and Grundfest (1957) have shown that it inactivates excitatory auxodendritic synaps in mammalian brain.

Recent studies made by Curtis, Phillis and Watkins (1959) and Eccles, Schmidt and Willis (1963) using micro electrode technique showed the depressant action of Gamma-aminobutyric acid in many of the neurons of the spinal cord,

Due to the potent depressant action on the nerve cells, it has been suggested that Gamma-aminobutyric acid and related amino acids might be inhibitory transmitters in the central nervous system of vertebrates. These substances have now been shown not to produce a hyper polarization of nerve cells as would be expected for the inhibitory transmitter, but to have a general depressant action of all neuronal responses (Curtis, Phillis and Watkins; 1959; Curtis and Watkins, 1960; Eccles, 1965).

The depressant action of Gamma-aminobutyric acid differs from that of the transmitter for post synaptic spinal inhibition in that it is not suppressed by strychnine (Curtis, Phillis and Watkins, 1959). However, the electrophoretic application of Gamma-aminobutyric acid to the Mauthner cells reveal a remarkable spatial specificity in that it exerts a depressant action only when it is applied to specific regions of the cells (axon hillock, soma and adjacent dendrites) that have inhibitory synapses (Diamond, 1963).

The powerful depressant action of Gamma-aminobutyric acid applied on cortical neurons resemble post synaptic inhibition in being resistant to a wide range of pharmacological substances. Since Gamma-aminobutyric acid is present in large quantities in the brain, Krnjevic, Randic and

Straughan (1964) have suggested that Gamma-aminobutyric acid may be a post-synaptic transmitter substance whereas Curtis, Phillis and Watkins (1959) and Eccles, Schmidt and Willis (1963) rule out a post-synaptic inhibition on the ground that it does not act on the same site as strychnine and believe that it may be involved in presynaptic inhibition.

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

In contrast to Gamma-aminobutyric acid, glutamic acid is belived to have an excitatory effect. There is no really 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 crustacian neuro-muscular junction and that of acetyl choline on the vertebrate end-plate (Takeuchi and Takeuchi, 1964).

In general acidic amino acids structurally related to glutamic acid depolarize neurons and function as excitatory substances (Krnjevic, 1964; Curtis, 1962,1965). In contrast

the neutral amino acids structurally related to Gamma-aminobutyric acid depress the firing of neurons.

Thus glutamic acid and Gamma-aminobutyric acid may be considered as transmitter substances causing excitatory and inhibitory effects in the central nervous system. The elementary criteria of a transmitter substance are that in the relevent tissue it must be produced at the appropriate time, stored, released to exert an appropriate action, and be removed (Elliot, 1965). All the criteria except that of release are met by both (Elliot, 1965). Recent experiments on crustacean nervous system show that during the inhibitory process Gamma-aminobutyric acid was found to leak from punctured cortical surface (Otsuka, Iversen, Hall and Kravitz, 1966). Whereas the rate of release of Gamma-aminobutyric acid was increased during the "sleep" condition, the rate for glutamate was increased about 50% when the electroencephalographic pattern indicated "arousal" (Jasper, Khan and Elliot, 1965).

Indirect evidence regarding the role of Gamma-aminobutyric acid as an inhibitory transmitter is to be found from the lowered concentrations of this amino acid in epileptic seizures and due to the action of convulsive agents in the brain (Roberts and Baxter, 1959).

Thus the concentrations of Gamma-aminobutyric acid and its parent substance glutamic acid in the central nervous system are probably very critical as any disturbance in them may change the electrophysiological status of the brain resulting in abnormal function of the brain.

It has been previously mentioned that the source of the brain glutamic acid is metabolized glucose. 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 the glutamic acid and Gamma-aminobutyric acid in the central nervous system (Cravioto, Massieu and Izquiero, 1951; Dawson, 1950, 1953). Systemically administered glutamic acid can restore the brain glutamate level to the normal.

A number of convulsive agents cause a reduction in the concentration of these amino acids. Thus convulsive agents such as methionine sulphoximine, petroxin, megamide etc. resulted in a low level of Gamma-aminobutyric acid and glutamic acid in brain slices (Tower, 1961).

It would also appear that the effects of vitamin deficiencies on the central nervous system are atleast in part due to their effects on glutamic acid metabolism. Thus thiamine deficiency in the pigeon is associated with a significant decrease in the glutamic acid content of the

brain (Peters, 1962). Convulsions caused by pyridoxine deficiency are associated with a decreased activity of glutamate decarboxylase and a lowered concentration of Gamma-aminobutyric acid (Tower, 1958a; Roberts, Younger and Frankel, 1951). The convulsions caused by the administration of hydrazides are also due to their inhibitory action on pyridoxine dependent enzymes such as glutamate decarboxylase (Roberts and Baxter, 1959; Baxter and Roberts, 1960).

As already pointed out, because of the importance of glutamic acid system in brain metabolism this was the parameter employed in studies on the effect of protein deficiency in rats on brain biochemistry in this laboratory. These studies showed a decrease in the activities of L-glutamate:NAD-oxidoreductase and L-glutamate:1-carboxy-lyase and associated changes in the aminoacids (Rajalakshmi, Govindarajan and Ramakrishnan, 1965). The animals were also found to be slower in learning a visual discrimination. The authors consider the results to be consistent with the metabolic and electrophysiological roles of glutamic acid and Gamma-aminobutyric acid in the brain.

The adverse effects of a low protein diet in the rat on visual discrimination learning and brain biochemistry reported previously from this laboratory raise the question

whether the differences, particularly those in L-glutamate:NAD-oxidoreductase and L-glutamate:1-carboxy-lyase are due to differences in the availability of nicotinic acid and pyridoxine and whether similar differences are found with differences in the quality of proteins in the diet. The present studies were designed in this context to investigate (a) whether the effects of a low protein diet can be reversed by appropriate supplements such as pyridoxine, nicotinic acid and glutamic acid, (b) whether diets containing poor quality proteins of the type usually present in cereals and millets exert an effect similar to that of a low protein diet and (c) whether overall improvement of deficient diets consumed by poor children of the postweaning period in this region, by correcting the protein calorie malnutrition and deficiencies of other nutrients such as vitamin A and riboflavin, is associated with an improvement in psychological performance and brain biochemistry. Aspects (b) and (c) were chosen because of their greater relevance to actual dietary patterns in pre-school children. Studies were also made of the effects of electroconvulsive shocks on animals fed low and high protein diets in order to see whether susceptibility to the same is altered by a low protein diet. The effects of prolonged calorie restriction were incidentally studied in an experiment designed otherwise. These studies are described in this thesis.