CHAPTER I

INTRODUCTION

I N T R O D U C T I O N

The brain is the chief integrative mechanism which coordinates the activities of the various parts of the organism and is thus the central headquarters responsible for the unified behaviour of the organism as a whole. It is not only an organ specialized in receiving and sending impulses but also one whose biochemical reactions have tremendous influence on physiological mechanisms of the whole body. The concept of the association of psychological functions with brain function is familiar even to the lay man.

The ontological development of any tissue is greatly influenced by the demand on its function by the whole body. Since the brain is needed as a centre for the control of the functions of the body, it is not surprising that the development of the brain precedes that of many other organs.

The development of the brain starts with the formation of the neural tube. In the embryonic development of all vertebrates three primary vesicles or swellings appear in the anterial end of the neural tube which are the three major regions of the brain itself. The secondary subdivisions and the associated structures

are shown in Table 1, with their functions in the adult brain. However, the association of different areas with different functions does not mean that the brain functions in water-tight compartments. Although there is a certain amount of localization of function within the brain, the brain acts as a whole, particularly in complex learning processes (Hebb, 1960).

The cells in the brain are of two types, neuronal and non-neuronal. The neuronal cells, constituting about 5% of the total number, have axons and dendrites which are responsible for nervous transmission. The supporting non-neuronal cells or glial cells constitute about 95%. It is well-known that the former do not undergo cell division so that loss of neuronal cells constitutes a permanent loss. The total DNA content of the brain does not increase after a certain period in early life indicating that the total number of cells reaches a maximum value during this period. This would mean that glial cells also do not increase in number. However glia are known to continue to proliferate in adult rats (Altman and Das,1964) and it has been shown that in the rat, the 'enrichment' of the environment leads to a greater glial/neural ratio (Diamond, Krech and Rosenzweig, 1964). The distribution of neuronal and non-neuronal cells in the different regions

Table 1: Structural components of brain and associated functions*

primary vesicle and subdivision	associated structures	selected function
PROSENCEPHALON: (forebrain)		
telencephalon (endbrain)	frontal, parietal, temporal and occipital lobes	sensory-motor functions, complex learning, emotion and motivation.
	corpus callosum	interhemispheric coordination and duplication of memory traces.
	olfactory lobes	amplification of olfactory stimuli.
	basal ganglia	major integrative centre for the extraphyramidal motor system, regulation of voluntary motor activity.
	hippocampus	olfactory and visceral functions, motivational mechanisms.
diencephalon (interbrain)	thalamus	relay station between the cortex and lower centers, co-ordination of sensory impulses.
	hypothalamus	primary control of autonomic functions, neural and endocrine mechanisms, regulation of fat, carbohydrate and water metabolism and of hunger and thirst, distribution of blood to the brain, sexual and emotional behaviour, temperature regulation.
MESENCEPHALON (midbrain)	collicular region and cerebral peduncles	the conduction of impulses between the higher and lower centers (audition and vision in lower vertebrates).
RHOMBENCEPHALON (hindbrain)		
metencephalon	cerebellum	regulation of muscular activity, motor coordination,
	pons	involved in sensations and movements of mouth and face.
myelencephalon	medulla	breathing, heartbeat and blood pressure, reflex functions.

*Walsh, 1957; Gardner, 1963; Grossman, 1967.

of the rat brain (Nurnberger and Gordon, 1957) is shown in Table 2.

Neurons are relatively large cells due to their elongated axonal processes which in large vertebrates may reach several feet. They are linked together to form conduction pathways which are supported or held together by a framework of specialized, non-conducting cells known collectively as neuroglia. A study of the minute anatomy of the adult nervous system reveals an extremely complex arrangement of cells. Electron microscopy reveals that nerve cells, nerve fibers and neuroglial cells are so closely packed that scarcely any intercellular space exists in the central nervous system (Fernandez-Moran, 1957; Cragg, 1968). Dendrites, which are protoplasmic outgrowth of the cell body, branch profusely but extend only to a short distance from the cell body. This arrangement increases the surface area of the neuron so that a large number of other neurons may be associated or linked with it. The axon is surrounded by the myelin sheath which is formed by glial cells.

The above facts reveal the heterogeneity of the brain in terms of morphology, anatomy and functioning. These differences in structure and function can be

Table 2: Distribution of neuronal and non-neuronal cell nuclei in rat brain*

region	no. of cells per g x 10^{-7}	
	neuronal	non-neuronal
visual cortex	1.40	8.0
hypothalamus	1.80	16.1
cerebellum	0.57	?
pons + medulla	1.78	9.5
corpus callosum	0	7.8

*Nurnberger and Gordon, 1957.

expected to be associated with differences in biochemical functions and chemical composition. The need to take into account the complexity and heterogeneity of this organ while studying its metabolism and to identify the biochemical correlates of functional and structural differences has been emphasized by several authors (Himwich, 1950; Waelsch,1959; Kety and Elkes, 1961; Zeman and Innes, 1963; and Richter, 1967).

As may be expected, the heterogeneity of different parts of the brain is less evident in the embryonic brain and is fully manifested with its development to maturity. This maturation takes place in several stages, the first stage being characterised by increase in the number of cells followed by subsequent changes in their size and structure and the development of axons, dendrites, myelination and the proliferation of glial cells. During this period of development there are several changes in chemical make-up as well as metabolic activity of the tissue. It can be presumed that these processes are associated with biochemical differentiation between different regions of the brain and that both are associated with the differences in function.

In the rat, brain weight increases dramatically until 21 days after birth and then at a slower rate

(Agrawal, Davis and Himwich, 1966). Body weight on the other hand increases slowly until 14 days of life and then proceeds at a rapid rate. Thus the ratio of brain weight to body weight increases rapidly during the first few weeks. Regional differences are also seen in the development of this organ. The weight of the cerebellum increases by 88 per cent between the ages of 14 and 35 days as compared to a 34 per cent increase in that of the cerebrum (Balazs, Kovacs, Teichgraber, Cocks and Eayrs, 1968). This would suggest that a longer period of maturation is required for this organ.

The increase in gross-weight during maturation of the brain is associated with a decrease in moisture content. In the kitten this decrease is found to take place at different rates in different regions. The pons-medulla and the superior-inferior colliculi show a relatively more rapid decrease. The cerebellum, thalamus, hypothalamus, caudate nucleus and hippocampus amygdala exhibit a less dramatic decrease with the least decrease in the cerebral lobes (Agrawal, Davis and Himwich, 1967).

There is an increase in the contents of DNA, RNA, protein, cholesterol, phospholipids and other substances. Because of differences in the rate of increase of these

substances their concentrations may fluctuate, as in the case of DNA. Many important enzymes show evidence of activity soon after birth and reach ceiling levels as the brain matures. The onset and rate of these changes vary in different regions of the brain.

For instance, myelination does not proceed at a uniform rate in all the regions of the brain. Rather, the rate of myelination would appear to be linked with the process of development and functional organization of different regions (Yakovlev and Lecours, 1967). The rate of synthesis of sulphatide which is an important constituent of myelin remains relatively high until 22 to 25 days after birth in the whole brain, but drops off much earlier in the midbrain (McKhann, Levy and Ho,1967). The in vivo labelling of sulphatides from labelled sulphate in the rat brain during early growth is greatest in the brain stem and least in the cerebellum. Further, no depletion of total lipid radioactivity was observed in any of the animals during a period of 60 days.

The changes in the free amino acid levels during maturation have been reported. Bayer and McMurray (1967) observed that in the developing brain, when expressed

relative to wet weight the concentrations of glutamic acid, GABA, glutathione, aspartic acid and cystathione increase between birth and maturity, whereas others such as alanine, citruline, glycine and tyrosine show decrease and yet others such as glutamine, serine ethanolamine and threonine show no change. Similar observations have been made in the mouse brain (Agrawal, Davis and Himwich, 1968) with regard to increase in concentrations of glutamate, GABA and aspartic acid. These authors report an increase in glutamine as well and a decrease in other amino acids. Changes in the free amino acid levels in the brain have been attributed to be partly due to a reduction of permeability to amino acids as the animal matures (Lajtha and Toth, 1961).

In this context, it should also be mentioned that, at least in the adult animal, a significant proportion of the amino acids of the brain are derived from glucose (Roberts, Flexner and Flexner, 1959). The rapid flux of glucose carbon to amino acids is found only at about 10 to 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.

The rapid growth of the brain during the early period involves a rapid synthesis of specific proteins at each stage. During the "critical period" (Peters and Flexner, 1950) while the neurons of the cortex develop their dendrites and become functionally active (Clouet and Gaitonde, 1956), the brain protein changes rapidly in content and nature (Block, 1937; Clouet and Gaitonde, 1956; Lajtha, 1958; 1959; Moore, 1965). It is not surprising that the proteins of the immature brain have a higher average turnover rate than those of the mature one (Gaitonde and Richter, 1956; Lajtha, Furst, Gerstein and Waelsch, 1957, Lajtha, 1964).

Thus the mean protein content of the brain of rat increases from 6.5% at birth to 11.1% in the adult animal (Richter, 1962). It varies in different regions of the brain because of differences in cell distribution and in the maturation of the cell (Randall, 1938; Clouet and Gaitonde, 1956; Richter, 1962). The increase takes place first in the medulla and hypothalamus which are first to become functionally active (Kelly, 1956; Richter, 1959, 1962). Changes in the amino acid distribution of the brain protein with age has been reported (Clouet and Gaitonde, 1956). The presence of different types of protein at different stages of functional and metabolic

maturity suggests the specificity of proteins contributing to different functional properties.

Pasquini, Kaplún, Garcia Argiz and Gomez (1967) have reported that the cerebral cortex has essentially completed its growth in terms of protein concentration by the 14th day after birth but the protein concentration of the cerebellum continues to increase upto 30 days of age. Vellis, Schjeide and Clemente (1967) report that the rate of ^{14}C - leucine incorporation into the protein of brain stem slices decreases with age.

In addition to the general pattern of decreasing protein synthesis, there is evidence that turnover of protein in the brain of the developing rat becomes progressively slower with increasing age (Lajtha et al., 1957). Further support for this can be obtained from the data of Yamagami, Fritz and Rappaport (1966) who showed that messenger RNA isolated from the brain of the young rat was more active in stimulating protein synthesis in cell-free systems than preparations isolated from the brains of adult rats. Recently Tewari and Baxter (1969) have shown that GABA stimulated the incorporation of amino acids into protein by a ribosomal system from immature rat brain, but not from immature rat liver.

The total brain DNA attains 90% of its adult level by 14 days post partum and reaches a plateau by 19 days (Winick and Noble, 1965; Adams, 1966). In the cerebellum, however, DNA concentration increases and a peak is reached at 15 days of age, which shows the non-homogeneous pattern of growth shown by different parts of this organ(Pasquini et al., 1967). The results of these studies confirm the observation that DNA synthesis has essentially ended in the whole brain by the time of weaning.

Oja (1966) has shown that the RNA/DNA ratio is constant up to 3 days of age and then increases rapidly up to 14 days after which the increase is less rapid until nearly adult values are reached at 30 days. Johnson (1967) has shown that the rate of synthesis of RNA in whole mouse brain suspensions is much less in the adult brain than at birth. It has also been shown that rat brain microsomes extracted from young animals had a greater intrinsic capacity for protein synthesis (Adams and Fox, 1969). These observations can be correlated with the overall decrease in protein synthesis in developing mammalian brain.

Glycolysis plays a greater part as energy-yielding reaction in the early stages of development, but after

birth the respiration increases. The rapid growth of the brain in early life is associated with a greater energy requirement relative to body weight in the new-born and young animal as compared to the adult animal.

The transition from anaerobic to aerobic oxidation does not proceed uniformly throughout the brain, but commences at the spinal cord and culminates in the cerebral cortex. The medulla showed the highest oxygen uptake, and the cortex, the least, during the first week of life. In the former case maximum value was reached in about 3 weeks whereas in other regions it was five weeks. In the cerebellum, however, oxygen consumption continued to increase after five weeks (Himwich, 1951).

As might be expected, changes in brain composition are associated with changes in the activities of the enzymes at different stages of development. Thus an increase in the cytochrome-cytochrome oxidase activity of the cerebral cortex takes place at the period critical for morphological change (Flexner, Flexner, and Straus, 1941). Succinic dehydrogenase and ATPase activities in rat brain are found to be at low levels upto 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 glutamine synthetase (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 (Kuhlman and Lowry, 1956). A similar observation was made with regard to glutamate decarboxylase (Roberts, Harman and Frankel, 1951). NAD-glycohydrolase activity was not evident at birth but became measurable within 1-3 days. It showed a rapid increase during the 2nd and 3rd weeks reaching ceiling levels at about one month of age when brain development is rapid (Burton, 1957; Baroda studies, unpublished).

Glutamine synthetase and glutamyl transferase activity have been studied in the neocortex, hippocampus, cerebellum, diencephalon, and pons-medulla of kittens and cats (Berl, 1966). The five regions showed a different pattern of development for the enzyme. The neocortex had the sharpest increase in enzyme activity and achieved the highest value.

Though 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 dehydrogenase does not increase with age (Kuhlman and Lowry, 1956).

The brain has to maintain a stable internal environment for efficient function and small changes in this environment can result in gross consequences in behaviour. For instance, the injection of NaCl into the region of the hypothalamus can make the animal drink itself to death (Andersson, 1952). The maintenance of a relatively stable chemical environment demands that the entry and exit of substances and the rates at which they take place are strictly regulated. The blood-brain barrier is a label for the differential extent and rate of accessibility of the brain to different substances present in 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. The immature brain uses substrates other than glucose more efficiently for respiration on the basis of studies on amino acid and acetate

oxidation (Dr. R. Balazs, personal communication to Dr. R. Rajalakshmi). The adult brain is readily accessible to substances such as glucose whereas it is believed to be almost inaccessible to substances such as glutamic acid. On the other hand it is readily accessible to substances present in the cerebrospinal fluid. The area of brain cells exposed to cerebrospinal fluid is in fact much larger than that exposed to blood, thus explaining the relative rapidity with which substances penetrate into the brain when injected into the cerebrospinal fluid (Bakay, 1957; Dobbing, 1961; Edstrom, 1964).

Unlike other capillary or cell membranes the blood-brain barrier is extremely impermeable to proteins, including antibodies and bacterial toxins. The brain manufactures most of its complex constituents some of which are uniquely present in the same, absorbing only relatively simple substances from the blood stream. This may also have something to do, with maintaining the internal 'mileu' of the brain. For instance, glutamate is an important constituent of the brain and it is almost entirely of local origin. The operation of the blood brain barrier against glutamate may have something to do with the fact that the formation of glutamate from 2-oxo-glutarate serves as an important ammonia removal mechanism in the brain.

The possibility that there might be regional differences in the function of the barrier was suggested by histological evidence showing that certain regions in the hypothalamus and midbrain are more accessible to vital staining. Radioactive adrenaline and noradrenaline have been found to penetrate into the hypothalamus but not into other parts of the mature cat brain (Weil-Malherbe,Whitby and Axelrod, 1961). Similarly studies made on the incorporation of radioactive lysine and leucine show regional differences (Lajtha, 1961).

The rates of exchange of substances may vary in different regions of the brain. Further, in any particular region, they are influenced, as may be expected by the concentration of the substance in that region. Similar observations have been made in the uptake of amino acids by brain slices (Kandera, Levi and Lajtha, 1968). For instance, taurine was found to be taken up by pons, medulla and spinal cord although very little was taken up by other regions. Thus the operation of the blood-brain barrier varies with regard to different substances in different regions.

Regional differences in the operation of the blood-brain barrier are consistent with differences of the flow

of blood (Sokoloff,1961). The average blood flow in grey matter was approximately five times that in white matter. In the conscious cat, the highest rates of blood flow were observed in regions associated with primary sensory functions. A reduction in blood flow to these regions was observed with light anesthesia which resulted in a relatively uniform cerebral cortical blood flow but at a decreased rate. The reduction was found to be appreciable in grey matter and negligible in white matter. Conversely, photic stimulation of the retina increased blood flow in discrete areas of the visual cortex, superior colliculus, and lateral geniculate ganglion (Sokoloff, 1961).

As mentioned earlier the RNA content of the neurons is higher than that in other types of cells. It has been suggested that the RNA molecules might be modified by cellular activity and thus be capable of storing memory traces (Hyden, 1959). RNA content is influenced by age (Hyden, 1959), neural activity (Hyden, 1959a) and learning (Hyden and Egyhazi, 1962). The age changes during growth and senescence correspond to those in intelligence test performance. Hyden (1959a) reported a marked increase in RNA and protein synthesis with intense neural activity. However, interference with either protein or RNA synthesis

by the administration of puromycin and actinomycin D has not been found to affect learning and memory although short term memory has been reported to be affected (Flexner, Flexner and Stellar, 1963; Cohen and Barondes, 1966). It would certainly seem premature to conclude that changes in the RNA and proteins of the nerve cell body are specific for learning (Richter, 1966).

As may be expected, the distribution of nucleic acids varies in different regions (May and Grenell,1959). In the rat brain DNA and RNA concentrations are highest in the cerebellum and hypothalamus. The lowest concentration of RNA was found in the medulla. A similar distribution has been found of RNA and DNA in the cat brain (Mihailovic, Jankovic, Petkovic and Mancic, 1958).

The turnover rates of nucleic acids as studied by the incorporation of radioactive adenine and orotic acid are also found to vary in cervical spinal cord, attached nerve roots, ganglia and hind brain, i.e. medulla, pons and cerebellum (Koenig, 1957; 1958). The ratio RNA/DNA which gives the average RNA content per cell is found to be highest in the grey matter of the cerebral cortex, the hippocampus, the thalamus, the corpus striatum and the hypothalamus. The smallest ratio was found in the cerebellum and olfactory bulb. The white substance of the

cerebrum, the mesencephalon and the spinal bulb give smaller values, which indicates that the glial cells are poorer in RNA than neuronal cells. Some of the regions having a high ratio of RNA/DNA also show a high turn over of RNA (Mandel, Harth and Borkowski, 1961).

It is generally agreed that the brain in situ under normal conditions respires almost exclusively at the expense of glucose (Gibbs, Lennox, Nims and Gibbs, 1942). Both in situ and in vitro, the oxidation of glucose can account for the major portion of oxygen consumed. However, fructose and mannose increase the oxygen uptake of excised minced brain tissues. Galactose had a small effect but mannitol and the pentoses were inert (Quastel and Quastel, 1961).

Elliott and Heller (1957) showed that the respiration of cerebral cortex is largely due to neurons. The average neuron in the cerebral cortex is metabolically much more active than that in cerebellar cortex. On the other hand, non-neuronal cells of white matter respire more actively than the average neuron in cerebellar cortex. Similar variations are found in an aerobic glycolytic activity.

As might be expected regional variations are also observed with respect to oxygen consumption. In the

adult animal, oxygen consumption was greatest in the midbrain and thalamus, least in the cortex and cerebellum and intermediate in the medulla and candate nucleii (MacIlwain, 1959).

The role of glycogen in the brain is far from clear as most of the glucose used for respiration is derived from the blood (Kety, 1957). However, the brain does possess the enzyme machinery for the utilization of glycogen and recent studies suggest a constant turnover of brain glycogen (Brodskaya, 1964). It has also been suggested that glycogen in brain has some role to play in its structural integrity. It is interesting to note that the distribution of glycogen varies in different regions of the rat brain (Mrsulja, Terzic and Varagic, 1968) with the brain stem containing much more than either the hypothalamus or the cerebral cortex. Woolley and Timiras (1963) observed that glycogen concentration was significantly reduced in the forebrain, brain stem and cerebellum of rats exposed for 3, 8 and 30 days at 12,500 ft altitude. After 60 days at this altitude, brain stem and cerebellum glycogen concentrations returned to normal, but forebrain glycogen was still significantly decreased. Differences of a smaller order in the mouse brain have been reported with regard to energy reserves in the form of

phosphocreatin, ATP, lactate and high energy phosphate (Gatfield, Lowry and Schulz, 1966).

As mentioned earlier, there is a high rate of turn-over of brain proteins (Gaitonde and Richter, 1955, 1956; Waelsch and Lajtha, 1961). The rate of incorporation of labelled methionine has been found to be high in the cerebellar cortex and low in white matter, with intermediate values for the cerebral cortex, thalamus and other parts examined (Richter, Gaitonde and Cohn, 1960).

Among the peptides present in the brain, glutathione has been studied extensively. The concentration of glutathione in the liver and brain is several times that in the blood plasma suggesting its importance in cerebral as well as general metabolism. It has been suggested that most of brain glutathione is locally synthesized (Douglas and Mortensen, 1956). It accounts for about one-third of total non-protein, acid-extracted nitrogen. Almost all of it is present in the reduced form (McIlwain, Martin and Tresize, 1957) and it may have an important role in keeping in reduced form the thiol compounds involved in enzyme activity. Incidentally, it has been shown in this laboratory (Rajalakshmi and Ramakrishnan, 1969), that in the liver it is a more

sensitive index of protein nutrition than other parameters such as protein, xanthine oxidase or succinate dehydrogenase. This is perhaps not surprising as glutathione requires glutamic acid,cysteine and glycine, the availability of which will be affected by protein deficiency.

It has been claimed that blood levels of glutathione are depressed in certain mental conditions and that the administration of glutathione has a beneficial effect (Eiduson, Geller, Yuwiler and Eiduson, 1964). It would be reasonable to expect that consistent with the pattern for other substances, the concentration of this substance also varies in different regions.

Another substituted amino acid occurring in high concentration is acetyl aspartic acid (Tallan, Moore and Stein, 1958). Although it has a slow turnover rate it has been shown that N-acetylaspartic acid actively donates acetyl groups during lipid synthesis, especially at the sight of myelination (D'Adamo and Yatsu, 1966). Marcucci, Mussini, Valzelli and Garattini (1966; 1968) have studied the distribution of this amino acid in different brain regions and showed that the concentration of the same is to be lower in some brain regions of a strain of aggressive mice.

It is well-known that 5-hydroxytryptamine is an important constituent of the brain. Studies on the distribution of the enzymes and intermediates of serotonin metabolism show the hypothalamus, midbrain and caudate nucleus to be relatively rich in serotonin (Amin, Crawford and Gaddum, 1954), and the distribution of the same to correspond roughly with that of 5-hydroxytryptophan decarboxylase (Udenfriend, Bogdanski and Weissbach, 1957).

The distribution of acetylcholine in the central nervous system of man, dog and cat has been studied (Feldberg, 1945). The greatest amount was found in the basal ganglia, and the least in cerebellum with the cortex and brain stem showing intermediate values.

The distribution of choline acetylase essentially parallels that of acetylcholine (Burgen and MacIntosh, 1955). Further there seems to be some correlation in most parts of the nervous system between the distribution of acetylcholinesterase, choline acetylase and acetylcholine. However, the cerebellar cortex has a disproportionately high concentration of esterase, although it has relatively less of the other two (Burgen and MacIntosh, 1955).

Twenty per cent of total nitrogen in the brain is made up of free amino nitrogen and 25% of this is contributed by glutamic acid and glutamine (Waelsch, 1952; 1955; Weil-Malherbe, 1952; Ansel and Richter, 1954).

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 *in vitro*. 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 glutamic acid showed that during the oxidation of glutamic acid no ammonia was evolved. 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. It has been suggested that glutamic acid is converted to 2:oxoglutarate and glutamine and that the former is oxidised via the TCA cycle.

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 (Walesch, 1951; Strecker, 1957). Glutamic acid administration to patients in insulin coma leads to consciousness being regained, a phenomenon which may also be due to the release of glucose by adrenaline after administration of the amino acid (Strecker, 1957). An increase in the activity of glutamate dehydrogenase and glutamate decarboxylase has been found when rats fed a low protein diet were supplemented with glutamic acid (Rajalakshmi, Pillai and Ramakrishnan, 1969).

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 as well as nuclear fractions (Tower, 1959; Weil-Malherbe, 1957).

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 (Krebs, 1935; Cohen and Hekhuis, 1941; Speck, 1949; Elliott, 1951; Roberts and Frankel, 1951; Roberts et al., 1951; Lajtha, Mela and Waelsch, 1953). 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.

The glutamine of brain has remarkable stability (Weil-Malherbe, 1957). The brain has the enzyme glutamine

aminohydrolase (Krebs, 1935a) but glutamine is seldom converted to glutamic acid. This is not surprising as its conversion to glutamic acid will result in the liberation of ammonia. The high rate of turnover of glutamine may be due to its continuous formation from glutamic acid and conversion to peptides. However, it is in a dynamic state with a rapid turnover rate both *in vitro* and *in vivo* (Tower, 1958; 1959).

The brain also has the enzyme machinery for the transamination of glutamic acid to alanine and aspartic acid (Cohen and Hekhuis, 1941). As already mentioned the metabolism of glutamic acid to GABA, succinic semi-aldehyde and succinic acid provides a shunt pathway from 2-oxoglutarate to succinate in the brain. The enzyme system which catalyses these reactions, namely, glutamate decarboxylase, GABA-transaminase and succinic semi-aldehyde dehydrogenase are present in the brain(Roberts and Bregoff, 1953; Albers and Salvador, 1958; Roberts, 1960; Albers, 1960).

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; Stem, 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 (Beloff-Chain, Cantanzaro, Chain, Masi and Pocchiari, 1955; Roberts,Flexner and Flexner, 1959; Busch, Goldberg and Anderson, 1956;Busch, Fujiwara and Keer, 1960). Under these conditions GABA also showed labelling. Similar results were obtained from in vitro studies (Tower, 1958). When radioactive glucose was administered to rat or cat 65-70% of total radioactivity 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; Gaintonde, Dahl and Elliott, 1965; Gaitonde, 1965). In the brain the activity was found to be mostly in glutamic acid, aspartic acid and to a smaller extent in glutamine, GABA and alanine (Waelsch and Lajtha, 1961; Gaitonde, 1965).

The formation of glutamic acid is catalyzed 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 raises questions regarding its presence in the brain in high concentrations. 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 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).

Unlike glutamate decarboxylase, GABA-transaminase 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 GABA present in some foods of vegetable origin as well as animal brain. Both glutamate decarboxylase and GABA-transaminase require pyridoxal phosphate as cofactor for their activity (Roberts and Baxter, 1959). In the former, the apoenzyme is loosely bound with its prosthetic group

whereas in the latter case it is firmly bound (Roberts et al., 1951; Killam and Bain, 1957; Tower, 1957). This difference accounts for the greater effect of pyridoxine deficiency on glutamate decarboxylase (Roberts and Baxter, 1959; Killam, 1957), an observation consistent with the decreased level of GABA in this condition (Killam, Dasgupta and Killam, 1960).

GABA present in the brain is solely derived from glutamate and recent studies have shown the operation of the blood brain barrier against this amino acid. The amount of GABA present in any region has been claimed to be linearly related to glutamate decarboxylase (Sisken, Roberts and Baxter, 1960). It would be 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 glutamic 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 succinate semialdehyde dehydrogenase is

highest in the basal ganglia, thalamus, hypothalamus and lowest in white matter.

As mentioned earlier, inspite of the presence in the brain of glutamine aminohydrolase the use of glutamine as fuel after its conversion to glutamic acid is not advantageous to the brain because of the formation of ammonia. However, both glutamate and glutamine may be used by cerebral tissues when they are incubated with glucose inhibitors or with low oxygen tension associated with hypoxia (Tower, 1959; 1963; Waelsch, 1959). Even normally glutamine may be utilized for energy purposes after deamination to glutamic acid on the basis of the observation that ammonia is formed during nerve activity (Richter and Dawson, 1948; Hyden, 1955; Richter, 1959a; Waelsch and Lajtha, 1961). However, 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.

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). On the other hand, a substantial portion of glucose is oxidised via the GABA shunt and estimates of the proportion so oxidised vary from 10% to 40% (McKhann, Albers, Sokoloff, Mickelsen and Tower, 1960; McKhann and Tower, 1959; 1961; Elliott, 1965). However, no net change in 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 transamination and the reductive deamination of 2-oxoglutarate.

Apart from the metabolic functions of glutamic acid and GABA, 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 powerful depressant action of GABA when applied on cortical neurons and its presence in large quantities in the brain (Krnjevic, 1964) have led to the suggestion that GABA may be a postsynaptic inhibitory transmitter

substance. 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.

In contrast to GABA, glutamic acid is believed to 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 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 GABA depress the firing of neurons.

The glutamic acid and GABA may be considered to have mutually complementary roles in the central nervous system. The elementary criteria of a transmitter substance are that (in the relevant tissue) it must be produced , stored, released and be removed at appropriate times

(Elliott, 1965). All the criteria except that of release are met by both (Elliott, 1965). However, recent experiments on the crustacean nervous system show that during the inhibitory process GABA is found to leak from punctured cortical surface (Otsuka, Iversen, Hall and Kravitz, 1966). Further, the rate of release of GABA is increased when the EEG pattern indicates 'sleep' and that for glutamate is increased by about 50% when it indicates "arousal"(Jasper, Khan and Elliott, 1965).

Several factors such as prolonged cold exposure (Andjus, Knopfelmacher, Russel and Smith, 1955; Schneider, 1957; Subbarao and Gupta, 1965), insulin shock (Cravioto, Massiew and Izquierdo, 1951), hypoxia (Himwich, 1951), psychotropic drugs (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 largely been ignored in spite of the dramatic effects of vitamin deficiencies on the central nervous system (Eiduson et al., 1964; Coursin, 1965; 1967). This was primarily due to the fact that short term calorie restriction specially in the adult animal was not found to result in changes in brain composition (Folch, 1947; Fulton,1949).

This is due to the preferential supply of nutrients to the brain from body stores. 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 even in the adult animal, the brain can continue to function normally in the face of local deficiency. The effects of insulin hypoglyceamia and hypoxia are dramatic instances of the susceptibility of the brain to a lack of glucose and oxygen. In the young child or animal, nutritional deficiency may affect not only function but also structural development.

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; Eiduson et al., 1964).

Pellagra, a nutritional deficiency disease (Goldsmith, 1953) primarily attributed to niacin and amino acids is characterized by psychic disturbances such as loss of recent memory, loss of clarity, general lassitude, depression, and other clinical manifestations (Goldsmith, 1956).

A deficiency of thiamine is also associated with mental depression and other behavioural changes apart from

neurological changes (Lowery, 1952; Brozek, 1957). Neurological symptoms seem to depend on the lowering of the rates of oxidative decarboxylation and cocarboxylase activity 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). It is also shown that convulsions associated with thiamine deficiency are due to a decreased ability of the brain to oxidize pyruvate, while liver enzymes are relatively unaffected (Bennett, Jones and Nelson, 1966).

Dreyfus and Victor (1961) studied the distribution of thiamine in different regions of rat brain (Table 3). The results show that the distribution of total thiamine in the brain of normal rats is relatively uniform with certain exceptions: the concentration of the vitamin is significantly higher in the cerebellar vermis and lower in the thalamus and the lateral pontine tegmentum although the latter is site of the most severe histopathologic alterations in thiamine deficiency. The high content of thiamine in the cerebellum of the human brain has also been shown. In thiamine deficient rats, the concentration was found to be decreased to the same extent in all the parts, although some parts showed more physiologic and pathologic vulnerability. The abnormalities found in thiamine deficiency include tissue destruction and

Table 3: Distribution of total thiamine in normal rat brain*

region	thiamine content (µg/g dry weight)
cortex	13.0
caudate nucleus	17.4
thalamus	9.2
hypothalamus	13.0
mammillary region	12.4
periaqueductal region	13.2
lateral pontine tegmentum	11.5
base of pons	13.1
vermis (cerebellum)	21.1
medulla	13.4

*Dreyfus and Victor, 1961.

a marked proliferation of glial cells. A decrease in brain glutamic acid has been reported in thiamine deficiency (Peters, 1962).

The effect of a deficiency of vitamin A on the functioning of the retina is well known. A decreasing efficiency of dark light adaptation, night blindness and finally total blindness are found to result (Dam and Scondergaard, 1964). In the young, disordered ossification follows vitamin A deficiency and causes 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). Further, vitamin A is necessary for the formation and closure of the neural tube and would also appe ar to exert an indirect effect on the central nervous system by its action on the thyroid hormone (Richter,1965). Incidently it has been observed that rats fed a vitamin A deficient diet showed a significant decrease in the activities of glutamate dehydrogenase and glutamate decarboxylase (Rajalakshmi and Ramakrishnan, 1969).

The effects of a deficiency of pyridoxine (Tower, 1958a; Coursin, 1960), riboflavin (Street, Cowgill 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 et al., 1941). Pantothenate deficiency caused malfunctioning of central nervous system involving paralysis, coma or convulsions in a number of animal species (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 human infants (Maloney and Parmelee, 1954; Hunt, Stokes, McRory and Stroud, 1954) during pyridoxine deficiency which also produces abnormal electroencephalographic pattern and reduced respiration of brain tissue slices (Roberts, Wein and Simonsen, 1964). A degeneration of neurons is also reported with riboflavin deficiency (Eiduson et al., 1964).

Deficiencies of certain minerals are also associated with neurological disorders. The association of iodine deficiency in the maternal diet with deaf-mutism in the

offspring is well-known. In experimental animals, a deficiency of iodine is aggravated by neurological stress (Milcu, 1960). Hypothyroidism induced by iodine deficiency has been found to affect glutamate decarboxylase in brain synaptosomal fraction (Balazs et al., 1968). Myelination is found to be impaired in copper deficiency in sheep (Innes and Shearer, 1940) and in guinea pigs (Everson, Shrader and Wang, 1968). A deficiency of manganese in the maternal diet is found to affect neuromotor development in the progeny in rats (Follis, 1958).

The wide spread prevalence of protein-calorie malnutrition in children in many areas of the world and clinical observations of extreme apathy in these children have led to several studies on the effects of protein and calorie deficiencies on CNS function in children as well as in experimental animals.

The effects 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. 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). Recently Joel, Moser, Majno and Karnovsky (1967) reported that certain polyunsaturated fatty acids of the adult brain of hen are more labile to starvation than has generally been considered to be the case with brain lipids.

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 (Lowry, Pond, Barnes,Krook and Loosli, 1962).

Undernutrition during early life is found to reduce brain weight as well as DNA content. Reports on changes in DNA concentration are conflicting but the general trend of the data does not suggest a statistically significant change in the same (Table 4). For instance, Dobbing (1968) reports a change in whole brain DNA but no change in DNA concentration in pigs inspite of a severe reduction in body weight and a less drastic reduction in brain weight. ~~in pigs.~~ Guthrie (1968) and Chase, Lindsley and Brien (1969) found no change in DNA concentration of rats undernourished from birth. An appreciable decrease in DNA concentration (18%) is derived from the data of Winick and Noble (1966)

Table 4: Effect of early undernutrition on DNA

investigators	species	period of treatment (days)	values as % control values			
			body weight	organ weight	DNA whole organ	DNA Mg/g
undernourished prior to weaning:						
Winick and Noble (1966)	rat	0-21	48	(a) 83	68	82
Rajalakshmi et al. (1967)	rat	0-28	51	(b) 88	80	90
Chase et al. (1969)	rat	0-18	51	(b) 90	102	113
				(c) 82	81	98
Dobbing (1968)	pig	14-365	3.5	(a) 66	81	108
undernourished from birth and rehabilitated:						
Winick and Noble (1966)	rat	22-133	79	(a) 85	78	92
Guthrie and Brown (1968)	rat	22-133	72	(a) 87	81	91
Dobbing (1968)	rat	22-133	77	(a) 93	88	95
Howard and Granoff (1968)	mouse	17-276	83	(b) 93	92	98
				(c) 86	78	92
Dobbing (1968)	pig	366-1278	80	(a) 86	86	100

Where necessary, the values have been calculated from the data given by the authors. Those deviating from 100 do not necessarily represent statistically significant differences.
(a) whole brain (b) cerebrum (c) cerebellum.

although this decrease might not have been statistically significant. It may also be added that the decreases derived from the data of Winick and Noble (1966) are generally greater than those reported by others.

Similarly, there was a reduction in protein content 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 Ramakrishan, 1967).

It appears on the basis of other reports that undernutrition in early life affects other parameters such as myelination and cholesterol (Dobbing, 1964; Dobbing and Widdowson, 1965) and RNA concentration (Winick and Noble, 1966). A decrease in RNA content of the cytoplasm in purkinje cells has been found by Novakova, Koldovsky, Hahn and Krocek (1967).

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, 1968). However, these differences largely disappeared at 45 days of age so that the effects do not seem to be permanent. 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 or conditioning (Mysiivecek, et al., 1968). In the case of all these parameters any differences found at younger age levels were largely found to disappear in the older animals.

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.

Poor nutrition during early childhood affects growth rate and consequently adult stature. It is also responsible in large measure for the high rate of infant mortality in poorly nourished communities. It is now being increasingly suspected that it may also affect intellectual development

although the operation of social and cultural factors complicates the picture to some extent in these studies (e.g. Stock and Smythe, 1963; Cravioto and Robles, 1965).

Since either malnutrition or undernutrition or both affect the majority of young children in poor countries the question arises as to what extent such children suffer from intelectual 'stunting' and to what extent the same is reversible by subsequent rehabilitation on a good diet. If such stunting is a fact, it raises questions regarding the educability of these children, their productivity as adults and their capacity to reap the benefits of being members of welfare state.

The study of this problem is a complicated one as children of poor families are not only poorly nourished but also live in impoverished environments. Their parents represent less successful members of society and as a group represent individuals with less formal education and less intellectual capacity. It is not possible to separate out the effects of these different variables easily in children. Nor is it possible to measure brain changes except for indirect measurements such as head circumference and EEG patterns except in autopsy cases. It is also not possible

to separate out the effects of a lack of specific nutrients in the diet as the diets given to children are lacking in several nutrients.

Animal studies under controlled conditions permit the variation of one factor at a time and the measurement of specific parameters. It is not surprising therefore that such studies are carried out to gain insights into the problem with the hope that atleast some of the results can be extrapolated to man.

Inspite of the high metabolic activity of the brain, no metabolic studies have been carried out on the effects of protein or other deficiencies. This is perhaps not so surprising as the choice of the metabolic parameter is indeed a problem.

The brain compares with plasma and liver in its concentration of essential amino acids, but has a much higher concentration of non-essential amino acids (Waelsch, 1957). Glutamic acid, glutamine and GABA account for a substantial portion of the latter. Most of brain glutamic acid is locally synthesized from 2-oxoglutarate and NAD is required for this synthesis (Strecker, 1957). It is reported that NAD content of the liver is affected in protein deficiency (Tulpule, 1959). If there is a similar reduction of

NAD in the brain, this may be expected to affect the activity of the enzyme needed for glutamate synthesis.

The role of glutamic acid, GABA and the pathways of their metabolism has already been discussed. Till recently conduction along the nervous system was believed to be an all-or-none affair, the brain functioning more or less like a telephone exchange. We now know that, in addition to this mode of transmission along the axons of nerve cells, there is another mode of transmission across the dendrites (Hebb, 1960). The axon either responds fully, or not at all, to stimulation (all-or-none response), whereas the response of the dendrite varies with the strength of the impulse (graded response). The latter results in diffused transmission which does not reflect the original nature of the nerve impulse, but is very necessary for maintaining background electrical activity in the brain. Dendritic activity is evident even in deep 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 (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 disorganized. We may therefore presume that an optimum level of dentritic activity is crucial for efficient CNS function. This may involve the maintenance of critical levels of glutamic acid and GABA.

It will be evident from the foregoing that the biochemical make up of the brain varies from region to region and is affected by nutritional deficiency. The biochemical and pathological changes with deficiency also seem to vary from region to region. Previous studies in this laboratory suggest that the certain enzymes of glutamate metabolism are affected in rats fed on a protein deficient diet. The deficient rats were also found to perform less well on visual discrimination and reversal learning (Rajalakshmi, Govindarajan and Ramakrishnan, 1965). The present studies were undertaken in order to identify the pattern of distribution of selected substances and enzymes in different regions of the brain and to investigate how this pattern is influenced by protein deficiency.

The regions studied were grossly distinguishable anatomic parts, namely, cerebellum, medulla, pons, midbrain,

olfactory lobes, visual cortex, basal ganglia, hypothalamus, corpus callosum, and residual brain. The biochemical parameters studied were:

(a) enzymes related to glutamate metabolism namely, glutamate dehydrogenase, glutamate decarboxylase, alanine aminotransferase, aspartate aminotransferase, glutamyltransferase and glutamine synthetase.

(b) oxygen consumption with glucose and glutamate as substrates.

(c) concentrations of glutathione, ascorbic acid and protein.

These investigations are incorporated in this thesis.