# CHAPTER I

# INTRODUCTION

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The importance of the nervous system in the homeostatic regulation of the body functions in higher vertebrates is well known. The nervous system in the same is made up of the central and peripheral systems, the former consisting of the brain and the spinal cord, and the latter, consisting of sensory and motor nerves which carry impulses between the brain and the sense organs and/or the effector organs.

The cells in nervous tissue have unique characteristics which enable them to carry out their functions of transmission of the nerve impulse, the storage and retrival of information and the use of the same for maintaining homeostasis, for regulating behaviour and for higher intellectual functions. Nervous tissue consists broadly of two categories of cells, namely, neurons and glia, the former, concerned with the above functions with the close cooperation of the latter (Hyden, 1967). The structure of the neuronal cell is well suited for its function, the same consisting of a cell body and fibrillar processes in the form of branched fibers called dendrites and a long fiber called axon which may have collaterals.

During the development of the CNS in vertebrates, the vertebral central canal gets lined by a ciliated columnar

epithelium termed ependyma. The formation of neuroblasts which give rise to neurons and spongioblasts or glioblasts which give rise to glia take place in the ependyma followed by their multiplication, polarization, differentiation and migration to appropriate regions. The changes at the cellular level include increase in the volume and diameter of the cell body, axonal growth, dendritic proliferation and synapse formation (Purpura et al, 1964). The above changes are associated with a decrease in the proportional volume of the cell body and increase in that of membrane because of the large increase in axons and dendrites (Brizze et al, 1964; Ford, 1973). The above changes are associated with myelination the onset of which is preceded by a remarkable increase in glial cell number and metabolic activity of the same (Jacobson, 1970). The myelin sheath is formed by the spiralling of myelin around the axon so as to form a lamellar structure by oligodendrocytes in the CNS. Macroneurons with longer axons are myelinated first. Myelination in the CNS proceeds from the phylogenetically older regions (Jacobson, 1970). Thus in the CNS, myelination starts first in the cord followed by the different areas of the brain in the caudocranial order (Jacobson, 1970; Smith, 1973; Banik and Smith, 1977).

Neuronal conduction is normally from cell body via the axon to the dendrites or cell body of the connecting neuron

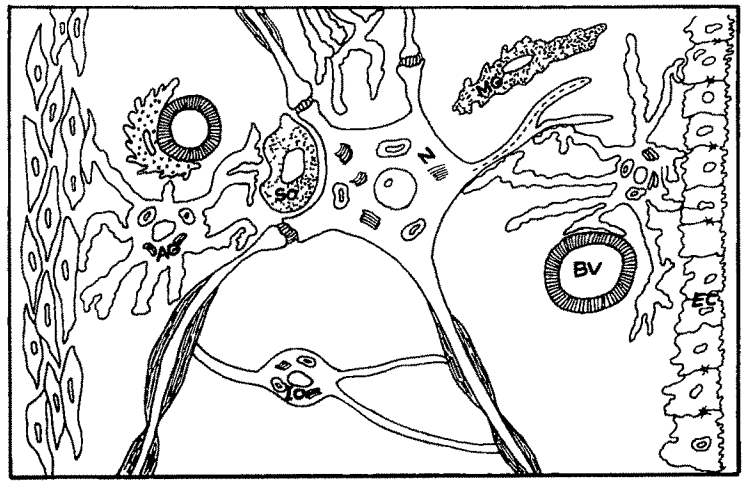
(Warwick and Williams, 1973). Transmission along the axon is made more efficient by the myelin sheath which is formed by oligodendrocytes surrounding the axon in the CNS and Schwann cells in the PNS and which not only offers insulation but also makes more speedier conduction because of the gaps in the sheath(nodes of Ranvier) where the axon comes into contact with the surrounding fluid causing changes in the polarity so that the impulse jumps from node to node (Norton, 1976; Moreil and Norton, 1980).

Not all the axons are myelinated in the CNS. In certain regions such as the cerebral cortex, the microneurons have short unmyelinated axons whereas in other regions such as the corpus callosum, the axons are highly myelinated. The axons of the same neuron may be unmyelinated in one region and become myelinated as it leaves that region. Depending upon the predominance of non-myelinated and myelinated nerve fibers, the CNS can be grossly differentiated as gray matter and white matter.

Gray matter consists of : (a) the cell bodies, dendritic trees and the initial segments of neurons; (b) the terminal segments and synaptic endings of axons, (c) variety of glial cells such as protoplasmic astrocytes, which are attached to the blood vessels and satellite oligodendrocytes associated closely with the surface of the neuron and (d) intercalated

FIG. 1. DIAGRAMATIC REPRESENTATION OF THE MAJOR COMPONENTS OF GRAY & WHITE MATTER. (ADAPTED FROM FIG 1-1, BASIC NEUROCHEMISTRY & FIG. 7-22, GRAY'S ANATOMY).

EC-EPENDYMAL CELL, AG-ASTROGLIA, MG-MICROGLIA, N-NEURON, BV-BLOOD VESSEL, SO-SATELLITE OLIGODENDROGLIA, IO-INTERFASCICULAR GLIGODENDROGLIA.



neurons with short axons which remain within the gray matter. The white matter mainly consists of : (a) myelinated axons; (b) interfascicular oligodendrocytes which makes the myelin sheath, and (c) fibrous astrocytes attached to the blood vessels (Fig. 1).

The gray matter (on a dry weight basis) consists of less than 8% of glial cells (astrocytes, microglia and oligodendrocytes) and 75-80% of cell body and axon dendritic mesh work (Brante, 1949). The white matter contains 40-50% myelin and the rest includes glial cells (oligodendrocytes and fibrous astrocytes), axons and blood vessels (Brante, 1949; Norton and Autilio, 1966). Thus gray matter contains predominantly of neuronal cell bodies and neuropil (network of dendrites, synaptic complexes and astrocytes) whereas white matter contains myelin and the myelin making oligodendrocytes.

The proportions of gray and white matter vary in different regions of the nervous system as the degree of myelination varies. In general, the peripheral nerves have a higher degree of myelination than the spinal cord, which in turn has a higher degree of myelination than the brain. Regions such as the brain stem and cerebellum are myelinated to a greater degree than higher regions such as the cerebral cortex. The proportions of gray and white matter are about equal in the adult human brain (Brante, 1949), but the same vary in the cerebral

hemispheres (60 and 40% respectively) (Jun and Feigin, 1973) and the spinal cord (20 and 80%) (Friede, 1975). Even in the same region the proportions of gray and white matter may vary e.g. in the rat spinal cord, the thoracic, cervical and lumber regions contain 75, 65 and 50% of white matter (Zeman and Innes, 1963). The proportions of the two also vary with age. For instance, in the human spinal cord the ratio of white to gray matter is 1:1 at the 4th month of fetal life, 2:1 at birth, and 4.5:1 in the adult (Friede, 1975).

The complexity of the nervous system increases as we go higher up on the phylogenetic scale and this is associated with a progressively increasing degree of myelination. In the lower vertebrates such as frogs and fishes white matter is not well developed. The myelin isolated is found to be immature, containing less lipid and more protein (Cuzner et al, 1965). It is only in mammals we find that gray and white matter are well formed and the CNS contains a well-knit neuronal network with long axons well insulated by myelin of white matter.

Some of the morphological features of the developing rat brain are summarized in Table 1. From the table it can be seen that the development of neuronal cell bodies, dendritic arborization, development of axons, formation of astrocytes and synaptic connections which comprise gray matter is completed by 30 days of age, whereas the differentiation of

Table 1 : Developmental periods in the rat brain*.

| Period | Duration | Cell growth characteristics | Development of gray and white matter |
|---|---|---|---|
| I (a) | 10-14 days of gestation | Formation and proliferation of neuroepithelial cells | |
| (b) | 14th day of gestation to birth | Rapid phase of neuronal cell division and attainment of adult number of long axon macro neurons at birth. | initiation of development of gray matter |
| II | Birth to 10 days of age | Outgrowth of dendrites and axons and establishment of neuronal connections (neuropil development), multiplication of microneurons and spongioblasts. | development of gray matter |
| III | 10-20 days after birth | Further extension of neuronal connections and synaptogenesis: differentiation and maturation of glial cells into astrocytes and oligodendrocytes; onset of myelination. | Peak period of gray matter development, onset of white matter formation |
| IV. | 20-30 days after birth | Establishment of adult number of oligodendrocytes; peak period of myelination; completion of gray matter development. | completion of gray matter development. Peak period of whitematter development. |

* data compiled from different sources.

Davison and Dobbing, 1968; Venier et al, 1971; Benjamins and Mckhann, 1976.

oligodendrocytes, which predominate in white matter begins only after 10 days of birth and the rate of myelination, which starts around this time, reaches a peak by 20 days of age, slows down by 30 days of age (Norton and Poduslo, 1973) but continues till 365 days. Thus the gray matter matures much earlier than white matter.

The high proportion of membrane to cell-body in nervous tissue and the presence of myelin renders nervous tissue unique in composition, characterized by a high concentration of lipids. The fact that practically all the lipids are membranal and that fat is not used by nervous tissue as fuel also distinguishes it from other tissues not only with regard to lipid content but also lipid composition (Tables 2 and 3). Further, the functions of nervous tissue necessitate the maintenance of a stable chemical composition and this, in turn, necessiates the maintenance of a membrane structure with high integrity as well as the capacity for speedy and highly selective ion transport for neural transmission. Not surprisingly, the lipids of neuronal membranes differ from those of other membranes not to mention the myelin sheath which has a unique composition. For instance, the lipid-protein ratio is about half or less in the plasma membrane of rat skeletal muscle or liver, about two thirds in human erythrocytes, one in the axonal membrane and around 3.5 in myelin (Rajalakshmi, 1980).

Table 2 : Chemical composition of tissues in adult man*.

| Tissue | Total weight (kg) | Water (%) | g per 100 g of fresh tissue | | Lipid/protein |
|---|---|---|---|---|---|
| | | | Ether extract (lipid) | Crude protein (N x 6.25) | |
| Liver | 2.3 | 72 | 3.1 (11) | 22.2 (79) | 0.14 |
| Striated muscle | 40.0 | 70 | 6.6 (22) | 22.0 (73) | 0.27 |
| Kidneys | 0.5 | 71 | 7.2 (25) | 19.3 (67) | 0.37 |
| Skin | 6.0 | 58 | 14.0 (33) | 27.0 (64) | 0.52 |
| Alimentary tract | 1.9 | 77 | 9.2 (40) | 12.8 (56) | 0.72 |
| Heart | 0.5 | 63 | 16.6 (45) | 17.5 (47) | 0.95 |
| Nervous tissue | 3.0 | 75 | 12.4 (50) | 11.5 (46) | 1.08 |
| Skeleton | 17.6 | 28 | 25.0 (35) | 19.7 (27) | 1.27 |
| Adipose tissue | 11.4 | 23 | 71.6 (93) | 5.6 (7) | 12.8 |

* according to Forbes, Cooper and Mitchell (1953).
% dry weight is shown in parentheses.

From the foregoing it should be obvious that gray and white matter differ appreciably in lipid content and composition, the lipids in the former being mainly in the membrane whereas those in the latter are derived from both the cell membrane and myelin. These differences are naturally associated with those in other constituents such as moisture and protein. Gray matter has a higher moisture content (82%) as compared to white matter (72%) whereas white matter has higher concentration of lipids, 16%, as compared to 6% in gray matter (Suzuki, 1976). Although the concentration of protein is similar in both, about 12%, the ratio of lipid to protein is ~~x~~ higher in white matter (1.3 as against 0.5) as can be expected from its higher lipid content (Toews and Horrocks, 1976).

Lipids are generally membranal constituents and play an important role in the maintenance of membrane-bound enzymes, changes in membrane permeability, cell to cell recognition and specific receptors for toxins (Suzuki, 1976). Further, lipids form the major chemical constituents of the myelin sheath which not only provides insulation for the axons but also enables a rapid transmission of the nerve impulse because of the saltatory conduction facilitated by the nodes of Ranvier present in the myelin.

Although certain other tissues such as adipose tissue, heart, bone and skin are also high in lipids (Table 3), the

Table 3 : Lipid composition of different tissues in the adult rats.

| Tissue | Total lipid (% fresh weight) | % of total lipid* | | | | Gangliosides* as NANA (mg/100 g dry wt.) |
|---|---|---|---|---|---|---|
| | | Phospho-lipid | Cholesterol | Neutral fat | Cerebro-sides | |
| Brain | 7.8 | 60 | 17 | - | 19 | 200.0 |
| Liver | 4.3 | 69 | 5 | 25 | 0.5 | 10.0 |
| Intestine | 6.5 | 50 | 10 | 33 | 7 | 18.0 |
| Kidney | 3.8 | 70 | 9 | 15 | 6 | 13.0 |
| Lung | 3.4 | 59 | 12 | 21 | 4 | 20.0 |
| Skin | 11.0 | 10 | 3 | 85 | - | - |
| Bone | 1.2 | 2 | 20 | 70 | - | - |

* according to Deuel (1955).

** according to Puro and coworkers (1964).

role and composition of lipids present in them differ from those in nervous tissue. The major lipid component of these tissues is triglycerides which serve as a lubricating and insulating surface lipid in the skin, as a reserve of food energy for the whole body in adipose tissue, and as an additional fuel valuable during vigorous muscular exercise in muscle. As glucose is the sole fuel used by the normal adult brain and practically all the lipid in the brain is either in the neuronal membrane or myelin and as the major classes of membrane lipids are cholesterol, phospholipids, galactolipids and gangliosides, it is not surprising that triglyceride is practically absent in the brain (Dickerson, 1968). On the other hand, nervous tissue has high concentrations of cholesterol, galactolipids and gangliosides. The lipid composition of gray and white matter also differs (Table 4) as may be expected as the membranal lipids predominant in gray matter have a role different from that in myelin lipids. Except for gangliosides and phosphoinositides, white matter has higher concentrations of all the lipid components. This is particularly true of galactolipids whose concentration in white matter is 10 times that in gray matter and which is therefore considered as a 'marker' for both white matter and myelin. Gangliosides whose concentration in gray matter is 7 times that in white matter are considered as 'markers' for gray matter. In this connection, the microsomal activity of the glucosyl transferase, one of the enzymes

Table 4 : Concentration, content and composition of different lipids in the adult human brain gray and white matter*

| | Concentration | | Content** | | Composition | |
|---|---|---|---|---|---|---|
| | Gray matter | White matter | Gray matter | White matter | Gray matter | White matter |
| | (mg/g fresh tissue) | | (mg/g fresh whole brain) | | (% of total lipids) | |
| Cholesterol (CHL) | 13.0 | 42.9 | 6.50 | 21.5 | 22.0 | 27.5 |
| Galactolipids (GL) | 4.32 | 41.2 | 2.16 | 20.6 | 7.30 | 26.4 |
| Phospholipids (PL) | 41.1 | 71.6 | 20.6 | 35.8 | 69.4 | 45.9 |
| mole ratio of CHL:GL:PL | | | | | 100:33:316 | 100:96:167 |
| Plasmalogens | 5.21 | 17.5 | 2.60 | 8.75 | 8.80 | 11.2 |
| Gangliosides | 2.72 | 0.37 | 1.36 | 0.19 | 4.60 | 0.24 |
| Phospholipid fractions | | | | | (% of total phospholipids) | |
| Ethanolamine phosphoglycerides (EPG) | 13.0 | 23.3 | 6.50 | 11.7 | 31.7 | 32.5 |
| Choline phospho-glycerides (CPG) | 15.7 | 20.0 | 7.85 | 10.0 | 38.3 | 27.9 |
| Sphingomyelin (SM) | 4.1 | 12.0 | 2.05 | 6.00 | 10.0 | 16.8 |
| Serine phospho-glycerides (SPG) | 5.1 | 12.2 | 2.55 | 6.10 | 12.4 | 17.0 |
| Inositol phospho-glycerides (IPG) | 1.64 | 1.56 | 0.82 | 0.78 | 4.0 | 2.20 |
| EPG/CPG | - | - | - | - | 0.83 | 1.17 |

* values taken from Suzuki (1976).
** values calculated assuming gray and white matter to contribute equally to adult brain. (Brante, 1949).

involved in the synthesis of gangliosides is shown to be higher in gray matter than in white matter (Deshmukh et al, 1974). Astrocytes and neurons isolated from the gray matter show a higher activity of this enzyme than oligodendrocytes isolated from white matter (Deshmukh et al, 1974). Similarly, the microsomal activity of galactosyl transferase, one of the key enzymes in galactolipid synthesis, is found to be higher in white matter than in gray matter. Further, the activity of this enzyme is higher in oligodendrocytes isolated from white matter than in astrocytes or neurons obtained from gray matter (Deshmukh et al, 1974).

The sharp differences between gray matter and white matter with regard to this lipids are consistent with their physicochemical properties and postulated roles. Galactolipids generally contain long chain hydroxy fatty acid components ($C_{24}$,h:0, $C_{24}$,h:1), have a high stability and transition temperature, a slow turnover in the adult and perhaps guard the integrity of the membrane and the myelin sheath. Gangliosides generally contain mainly stearic acid ($C_{18}$:0) as a fatty acid component are highly concentrated in the synaptic membrane, and have been implicated with synaptic activity and learning (Weigandt, 1968; Irwin and Samson, 1971; Rehman, 1977). Polyphosphoinositides, which contain stearic and oleic acids as the major fatty acid components (Kerr and Read, 1963), have a high turnover rate and seem to play a structural as well as

functional role which involves their binding with cations at the membrane surface (Hendrickson and Reinertsen, 1971; Michell, 1975).

The differences in the lipid composition of gray and white matter are reflected in the molar ratios of cholesterol: galactolipids:phospholipids, the same being 100:33:316 in the former, and 100:96:167, in the latter. Regarding the relative contribution of different phospholipids, the ratio of EPG to CPG is 0.83 in gray matter whereas it is 1.17 in white matter. The percentage contribution of inositol phosphoglyceride is almost twice as much in gray matter as in white matter (Table 4).

Histochemical observations suggest that most of the lipids are located in the neuropil in gray matter and myelin in white matter (Lowry et al, 1954; Friede, 1966). Data available on the lipid composition of different cell types in the brain (rat and bovine) are given in Table 5. It can be seen from the same that gray matter lipids may reflect to a considerable extent the lipid pattern in neuronal cell body and astrocytes whereas white matter lipids reflect those in myelin. In this connection it is of interest to note that the specific activity of CNP, which is considered a 'marker' enzyme of myelin is 5-8 times as much in white matter as in gray matter in the rat (Deshmukh et al, 1974; Sabri and Davison, 1977), rabbit (Kurihara and Tsukada, 1973) and human (Toews and Horrocks, 1976). The

Table 5 : Lipid composition of different cell types, axons and myelin of the brain.

| | Neuronal perikarya or cell body (RAT)* | Astrocytes (RAT)* | Oligo-dendrocytes (BOVINE)** | Axons from white matter (BOVINE)** | Myelin (BOVINE)** |
|---|---|---|---|---|---|
| Total lipids (% of dry weight) | 24.1 | 38.9 | 29.5 | 13.4 | 75.3 |
| Individual lipids (% of total lipids) | | | | | |
| Cholesterol (CHL) | 10.6 | 14.0 | 14.1 | 20.0 | 38.1 |
| Galactolipids (GL) | 2.1 | 1.8 | 10.0 | 20.0 | 29.3 |
| Phospholipids (PL) | 72.3 | 70.9 | 62.2 | 60.0 | 43.0 |
| mole ratio of CHL:GL:PL | 100:20:682 | 100:13:506 | 100:71:441 | 100:100:300 | 100:104:153 |
| Phospholipid fractions (% of total phospholipids) | | | | | |
| Ethanolamine phospho-glycerides (EPG) | 25.2 | 28.4 | 22.5 | 24.5 | 40.5 |
| Choline phosphoglycerides (CPG) | 55.2 | 51.2 | 47.3 | 31.7 | 25.3 |
| Sphingomyelin (SM) | 4.4 | 5.2 | 11.4 | 15.5 | 16.5 |
| Serine phosphoglycerides (SPG) | 5.4 | 7.3 | 6.6 | 9.3 | 15.1 |
| Inositol phosphoglycerides (IPG) | 6.8 | 4.9 | 7.6 | 5.7 | 1.9 |
| EPG/CPG | 0.46 | 0.56 | 0.48 | 0.77 | 1.60 |

* values taken from Poduslo and Norton (1972).

** values taken from Devries and Norton (1974).

oligodendrocytes and axons which also form part of the white matter have a lipid composition different from that of myelin. They also differ from neurons (Table 5).

Since lipids form a major component of the brain, several studies have been made on changes in their composition during development in different species such as the rat (Brante, 1949; Kishimoto et al, 1965; Wells and Dittmer, 1967; Cuzner and Davison, 1968; Vanier et al, 1971; Alling and Karlsson, 1973; Norton and Peduslo, 1973; deSousa and Horrocks, 1979; Karlsson and Svennerholm, 1978), mouse (Folchi, 1955), rabbit (Dalal and Enstein, 1969) and man (Brante, 1949; Rouser and Yamamoto, 1969; Yasuf and Dickerson, 1977; Martins and Ballabrica, 1978). The concentration of gangliosides in the rat brain increases approximately 3 fold from birth to 17 days of age, the maximum rate of increase being between 11 and 13 days of age (Suzuki, 1965b; Vanier et al, 1971). Similarly, in man the concentration of gangliosides increases significantly in gray matter during the first two postnatal months and reaches adult levels by 2 years of age (Vanier et al, 1971; Svennerholm and Vanier, 1972). This increase in gangliosides coincides with the peak period of development of gray matter with regard to dendritic arborization and synaptogenesis in the rat brain (Vanier et al, 1971). During this period the activity of glucosyltransferase, one of the enzymes involved in the synthesis of gangliosides

is also found to be very active (Shah, 1971). Incorporation of labelled glucosamine into brain gangliosides is also found to be maximum during this period (Suzuki, 1967; De Maccioni and Caputto, 1968).

In the rat the concentration of cholesterol increased by 60% between birth and 10 days of age (Cuzner and Davison, 1968; Alling and Karlsson, 1973; Karlsson and Svennerholm, 1978; deSousa and Horrocks, 1979) and by 300% between 10 and 140 days of age (Norton and Poduslo, 1973). These increases are associated with increases in the activities of enzymes involved in cholesterol synthesis and in the rate of incorporation of labelled precursors into brain cholesterol. The same are found to be very low in the adult brain (Smith, 1965 and 1967; Jones et al, 1975).

Galactolipids which are present in higher concentrations in white matter are not detected before 10 days of age in the rat brain, an observation consistent with the onset of myelination during this period (Cuzner and Davison, 1968; Alling and Karlsson, 1973; Karlsson and Svennerholm, 1978). The percentage increase in the same during development is much higher than in the case of other lipids (Kishimoto et al, 1965; Wells and Dittmer, 1967; Cuzner and Davison, 1968; Norton and Poduslo, 1978; deSousa and Horrocks, 1979). Similar observations have been made with regard to galactolipids in human brain white

matter during development (Vanier et al, 1971; Svennerholm, and Vanier, 1972). The increase in galactolipid concentration is 40 fold between 10 and 140 days of age in the rat brain and is associated with a 20 fold increase in myelin content (Norton and Poduslo, 1973) and a marked increase in the activity of CNP. which is manifest just before the onset of myelination i.e. before 10th day after birth (Olafson et al, 1969). A marked increase in the activities of the enzymes concerned with the synthesis of galactolipids (Brenkert and Radin, 1972; Constantiocecccerini and Morell, 1972) and the increased incorporation of labelled precursors into whole brain galactolipids has been observed during this period (Burton et al, 1958; Kishimoto et al, 1965; Mckhann and Ho, 1967).

A 30% increase is found in the concentration of total phospholipids between birth and 10 days of age in the rat (Cuzner and Davison, 1968; Alling and Karlsson, 1973; Karlsson and Svennerholm, 1978; deSousa and Horrocks, 1979) and a 200% increase between 10 days and 140 days (Cuzner and Davison, 1968; Norton and Poduslo, 1973; deSousa and Horrocks, 1979). Similarly the concentration of phospholipids in the developing human brain increases by 150% and 250% respectively in gray and white matter between birth and 2 years of age (Vanier et al, 1971; Svennerholm and Vanier, 1972). As may be expected, the concentration of different phospholipids also increase with age,

the increase being more in the case of EPG and CPG. The ratio of EPG to CPG increases from 0.56 at 10 days to 1.0 in the adult (Norton and Poduslo, 1973). This increase is accounted for by that in white matter as the value remains less than one in gray matter at all ages (Vanier et al, 1971; Svennerholm and Vanier, 1972). Plasmalogens which are myelin specific phospholipids increase significantly during maturation (Cuzner and Davison, 1968; Norton and Poduslo, 1973; deSousa and Horrocks, 1979).

The increase in the concentrations of EPG and CPG are associated with increases in the activities of phosphoethanolamine transferase (rat brain) and phosphocholine transferase (rabbit brain) respectively (McCaman and Cook, 1966; Ansell and Metcalfe, 1971). Recently, it has been found that in the developing chick brain microsomes, a doubling in the specific activities of phosphoethanolamine and phosphocholine transferases occurs during glial proliferation which is also associated with a higher accretion of EPG and CPG during that period (Freysz et al, 1978 and 1980). It has also been shown that the phosphotransferases (Strosznajder et al, 1977a) and phospholipases $A_1$ and $A_2$ (Woelk et al, 1973) are more active in neuronal cell bodies than in astrocytes obtained from rabbit brain gray matter. Activities of enzymes which synthesize and degrade plasmalogens also increase during myelination (Ansell, 1973; Dorman et al, 1977; Horrocks et al, 1978).

Some of the changes in the lipid composition of the developing rat brain are summarized in Table 6. The changes in the first 10 days reflect the development of gray matter and maturation of cells present in it. Those between 10 and 30 days reflect both myelination and the development of neuropil whereas those after 30 days reflect the progress of myelination.

Since gray and white matter follow different patterns of maturation the effects of nutritional deficiencies on the morphological and biochemical development of gray matter and white matter may be expected to vary with the timing and duration of the nutritional stress. Further, it may well be that the two are differentially affected if the maturation of gray matter enjoys ontogenetic and metabolic priority. Since the maturation of the brain is associated with changes in morphological features and chemical composition several studies have been made on the effects of nutritional deficiencies during the prenatal, neonatal and postweaning periods on the whole brain and isolated myelin. Except for limited studies on malnourished children [illegible] studies have been made on the comparative effects of nutritional stress on gray matter and white matter.

Prenatal undernutrition produced by feeding the mother a low protein diet during gestation was associated with deficits

Table 6 : Changes in the lipid composition of developing rat brain*.

| Lipid component | % increase | | |
|---|---|---|---|
| | 0-10 days | 10-30 days | 30-140 days |
| Total lipid | 38 | 95 | 29 |
| Cholesterol | 86 | 125 | 31 |
| Galactolipids | - | 1373 | 140 |
| Phospholipids | 22 | 71 | 8 |
| Gangliosides | 58 | 63 | 0 |
| Plasmalogens | 66 | 152 | 24 |
| Ethanolamine phosphoglycerides | 11 | 97 | 5 |
| Choline phosphoglycerides | 81 | 42 | 0 |
| Sphingomyelin | 102 | 195 | 17 |
| Inositol phosphoglycerides | 10 | 89 | 4 |
| Serine phosphoglycerides | 69 | 63 | 7 |

* values calculated from Karlsson and Svennerholm, 1978; Norton and Poduslo, 1973; Cuzner and Davison, 1968.

in body and brain weights of newborn pups but no significant changes were observed in the concentrations of DNA, RNA, protein (Zamenhof et al, 1968; Envonwu et al, 1973; Karlsson and Svennerholm, 1978) and lipids (Rajalakshmi and Nakhasi, 1974; Karlsson and Svennerholm, 1978). The reduced brain size and the normal concentration of DNA, RNA and protein suggest a reduction in cell number rather than cell size. However, these deficits were abolished with normal nutrition during the postnatal and postweaning period (Zamenhof et al, 1973).

Since the adult number of long axon macroneurons of the brain is reached even at birth neonatal undernutrition may not affect the number of macroneurons but may affect their development (Sugita, 1918; Shoemaker and Bloom, 1976), but the number of glial cells is found to be decreased (Siassi and Siassi, 1973). The reduction in brain weight has been explained in terms of the retardation of neuronal development and delayed myelination (Bass et al, 1970a and b; Krigman and Hugan, 1976; Stewart et al, 1976; Griffins et al, 1977).

Histological examination of the cerebral cortex of rats undernourished during the neonatal period suggests increased cell density (increase in cell number/unit area) and reduction in dendritic arborization (Eayrs and Horn, 1955; Sima and Persson, 1975; Cardero et al, 1976) and dendritic area in the Purkinji cells (Pysh et al, 1979) and non-neuronal cell count

(Siassi and Siassi, 1973). Electron microscopic studies confirm a greater neuronal cell density suggestive of a retardation in neuropil development of the rat cortex (Cragg, 1972). A decrease in the size and density of presynaptic endings (Gambetti et al, 1974) and deficits in the number of synapses per unit area of the brain have also been reported (Burns et al, 1975; Shoemaker and Bloom, 1976).

Electron microscopic studies also showed that the brain of the undernourished rat is characterised by a decrease in the number of astrocytes and oligodendrocytes in white matter (Krigman and Hogan, 1976). A significant reduction in the myelinated axons and an increase in promyelinating axons have been observed (Krigman and Hogan, 1976). Thus prenatal undernutrition is found to affect the cell number but not cell size whereas neonatal undernutrition affects the maturation of neuronal cells and both cell division and cell growth in non-neuronal cells.

The morphological changes have been found to be correlated with chemical changes as might be expected. The reduction in cell number is associated with a reduction in total DNA content (Winick and Noble, 1965 and 1966), although DNA concentration is not generally affected (Enwonwu et al, 1973; Patel et al, 1973; Sobotka et al, 1974; Karlsson and Svennerholm, 1978). The concentration of RNA (Guglielmone et al, 1974) and protein

(Gambetti et al, 1972; Enwonwu et al, 1974; Karlsson and Svennerholm, 1978) are found to be unaffected with neonatal undernutrition. The decrease in cell size is evident in the decreased ratio of RNA to DNA and protein to DNA (Gambetti et al, 1972; Enwonwu et al, 1974).

Some of the morphological changes observed such as poor axonal and dendritic growth and delayed myelination would lead us to expect changes in lipids. The lipid concentration of the brain is affected in undernutrition the effects being most evident between 2-3 weeks of age in rats, a fact consistent with the sequence of events in the CNS. A significant decrease in the concentration of gangliosides has been reported by several authors (Bass et al, 1970; Ghittoni and DeRaveglia, 1972; Merat and Dickerson, 1974; Krigman and Hogan, 1976; Rajalakshmi and Nakhasi, 1974; Reddy and Sastry, 1978). But such decrease was not found by Gaison and Waisman, (1970) and Karlsson and Svennerholm (1978). The inconsistency observed in the above studies with regard to the concentration of gangliosides may be because of the differences in the strain of the rats used and the severity of the undernutrition achieved and variations in the methodology. Even in studies reporting a deficit in gangliosides, the constitution of different ganglioside fractions to the total was not affected (Merat and Dickerson, 1974; Reddy and Sastry, 1978; Karlsson and Svennerholm, 1978).

This suggests that only a quantitative and not a qualitative decrease has been observed by some investigators. Studies carried out on the human brain are not consistent. Fishman et al (1969) found the ganglioside concentration in the white matter of malnourished children who had died between 4 and 12 months of age to be not affected. On the contrary, Kokrady et al (1972) reported a significant decrease in the concentration of gangliosides in both gray and white matter of kwashiorkor children who died at the age of 9 years. The differences observed in the above two studies may be because of differences in the age, severity, duration and type of malnutrition.

The enzyme CMP-NANA synthetase, which is involved in the synthesis of gangliosides, and sialidase, an enzyme involved in the degradation of gangliosides were both found to be greater with neonatal undernutrition, even though no differences were observed in the concentrations of the gangliosides (Morgan and Naismith, 1975). Since the above enzymes showed higher activities between 6 and 16 days after birth and falls to a constant level by the 16th day (Roukema et al, 1970), the authors attributed the higher activities of these enzymes to delayed maturation. In vivo incorporation of $^{14}$C-glucosamine into gangliosides has not been affected with undernutrition (Reddy and Sastry, 1978).

Neonatal undernutrition has been found to decrease significantly the concentration of cholesterol in the rat brain (Culley and Mertz, 1965; Dobbing and Sands, 1971; Rajalakshmi and Nakhasi, 1974; Krigman and Hogan, 1976; Reddy and Sastry, 1978) and spinal cord at 21 days of age (Rajalakshmi and Nakhasi, 1976; Sharma, 1979). The *in vivo* incorporation of labelled glucose into brain cholesterol (specific activity) is also found to be decreased (Agrawal *et al*, 1972; Chase *et al*, 1976; Jailkhani and Subramanyam, 1977). In the case of malnourished children the concentration of cholesterol is not found to be affected in white matter (Fishman *et al*, 1969). It is possible that the amount of white matter rather than its composition gets affected first.

Among the other lipids, the deficit in galactolipids is found to be greater (Culley and Mertz, 1965; Geison and Waisman, 1970; Rajalakshmi and Nakhasi, 1974; Krigman and Hogan, 1976; Reddy and Sastry, 1978; Karlsson and Svennerholm, 1978). Similar deficits in galactolipids in both gray and white matter have been found in autopsy studies of malnourished children (Fishman *et al*, 1969; Kokrady *et al*, 1972). The latter is consistent with the decreased amount of myelin in malnourished children (Fox *et al*, 1972) and neonatally undernourished rats (Fishman *et al*, 1971; Nakhasi *et al*, 1975; Simons and Johnston, 1976; Wiggins *et al*, 1974 and 1976; Reddy *et al*, 1979;

Wiggins and Fuller, 1979). A decrease in the concentration and specific activity of CNP in the whole brain (Nakhasi et al, 1977; Reddy et al, 1979) as well as isolated myelin (Nakhasi et al, 1975; Simons and Johnston, 1976; Reddy et al, 1979) has been reported in rats.

Both in rats and children decreased concentration of galactolipids found in malnutrition is associated with a lower concentration of the activity of galactolipid sulfotransferase, an enzyme involved in the synthesis of sulfatides (Chase et al, 1967 and 1972). In rats in vivo incorporation of labelled precursors into galactolipids is also found to be decreased (Chase et al, 1967; Agrawal et al, 1972; Chase et al, 1976; Jailkhani and Subramanyam, 1977). Using ($^{3}H$) and ($^{14}C$) acetate, choline or glycerol as precursors, in vivo incorporation of isotopes into lipids of isolated myelin is depressed by about 60% as compared to incorporation in other subcellular fractions (Wiggins et al, 1976). The decrease was also observed in the case of myelin isolated from different brain regions (Wiggins and Fuller, 1979).

Neonatal undernutrition is found to decrease the concentration of total phospholipids in the rat brain at 21 days of age (Culley et al, 1966; Ghittoni and DeRaveglia, 1972 and 1973; Rajalakshmi and Nakhasi, 1974; Krigman and Hogan, 1976; Reddy and Sastry, 1978). Among the different phospholipid components,

plasmalogens which are considered to be the marker lipids for myelin phospholipids are found to be decreased in the whole brain as well as myelin in rats (Culley et al, 1966; Geison and Waisman, 1970; Ghittoni and deRaveglia, 1972 and 1973; Reddy and Sastry, 1978; Ghittoni, 1979) and in white matter in malnourished children (Fishman et al, 1969) Fishman et al, 1971; Nakhasi et al, 1975; Simons and Johnston, 1976). Ethanolamine and choline phosphoglycerides are also found to be affected the former being affected to a greater extent. Consequently the EPG to CPG ratio is decreased (Ghittoni and deRaveglia, 1972; Reddy and Sastry, 1978; Ghittoni, 1979). Regarding phosphoinositides, while monophosphoinositides are not affected, the concentrations of polyphosphoinositides (DPI and TPI) are found to decrease with undernutrition (Sharma et al, 1980). The incorporation of $^{14}C$ glucose into different phospholipids is found to be reduced in the undernourished rats (Agrawal et al, 1972). On the other hand the incorporation of $^{32}P$ into phospholipids is found to be increased (Reddy and Sastry, 1978; Sharma et al, 1980).

While neonatal undernutrition affects the concentration of brain lipids, postweaning undernutrition or protein deficiency has not been found to alter the concentrations of different lipids in the whole brain (Dobbing and Widdowson, 1965; Rajalakshmi et al, 1974) or different parts of the brain

(Dickerson et al, 1972; Rajalakshmi et al, 1974). It has also been shown that postweaning protein deficiency has no effect on the lipid concentration or composition in the white matter in the case of pig brain (Sun and Tumblesson, 1972). This may be because the peak increases in lipids take place in the neonatal period although they continue to show increases in the post-weaning period.

Many studies have been carried out to find out whether the effects of neonatal undernutrition on the structure and chemical composition of the brain can be reversed by postweaning rehabilitation. It is generally observed that animals undernourished in early life fail to catch up with control animals with regard to body weight and this is also true of brain weight although the deficits are much less in the rehabilitated animals (Guthrie and Brown, 1968; Culley and Linenberger, 1968; Rajalakshmi et al, 1974; Reddy and Sastry, 1978). With regard to brain lipids, however, the findings are conflicting and the reversibility perhaps depends on the size of the initial deficit as well as the extent of rehabilitation. In some studies deficits are reported to persist even after rehabilitation in the case of brain cholesterol (Culley and Linenberger, 1968; Dobbing, 1968; Geison and Waisman, 1970; Dickerson and Jarvis, 1970; Smart et al, 1973; Rajalakshmi et al, 1974; Reddy and Sastry, 1978), galactolipids (Culley and Linenberger, 1968; Geison and Waisman, 1970; Reddy and Sastry, 1978), gangliosides

(Dickerson and Jarvis, 1970), phospholipids (Culley and Linenberger, 1968; Rajalakshmi et al, 1974; Reddy and Sastry, 1978) and plasmalogens (Geison and Waisman, 1970; Reddy and Sastry, 1978).

Consistent with this is the observation that the deficit in the amount of myelin was found to persist although it increased from 70% to 80% of control value in terms of amount per gram brain (Simons and Johnston, 1976; Reddy et al, 1979). In other studies complete 'catch-up' regarding lipid concentrations has been reported (Benton et al, 1966; Guthrie and Brown, 1968).

Most of the studies on the effects of nutritional deficiencies in experimental animals have been carried out on the effects of protein or calorie deficiencies and only a few studies are concerned with vitamin deficiencies, in spite of the well known CNS and PNS symptoms associated with the same even in adults.

Thiamine has received greater attention than other B-vitamins because of the known neurological involvement in beriberi in both infants and adults. Perinatal thiamine deficiency has been found to decrease the body and brain weights of weanling rats (Geel and Dreyfus, 1975; Trostler et al, 1977). However, as food intake also decreases in

thiamine deficiency a question arises as to whether the changes observed are due to undernutrition or thiamine deficiency. The thiamine content of the brain is also found to be reduced and specific sites of histological lesions have been identified (Dreyfus and Victor, 1961).

A reduction in brain transketolase was observed in a number of studies (Dreyfus and Hauser, 1965; Kaufmann, 1972; Geel and Dreyfus, 1974; Trostler et al, 1977; Prasanna, 1978). In the studies of Geel and Dreyfus (1975), in which a pair fed control group was used a significant decrease in the concentration of cholesterol was observed in the food restricted group but not in the thiamine deficient group. An increase was found in ganglioside concentration in both groups. This observation is certainly surprising in the case of the food restricted group as it is in variance with the observations of others (Merat and Dickerson, 1974; Reddy and Sastry, 1978). Recently Trostler et al (1977) reported a decreased concentration of of cholesterol, galactolipids and phospholipids in both pair fed and thiamine deficient rats at weaning. But the deficits were greater in the thiamine deficient group. These observations suggest that the deficits observed in the case of thiamine deficiency may not be entirely due to food restriction. However, the degree of undernutrition was somewhat more severe in thiamine deficiency, the values for body and brain weights

being 45% and 86% of control values as compared to 53% and 89% in the food restricted group (Trostler et al, 1977). The deficits in galactolipids perisisted in the thiamine deficient rats even after rehabilitation for 3 weeks, whereas they were abolished in the food restricted group (Trostler et al, 1977).

Pyridoxine has come in for some attention perhaps because of its role in the synthesis of GABA, transamination reactions and lipid metabolism. Reduced levels of GABA have been found in pyridoxine deficiency associated with convulsions (Dakshinamurti, 1977). Pyridoxal phosphate concentrations in different areas of the rat brain are also found to be reduced with pyridoxine deficiency (Bhagavan et al, 1977). Reduced concentrations of linoleic, arachidonic, lignoceric and nervonic acid in the rat brain with deficiency during the suckling period have been reported (Dakshinamurti, 1973; Thomas and Kirksey, 1976). Similar observations have been made with regard to gangliosides and galactolipids (Thomas and Kirksey, 1976). Significant decreases have also been found in the incorporation of $^{14}$C-acetate into cholesterol, galactolipids and phospholipids in the rat brain (Stephens and Dakshinamurti, 1975). The number of oligodendrocytes is found to be reduced (Yonezawa et al, 1969), an observation consistent with a reduced myelin content (Kurtz and Kanfer, 1973). Abnormal EEG patterns, convulsions and delayed auditory evoked potentials are known to result from pyridoxine deficiency (Stephens, et al, 1971).

The role of pantothenic acid in the formation of acetyl CoA which occupies a key position in the metabolism of amino acids, fatty acids and carbohydrates is well-known. Mitchell (1964) has pointed out the critical need for pantothenic acid for fetal development and the fact that fetal tissues contain much greater concentrations of the same than maternal liver, a pattern not found in the case of other vitamins. Pantothenic acid deficiency during gestation is found to result in severe impairment of reproductive performance in rats and guinea pigs (Nelson et al, 1957). Mental depression and neuromotor disorders have been found in man due to pantothenate deficiency (Bean et al, 1955). Though there was a significant reduction in the concentrations of cholesterol, galactolipids and phospholipids in the pantothenate deficient rat brain, no differences were observed between the pair-fed and pantothenate deficient group (Rajalakshmi and Nakhasi, 1975). However, subsequent studies in this laboratory showed a significant decrease in the concentrations of EPG and CPG when compared to pair-fed group (Jarori, 1976).

Although the role of vitamin $B_{12}$ in the metabolism of myelin in the nervous system is not identified, a lack of the same in man has been found to result in a demyelinating disease of the spinal cord referred to as "subacute combined degeneration" (Dayan and Ramsey, 1974). A decrease in the

ethanolamine phosphoglycerides and sphingomyelin in white matter and a marked fall in the content of unsaturated fatty acids in all the phospholipids of gray matter have been reported in the brain of a 7 year old child who died of methyl malonic aciduria and megaloblastic anemia (Dayan and Ramsey, 1974). Induction of vitamin $B_{12}$ deficiency in experimental animals is rather difficult but has been induced in rats by feeding them a $B_{12}$ free diet for 6-8 months from weaning (Fehling et al, 1978a). The deficiency was judged by estimating the methyl-melonic acid in urine and the tissue concentrations of vitamin $B_{12}$. No significant differences were found in the concentrations of of lipids but a significant increases were found in old chain ($C_{15}$ and $C_{17}$)fatty acids (Fehling et al, 1978a and b). A condition similar to vitamin $B_{12}$ deficiency has been sought to be induced by injecting massive doses of cycloleucine (a compound which inhibits 5-methyl tetrahydrofolate-methyl-transferase which requires vitamin $B_{12}$ as a cofactor) in rats during the suckling period. This is found to result in significant decrease in total phospholipids and plasmalogen concentrations in the brain and spinal cord (Ramsey and Fischer, 1978). Decreased contents of protein and sulfatide have also been reported in the adult mice brain (Nixon, 1976).

Vitamin A is necessary for the formation and maturation of the neural tube. In experimental animals vitamin A deficiency

is associated with the poor development of the vertebrae and the skull and a resulting pressure on the nervous system (Fell, 1960). Degenerative changes also occur independently of the malformation of bones. Degeneration of the nerve in the optic thalamus, the optic, femoral and sciatic nerves and spinal cord was also observed (Rao, 1936; Ridgon, 1962).

Decreased concentrations of cholesterol, galactolipids and gangliosides in the rat brain (Bhat and Rama Rao, 1972, 1978) and a significant reduction in the concentration of galactolipids in the rat spinal cord (Sharma, 1979) have been observed at 21 days of age in vitamin A deficient rats. Decreased incorporation of $^{14}$C-glucose into brain cholesterol, galactolipids and gangliosides has also been observed in the vitamin A deficient rats (Bhat and Rama Rao, 1976). Impairment of myelination in the brain has been shown by histological studies (Bhat and Rama Rao, 1978). A reduction in specific activity and concentration of the myelin marker enzyme, CNP (Nakhasi, et al, 1977) and a lower yield of myelin (Bhat and Rama Rao, 1978) have been reported.

Essential fatty acid (EFA) deficiency was induced by feeding rats for two generations on an EFA deficient diet (Alling et al, 1972). In the third generation a reduction in body and brain weights could be observed (Karlsson and Svennerholm, 1978). Paoletti and Galli (1972) have found with

a prolonged EFA deficiency, a reduced brain weight and lipid content and altered fatty acid composition of phospholipids, particularly EPG (Galli et al, 1970; White et al, 1971). Similar observations were made by Svennerholm and associates, who also found deficits in the concentration of galactolipids (Svennerholm et al, 1972; Alling et al, 1972; Alling et al, 1974; Karlsson and Svennerholm, 1978). No differences were found in the lipid composition of isolated myelin and synaptosomal plasma membrane but the amount of myelin was found to be reduced (Karlsson, 1975).

Effects of mineral deficiencies on the chemical composition of the CNS have received very little attention. Copper deficiency is known to lead to a condition called "Sway back" in the developing lambs (Lewis et al, 1967), pigs (McGavin et al, 1962) and goats (Owen et al, 1965). In experimentally induced copper deficiency, a significant decrease in the concentration of cholesterol and galactolipids (Dipaolo et al, 1974) and reduced concentration and specific activity of CNP (Prohaska and Wells, 1974) have been reported in the rat brain.

The lipid studies described above were carried out on either the whole brain or myelin. Studies on the comparative effects of deficiency in gray matter and white matter would be of interest as the two differ in lipid composition, mature at different rates and at different stages and are likely to have

different metabolic priorities. Svennerholm and Vanier (1972) pointed out in this connection that "the use of whole brain instead of separated cerebral cortex and white matter has several disadvantages. The concomitant determination of the lipid composition of gray and white matter during maturation will provide fundamental knowledge on the lipid biochemical events at the outgrowth of the neurons and the myelination. That information will be missed if total brain is used."

The present studies were designed in this context to understand the patterns of maturation of gray and white matter with regard to lipid composition, changes in the proportions of the same with development and the effects of nutritional deficiencies during the neonatal period on the above.

The lipid components studied were cholesterol, galactolipids, gangliosides, total phospholipids and phospholipids components. Since CNP is known to be a marker enzyme for myelin which is a major source of lipids in white matter, the activity of this enzyme was also studied. Since EPG and CPG form around 80% of phospholipids in gray and white matter and phosphoethanolamine and phosphocholine transferase are the key enzymes involved in the synthesis of these lipids, detailed kinetic studies of these two enzymes were also carried out.

Studies were also made of the effects of thiamine deficiency and undernutrition during the neonatal period on the lipid composition of the gray and white matter. Additional studies were also made of the effects of thiamine deficiency on the whole brain and spinal cord lipids. The details of these studies are incorporated in this thesis.