CHAPTER IV

SUMMARY

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Some distinctive features of the nervous system are that the neuronal cells have axons which along with dendrites in the connecting neuron enable neuronal transmission and these axons are myelinated to various degrees in different parts of the nervous system. Because of the myelin sheath which is white, parts of the brain which are heavily myelinated appear white in contrast to other parts which appear gray even to the naked eye. Gray matter mainly consists of neuronal cells bodies, dendritic trees, synaptic endings and protoplasmic astrocytes whereas white matter contains myelinated axons, oligodendrocytes and fibrous astrocytes. The degree of myelination varies with phylogenetic and ontogenetic development and this is reflected in the proportions of gray and white matter and consequently in chemical composition.

Among the major chemical constituents lipids form one of the important components of the central nervous system (CNS) and are believed to have several functions at membranal level. In the CNS, white matter contains higher percentage of lipids than gray matter. This is because of the preponderance of the lipid rich myelin present in it. Among the lipids cholesterol, galactolipids, phospholipids and gangliosides are the major ones present in CNS. Gray matter is rich in gangliosides whereas all other lipids are more in white matter.

Several studies have been concerned with changes with development on the composition of the whole brain or isolated myelin and the effects of nutritional stress on the same. While the study of the whole brain gives us an idea of the overall processes, the study of changes in gray and white matter will provide some additional insights regarding the development of neuronal cells, glial cells and myelination. However there are not many reports on changes in the lipid composition of gray and white matter with development and nutritional stress. It was therefore considered worthwhile to investigate the changes in the lipid composition of gray and white matter during the development of the rat brain as well as the effects of nutritional deficiencies on the same.

Studies were carried out on the changes with development and nutritional stress in early life on the lipid composition of gray and white matter in the rat brain. For the former aspect studies were made at 2,3,4,6,9,12,20 and 52 weeks of age. Nutritional stress was induced in the form of maternal protein or calorie or thiamine deficiency. Studies were also made of the reversibility of effects of neonatal undernutrition with realimentation during the postweaning period. The lipids studied were cholesterol, galactolipids, phospholipids, gangliosides and various phospholipid fractions.

Additional studies were made on 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP) which is considered as a myelin

marker enzyme and on phosphoethanolamine and phosphocholine transferases which are involved in the synthesis of EPG and CPG respectively the proportions of which are found to be altered with maturation and nutritional stress. The studies on thiamine deficiency were extended to the whole brain and spinal cord in order to get comparative data.

During the development of the rat brain, gray matter reaches 100% of its adult weight by three weeks of age whereas white matter shows a steady increase with age. The lipid concentrations (mg/g fresh tissue) in white matter were much higher than in gray matter as expected, the respective values in the mature gray matter being 61, 15, 5 and 42 for total lipids, cholesterol, galactolipids and phospholipids and the corresponding values for white matter being 138, 33, 33 and 71. The adult values were found to be attained much earlier in gray matter (by 4 weeks) whereas in white matter there was a rapid increase between 2 and 4 weeks of age followed by a progressively declined rate of increase even after 4 weeks.

The composition of different lipids as percentage of total lipids reaches a stable value by 4-6 weeks of age in both grey and white matter. Thus the increasing concentration of lipids in the white matter with age seems to be due to increase in the amount of myelin rather than a change in the composition. The proportions of white and gray matter at different ages were derived from the galactolipid concentrations of the whole brain,

gray matter and white matter. The proportion of white matter was found to increase with age as expected the values being 19% of whole brain at 3 weeks, 36% at 9 weeks and 54% at one year of age. As a corollary to this the contribution of different lipids by gray matter to the whole brain was more at early ages and decreased with age. Computations were made of the proportions of myelin and nonmyelin components of the white matter from the whole brain myelin values assuming that all the myelin in whole brain is present in white matter. White matter was found to contain about 45% of myelin and 55% of nonmyelin on dry weight basis at all the ages except at 2 weeks of age. However, the lipid rich myelin was found to contribute more (65%) to the total lipids in white matter. This was found to be specially the case with regard to galactolipids with myelin contributing about 85% of the total galactolipids present in white matter.

Although a considerable amount of work has been reported on the impact of neonatal undernutrition on the development of whole brain, not much is known about the same on the chemical maturation of gray and white matter. The amount of gray matter reaches adult value by 3 weeks of age whereas the white matter showed steady increase even after this period. Therefore it is reasonable to expect that neonatal undernutrition may have differential effects on the content and lipid composition of gray and white matter.

Neonatal undernutrition was found to lower the amount of both gray and white matter but the latter was found to be affected more, the value being 82% and 53% of controls for gray and white matter respectively. The concentrations of different lipids were less affected in gray matter than in white matter, the deficits being observed only with regard to galactolipids in gray matter and with regard to most lipids in white matter. The proportion of white matter was also found to decrease, the values being 19 % and 13% of the whole brain in control and undernourished rats. In the white matter both myelin and nonmyelin components were equally affected by undernutrition. Thus the changes observed in the whole brain lipids were due to both qualitative and quantitative changes in gray and white matter.

When neonatally undernourished rats were fed normal diet (i.e. 20% protein diet) ad lib. after weaning a complete catchup was observed in the weight of gray matter but not in that of white matter. Thus the persisting deficits observed in whole brain weight are deemed to be due to the reduced amount of white matter. The concentrations of different lipids were, however, restored to normal values by rehabilitation. These results suggest that gray matter is less affected with neonatal undernutrition and the deficits can be more readily reversed by rehabilitation. On the contrary white matter was found to be affected more by neonatal undernutrition and the deficits were irreversible. In white matter lipids the

'catchup' by rehabilitation was more in myelin rather than in nonmyelin component, the values being 69% and 45% of controls for myelin and nonmyelin components respectively.

When the neonatally undernourished rats were subjected to protein deficiency or undernutrition during the postweaning period, the deficits observed in the concentration of different lipids at weaning were abolished. The bridging of deficits in both gray and white matter lipids even in rats undernourished beyond weaning is in line with observations of an extended span of development in undernourished rats. Similar observations have been made previously in this laboratory with regard to GDH and GAD activities. The reported deficits in the concentration of different lipids in the whole brain were due to the quantitative changes observed in gray and white matter.

Maternal thiamine deficiency is known to cause infantile beriberi which is associated with neurological symptoms and specific CNS lesions. Not many studies have been carried out on the chemical composition of the CNS in spite of known lesions. Studies were therefore carried out to find out the effects of maternal thiamine deficiency during late gestation through lactation on lipid composition in different parts of CNS. Since thiamine deficiency is known to cause anorexia and reduced food intake an additional group was pair fed on normal diet with the thiamine deficient group.

The thiamine deficient group(TD) had lower body, brain and spinal cord weights than not only the ad lib. fed rats given the complete diet but also the pair fed animals. Transketolase, a marker enzyme of thiamine deficiency, was found to be less in TD rats but not in the pair fed rats in all parts of the CNS studied viz., brain, spinal cord, gray matter and white matter, confirming the thiamine deficiency per se in TD rats.

In the case of brain, in both thiamine deficient (TD) group and the pair fed group (PFC), deficits were found in the concentrations of total lipids, galactolipids and phospholipids but the two groups differed with regard to cholesterol concentration. cholesterol concentration decreased in PFC group whereas there was no change in TD group. Among the phospholipids plasmalogens and ethanolaminephosphoglycerides (EPG) were found to decrease significantly in TD group. Spinal cord showed similar changes but the decrease in plasmalogens and EPG were significant even in PFC group. An additional decrease in the concentration of choline phosphoglycerides (CPG) in both PFC and TD groups was observed in the spinal cord. Thus both the thiamine deficiency and calorie restriction were found to have more adverse effects on the lipid composition of spinal cord as compared to brain. Similar observations have been made previously in this laboratory with regard to the effect of maternal protein deficiency on lipid concentrations in spinal cord.

Thiamine deficiency was found to have no significant effects on the lipid composition of gray matter. But the white matter is found to be affected similar to the brain and spinal cord. However, the deficits observed in galactolipids and plasmalogens in TD group were significantly higher than in PFC group. Thus these studies suggest that calorie restriction or thiamine deficiency have more severe effects on myelin rich areas of the CNS. i.e. spinal cord and white matter. From these studies it can be concluded that the changes observed in TD group are partly due to thiamine deficiency and partly due to calorie restriction.

From the foregoing it is clear that white matter is more vulnerable to nutritional stress than gray matter. Since most of the lipids in white matter are contributed by myelin the question arises whether myelin formation is affected under-nutritional stress. It is known that the activity of 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP), a marker enzyme of myelin can provide a fair index of myelination. Studies were undertaken to investigate the variations with age and nutritional stress on the activity of this enzyme.

The specific activity of CNP was found to be 5-8 fold higher in white matter than in gray matter. The activity did not show any appreciable change with the age either in gray matter or in white matter. Neonatal undernutrition produced by maternal protein deficiency was associated with a significantly

reduced specific activity of this enzyme both in gray and white matter suggesting reduced myelination. However, thiamine deficiency and calorie restriction do not seem to have any effect on the activity of CNP in brain, spinal cord, gray matter and white matter.

As mentioned earlier severe undernutrition during neonatal period was found to reduce the concentrations of total phospholipids, EPG and CPG significantly in the white matter. Therefore studies were undertaken to find out the effects of severe neonatal undernutrition on the key enzymes involved in the synthesis of these lipids namely phosphoethanolamine (PET) and phosphocholine transferase activities (PCT) in gray and white matter microsomes.

The content of microsomes in the white matter was higher than that in the gray matter. The amounts of microsomes in both gray and white matter were not affected by neonatal undernutrition. The activities of phosphoethanolamine and phosphocholine transferases were significantly lowered with neonatal undernutrition in both gray and white matter. The lower activities of these enzymes in white matter are in agreement with lower concentrations of EPG and CPG. But lower activities of enzymes in gray matter without any change in the concentrations of EPG and CPG need explanation and can perhaps be accounted for by a slow turnover of these lipids. The data on the kinetic constants of these enzymes suggest that undernutrition might cause an alteration in the properties of these two enzymes.