CHAPTER VI

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Growth and development, which occurs generally during the early part of the life span in any given species of animal, is characterised by two distinguishable biochemical phenomena - (a) hyperplasia where there is a rapid formation and accumulation of new cells and (b) hypertrophy where deposition of various biochemicals in the newly formed cells occurs. It is generally believed that, by adulthood each cell attains a stable composition and much of the metabolic processes at this stage are diverted towards maintaining such a stable composition. After this adulthood, the process of aging becomes apparent.

The nature of aging process has been the subject of considerable speculation and several theories have been proposed to explain this phenomenon (Szilard, 1959; Orgel, 1963; Kanungo, 1975; Reiner, 1978; Sharma and Rothstein, 1980; Mekerrow, 1979; Harman, 1981). In general it is considered as a progressive accumulation of changes with time which are responsible for the increasing susceptibility to disease and death with advancing age. Since growth of any tissue could be followed by studying the changes in the levels of the macromolecules like DNA, RNA and protein, several researchers have examined the changes in these biochemicals in a variety of organs including brain, the organ which is likely to have a direct relation with the process of aging. However, these earlier studies were not complete enough to reach any meaningful conclusions on the subject. Some of these details have already been mentioned in Chapter I.
It is necessary that a full spectrum of changes in these macromolecules from ontogenesis to old age are to be examined if one wants to correlate the metabolism of these macromolecules with the process of aging.

Thus earlier studies from this laboratory (Shrivastaw and Subba Rao, 1975; Subba Rao and Shrivastaw, 1976) on the possible changes in the DNA, RNA, protein along with the deoxyribonucleases in different regions of chick brain have revealed that after reaching the plateau levels during adulthood, the DNA content increases significantly in the old age. It is also evident from these studies that the activity of acid DNase decreases significantly in the aging brain, whereas the alkaline DNase, although decreased remained still at a significant level in the old age. Similar type of results as far as the DNA contents are concerned have also been observed from a different laboratory, where it is shown that the DNA content increases significantly in the old chick brain (Vernadakis, 1973).

The results presented in Chapter III point out that the enhancement in DNA content of brain during the late stages of the life span occurs even in rat also. Specifically three phenomena have been noticed during these studies. Firstly, the region of white matter appears to exhibit growth and development, although at different rates, continuously up to old age. In contrast, the increase in grey matter and cerebellar region stops at 30 days. A steady increase in protein/DNA ratio from 7 days to 60 days in all the regions studied could probably be taken as cell size increase following earlier hyperplasia. Similar studies conducted earlier showed that the profile of changes in the wet wt of brain with aging varies in different
species and also depends on the regions studied. (Burger, 1957; Himwich, 1973; Howard, 1973; Somarjski and Rosten, 1973; Subba Rao and Shrivastaw, 1979). From these reports it is clear that the wet wt of brain in the case of humans, monkeys and dogs decreases with the onset of aging whereas in the case of mouse and rat, the wet wt either increased continuously with age or remained static during aging. Thus the present results which show a continuous increase in the wet wt of white matter is in good agreement with the above results. Some of the above mentioned studies where no difference in the wet wt of the brain during aging was noticed, may be because the changes in the total brain wts were examined rather than those of different individual regions.

Another feature of the present investigation is the DNA accretion found to be taking place predominantly at two stages in the life span of the rat brain. The first stage being the early developmental period and the second somewhere beyond the adult life and before 2 years of age. In all the regions studied (White matter, Grey matter and Cerebellum), the DNA content increased significantly between 225 and 750 days showing a second peak of cell proliferation. It is to be noted that, among the regions studied, the white matter and cerebellar regions appear to be characterised by pronounced cell proliferation during this period as indicated by simultaneous DNA increments with a marked decrease in the protein/DNA ratio (Tables 10, 11 & 13). Working on the same subject Caron and Unsworth (1978), Wintzerith et al., (1978), and Porta et al., (1980) have also shown significant rise in the DNA content in the old brain in the case of mouse and rat. Thus, the DNA content in mouse cerebellum increased
from 0.031 mg at birth to an adult level of 0.235 mg at 18 days of age. After slight variation during adulthood, the DNA content rose sharply in 23 months old animals to 0.35 mg (Caron and Unsworth, 1978). Similarly, the content of DNA and RNA in various parts of the adult brain (3 month old) and old brain (30 month old) were also measured in male wistar rats (Wintzerith et al., 1978). The amount of RNA rose in cerebellum (+22%) and brain stem (+18%) of old rats, whereas the content of DNA rose in cerebrum (+24%), cerebellum (+22%), brain stem (+26%) and whole brain (+23%) of old animals, as compared to the corresponding parts of the younger rats. Therefore it appears that the rise in DNA content in the case of aging mouse and rat brain seems to be a consistently observed phenomenon.

If it is accepted that the DNA content of a diploid cell is constant, the increase in DNA amount in old brain region must reflect formation of new cells. In such a case the question arises as to which type of cells are undergoing replication at this stage of the life span. Morphological and biochemical studies conducted in this laboratory as well in other laboratories do point out that probably it is the glial cells that are proliferating in the old age (Brizzee, 1973; Vernadakis, 1973; Altman, 1969; Subba Rao and Shrivastaw, 1979). The present results concerning the continued accretion of DNA in white matter with age adduce support for the glial cell proliferation throughout the life span. The third phenomenon that became apparent in the present studies is the positive correlation between the activities of acid and alkaline DNases and the accretion of the DNA content in all the regions studied. Both these enzymes, in particular acid DNase, show high activity during early stages of development, that is
at a time when DNA synthesis must also be occurring at a high rate (Giuffrida et al., 1970; Brasel et al., 1970; Bakshi and Kumar, 1978). Acid DNase activity decreases with the age up to 225 days, whereas alkaline DNase showed maximum activity around 30 days and then falls up to 225 days. However, a significant increase in the activity (both specific as well as total activity) in all the regions was observed between 225 and 750 days, a period where a significant accumulation of DNA was also observed.

Sung (1968) has reported the presence of two deoxyribonucleases (acid and alkaline) in rat brain. From his studies, it was clear that the cerebellum from adult rat has a lower acid DNase activity and higher alkaline DNase activity and therefore has a higher ratio of alkaline DNase/acid DNase. It was postulated, since cerebellar region is known to contain higher concentration of DNA than the other areas of the brain (Heller and Elliot, 1954; May and Grevelle, 1959), there may be some relation between the high concentration of DNA and higher activity of alkaline DNase in cerebellum. Similar type of results were also observed by later workers (Chanda et al., 1975). Our results on acid and alkaline DNase in different regions of rat brain also substantiate the above concept in that alkaline DNase may have a major role in the matured or aged rat brain. Although DNases are supposed to be degradative in nature, it has been proposed by several researchers that they may have a role in actual DNA synthesis. Thus Lehman (1967), Laskowiski (1967) and Bernardi (1971) have postulated that DNases enhance the DNA synthesis by providing required nicks, and thus play an important role in the process. Examining the activity of DNase II (acid DNase) in relation to cell cycle in synchronised HeLa S3 cells Slór et al., (1973) have shown that DNase II exhibits 2 to
7 fold increase in activity at those times when DNA synthesis is taking place. It has also been noticed that the peaks of DNase II activity coincide with the peaks of DNA synthesis and the increase in the activity of DNase II is not due to the activation of the already existing molecules, but due to the formation of new molecules. Similarly Stambolova et al., (1973) have found correlation of DNase activity with DNA polymerase in different cell fractions of rat brain. From all this, along with the present findings it does appear that DNases play an important role in DNA synthesis and/or repair.

The results presented in Chapter IV show the effect of early postnatal nutritional deprivation on DNA, RNA, protein and DNases, in different regions of rat brain during suckling period. Firstly, there is a marked difference between the regions in their susceptibility to nutritional deprivation. Thus, while grey matter is not affected by postnatal nutritional deprivation white matter appears to be quite vulnerable under the conditions. Cerebellum is intermediary between grey and white matter regions in its response to imposed caloric restriction (Tables 14 & 21). In respect to the various biochemical parameters studied, viz., DNA, RNA and protein content it is in the white matter that significant and marked reductions were noticed. Cerebellar region, although not affected up to the extent as that of white matter, showed deficits in the above mentioned biochemical parameters. However, grey matter was found to be unaffected by early postnatal nutritional deprivation. The present results also show that timely rehabilitation for a sufficient period can correct these deficits in cerebellum but not in white matter region, (Tables 15-17 & 21, 22).
The differential behaviour of these regions, white matter, grey matter and cerebellum, may be due to the difference in the cell types that the regions are made up of and also the growth schedule of these particular cell types. White matter is largely composed of oligodendrocytes and astrocytes along with the axonal processes, whereas grey matter can be considered as a region largely made up of neurons and some astrocytes. The cerebellar region of brain is known to contain a variety of neuronal cells and astrocytes. In rat brain vigorous cell proliferation proceeds during early postnatal period and accounts for almost 50% and 90% of final cell number in the forebrain and the cerebellum (Altman, 1969; 1972 a,c) respectively. Thus the period of extensive cell proliferation is quite limited and the process is completed in about 3 weeks after birth in the rat and 1.5 to 2 years in man (Dobbing and Sands, 1973). Predominantly it is the glial cells that are formed in the forebrain during postnatal period (Altman, 1969). As far as the cerebellum is concerned the nerve cells in this particular region continue to replicate even after birth in rat (Altman, 1972 a,b,c). It is interesting to note from the studies of Patel et al. (1973) that prenatal undernutrition has no effect on the acquisition of cells in fetal rat brain, while undernutrition during suckling period results in reduced DNA content in brain. Rehabilitation of these undernourished animals rectifies the cell number up to some extent and full recovery was not possible when the rats are rehabilitated from 21st day. Other studies, in which different modes of undernutrition were employed, rehabilitation for a long period after weaning could not bring back the deficits to normalcy (Howard and Granoff, 1968; Dobbing et al., 1971 reviews: Dobbing, 1974, Winick, 1976). Thus, in all these investigations it has become clear that
brain is affected by early postnatal undernutrition and rehabilitation after 21 days in case of rat could not correct the deficits. The present results, along with the earlier investigations mentioned above, are taken to indicate the proliferative nature of cells in white matter during early postnatal weeks thus making this region most vulnerable to nutritional restriction. It is pertinent to mention here that recently Reddy et al., (1982) have also reported similar findings. Grey matter was unaffected by undernutrition because of the post-mitotic stage that a large number of cells in this region have reached by birth itself. Cerebellum, on the other hand, is intermediary in its behaviour since some significant replicative activity is still going on in this region during the first two postnatal weeks. The lack of significant effect of prenatal undernutrition (Patel et al., 1973) although fetal brain cells are actively undergoing division at this stage, may be due to the protective nature of the maternal nutritional resources.

Several recent studies on the same subject on the individual fractions also revealed that glial cells are the cells most vulnerable during undernutrition (Hamberger et al., 1975; Pasquini et al., 1981; Giuffrida et al., 1980). Since oligodendroglial cells are responsible for the myelin synthesis the present results also substantiate the decreased synthesis and content of myelin observed in a variety of earlier studies (Benton et al., 1966; Chase et al., 1967; Fishman et al., 1971; Nakashl et al., 1975; Krigman and Hogman, 1976; Wiggins et al., 1976; Klm and Pleasure, 1978; Reddy and Horrocks, 1982; Reddy and Sastry, 1978).

It is rather intriguing the way in which the acid and alkaline DNases are maintained in different regions of undernourished rat brain
(Tables 18-20 & 23,24). It appears that alkaline DNase is the enzyme which retains its activity at a high level, in spite of the limited energy and protein available under these conditions. Rehabilitation of these under-nourished rats up to 150 days resulted in higher amounts of these enzymes as compared to the age matched controls. Thus, the two DNases are synthesized in a preferential manner during rehabilitation and must be playing important role in DNA metabolism in adult and aging brain.

The increased DNA in aging brain, so pointedly indicated by the results in Chapter III could be due to any one or more of the following events: (a) replication of glial cells (b) repair of DNA in both neurons as well as glial cells (c) an increase in the intracellular DNA not necessarily connected with the cell proliferation. In all these cases one must be able to see an increase in such enzymes connected with replication and/or repair of DNA. Indeed the acid and alkaline DNases, presumably participating in the DNA repair and replication processes, have been found to increase in the present studies. In addition the findings presented in Chapter V further show that DNA polymerase activity also shows a second peak of activity around 540 days. It is also clear that, in all the brain regions studied, the predominant DNA-polymerase present, after 7 days of postnatal age, is the polymerase B type.

Cogent evidence has already accumulated to show that mammalian cells have at least three different types of DNA-polymerases; α, β and γ, the last one being present both in mitochondria and nucleus while the first two in nuclear fraction and can be released into cytosol fraction
(Weissbach et al., 1975) under appropriate conditions. Enough experimentation exists today to indicate that α polymerase responds to variations in the rate of DNA synthesis (Falaschi and Spadarò, 1978; DePamphilis and Wassarman, 1980; Kornberg, 1980) while polymerase β is considered to be essentially a repair enzyme (Waser et al., 1979; Kornberg, 1980). In such an event, the present results, showing a second bout of polymerase β activity in aging brain, bring forth a pertinent question whether β-polymerase is also involved with replication process at least during the later stages of life span when the levels of α-polymerase are at an insignificant level. Indeed, there appears to exist a role for DNA-polymerase β in DNA replication.

Thus, Weissbach and other workers have shown increasing levels of β and γ polymerases during replication (Weissbach, 1977; de Recondo and Abadieedebat, 1976; Wang and Popencoe, 1977). Also, Butt et al. (1978) found that when DNA polymerase α was selectively extracted out with 0.2 M KCl in 5 phase L cells (where 20% of β-polymerase is also extracted out) the DNA synthesis was inhibited only by 40% in the isolated nuclei. Subsequent removal of 70% β-polymerase lead to 80% inhibition of DNA replication. These results are suggestive of the fact that both α and β polymerases are involved in a concerted manner in the DNA replication.

Recent studies from different laboratories have shown extra DNA content in neurons of rat brain (Bohm et al., 1981; Heizmann et al., 1981). Waser et al. (1979) have also shown that β-polymerase is the only enzyme present in the adult neurons which would take care of the DNA repair process in neurons. Thus it appears that whatever DNA that was
observed in neurons might have been synthesized by β polymerase. It is possible that β-polymerase in its capacity as repair enzyme also could cause increments in DNA content, as shown by Mosbaugh and Linn (1982) that even in the repair process, strand displacement DNA synthesis occurs which may account for the extra DNA amounts found in the individual cells. It is pertinent to note that Mosbaugh and Linn (1983) have also shown that using the gaps produced by the action of HeLa DNase V, larger fragments of DNA are synthesized by HeLa DNA polymerase β by nick translation. Moreover, the HeLa DNase V stimulated both extent and rate of DNA synthesis by β-polymerase.

Be that as it may, the findings of present investigation clearly demonstrate an increase in DNA polymerase β and the two DNases in aging brain which in all probability related to the observed rise in DNA content in the brain at the same time. The exact molecular relationship between these three events is a challenge to the future workers in this field.