DNA METABOLISM IN DIFFERENT REGIONS OF DEVELOPING AND AGING RAT BRAIN

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By

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This is to certify that I, **K.V. Subba Rao**, have carried out the research work embodied in the present thesis under the guidance of **Prof. Kalluri Subba Rao** for the full period prescribed under Ph.D. Ordinances of the University.

I declare to the best of my knowledge that no part of this thesis was earlier submitted for the award of research degree of any University.

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K.V. SUBBA RAO

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CHAPTER I

GENERAL INTRODUCTION

The process of aging is one of the most common and unavoidable phenomenon in all living beings. A major issue in the field of gerontology is to relate the changes in behavioural, physiological and biochemical properties with aging. Though, at present, many theories of aging exist (Szilard, 1959; Orgel, 1963; Kanungo, 1975; Reiner, 1978; Sharma and Rothstein, 1980; Mckerrow, 1979; Harman, 1981), much of the emphasis has been to understand the process of aging on the basis of cell loss, cell alterations or both. In general, the cell population of mammalian system in adults has been classified into 3 categories depending on their mitotic activity and their life span (Goldstein, 1971). The three groups include continuous, intermittent and non-mitotic or post-mitotic cells. Since DNA, RNA and protein are the fundamental macromolecules of any cell, it has been proposed that accumulation of errors in these macromolecules may be the main source of biological aging (Orgel, 1963; Strehler and Borrows, 1970; Hahn, 1971; Strehler et al, 1971; Gensler and Bernstein, 1981). However, some results opposing this concept have also been reported (Yagil, 1976; Evance, 1976; Pendergrass et al, 1976; Kanungo, 1979; Sharma and Rothstein, 1980).

There are highly significant differences in the maximum life span of mammalian species, ranging from 2 to 4 years for rodents and 100 years for man. Despite the wide range of life span among the species, it seems that different species may age by similar mechanism. In order to understand

the factors responsible for the similarities and differences in longevity, various organs have been studied carefully and correlated with the maximum life span. It has been established that there are significant correlations among the life span, brain wt and metabolic rate (Sacher, 1959; 1973; Holliday, et al.,1967; Timiras, 1972). Although aging may be a generalized phenomenon throughout the mammalian body, attention has frequently been focussed on such controlling and integrative organs like 'brain' and 'endocrines' in which age dependent changes may have widespread consequences throughout the body. Due to the significant correlation between the brain wt and the life span it has been proposed that the brain may play an important 'pacemaker' role in aging or it may be an 'organ of longevity' due to its unique role in adaptation to the external environment and its homeostatic integration of all the organs of the body (Sacher, 1973).

Although the actual work on the brain has started a century ago, much of the early attention has been paid to anatomical, morphological and histological studies. The real impetus to the subject of brain biochemistry has come only during the last two decades. Studies by several workers have shown that there are various stages in the development of the brain (McIlwain, 1959; Himwich, 1962; Davison and Dobbing, 1968). Davison and Dobbing have proposed the following sequence for the development of the brain:

(i) embryogenesis with subsequent differentiation of the brain and the appearance of neuronal and glial cells.

(ii) axonal and dendritic growth and initiation of myelination.

(iii) enlargement of cells, continued maturation, and increase in brain size.

(iv) the matured brain without much increase in size.

(v) senile degeneration of the brain.

Discussing the same subject Winick (1971) has proposed the following stages about the brain development:

(a) rapid cellular proliferation (hyperplasia)

(b) cellular proliferation with simultaneous deposition of biochemicals in the already formed cells (hyperplasia & hypertrophy).

(c) little cell proliferation with cell size increase (hypertrophy).

During these studies it also became evident that certain biochemical parameters like DNA, RNA and protein can be used as indices for the cell proliferation and cell size increase. It is well known that the DNA content for a diploid cell is constant in any tissue of particular species (Leslie, 1955), Gray and Deluca, 1956). Thus the total DNA content in any tissue of any animal gives a measure of the total number of cells. It is generally agreed that majority of the brain cells are diploid, although a small percentage of them are reported to exhibit polyploidy (Viola, 1963). Several workers have shown that the DNA content of brain cells is constant and shown to be 6.1 to 7.1 pg per cell (Heller and Elliott, 1954; Kissane and Robins, 1958; Mandel et al, 1948). Thus by measuring the DNA content, it would be possible to follow the cell proliferation in any tissue. Since the dry wt of the tissue is mainly due to protein and RNA, the levels of these chemicals in any tissue could be taken to represent the cell size (Pope et al, 1964).

BIOCHEMICAL CHANGES DURING BRAIN DEVELOPMENT

The most important index of brain development is the wet weight. Studies by Dobbing and Sands (1970, 1973) on brain development of different species

have revealed that the wet weight of brain increases before, after or around the birth, depending on the species. From these studies, it is clear now that the rapid growth of the brain in the case of rat is largely postnatal, while in the case of guineapig it is essentially prenatal. However, high velocity of brain development in pig and human is both before and after birth. In general brain wt reaches its maximum before body wt and the period of maximal rate of wet weight increase takes place at various time periods in different regions of the nervous system.

Changes in nucleic acids

As already mentioned above, the levels of DNA and RNA contents in any tissue represent the cell number and cell maturation in that particular tissue. Mandel and Coworkers (1948, 1964) have shown that the levels of nucleic acids in the developing brain varies with different animals. In the guineapig brain, the RNA content increases up to 10th day postnatal, while the DNA remains constant from birth and unaltered throughout adulthood. In the rat, the nucleic acids increase up to 10th-15th day postnatal and subsequently remain constant. In the rabbit brain, the nucleic acids increase rapidly for 30 days after birth; then they increase slowly until 90th day, at which time the DNA and RNA reach adult levels. The changes in DNA and RNA in chick brain were studied by Leslie and Davidson (1951) where they have shown that the DNA concentration decreases from 8th day incubation to 2 days after hatching, whereas the RNA content fluctuates during the same time. Margolis (1969) followed the changes in DNA content in various regions of chick brain starting from 11 days of embryonic stage to 6 weeks after hatching and showed a decrease in DNA concentration with age in

all the regions studied except in the cerebellum where maximum DNA content was observed just before hatching. Studies by Zamenhof and Van Marthens (1971) on chick brain DNA content revealed marginal DNA increments starting from 12th day embryonic to 40 days old post-hatching.

However, in many of the experiments carried out on the same subject, the most well studied animal was the rat. Thus Keup (1957) has found that the DNA content in rat cerebral cortex was higher as compared to RNA content during prenatal period. However, this relation was reversed shortly after birth and the RNA/DNA ratio increased in whole brain with age. Adams (1966) has also shown an increase in total DNA content, nuclear and transfer RNAs in rat brain from birth up to 18th day postnatal and then the levels of nucleic acids remained relatively constant throughout the adulthood. A systematic study concerning the changes in nucleic acids as well as protein in different regions of developing rat brain was conducted by Fish and Winick (1969). When the wet wt alone was measured little difference was seen between different regions. However, when the cell number (on the basis of DNA) and cell size (Protein/DNA ratio) were checked, striking differences were found. Thus, in cerebellum the DNA content increased 8.5 fold between 6 and 17 days and subsequently tappered off. The protein to DNA ratio declined during the same period. Although the increase in the cell number in the cerebrum was only 2.8 fold between 6 and 17 days it continued until 21 days, at which time the experiments were terminated. After 10 days postnatal there was also an increase in the protein to DNA ratio. In the brain stem, there was a moderate increase in cell number between 6 and 14 days. Either the whole brain or in different regions, the

RNA content increased up to 14 days postnatal and then the values plateaued off. Many recent studies on the same subject have also revealed similar pattern of DNA and RNA levels in developing rat brain (Mori et al, 1970, Seiler and Lamberty, 1975; Weichsel and Davison, 1976; Griffin et al, 1977).

Studies on human brain nucleic acids have revealed that both the DNA and RNA contents increase rapidly before birth and continue to increase slowly up to 2 years (Howard, et al, 1969; Dobbing and Sands, 1970). However, studies conducted by Dobbing and Sands (1970) on human brain point out high levels of DNA content in two major periods, first period lasting up to 18 weeks of gestation - a period which corresponds to neuroblast proliferation. The second phase begins around 25th week of gestation and continues during postnatal life representing glial multiplication. Similar biphasic cellular proliferation in human brain was later reported by Subba Rao and Janardana Sarma (1976).

Several workers have simultaneously studied various enzymes involved in DNA metabolism during the brain development. Three types of DNA polymerases were reported in developing rat brain by Bharucha and Murthy (1971), a soluble enzyme in the nuclear extract, an insoluble enzyme bound to the nuclear residue, and a soluble cytoplasmic DNA polymerase. The activity of DNA polymerase was maximum at birth and rapidly decreased by 6 weeks and remained at a very low level even by 12 weeks of age in rat. Studies by other workers on thymidine incorporation into rat brain DNA (Sung, 1969; Mori et al, 1970) have shown that the uptake of ^{14}C or ^{3}H-thymidine was maximum in early stages and decreased to a very low

level in adult. It is also clear from these studies that cerebellum incorporates thymidine 20 times more as compared to the cerebral cortex in developing rat brain (Sung, 1969). Other studies on thymidine incorporation have also revealed similar results (Smart, 1961; Winick, 1965; Altman, 1967; Burdman, 1972; Bakshi and Kumar, 1978). Chiu and Sung (1973) reported two types of DNA polymerases (i) cytoplasmic (ii) nuclear in rat brain. It has also been shown by these workers that the cytoplasmic DNA polymerase was high as compared to nuclear DNA polymerase in young rat brain and vice versa was the case in the adult rat brain. These studies were supported subsequently by Stambolova et al, (1973, 1974), where they studied the DNA polymerase activities in different types of cell fractions (neurons, astrocytes and oligodendroglia). It was apparent from these studies that the two forms of DNA polymerase (A and B as earlier reported by Chiu and Sung, 1973) are present in all types of cells from both 10 day old and adult rats. Both the DNA polymerases A and B are found in the so called soluble fraction and also in particulate fraction. The larger part of the DNA polymerase activity of all nuclear fractions, with the exception of oligodendrocytes from infant rats, was due to the action of enzyme B. However, the DNA polymerase activity in soluble fraction (form A) is maximum in oligodendrocytes as compared to neurons and astrocytes. This high level of DNA polymerase A in oligodendroglial cells enable the incorporation of ^{3}H-thymidine in vivo, 3 times more as compared to the other fractions (Stambolova et al, 1973; 1974). Similar type of results were obtained by Inoue et al.,(1976) in the case of guineapig. The activity of DNA polymerase and also other enzymes like, thymidine kinase and thymidylate synthetase which are also involved in DNA synthesis, were found to be high, at the

time when maximum DNA synthesis was going on in rat brain (Chiu and Sung, 1970; Brasel et al, 1970; Mori et al, 1970; Giuffrida et al, 1970; Yamagami et al, 1972; Millard, 1972; Weichsel, 1974, Patel et al, 1977; Bakshi and Kumar, 1978).

All these studies concerning various enzyme activities responsible for biosynthesis of DNA, thus revealed that these activities are at their highest during very early development of brain - at a time when rapid accretion of DNA is also found to occur. It should be also noted that generally cerebellum shows higher activity during postnatal period. One other type of enzyme relevant to DNA metabolism but whose physiological role remains obscure is the deoxyribonuclease. The first report on the presence of two deoxyribonucleases from rat brain was by Sung (1968), where it was shown that the first type of DNase "Acid DNase" has a pH optimum of 5, hydrolyzes native DNA better compared to denatured DNA, and has a high requirement for Mg^{+2}. The second one, "alkaline DNase" has its pH optimum between 7.4 to 8.9, prefers denatured DNA as a substrate and requires low Mg^{+2} concentration. During these studies, it was obvious that both acid and alkaline DNases were present in equal proportions in young brain, i.e., in 2 day old rat and the ratio of alkaline DNase to acid DNase increased with the age showing higher levels of alkaline DNase in every region of the brain. Later studies by Chanda et al (1975) on deoxyribonucleases also revealed similar pattern of these enzyme levels in young and adult rat cerebellum. The presence of acid DNase in human brain was first reported by Subba Rao (1973) where acid DNase exhibited high activity during the early gestation period and decreased continuously with the age and remains at a low level by birth.

Studies on deoxyribonucleases along with the DNA polymerases by Stambolova et al., (1973) in different types of cell fractions (neurons, astrocytes and oligodendroglia) revealed a general correspondence in the pattern of the relative activities of nucleases in all cell fractions with the DNA polymerase. Thus all these studies mentioned above have shown high levels of DNases at a time when rapid DNA synthesis is occurring in the brain tissue.

Proteins

In developing brain protein increases correspondingly with the wet weight. The ratio of protein to DNA indicates cell size and the ratio is found to be increasing during development. The time at which the protein level reaches the maximum varies in different animals - in rat brain 99 days, mouse 80-90 days, in the case of guineapig 55 to 76 days and in man by 2 years. The levels of protein in rat brain was determined by Simons and Johnston (1976) from 21st day to 120th day of postnatal life and it is found that the increase in the total protein content in cerebellum and the whole brain continues up to 60 days of age. The total protein content in whole brain as well as in different regions of rat brain was also determined by Fish and Winick (1969). It is clear from these studies that a continuous increase in total protein content from 6 days to 21 days in whole brain as well as in cerebrum and brain stem occurs. The increase in total protein content in both cerebellum and hippocampus showed a different pattern exhibiting saturating levels of protein at 14 and 17 days postnatal respectively. Later studies by others on the protein content in rat brain have also showed similar pattern in the protein increase with the age (Gaitonde et al, 1978; Kisler, 1979).

UNDERNUTRITION

Although the chemical aspects of the brain, both in developing and adult stages, became the subject of research during the last two decades, much of the attention has been diverted to see the possible effects of various environmental factors, particularly early malnutrition and undernutrition on brain development and function. Since malnutrition is a prevalent phenomenon in vast areas of the world, it has become important to know as to what happens to the brain of malnourished infant or child so that the chances for proper rehabilitation of previously malnourished child could be ascertained. Various investigators started working on this interesting and important problem and the detailed picture of the effects of undernutrition of the brain development has first come from Dobbing's group (1971, 1977). It is clear from these studies that the rate of development and maturation of the central nervous system varies considerably from one species to other. Thus it has been shown that the development and maturation in the case of rat is postnatal, prenatal in the case of guineapig, while it is perinatal in the case of human beings. On the basis of these results Dobbing has proposed the hypothesis of the "Critical growth period" or "Growth spurt period" at which time the brain growth is fastest and is also the most vulnerable period and any restriction imposed on the brain during this period leads to permanent deficits in brain development. This concept has been substantiated not only by the data obtained from Dobbing's laboratory but also by the results simultaneously obtained independently from other laboratories (Winick, 1969; Chase, 1973).

Body wt and Brain wt

In rat, brain reaches about 80% of its adult weight by 4 weeks of age, while the same percentage of adult brain wt is reached in pig by 8-10 weeks. Recent studies by Dodge et al.,(1975) have shown that nutritional deprivation during the critical periods of brain growth results in irreversible reduction in the brain wt. Cerebellum is the most affected region of the brain.

DNA, RNA, Protein and Lipids

Since the DNA content within any diploid cell in a given species is constant, the amount of DNA in any organ could be taken as a reflection of the cells in that organ, and the protein/DNA ratio could be taken as an index for the cell size increase. Therefore, nutritionally deprived animals have been examined in different laboratories. Winick and Noble (1966) have shown that nutritional restriction during critical growth period interferes with the cell division and this deficit was permanent and irreversible inspite of subsequent proper rehabilitation. However, at the same time, the effect of nutritional deprivation is minimum in the case of adult rats, affecting only to a very small extent the DNA and cholesterol contents. Proper rehabilitation of these animals brought back the deficits to normalcy. At the same period, two other groups (Culley and Lineberger, 1968; Guthrie and Brown, 1968) have shown reduced levels of brain wt, DNA content and cholesterol in undernourished rats during the preweaning period. A systematic study by Culley and Lineberger (1968) on body wt, brain wt, total DNA, RNA, protein and lipids in different regions of rat brain exposed to nutritional restriction for different periods (5,11,17 and 60 days) revealed that the

nutritional deprivation caused irreversible deficits in these biochemical parameters and of the three regions studied, cerebellar region was found to be the most affected region. Zamenhof et al.,(1972) studied the effect of prenatal undernutrition on DNA and protein contents in rat brain. It was noticed that the nutritional insult suffered by females was sustained over one or two generations and exposed in the off-springs. A variety of studies carried out in recent years on the same subject not only confirmed the earlier observations but also extended to several other species like pig, guineapig and monkeys (Ghittoni and Faryna, 1972; Patel et al, 1973; Kerr and Helmuth, 1973). Studies on myelin specific lipids and myelin content (Benton et al, 1966; Fishman et al, 1971; Davison and Dobbing, 1966) during the critical growth period, have revealed that the nutritional deprivation caused decrements in the levels of myelin content and myelin specific lipids. However, the quality of myelin was not affected by undernutrition. These studies on myelin and myelin specific lipids in undernourished rats was supported later by other workers (Krigman and Hogan, 1976; Wiggins et al, 1976; Reddy and Sastry, 1978; Reddy et al., 1979)

Though many studies have thus indicated that nutritional deprivation after the 'critical growth period' of brain development does not produce permanent deficits in the brain, a few workers did find some irreversible biochemical deficits even if nutritional restriction was imposed on adult animals. Studies by Graystone and Cheek (1969), and Mehta and Chakravarty (1973) on rat brain, revealed calorie or protein restriction from 3 weeks to 7 weeks causes marginal deficits in DNA and RNA contents. On the other hand not all workers have found undernutrition during preweaning

period to affect the brain size and its biochemical composition. Thus, Rajalakshmi et al.,(1967) did not find any changes in the rat brain size or in the DNA, RNA and protein contents of the brain, although the animals were exposed to undernutrition during suckling period. Benton et al.,(1966) found that the biochemical deficits produced in brain as a result of preweaning dietary restriction could be overcome by rehabilitation from 3 weeks to 6 weeks of age. Cheek et al.,(1976) working with rhesus monkey have shown that protein calorie or protein deprivation almost throughout the gestation period did not result in any significant change in the protein, DNA, RNA, cholesterol, phospholipid and water contents of the fetal brain. Indeed this observation was in sharp contrast to the numerous earlier findings and it was argued that the difference observed after maternal restriction of protein and/or energy in sub primate mammals are not necessarily applicable to man. However, recent studies from different laboratories have substantiated the fact that undernutrition in early life causes a reduction in DNA and RNA content (Winick, 1976; Griffin et al, 1977), cell size and number (Patel, et al, 1973) a delay of myelination (Krigman and Hogan, 1976; Wiggins et al, 1976; Reddy et al., 1979).

A series of painstaking studies by Winick and his Coworkers (1968-1970, 1972) on human brain revealed that children dying of severe Marasmus had reduced number of cells (as indicated by DNA content) in different regions of the brain as compared to the brains of children of the same age who died due to non neurological reasons. In these cases, the cell size (as indicated by protein/DNA) was also reduced. However, children dying due to Kwashiorkor did not show any significant decrease in the DNA content, but had smaller cell size. Data from other laboratories also suggested that in severe

protein/calorie malnutrition DNA is reduced in cerebrum and cerebellum (Dodge et al, 1975). Later studies on human brain by Subba Rao and Janardana Sarma (1976) revealed significant reduction in the weights of cerebellum and brainstem on percentage basis in small for date infants. In these studies, cerebellar DNA was unaffected but slight lowering of cell number (15%) in cerebrum was noticed. The cell size was unaltered. The concentration of various constituents, viz., proteins, lipids of different classes, total lipids, studied in different regions of brains of 'small for date' infants remained unaffected. These studies along with the recent study by the same authors, Janardana Sarma et al., (1983) indicate that the brain biochemistry of small for date infants does not seem to be affected drastically and the changes observed could probably have been corrected by suitable rehabilitation.

Very few studies were conducted on the possible changes in the various enzymes involved in DNA metabolism during undernutrition. Thus, studies by Bakshi and Kumar (1979) have shown that the activity of DNA polymerase was affected by preweaning undernutrition and rehabilitation of these undernourished rats from 21 days to 110 days could not bring back the activity of DNA polymerase to normalcy. It has also been shown by these workers that the rate of thymidine incorporation into rat brains of undernourished animals was reduced. Studies from this laboratory by Subba Rao et al.,(1980) on DNA, RNA and protein along with the deoxyribonucleases in undernourished animals revealed that early nutritional deprivation resulted in lesser amounts of DNA but the concentration of DNA was unaffected. DNA, RNA and protein values in the brains of those rats undernourished

from birth to 14 days but subsequently rehabilitated up to 130 days, exhibited complete recovery. Both specific activity as well as the total activity of acid and alkaline DNases showed a significant reduction in 14 days undernourished brain. However, with continued nutritional restriction up to 24 days the specific activity of these enzymes returned to normal, although the total activities were still in deficit.

BIOCHEMICAL CHANGES DURING AGING

Although there is no evidence to relate specific molecular changes directly to aging process, it is believed that biochemical or morphological alterations in the nervous system may have some role in the process of overall aging. Thus many workers have attempted to find out the possible biochemical parameters in different species during aging. The early work of Burger (1957) on human brain showed that while many lipid constituents like cerebrosides, phosphatides and total sterols, total nitrogen and phosphorus content are unaltered or only slightly decreased with the age, a significant decrease was observed in brain wt, oxygen consumption and sulphur content during aging. Discussing the changes in the brain wt with aging, Himwich (1973) pointed out that in humans and dogs there was a decrease of 10-13% in aged brain as compared to the matured animals. However, no significant differences in the brain wt were observed in the case of rat and mouse as a result of aging (Howard, 1973). A systematic study by Somarjski and Rolsten (1973) on nucleic acids and brain wt in different species revealed that the brain wt attains a peak value by adult in the case of rhesus monkey and humans and declines with age, whereas the brain wt in the case of mouse continuously increases up to old age.

As far as the nucleic acids are concerned, the RNA content slightly decreases in relation to fresh wt between the late development and maturity with no change observed with the onset of aging in the mouse brain. Further, the concentration of DNA in mouse brain remains constant with increasing age. In the human brain the concentration of DNA in the frontal cortex decreases with the attainment of senescence. However, no change could be noticed in the region of caudate and putamen. In rhesus monkey, different regions showed different trends with a general tendency of no significant change in the concentration of DNA or RNA with aging.

Howard (1973) while studying the DNA content in rodent and monkey brains found that after reaching the adult level, DNA content in different regions of rat and mouse brain did not change at least up to 425 days of age. At the same period, several workers have studied the quantitative neuronal and glial populations in the cerebral hemispheres of different species. Thus Brizzee (1973) has reported that the glial packing density and the ratio of glia/neuron in the cortex of monkeys was significantly higher in aged animals than in young and adult animals. Previous studies by the same authors (Brizzee et al, 1968) on cell enumeration by three different techniques on rat brain neuronal and glial cells revealed that glia/neuron ratio increases significantly between ages 425 days and 750 days of rat, where glial cells have increased, neurons being constant throughout the age period studied. Johnson and Erner (1972) employing the ultrasonic cell separation technique found that the total glial population in the whole brain decreased from young adulthood to about middle age, then increased significantly in the old age. From these observations Brizzee (1973) concluded that the increase in glial

population and in the ratio of glial to neuron in the absence of any frank disease condition may be considered as a consistent phenomenon of aging in the cerebral cortex of rats and monkeys. A systematic study to find out biochemical changes during aging in chicken brain has come from the laboratory of Vernadakis (1973) where it is shown that there is a significant increase in the DNA content in different regions of chick brain even beyond the adult age. The author postulated that this increment in the DNA content in the old age may be due to newly forming glial cells. In these studies DNA was expressed as 'per g of tissue' and total DNA contents were not given. It is also clear from these studies that the butyrylcholinesterase activity shows marked increase in both cerebrum and cerebellum of chick brain beyond 20 months of age. Regarding the changes in the neurotransmitters during aging, the values were found to fluctuate and regional differences were also observed.

Recent studies from this laboratory (Shrivastaw and Subba Rao, 1975; Subba Rao and Shrivastaw, 1976) on chick brain have revealed high levels of DNA content in the embryonic stages in both cerebrum and cerebellum, along with high activities of acid and alkaline DNases. However, with the advancement of age the increase in DNA content was slower and once again marked increase in the DNA content was observed in the old chick brain (2 years). As far as the DNases are concerned, the specific activity of acid DNase markedly decreased with the age, alkaline DNase remaining at a significant level even in the old age. Later studies by the same authors (1979) on white and grey matter regions of chick brain have also revealed a similartrend of changes in DNA and DNases.

SCOPE OF THE PRESENT INVESTIGATION

It is evident from the literature reviewed above that a majority of the investigations to examine the biochemical changes in developing animal brain were conducted on rat. However, studies conducted so far were largely confined either to one macromolecule or the other but not all of them at the same time. Further, these studies were largely restricted either to the early developmental period of the brain or to the period of aging. For these regions, rat brain was chosen as the tissue of study in the present investigation so that the present results can usefully supplement the already existing information. From the review of the literature a need for studying the changes in all the major macromolecules viz., DNA, RNA and protein in different regions of rat brain starting from its early developmental stages through old age has become apparent. It is envisaged that while DNA would reflect on the cell number, RNA and protein contents together with that of DNA will be useful in assessing the process of hypertrophy or cell size increase, which in its turn will reflect on the general metabolic potential of the cell.

Recent findings from this laboratory indicated that acid DNase, an enzyme supposed to be involved in the catabolism of DNA shows very high activity during the very early stages of human as well as chick brain development, a time when rapid cellular proliferation (DNA synthesis) is also occurring in these tissues. Thus the possibility that this enzyme might be playing an important role, other than degrading DNA, is evident. It is also clear now that the central nervous system is made up of different types of cells, viz., neurons, astrocytes and oligodendroglial cells. The ratio of these cell

types in different regions in the CNS differ and the developmental schedules of these regions are also known to be different. Since white matter consists mainly of oligodendroglial cells with few fibrous astrocytes and grey matter is considered to be a region containing mainly neurons and some protoplasmic astrocytes (Clausen, 1969 ; Raine 1981), the changes in DNA, RNA and protein along with the acid and alkaline deoxyribonucleases were studied in different regions of brain at various stages of the life span of rat.

The results obtained revealed that the region of white matter, as opposed to grey, exhibited continued growth throughout the period studied (from 1 to 750 days of age). By various biochemical parameters such as DNA, RNA and protein content and the ratio of protein to DNA, it could clearly be seen that between the ages of 225 and 750 days there was a second bout of DNA accumulation in the regions examined. The putative DNA degrading enzymes, acid and alkaline deoxyribonucleases, show a positive correlation with the rapid DNA accretion noticed during developmental stages and again between 225 and 750 days of age. All the above mentioned parameters were also studied in another region, cerebellum, whose growth schedule is found to be different from the rest of the brain. These results are presented in Chapter III.

We have extended these studies to see the possible effects of early postnatal nutritional deprivation and subsequent rehabilitation on the growth of different regions, viz., white matter, grey matter and cerebellum, by studying the DNA, RNA and protein contents. Since, acid and alkaline DNases

have shown positive correlation with the DNA accumulation, the activities of these enzymes were also studied in the nutritionally deprived animals. In respect to the various parameters studied, white matter was found to be markedly vulnerable to undernutrition, but grey matter region was unaffected. However, the cerebellar region in terms of developmental schedule and response to the imposed calorie restriction, is intermediary between grey and white matter regions. It has also been observed that the earlier the initiation of nutritional rehabilitation, the better was the recovery. The specific activity of acid and alkaline DNases were not affected by nutritional deprivation, whereas the total activities were found to be affected significantly. Rehabilitation of the deprived groups up to 150 days resulted in higher amounts of these enzymes as compared to those of age matched controls. The results are presented in Chapter IV.

Since, the present results revealed a significant increase in the amounts of DNA in different regions of aging rat brain, DNA polymerase activity in white matter, grey matter and cerebellar regions of developing and aging rat brain has been studied. The enzyme exhibited its highest activity during the early developmental stages with a decline to a low adult value by 225 days of age. However, the activity once again increased between 225 and 540 days thus showing a second peak in the later part of the life span. Studies, with specific inhibitors like aphidicolin and ddTTP on the enzyme activity have indicated that this rise in the DNA polymerase activity in various regions studied was mainly due to an increase in the polymerase β type. The results are presented in Chapter V.

CHAPTER II

Materials and Methods

CHAPTER II

MATERIALS AND METHODS

Animals

Rats of specified age (Wistar strain) were obtained from the Animal house of Indian Drugs and Pharmaceuticals Limited (I.D.P.L.), Hyderabad, and also from the Animal house of this University. Rats were fed on Hindustan Lever (New Delhi) "Rat and Mice Feed", which is complete in all nutritional aspects.

Materials

Highly polymerized calf thymus DNA, yeast RNA, bovine serum albumin, 2',3'-cyclic AMP, unlabelled nucleotides dATP, dGTP, dCTP and dTTP were purchased from Sigma Chemical Company, St. Louis, MO, USA. (^{3}H-methyl) dTTP (Sp. act. 46 Ci/m mole) was purchased from the Radiochemical Centre, Amersham, England, 2',3'-dideoxythymidine triphosphate (ddTTP) was purchased from P.L. Biochemicals. Aphidicolin was a gift from Dr. A.H. Todd of I.C.I. Ltd., U.K. All the other chemicals used were of analytical grade.

Separation of White and Grey Matter Regions

Animals were sacrificed by decapitation at various stages of life. Cerebral hemispheres were carefully removed from the brain (i.e., Whole brain excluding brain stem, cerebellum and optic lobes). Starting from the dorsal side, the outer grey matter was gently scraped off with a scalpel until no more grey matter could be visualized. Then the underlying white

matter portion was collected separately. The whole separation was achieved with consistency after a few trail experiments. The separation was carried out at 0-4°C. Cerebellum was taken separately for the studies.

Preparation of Homogenates

White, grey and cerebellar regions of the rat brain were separately homogenized in 9 volumes of cold distilled water with Potter-Elvehjem type homogeniser. A portion of the homogenate was taken for the extraction of nucleic acids and the other was used for the assay of acid and alkaline DNases. For the assay of DNA polymerase, the tissues were homogenized in 0.5 M Tris-HCl buffer, pH 7.5 containing 0.1 mM β -mercaptoethanol, 1 mM $MgCl_2$, 0.1 mM EDTA, 5% glycerol, 1% Triton X-100 and 0.5 M KCl. After the homogenization the sample was kept at 0-4°C for 1 hr and centrifuged at 1,00,000 g for 1 hr. The clear supernatant thus obtained was used as the source of DNA polymerase.

Extraction of Nucleic Acids

The nucleic acids were extracted according to the procedure of Schmidt and Thannhauser (1945) slightly modified as per the suggestions of Munro (1966).

Two millilitres of 10% homogenate (w/v) was mixed with 2.5 ml of ice cold 10% TCA and centrifuged to remove acid soluble compounds. The sediment was washed once with 2.5 ml of ice cold TCA. The final sediment remaining after removal of the acid soluble compounds was extracted twice with 5 ml of 95% ethanol and the extract was removed by centrifugation.

An alcohol-ether (3:1) wash was given to the sediment to remove any traces of lipids. The lipid free pellet was suspended in 2 ml of 1 N KOH and incubated for 2 hours at 37°C. This incubation with 1 N KOH was sufficient to hydrolyse the brain RNA using rat brain. DNA and protein were then precipitated by addition of 0.4 ml of 6 N HCl and 2.6 ml of 5% TCA and allowed to stand in ice for 10 min and centrifuged. The supernatant fraction was collected separately to estimate RNA content. The sediment was suspended in 2.5 ml of 5% TCA and boiled at 90°C for 15 min with occasional shaking. The mixture was centrifuged and the supernatant was collected in a test tube. Now, the sediment was washed with 1.5 ml of 5% TCA and both the supernatants were taken for the estimation of DNA.

Estimation of DNA and RNA

DNA was estimated by the diphenylamine method while RNA was estimated by the orcinol reaction (Burton, 1956; Schneider, 1957) . The DNA and RNA were also estimated by measuring the U.V. absorption of the acid soluble fraction at 260 nm.

Preparation of standards

DNA and RNA were first dissolved in water at a concentration of 2 mg/ml. Then a portion of this solution was diluted with 5% TCA and heated for 15 min at 90°C. The volume of the solutions so obtained was made up with 5% TCA in such a way that the final concentration became 200 µg/ml.

Estimation of Protein

Protein was estimated either by Biuret Method (Gornall et al, 1949) or as per the method of Lowry et al.,(1951), depending upon the concentration of the protein in the sample.

Estimation of Phosphorus

Phosphorus in the standard DNA sample was measured by the method described by Bartlett (1959).

Assay Procedure for DNases

Acid and alkaline DNases were assayed essentially as described by McDonald (1955).

Acid DNase

The 3.0 ml reaction mixture consisted of 2 mg of DNA in 1 ml of water (highly polymerized, calf thymus), 0.1 M sodium acetate buffer, pH 5.1 and brain homogenate. At the end of 2 hr incubation at 37°C in a Dubnoff metabolic shaker water bath, the reaction was terminated by adding 2 ml of 1.4 N perchloric acid followed by chilling. The whole mixture was filtered through Whatman No. 42 filter paper and the absorbance of the filtrate (acid soluble deoxynucleotides) was measured in spectrophotometer at 260 nm against an appropriate blank. The enzyme activity was also followed by estimating the acid soluble deoxyribose by the diphenylamine colour reaction according to the procedure of Burton (1956). It was found that both these methods agreed well and therefore routine assay procedure consisted of spectrophotometer measurements with occasional cross checking with the colorimetric procedure.

The enzyme activity was expressed as μg of acid soluble DNA-phosphorus (DNA-P) liberated in two hours of incubation.

Alkaline DNase

The reaction mixture volume, method of assay and expression of activity and other details are the same as in the case of acid DNase, except that the reaction mixture consisted of heat denatured DNA as substrate (2 mg in 1 ml of water), 0.05 M Tris-buffer, pH 8.25, and the enzyme.

Preparation of Substrate

Heat denatured DNA was prepared by keeping a solution of DNA (2 mg/ml) at 100°C for 10 min and cooling it rapidly in an ice bath as described by Sung (1968).

DNA Polymerase Assay

The reaction mixture contained in a total volume of 50 μl, 40 mM Tris-HCl, pH 8.0, 1 mM β-mercaptoethanol, 7.5 mM $MgCl_2$, 4 mM ATP, 10 μg of 'activated DNA', 0.1 mM each of dATP, dGTP, dCTP and 20 μM dTTP (1 μCi). Incubation was carried out at 37°C for 20 min. At the end of the incubation, 1 mg of DNA as a carrier was added and the reaction was stopped by adding 2 ml of cold 10% TCA. The samples were kept in ice for 10 min and centrifuged at 4,000 rpm. The precipitate thus obtained was washed thrice with 5% cold TCA and thrice with 95% ethanol. The precipitate after washings was dissolved in 0.1 ml of 0.05 M NaOH and aliquots were taken into radioactive vials containing 10 ml of Bray's mixture and were counted in a Beckman LS-3133P liquid scintillation counter.

The enzyme activity was linear up to 60 ug of protein. Specific activity was expressed as picomoles of TMP incorporated into DNA per mg protein per hour.

CNPase Enzyme Assay

2',3'-cyclic nucleotide 3'-phosphohydrolase (CNPase) was assayed as described by Prohaska et al.,(1973).

Preparation of Homogenates

Cerebral hemispheres were removed from the brain after decapitation, white and grey matter regions were separated physically. Ten percent homogenates were prepared in distilled water. The homogenate (0.1 ml) was solubilized by adding 0.1 ml of 0.2 M Tris-HCl, pH 7.5 and 0.2 ml of 1% sodium deoxycholate solution. After 10 min at 0-4°C, approximate amounts of distilled water were added and homogenized, so that the protein was of an approximate dilution of 0.1 mg/ml.

Final CNPase Assay Conditions

In 0.2 ml of final volume, 5-25 µg of protein were added to 7.5 mM 2',3'-cyclic AMP, 50 mM Tris-maleate buffer, pH 6.2. The substrate initiated reaction was carried out at 30°C for 10 min. A sample containing substrate and buffer with boiled enzyme served as a reagent blank. The reaction was terminated by placing the tubes in boiling water bath for 30 sec. The mixture was returned to the 30°C water bath when 0.1 ml of 0.3 M Tris-HCl containing 21 mM $MgCl_2$, pH 9.0 was added along with 60 ug of E. coli alkaline phosphatase. After the inorganic phosphate was

liberated the volume was made upto 3 ml by addition of water. 5 ml of isobutanol : benzene (1:1) and 1 ml of 1% ammonium molybdate were added to the above solution with shaking for 30 sec to get the yellow chromophore into the upper layer. The phosphomolybdic acid thus formed was reduced by the addition of stannous chloride solution (0.2 ml) in presence of alcoholic sulphuric acid (7 ml) and the stable blue colour thus obtained was measured at 720 nm.

The enzyme activity was expressed as μ moles of inorganic phosphate liberated per min.

CHAPTER III

Changes in DNA, RNA protein and the activities of acid and alkaline deoxyribonucleases in different regions of developing and aging rat brain

CHAPTER III

CHANGES IN DNA, RNA AND PROTEIN AND THE ACTIVITIES OF ACID AND ALKALINE DEOXYRIBONUCLEASES IN DIFFERENT REGIONS OF DEVELOPING AND AGING RAT BRAIN

PART A

CEREBRUM
(WHITE AND GREY MATTER REGIONS)

INTRODUCTION

This laboratory, for the last several years, has been engaged in studying certain biochemical aspects of developing and aging brain in different species (Subba Rao and Janardana Sarma, 1972; Subba Rao, 1973; Shrivastaw and Subba Rao, 1975; Subba Rao and Shrivastaw, 1976; Subba Rao and Janardana Sarma, 1976; Subba Rao and Shrivastaw, 1979). During our studies on chick brain it has been observed that the DNA content of the brain continues to increase even beyond the adult stages, indicating that there is some cell proliferation occurring even in adult rat brain. It also became clear during these studies that the putative DNA degrading enzymes, the acid and alkaline deoxyribonucleases (DNases), exhibit maximum activity during the embryonic

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Part A of this chapter is written based on the paper, entitled "Changes in DNA, RNA and protein and the activities of acid and alkaline deoxyribonucleases in grey and white matter regions of developing and aging rat cerebrum", published in Mech. of Aging Dev., 18, (1982) 225-238., and Part B based on the publication in J. Biosci., 4 (1982) 139-144, entitled "Changes in DNA, RNA, protein and the activities of acid and alkaline DNases in developing and aging rat cerebellum".

stages of the brain. Thus, these enzymes, although degradative in nature, showed a positive correlation with the DNA accumulation (Shrivastaw and Subba Rao, 1975; Subba Rao and Shrivastaw, 1976).

We have taken up this study to check whether a similar pattern of biochemical changes would be observed in a different species, the rat. In view of the complexity of the type of cells that are present in the brain (Dunn and Bandy, 1974) and since white matter could be considered as a material predominantly made up of oligodendroglial cells and fibrous astroglia while the grey matter could be considered as a mixture of neuronal and protoplasmic astroglial cells, the present studies were carried out separately in grey and white matter regions of cerebrum. DNA, RNA and protein content and the activities of acid and alkaline DNases are estimated in the above mentioned regions during the life span of rat. The present results show that there is definitely a second bout of DNA accretion occurring in the aging rat brain in the regions studied. It is also noticed that the activities of acid and alkaline DNases show a simultaneous rise in aging brain.

MATERIALS AND METHODS

Rats of specified age (Wistar Strain) were obtained from Indian Drugs and Pharmaceuticals Limited (I.D.P.L.) Animal House, Hyderabad. Highly polymerized calf thymus DNA, yeast RNA and bovine serum albumin were purchased from Sigma Chemical Company, St. Louis, MO, USA. All the reagents used were of analytical grade.

The cerebral hemispheres were separated from the rest of the brain, and the white and grey matter regions of the cerebral hemispheres were carefully separated. Ten percent homogenates of the tissues were prepared in cold glass distilled water using a Potter Elvehjem homogeniser. Immediately a portion was taken for the extraction of DNA and RNA according to the procedure of Schmidt and Thannhauser (1945), except that the digestion was carried out at 37°C for 2 hr. DNA was estimated by the diphenylamine method while RNA was measured by the orcinol reaction. DNA and RNA were also estimated by measuring the UV absorption at 260 nm. Acid and alkaline DNases were assayed as described by McDonald (1955), by measuring the acid soluble deoxyribose at the end of incubation. The activity was also checked by following the increase in ultraviolet absorption (UV) at 260 nm of the acid soluble fraction. Protein was estimated by the biuret method (Gornall et al, 1949). Phosphorus was measured by the procedure of Bartlett (1959).

Studies on some of the properties of acid DNase in homogenates revealed a pH optimum of 5.1 in acetate buffer. The activity was linear up to 4 mg of protein and up to 2 hr of incubation time. Alkaline DNase showed a broad optimum pH around 8.25 in Tris buffer. Here also the enzyme showed linearity up to 2 hrs of incubation and to about 5 mg of enzyme protein. Native DNA (2 mg) was used as substrate for acid DNase while the same amount of denatured DNA was used as substrate for alkaline DNase. For those studies concerned with establishing the optimal conditions for assay, 1 day old cerebral homogenates for the acid DNase and 1 month old cerebral homogenates in the case of alkaline DNase were used (Young). Similar type of optimal conditions for acid and alkaline DNases from homogenates of old brain (750 days) were observed (Fig. 1 to 10).

In order to check the purity of white and grey matter portions of cerebrum, the activity of 2',3'-cyclic nucleotide-3'-phosphodiesterase, supposed to be localized in myelin or myelin synthesizing cells, was studied, according to the procedure of Prohaska et al.,(1973).

All the data obtained in this study were statistically treated and the significance of difference between any two values was calculated according to Student's t-test.

RESULTS

The distribution of 2',3'-cyclic nucleotide-3'-phosphodiesterase in white and grey matter regions of rat cerebrum is presented in Table 1. As can be seen from Table 1, the enzyme CNPase exhibits 4 times higher specific activity in white matter as compared to the grey matter both in young and old ages (20 days and 540 days). Thus it appears that the grey and white matter regions are pure at least up to 80%.

Table 2 shows the wet weights of white matter and grey matter regions with advancing age of rat brain. The wet weight of white matter increases steadily up to the last age point studied (750 days), whereas grey matter reaches a peak level at 30 days after birth and is maintained thereafter.

The DNA and RNA contents of white and grey matter regions of rat cerebrum at different stages of the life span are presented in Tables 3 and 4. It can be seen that the total DNA content of white matter continues to increase throughout the age period studied. It is to be noted that there

Fig. 1 Effect of pH on the activity of acid DNase in young (1 day old) and old (750 days) rat cerebrum. Each point represents the average of two experiments. Enzyme protein, 1.0 to 1.2 mg in the case of young and 1.5 to 1.75 mg in the case of old, was used.

Fig. 1

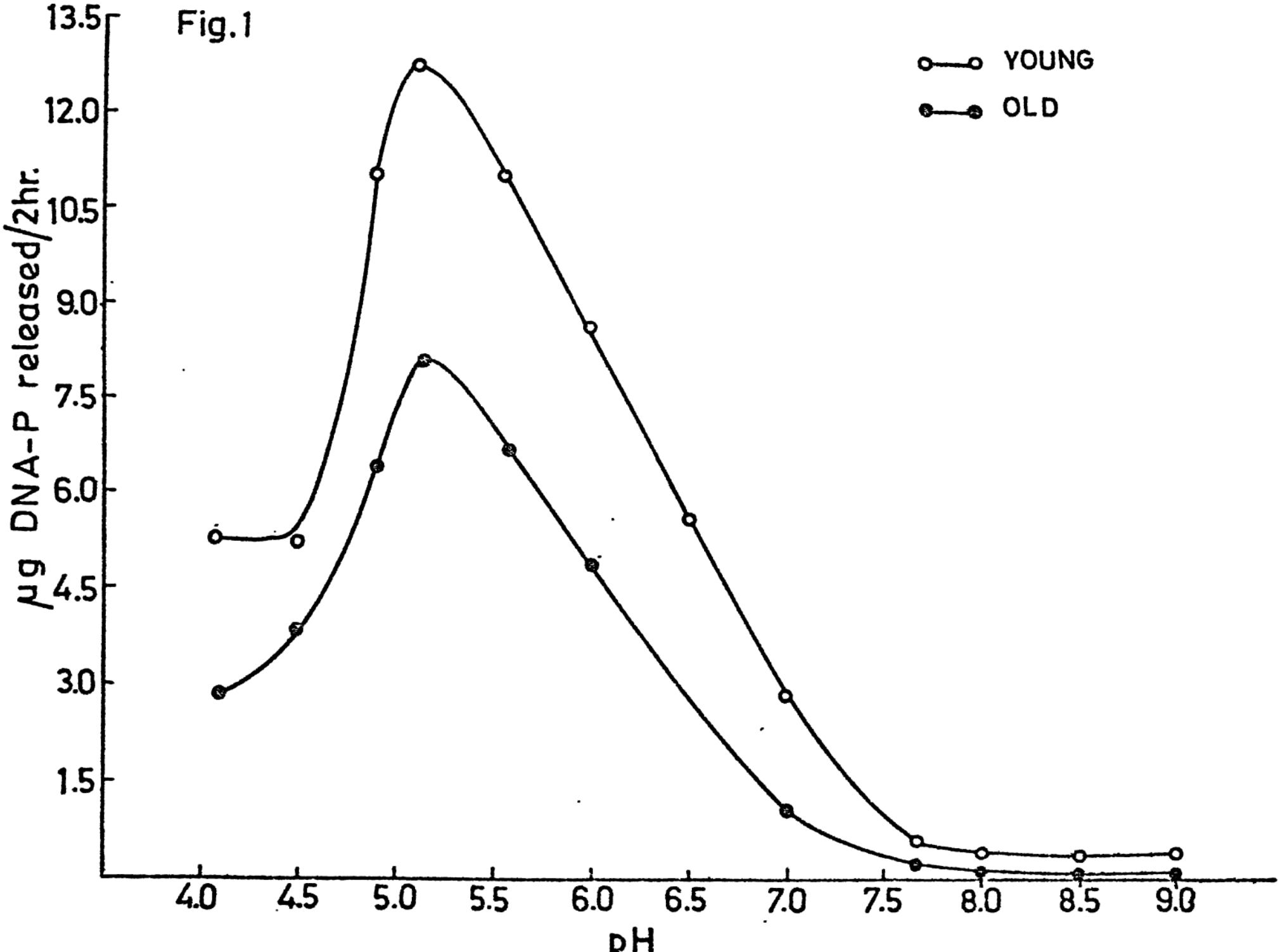
YOUNG
OLD
µg DNA-P released/2hr.
13.5
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
pH

Fig. 2. Effect of enzyme concentration on the activity of acid DNase in young (1 day old) and old (750 days) rat cerebrum. Each point represents the average of two experiments.

Fig. 2

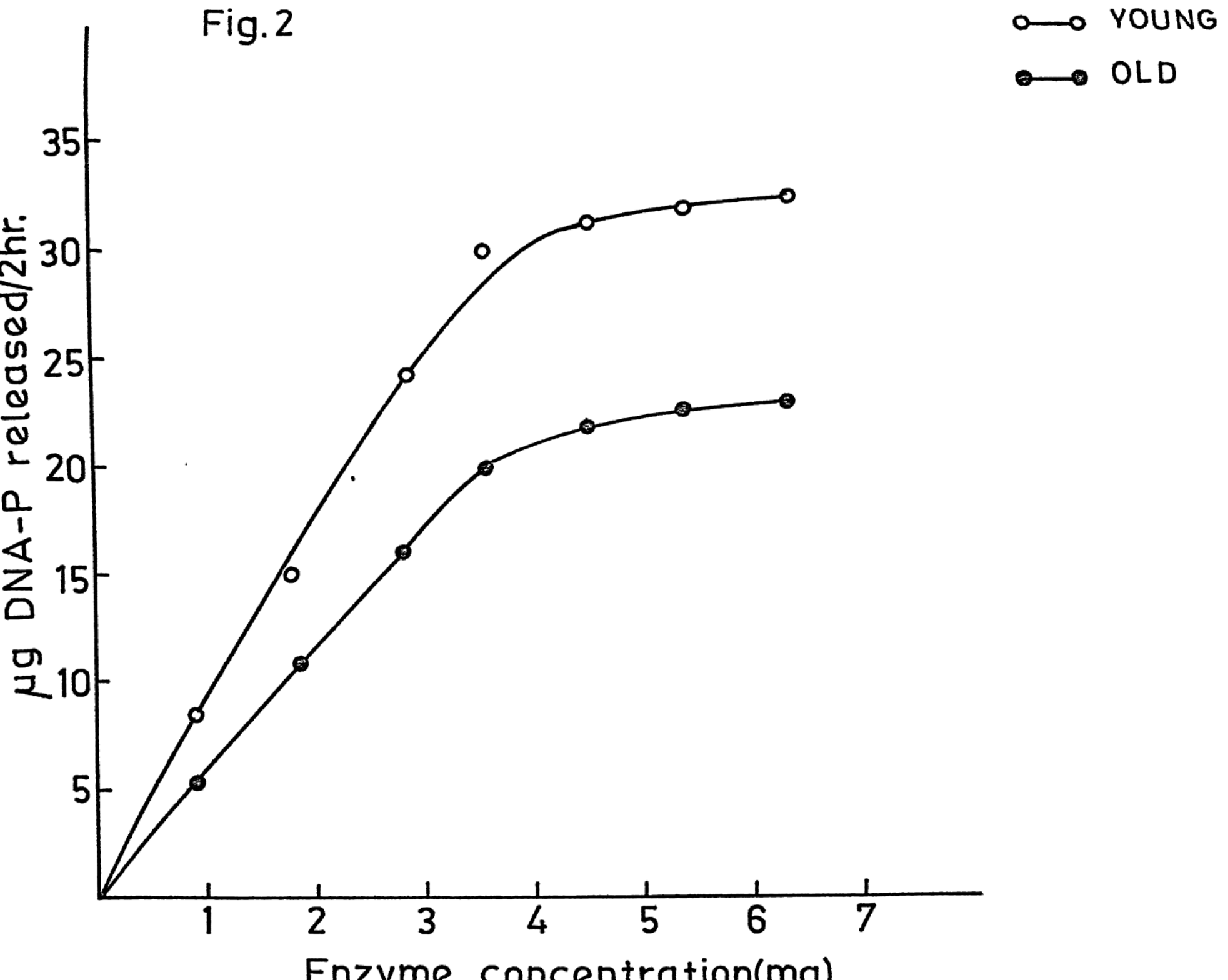
YOUNG
OLD
35
30
25
20
15
10
5
µg DNA-P released/2hr.
1
2
3
4
5
6
7
Enzyme concentration(mg)

Fig. 3. Effect of substrate concentration on the activity of acid DNase in young (1 day old) and old (750 days) rat cerebrum. Each point represents the average of two experiments. Enzyme protein, 1.0 to 1.2 mg in the case of young and 1.5 to 1.75 mg in the case of old, was used.

Fig.3

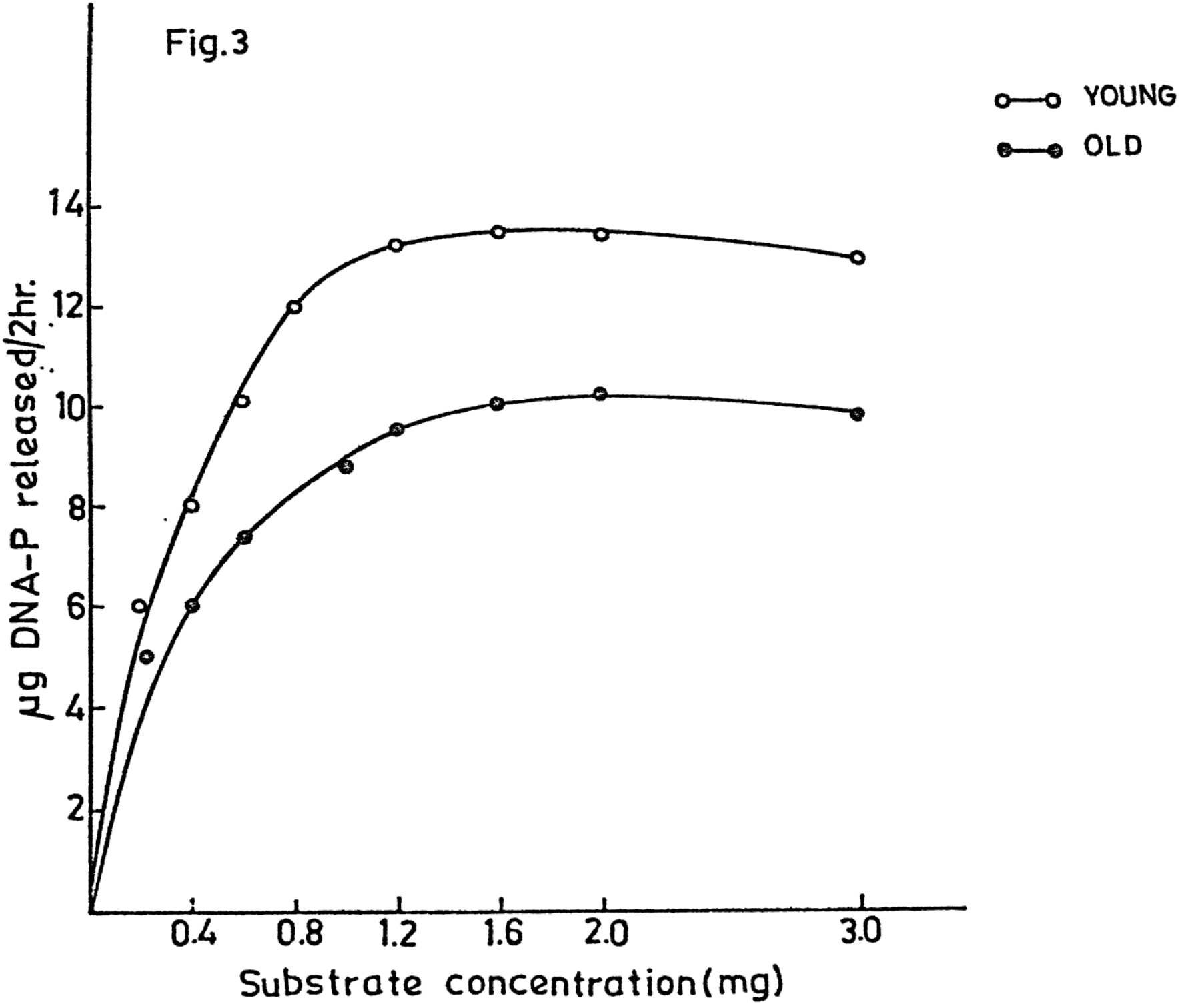
YOUNG
OLD
μg DNA-P released/2hr.
14
12
10
8
6
4
2
0.4
0.8
1.2
1.6
2.0
3.0
Substrate concentration(mg)

Fig. 4. Effect of substrate concentration on the activity of acid DNase in young and old rat cerebrum (Lineweaver-Burk plot, assuming the molecular weight of calf thymus DNA to be 8.6×10^6).

Fig.4

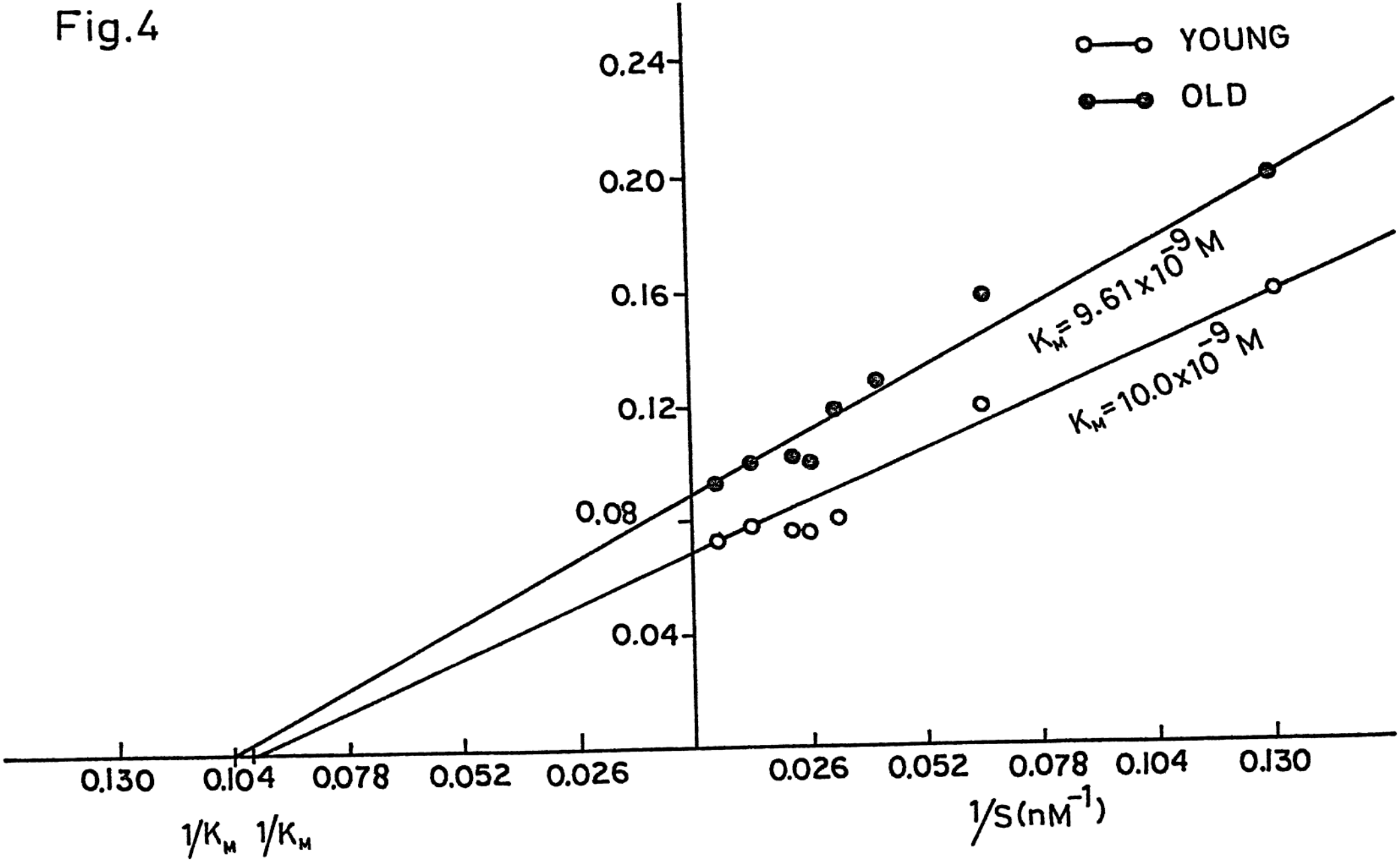
YOUNG
OLD
0.24
0.20
0.16
0.12
0.08
0.04
$K_M = 9.61 \times 10^{-9}$ M
$K_M = 10.0 \times 10^{-9}$ M
0.130
0.104
0.078
0.052
0.026
0.026
0.052
0.078
0.104
0.130
$1/K_M$ $1/K_M$
$1/S(nM^{-1})$

Fig. 5. Effect of time on the activity of acid DNase in young (1 day old) and old (750 days) rat cerebrum. Each point represents the average of two experiments. Enzyme protein, 2.0 to 2.2 mg in the case of young and 2.0 to 2.5 mg in the case of old, was used.

Fig.5

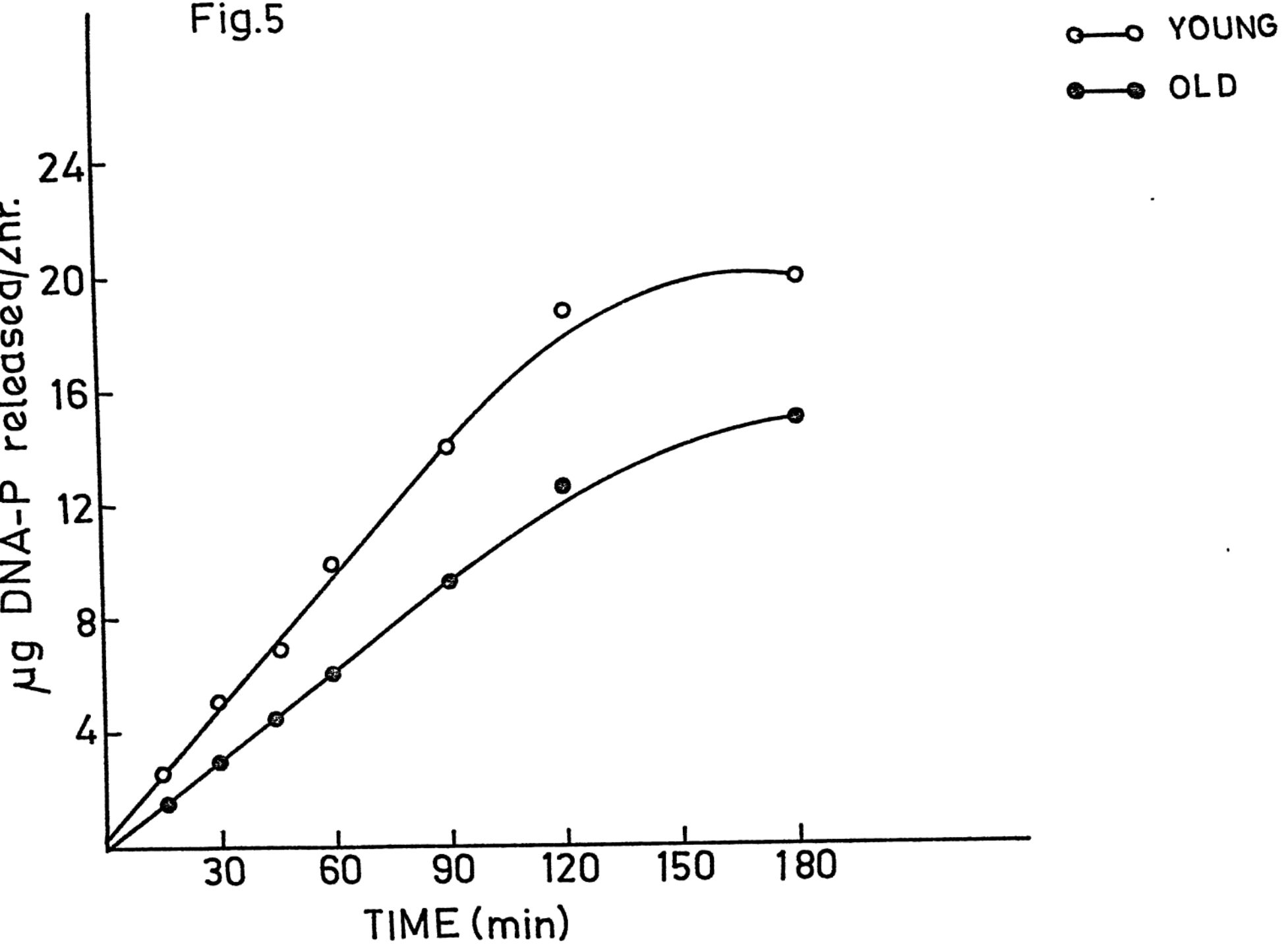
YOUNG
OLD
µg DNA-P released/2hr.
24
20
16
12
8
4
30
60
90
120
150
180
TIME (min)

Fig. 6. Effect of pH on the alkaline DNase activity in young (30 days and old (750 days) rat cerebrum. Each point represents the average of two experiments. Enzyme protein, 3.2 to 3.4 mg in the case of young and 3.75 to 4.0 mg in the case of old, was used.

Fig.6

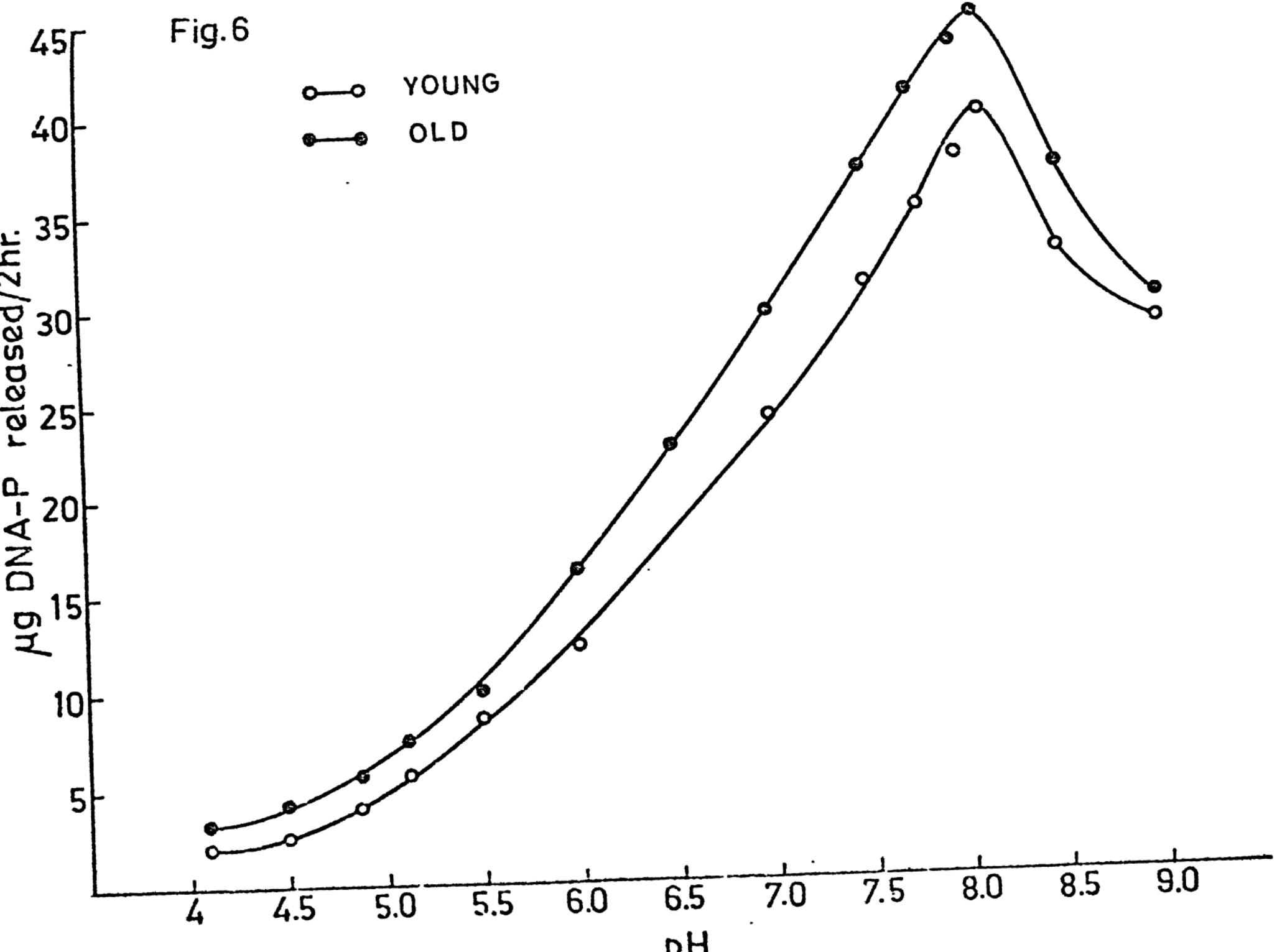
YOUNG
OLD
μg DNA-P released/2hr.
45
40
35
30
25
20
15
10
5
4
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
pH

Fig. 7. Effect of enzyme concentration on the alkaline DNase activity in young (30 days) and old (750 days) rat cerebrum. Each point represents the average of two experiments.

Fig. 7

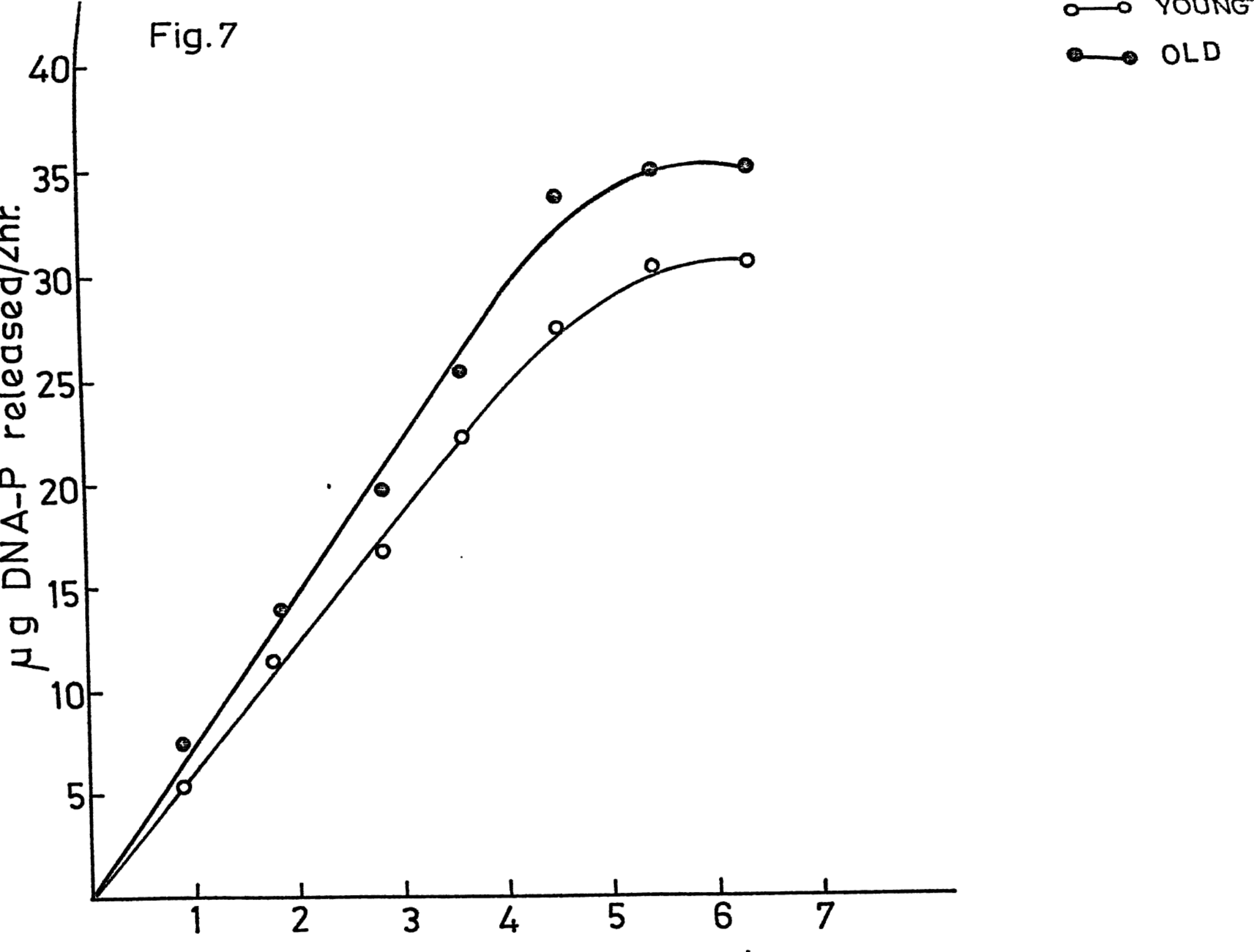

Fig. 8. Effect of substrate concentration on the alkaline DNase activity in young (30 days) and old (750 days) rat cerebrum. Each point represents the average of two experiments. Enzyme protein, 1.5 to 1.7 mg in the case of young and 2.0 to 2.3 mg in the case of old, was used.

Fig. 8

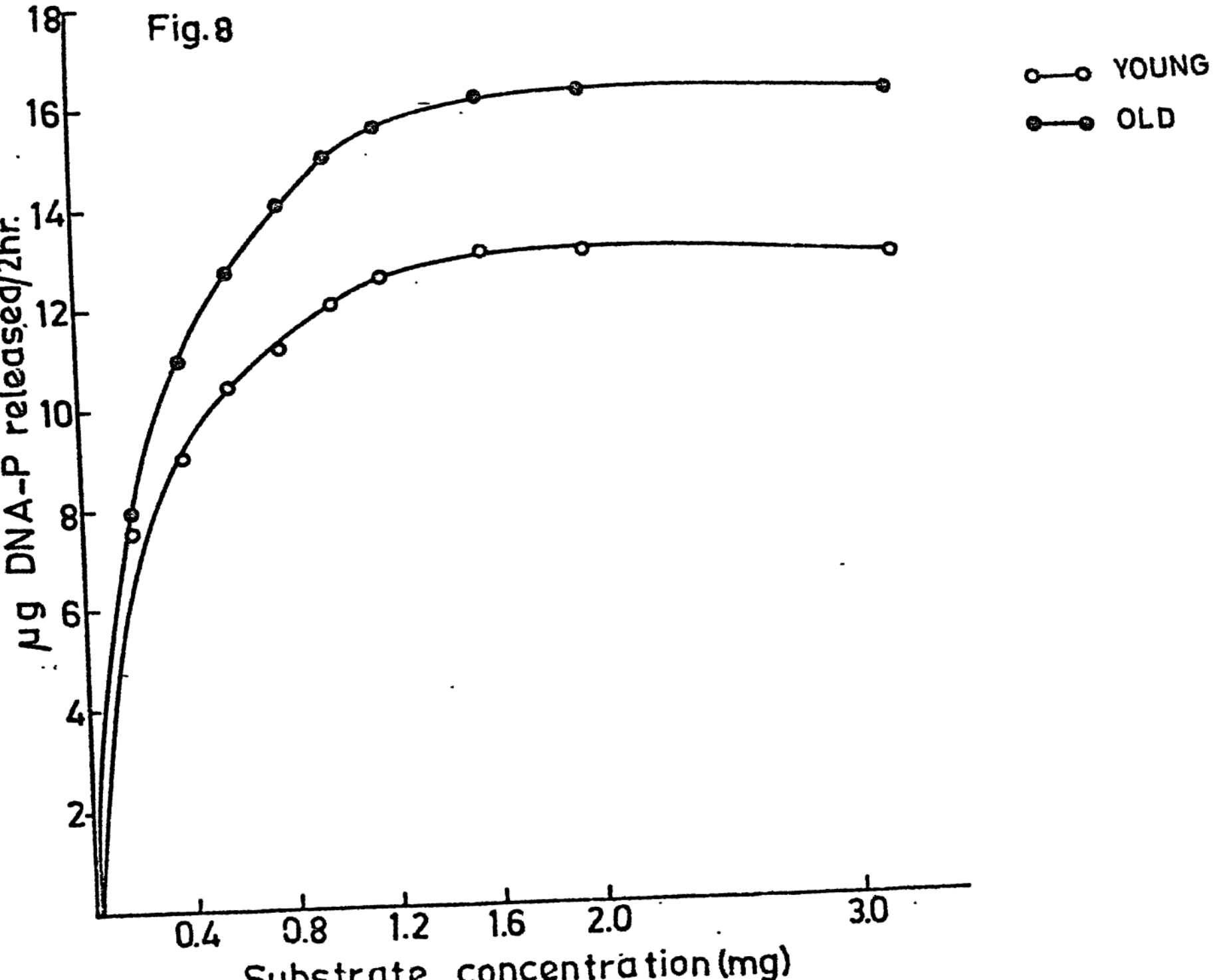
YOUNG
OLD
µg DNA-P released/2hr.
18
16
14
12
10
8
6
4
2
0.4
0.8
1.2
1.6
2.0
3.0
Substrate concentration (mg)

Fig. 9. Effect of substrate concentration on the alkaline DNase activity in young and old rat cerebrum. (Lineweaver-Burk plot, assuming the molecular weight of calf thymus DNA to be 8.6×10^6).

Fig.9

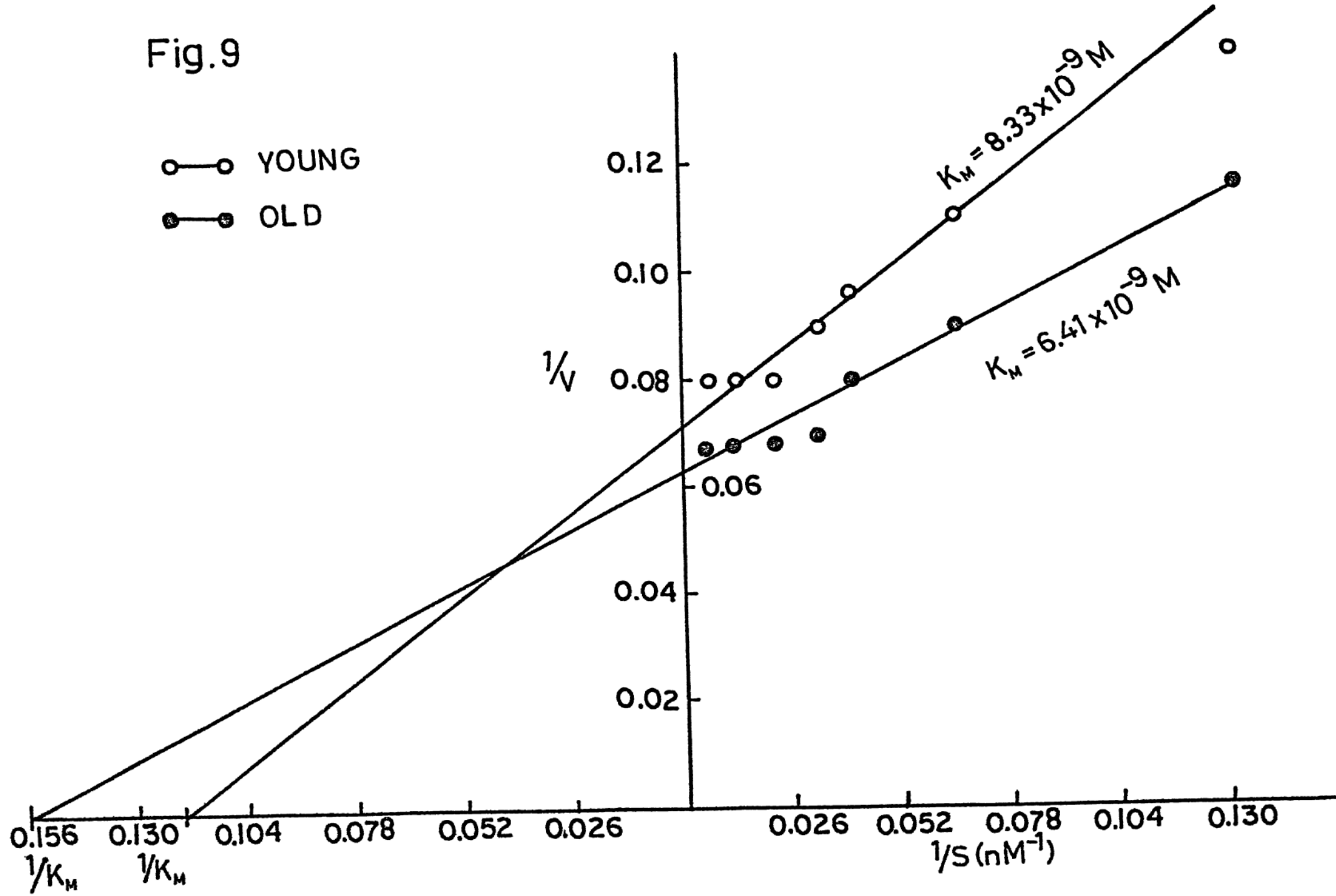
YOUNG
OLD
$K_M = 8.33 \times 10^{-9}$ M
$K_M = 6.41 \times 10^{-9}$ M
1/V
0.12
0.10
0.08
0.06
0.04
0.02
0.156
0.130
0.104
0.078
0.052
0.026
1/K_M
1/K_M
0.026
0.052
0.078
0.104
0.130
1/S (nM^-1)

Fig. 10. Effect of time on the alkaline DNase activity in young (30 days) and old (750 days) rat cerebrum. Each point represents the average of two experiments. Enzyme protein, 2.3 to 2.5 mg in the case of young and 3.0 to 3.3 mg in the case of old, was used.

Fig.10

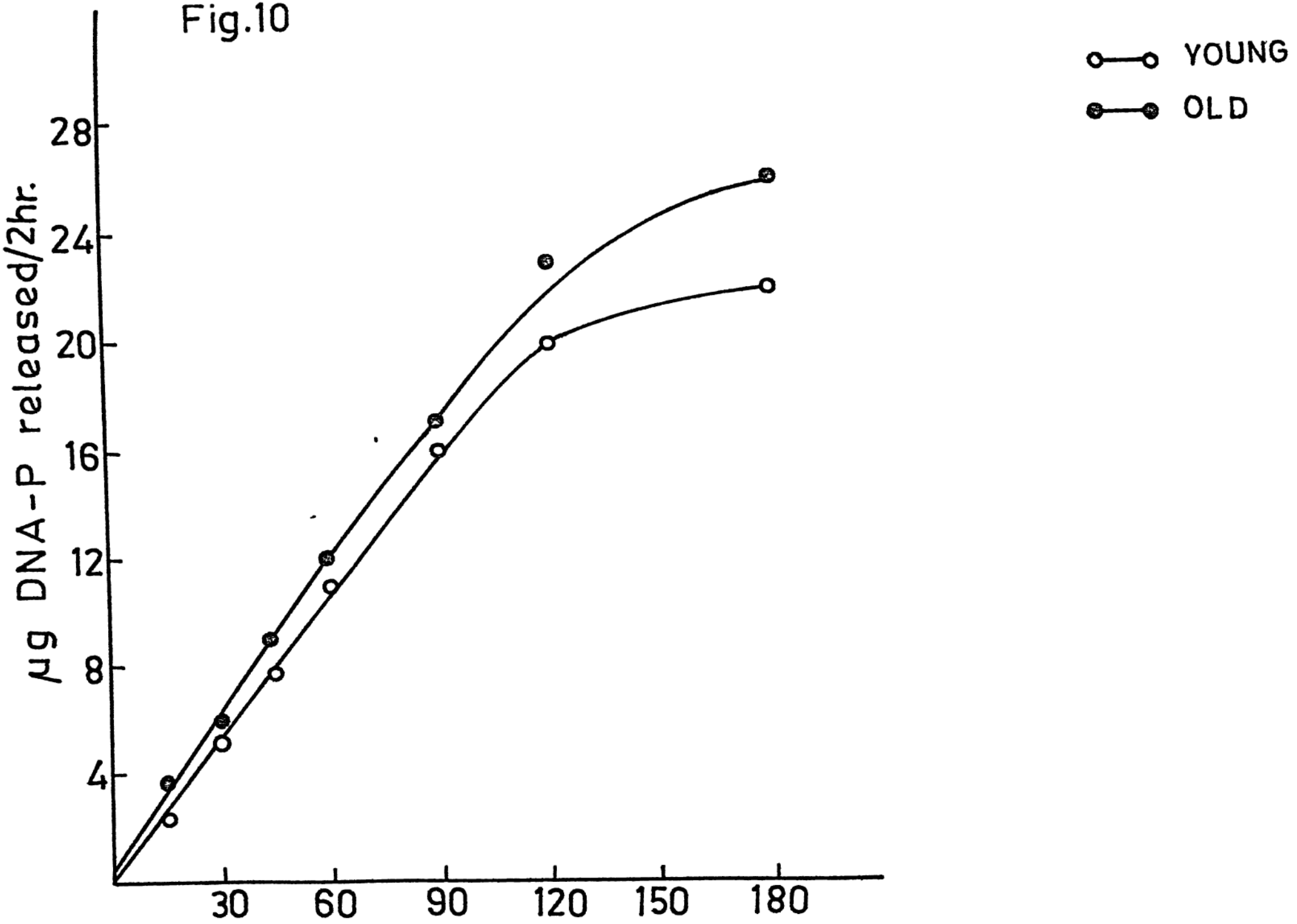
YOUNG
OLD
μg DNA-P released/2hr.
4
8
12
16
20
24
28
30
60
90
120
150
180

TABLE 1. DISTRIBUTION OF CNPase ACTIVITY IN WHITE AND GREY MATTER REGIONS OF RAT BRAIN

Age in Days	White Matter	Grey Matter
20 (6)	4.70 ± 0.48	1.20 ± 0.14
540 (6)	6.78 ± 0.48	1.92 ± 0.25

All the values are the means ± S.D. and expressed as μ moles of inorganic phosphate liberated/mg protein /min. The number of samples analyzed at each age are given in the parentheses.

TABLE 2. CHANGES IN WET WEIGHT OF WHITE AND GREY MATTER REGIONS AT VARIOUS AGES OF RAT CEREBRUM

Age in days	Cerebrum	
**1 (8)	0.22 ± 0.02	
**7 (8)	0.50 ± 0.04	
	White	Grey
15 (15)	0.29 ± 0.02	0.48 ± 0.07
(30 (8)	0.49 ± 0.03	0.73 ± 0.05
60 (10)	0.55 ± 0.05	0.68 ± 0.08
225 (16)	0.63 ± 0.05	0.77 ± 0.08
750 (17)	0.66 ± 0.09*	0.75 ± 0.10

All the values are expressed in gms and as means ± S.D. The number of samples analyzed are given in the parentheses.

** These values are for the whole cerebrum as it was not possible to separate white and grey matter regions at this age.

* This value is significantly different from the corresponding value at 60 days of age ($P < 0.001$).

is a significant increase between the ages of 225 and 750 days. However, the DNA content of grey matter reached a peak value at 30 days after birth and this value was maintained more or less constant up to 225 days of age. But as in the case of white matter, here also there is a significant increase in the DNA level between 225 and 750 days. When the DNA content is expressed per gram of tissue the values declined from day 7 onwards up to 30 days, but at later stages (60, 225 days) the values remained constant. Once again a significant increase in DNA concentration was observed in old age (750 days) in both the regions studied (Table 3). The total RNA content in both white and grey matter regions reaches a high value at 30 days of age (Table 4). Thereafter, a slight decrease was observed up to 225 days, but by 750 days of age there was a significant increase in the amount of RNA in both the regions. The concentration of RNA per gram of region steadily decreased up to 225 days of age. However, there was a significant rise in the RNA concentration in old age in both the regions.

Table 5 shows the changes in total protein content and protein expressed per gram of wet weight in white and grey matter regions of developing and aging cerebrum. The total protein content increases steadily up to old age in white matter, whereas in grey matter the maximum protein content was found at 60 days. At 225 days this value decreases and then shows a rise by 750 days. Thus as can be seen, from 30 days onwards the protein content in grey matter shows fluctuations with no net increase by 750 days of age. Protein expressed per gram of wet weight increases up to 15 days after birth in both white and grey matter regions and thereafter reaches a lower plateau. As shown in Table 6, the protein/DNA ratio increases continuously

TABLE 3. CHANGES IN DNA CONTENT OF WHITE AND GREY MATTER REGIONS IN DEVELOPING AND AGING RAT CEREBRUM

Age in Days	CEREBRUM			
	DNA/g. tissue		DNA/region	
1 (8)	3.4 ± 0.50		0.67 ± 0.1	
7 (8)	4.8 ± 0.30		2.50 ± 0.3	
	White	Grey	White	Grey
15 (15)	1.2 ± 0.30	1.4 ± 0.26	0.33 ± 0.1	0.65 ± 0.10
30 (8)	0.7 ± 0.10	0.8 ± 0.14	0.36 ± 0.05	0.68 ± 0.13
60 (10)	0.9 ± 0.06	1.0 ± 0.12	0.45 ± 0.03	0.63 ± 0.06
225 (16)	0.75 ± 0.20	0.85 ± 0.10	0.48 ± 0.06	0.68 ± 0.10
750 (17)	1.22 ± 0.18	1.43 ± 0.20	0.85 ± 0.10	1.10 ± 0.13

All the values are expressed in mg and as means ± S.D. Other details are same as given in Table 2. Values at 750 days are significantly different from the corresponding values at 225 days ($P < 0.001$).

TABLE 4. CHANGES IN RNA CONTENT OF WHITE AND GREY MATTER REGIONS IN DEVELOPING AND AGING RAT CEREBRUM

Age in Days	CEREBRUM			
	RNA/g. tissue		RNA/region	
1 (8)	2.8 ± 0.16		0.60 ± 0.10	
7 (8)	3.0 ± 0.50		1.50 ± 0.30	
	White	Grey	White	Grey
15 (15)	2.76 ± 0.35	3.10 ± 0.30	0.72 ± 0.16	1.47 ± 0.27
30 (8)	2.08 ± 0.37	2.10 ± 0.20	1.00 ± 0.17	1.60 ± 0.10
60 (10)	1.76 ± 0.09	1.90 ± 0.20	0.95 ± 0.12	1.25 ± 0.12
225 (16)	1.46 ± 0.10	1.65 ± 0.15	0.80 ± 0.20	1.30 ± 0.10
750 (17)	2.10 ± 0.17	2.45 ± 0.16	1.25 ± 0.19	1.96 ± 0.17

All the values are expressed in mg and as means ± S.D. Other details are same as given in Table 2. Values at 750 days are significantly different from the corresponding values at 225 days ($P < 0.001$).

TABLE 5. CHANGES IN THE PROTEIN CONTENT OF WHITE AND GREY MATTER REGIONS IN DEVELOPING AND AGING RAT CEREBRUM

Age in Days	CEREBRUM			
	Protein/g. tissue		Protein/region	
1 (8)	92.0 ± 8.0		19.0 ± 2.7	
7 (8)	85.0 ± 3.3		42.5 ± 2.2	
	White	Grey	White	Grey
15 (15)	106.0± 16.6	133.7 ± 27.0	30.6 ± 6.8	64.8 ± 19.0
30 (8)	80.0 ± 8.0	102.0 ± 10.0	39.0 ± 5.0	77.5 ± 8.2
60 (10)	110.0 ± 7.6	128.0 ± 10.7	62.3 ±10.3	91.3 ± 10.3
225 (16)	100.0 ± 12.0	97.5 ± 8.0	63.3 ± 8.0	71.7 ± 7.0
750 (17)	106.0 ± 16.8	117.0 ± 19.0	67.3 ± 12.3	82.7 ± 9.0

All the values are expressed in mg and as means ± S.D. Other details are same as in Table 2. Values at 750 days are significantly different from the corresponding values at 225 days only in the case of grey matter ($P < 0.025$).

up to 60 days after birth in both white and grey matter, thereby showing a steady increase in cell size. However, between 225 and 750 days of age there was a marked decrease in this ratio in white matter, thus suggesting relatively more hyperplasic activity during this period in this region. The RNA/DNA ratio showed a high value at both 15 and 30 days after birth in both white and grey matter. Thereafter, the values decrease up to 225 days. There was only a slight increase with further advancing age, which was found to be statistically significant only in the case of grey matter ($P < 0.025$).

Examining the RNA/DNA ratios in various regions of mouse brain, Chaconas and Finch (1973) observed no change in these values in aging hypothalamus, hippocampus and septum. In the striatum there was a slight but statistically significant decrease. It is difficult to compare these results with the present data since the present studies are carried out on a different regional basis (i.e., White matter and Grey matter). It must be mentioned, however, that in a recent study of Shaskan (1977) the RNA/DNA ratio in cerebral cortex of 20 month old rats was slightly less than the adult value but this difference was found to be not significant. It thus appears that our present results with white matter agree with these findings while the grey matter results are in slight variance.

The activities of acid DNase in different parts of rat cerebrum at various points of the life span are shown in Table 7. The specific activity of this enzyme was found to be maximum on the first postnatal day with a decline in activity at later stages of life up to 225 days. But the enzyme

TABLE 6. CHANGES IN PROTEIN/DNA AND RNA/DNA RATIOS IN WHITE AND GREY MATTER REGIONS OF DEVELOPING AND AGING RAT CEREBRUM

Age in Days	CEREBRUM			
	Protein/DNA		RNA/DNA	
1 (8)	29.4 ± 5.2		0.90 ± 017	
7 (8)	17.2 ± 2.1		0.60 ± 0.09	
	White	Grey	White	Grey
15 (15)	85.5 ± 17.6	98.4 ± 22.6	2.3 ± 0.50	2.7 ± 0.54
30 (8)	102.3 ± 24.6	122.7 ± 23.7	2.7 ± 0.75	2.6 ± 0.45
60 (10)	131.0 ± 17.0	139.7 ± 20.5	1.8 ± 0.20	2.1 ± 0.38
225 (16)	129.7 ± 24.5	78.0 ± 23.3	1.6 ± 0.36	1.6 ± 0.33
750 (17)	79.2 ± 12.8	77.8 ± 10.8	1.8 ± 0.30	1.8 ± 0.2

Values at 750 days are significantly different from the corresponding values at 225 days of age only in the case of white matter protein/DNA ratio ($P < 0.001$). For other details please see Table 2.

activity shot up to a significant level (80%) at 750 days. The pattern was similar in both white and grey matter. The total amount acid DNase activities is also presented in Table 7, and it can be seen that there is a marked increase in this activity in old age (750 days) compared to the value at 225 days. In fact, the activities at old age are also the maximum activities. On the other hand both the specific activity and total activity of alkaline DNase was highest in old age (Table 8). During the period of development, the activities gradually increase and reach close to the peak value at 30 days. The point to be noted is the marked increase of the activity between the ages 225 and 750 days.

In Table 9, both acid and alkaline DNase activities are expressed per mg of DNA since such an expression would reflect these activities per cell. As can be seen, at 30 days the activity of acid DNase was highest both in white and grey matter which showed a notable decline by 225 days. However, between 225 and 750 days the activities showed only a marginal increase and this increase was found to be statistically significant only in the case of grey matter. The pattern of changes in alkaline DNase was similar to that of acid DNase up to 225 days. Between 225 and 750 days there was a significant increase of this value in both the regions.

Among the changes in several biochemical parameters that were examined in this study, the most important and significant changes were found to be between ages 225 and 750 days. Therefore, a summary Table 10, is provided indicating the magnitude and significance of these changes. As can be seen, growth, apart from that during early stages, seems to occur

TABLE 7. ACID DNase ACTIVITY IN WHITE AND GREY MATTER REGIONS OF DEVELOPING AND AGING RAT CEREBRUM

Age in Days	CEREBRUM			
	Specific Activity		Total Activity	
1 (8)	8.0 ± 1.3		146.2 ± 36.8	
7 (8)	7.3 ± 1.0		309.5 ± 43.0	
	White	Grey	White	Grey
15 (15)	4.8 ± 0.45	4.3 ± 0.77	146.7 ± 35.0	285.0 ± 63.0
30 (8)	5.0 ± 0.50	3.9 ± 0.70	180.0 ± 17.0	282.0 ± 48.0
60 (10)	3.4 ± 0.27	3.0 ± 0.30	200.0 ± 25.0	274.0 ± 33.2
225 (16)	3.0 ± 0.20	3.2 ± 0.20	190.0 ± 20.2	227.0 ± 40.0
750 (17)	5.4 ± 0.80	5.5 ± 0.60	350.0 ± 58.0	468.0 ± 51.0

Specific activity is defined as μg of DNA-P liberated into acid soluble fraction per mg protein/2hr. Total activity is obtained by multiplying the specific activity with the amount of protein in mg in the region. For other details please see Table 2. Values at 750 days are significantly different from the corresponding values at 225 days ($P < 0.001$).

TABLE 8. ALKALINE DNase ACTIVITY IN WHITE AND GREY MATTER REGIONS OF DEVELOPING AND AGING RAT CEREBRUM

Age in Days	CEREBRUM			
	Specific Activity		Total Activity	
1 (8)	3.7 ± 0.50		72.0 ± 15.2	
7 (8)	4.7 ± 0.60		200.0 ± 16.0	
	White	Grey	White	Grey
15 (15)	5.5 ± 0.7	4.8 ± 0.7	160.0 ± 26.0	285.7 ± 72.0
30 (8)	8.0 ± 1.1	5.3 ± 1.1	291.0 ± 19.7	557.0 ± 104.0
60 (10)	5.4 ± 0.5	5.5 ± 0.5	310.0 ± 40.7	551.0 ± 60.0
225 (16)	3.4 ± 0.6	3.8 ± 0.1	204.0 ± 20.0	275.0 ± 30.0
750 (17)	8.3 ± 1.1	7.6 ± 0.1	600.0 ± 98.0	682.0 ± 65.2

Specific activity is defined as μg DNA-P liberated into acid soluble fraction/mg protein/ 2 hr. Total activity is obtained by multiplying the specific activity with the total protein in mg in the region. For other details please see Table 2. Values at 750 days are significantly different from the corresponding values at 225 days ($P < 0.001$).

TABLE 9. ACTIVITIES OF DNases EXPRESSED PER mg OF DNA IN WHITE AND GREY MATTER REGIONS OF DEVELOPING AND AGING RAT CEREBRUM

Age in Days	CEREBRUM			
	Acid DNase		Alkaline DNase	
1 (8)	210.5 ± 42.0		111.0 ± 27.0	
7 (8)	126.6 ± 17.9		81.3 ± 10.0	
	White	Grey	White	Grey
15 (15)	404.5 ± 79.7	423.5 ± 90.5	457.6 ± 98.0	469.3 ± 105.8
30 (8)	511.6 ±152.3	473.0 ±123.5	928.8 ±150.0	767.7 ± 77.2
60 (10)	425.0 ± 70.8	416.0 ± 94.9	664.5 ±110.8	689.3 ± 166.1
225 (16)	394.0 ± 54.0	348.0 ± 88.0	526.0 ± 70.0	380.0 ± 74.3
750 (17)	424.0 ± 62.3	423.0 ± 66.5	728.0 ±149.0	672.0 ± 105.6

All the values are the means ± S.D. and expressed as μg DNA-P liberated into acid soluble fraction/mg DNA/2 hr. For other details please see Table 2. Values at 750 days are significantly different from the corresponding values at 225 days only in the case of alkaline DNase ($P < 0.001$).

TABLE 10. SUMMARY TABLE TO SHOW BIOCHEMICAL CHANGES BETWEEN AGES 225 AND 750 DAYS IN RAT CEREBRUM

Age (days)	Region	DNA		RNA		PROTEIN		Protein/ DNA ratio	Acid DNase		Alkaline DNase		DNase activity per mg DNA	
		mg/g	mg/ brain	mg/g	mg/ brain	mg/g	mg/ brain		specific Activity	Total Activity	Specific Activity	Total Activity	Acid DNase	Alkaline DNase
225 (16)	White matter	0.75 ± 0.2	0.48 ± 0.06	1.46 ± 0.1	0.80 ± 0.2	100 ± 12	63.3 ± 8.0	130 ± 24.5	3.00 ± 0.2	190 ± 20.2	3.40 ± 0.62	204 ± 20	394 ± 54	526 ± 70
750 (17)	White matter	1.22 ± 0.18	0.85 ± 0.1	2.10 ± 0.17	1.25 0.19	106* ± 16.8	67.4* ± 12.3	79.2 ± 12.83	5.36 ± 0.8	350 ± 58.0	8.26 ± 1.1	600 ± 98.0	424* ± 62.3	728 ± 14.9
225 (16)	Grey matter	0.85 ± 0.1	0.68 ± 0.1	1.64 ± 0.15	1.30 ± 0.1	97.5 ± 8.0	71.7 ± 7.0	78.1 ± 23.3	3.20 ± 0.2	227 ± 40.0	3.84 ± 0.15	275 ± 30.0	348 ± 88.0	380 ± 74.3
750 (17)	Grey matter	1.43 ± 0.2	1.10 ± 0.13	2.45 ± 0.16	1.96 ± 0.17	117 ± 19.0	82.7 ± 9.0	77.8* ± 10.9	5.5 ± 0.56	468 ± 51.0	7.60 ± 0.1	682 ± 65.2	423 ± 66.5	672 ± 105.6

All the values are the means ± S.D.

*Except for these values all other values at 750 days are significantly different from those at 225 days ($P < 0.005$). The number of samples analyzed are given in the parenthesis. For other details please see text.

again between ages 225 and 750 days. The DNA content expressed either per gram or per whole region increased significantly during this period in both the regions, indicating cell proliferation even beyond the adult stages of life. Similarly, a considerable accumulation of RNA could also be observed during this period. The marked decrease in protein/DNA ratio between these two stages of life (225 and 750 days) only in the case of white matter is to be noted. Although DNases are supposedly degradative enzymes, there is a significant increase in their activities along with the DNA accretion during the period.

DISCUSSION

Several studies have been conducted earlier on the changes in nucleic acids and protein levels in brain (Winick, 1968; Fish and Winick, 1969; Margolis, 1969; Rapport et al, 1969; Winick, 1970; Bass et al, 1970; Dobbing and Sands, 1970; Patterson et al., 1971; Vernadakis, 1973; Howard, 1973; Weichsel, 1974; Seiler and Lamberty, 1975; Badger and Tumbleson, 1975; Chanda et al, 1975; Marges et al, 1977; Griffin et al, 1977; Caron and Unsworth, 1978; Gaitonde et al, 1978; Kistler, 1979; Porta et al, 1980). Unfortunately, inspite of the large body of such information, only a few data are available on the possible shift of these changes during later stages of development and old age (Vernadakis, 1973; Howard, 1973; Seiler and Lamberty, 1975; Caron and Unsworth, 1978; Porta et al, 1980). However, even in these experiments not all the above mentioned parameters were studied at the same time in different regions of the brain. In order to examine the biochemical changes in aging brain, it is only proper that the full spectrum of changes starting from early ontogenesis to old age are studied. Moreover, it is also important to conduct

such studies in different regions of the brain since it is now becoming increasingly clear that different regions of the brain may have different schedule of development and perhaps of aging and also that nervous tissue composed of a variety of cells (neurons, astroglia and oligodendroglia, etc.). To our knowledge there has only been one earlier study where nucleic acid changes in grey and white matter regions were measured separately (Bass et al, 1970). However, the experiments in those studies were terminated when the rats were 50 days old. The present investigation therefore, represents a single study where the changes in nucleic acids and protein were followed in white and grey regions of rat cerebrum beginning from the first day of life through old age. In addition, since our previous studies on chick brain revealed an intriguing relationship between DNA accumulation and the activity of DNases, these enzyme activities were also measured in the present experiments.

Three biochemical phenomena could be visualized from the data presented here. 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 stops at 30 days (Table 2). A steady increase in the protein/DNA in both white and grey matter from 7 days to 60 days could probably be taken as a cell size increase followed by earlier hyperplasia.

Secondly, DNA accretion appears to be taking place predominantly at two stages of the life span of rat brain, the first being the early developmental period and the second somewhere beyond adult life and before 2

years of age. Both white and grey matter regions exhibit the same trend. In both these regions, there was a marked increase of DNA content between the ages of 225 and 750 days. However, it should also be noted that, between the ages 225 and 750 days, the white matter region seems to be characterized by a more pronounced cell proliferation, as indicated by DNA accumulation (Table 3), and a marked decrease in the protein/DNA ratio (40%) during this period (Table 6). It must be admitted here, that the increase in DNA is being interpreted as increase in cell number since it is now well accepted that a constant amount of DNA exists in a mature diploid cell in any given species (Peng and Lee, 1979). All these results strongly indicate that during this later part of life the process of cell proliferation is occurring at a considerable rate. The question then arises, which cells are undergoing replication at this stage of life of the brain. Morphological and biochemical studies conducted in other laboratories as well in this laboratory (Altman, 1969. Vernadakis, 1973; Brizzee et al, 1964; Subba Rao and Shrivastaw, 1979) reveal that glial cells are the cells that probably replicate in this old age. Thus certain enzymic activities known to be located in glial cells are found to increase in old chick brain (Vernadakis, 1973; Subba Rao and Shrivastaw, 1979). A continuous increase in white matter DNA content observed in the present study also substantiates this contention.

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 DNA in both the regions studied. Both these enzymes, in particular the acid DNase show high activity during the 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 age (Table 7 & 8) while alkaline DNase shows increasing activity up to 30 days and then a fall up to 225 days. However, in both the regions the two enzymes exhibited a marked increase in their activities by the time the brain was 750 days old, a period during which significant accumulation of DNA was also noticed. It is difficult to predict the exact role being played by these putative degradative enzymes. However, several suggestions were made earlier that the DNases might be playing an important role, not only simply degrading DNA, but actually in the synthesis and/or repair of DNA (Lehman, 1967; Laskowiski, 1967; Bernardi, 1971).

Be as it may, the present results do indicate that the DNA increase in a given region of brain and the simultaneous increase in DNase activities cannot be brushed aside by simply considering that the two phenomena are coincidental.

PART B

CEREBELLUM

INTRODUCTION

Earlier studies from this laboratory have shown increased levels of DNA beyond the adult stages indicating some cell proliferation during the old age both in chick and rat brain (Shrivastaw and Subba Rao, 1976; Subba Rao and Subba Rao,1982 a). It also became clear from these studies that the putative DNA degrading enzymes, the acid and alkaline DNases show positive correlation with the DNA accumulation. It is increasingly becoming evident that different regions of brain have different schedules of development and the cerebellar region of the rat brain is known to develop during the early part of the postnatal period and hence represents another area of brain having a developmental time schedule different from both grey and white matter regions. We have therefore extended our earlier studies (Subba Rao and Subba Rao, 1982 a) to check certain biochemical parameters during development and aging rat cerebellum. The possible changes in DNA, RNA, protein along with the DNases during developing and aging rat cerebellum are presented here.

MATERIALS AND METHODS

Materials and methods are same as described in Part A.

RESULTS AND DISCUSSION

There was a steady growth in the wet weight of the cerebellum with advancing age up to 60 days. This weight was maintained constant

up to 225 days but between 225 and 750 days there was again a significant increase in the weight (Table 11).

It can be seen from the Table 11 that the total DNA content of cerebellum increased 4 fold between day 1 and 7 after birth, while during the same period the increase in protein content was only 2.5 fold and that of RNA was about 3 fold. This pattern suggests higher rate of cell proliferation as compared to the increase in cell size during this period. Similar higher rate of cell proliferation (as indicated by DNA increase) in the cerebellum during the few days after birth in rat was observed by earlier workers (Balazs and Patel, 1973; Weichsel, 1974; Griffin et al, 1977, Clark and Weichsel, 1977; Gaitonde et al, 1978; Clark et al, 1978; Litteria, 1980). However, from the 7th day onwards the rate of increase in protein was significantly higher than that of DNA indicating that beyond the 7th day the extent of cell size increase was greater than that of cell proliferation. From 15th day the levels of DNA remained essentially constant up to 225 days but between 225 and 750 days there was once again a significant increase in DNA content (expressed either as a total content or per gram of the region). In fact the values found in old cerebellum were the highest compared to the values at any other earlier ages. Even in the case of RNA and protein, there was a significant increase in their total content between 225 and 750 days although the values in old cerebellum were not the highest as compared to the values at earlier stages. It should also be noted that the magnitude of accumulation of DNA (100%) between adult and old ages was much greater than those in the case of either RNA or protein (30% and 20% respectively) during the period. These observations point to a second peak of cell proliferating

TABLE 11. CHANGES IN WET WEIGHT, DNA, RNA, AND PROTEIN LEVELS IN DEVELOPING AND AGING RAT CEREBELLUM

AGE IN DAYS	WET WEIGHT	DNA		RNA		PROTEIN	
		Per g. of tissue	Per region	Per g. of tissue	Per region	Per g. of tissue	Per region
1 (6)	24 ± 1	5.75 ± 0.2	0.13 ± 0.01	5.73 ± 0.50	0.13 ± 0.01	69.8 ± 3.6	1.62 ± 0.12
7 (12)	55 ± 4	9.53 ± 0.9	0.53 ± 0.07	6.62 ± 0.07	0.37 ± 0.03	75.3 ± 4.3	4.18 ± 0.32
15 (6)	210 ± 40	5.32 ± 1.2	1.20 ± 0.30	4.03 ± 1.10	0.80 ± 0.20	104.6 ± 13.5	22.5 ± 3.60
60 (10)	240 ± 40	4.40 ± 1.1	1.10 ± 0.28	1.98 ± 0.22	0.49 ± 0.05	126.4 ± 25.4	32.0 ± 6.20
225 (16)	240 ± 50	2.70 ± 0.50	0.82 ± 0.28	2.40 ± 0.25	0.59 ± 0.09	107.4 ± 17.3	25.20 ± 4.600
750 (17)	290 ± 30*	5.41 ± 0.90*	1.60 ± 0.16*	2.76 ± 0.26	0.79 ± 0.07*	114.5 ± 15.4	32.62 ± 3.01*

All the values are expressed in mg and as mean ± S.D. The number of samples analyzed at each stage is indicate parentheses. For other details please see text.

* These values are significantly different from the corresponding values at 225 days of age ($P < 0.001$).

activity during the life span of rat cerebellum confirming our earlier similar findings in the case of chick cerebellum (Subba Rao and Shrivastaw, 1976). The above observation is also in good agreement with the other findings with mouse cerebellum (Caron and Unsworth, 1978).

Highest specific activity of acid DNase was found on the 1st day postnatal. The values decreased steadily with increasing age up to 225 days. But there was a significant increase in this value by the time the animal became 750 days old, although this value was much lower than the activities observed during early developmental stages (days 1 and 7). A similar increase in the total activity of acid DNase between 225 and 750 days could also be seen and the value at 750 days was the highest (Table 12). Previous studies from this laboratory with chick cerebellum showed that there was no increase of either the specific activity or of total activity of acid DNase in old age (Subba Rao and Shrivastaw, 1976). It thus appears that there is species variation as far as the activity of acid DNase in aging brain is concerned.

On the other hand, highest specific activity and total activity of alkaline DNase were observed in aging cerebellum (Table 12). The specific activity remained at the same level from day 1 to 225 days with an approximately 100% increase between 225 and 750 days. A similar magnitude of increase is also found in total activity during this period. The values between 15 and 225 days showed some fluctuations but it should be noted that the value at 750 days were significantly higher even when compared to the earlier highest activity found at 60 days. It, therefore appears that alkaline

TABLE 12. ACID AND ALKALINE DNase ACTIVITIES IN DEVELOPING AND AGING RAT CEREBELLUM

AGE IN DAYS	ACID DNase		ALKALINE DNase	
	Sp. Activity	Total activity	Sp. Activity	Total Activity
1 (6)	17.0 ± 1.4	27.7 ± 3.4	8.7 ± 0.3	14.0 ± 1.0
6 (12)	15.7 ± 1.5	65.7 ± 5.3	9.3 ± 1.0	38.9 ± 5.9
15 (6)	4.9 ± 0.6	110.0 ± 27.0	7.6 ± 2.3	204.2 ± 50.9
60 (10)	3.0 ± 0.7	92.5 ± 12.4	8.1 ± 1.3	264.8 ± 47.2
225 (16)	3.2 ± 0.6	81.3 ± 1.6	7.8 ± 1.6	200.4 ± 36.6
750 (17)	5.5 ± 0.7*	176.5 ± 23.0*	14.6 ± 3.1*	445.0 ± 107.0*

Specific activity is expressed as μg of DNA-P liberated into acid soluble fraction/mg protein / 2 hr. Total activity is obtained by multiplying the sp. activity with total amount of protein in mg. For other details please see Table 11.* These values are significantly different from the corresponding values at 225 days ($P < 0.001$).

TABLE 13. PROTEIN/DNA, RNA/DNA and ACTIVITIES OF DNases/mg OF DNA IN DEVELOPING AND AGING RAT CEREBELLUM

AGE IN DAYS	PROTEIN/DNA	RNA/DNA	ACID DNases / mg/DNA	ALKALINE DNases / mg/DNA
1 (6)	11.9 ± 0.6	1.0 ± 0.1	203.5 ± 22.2	102.2 ± 3.9
7 (12)	8.0 ± 0.06*	0.7 ± 0.06*	131.8 ± 14.1*	73.6 ± 7.3*
15 (6)	20.7 ± 4.8	0.8 ± 0.2	98.2 ± 16.0	139.0 ± 40.2
60 (10)	30.7 ± 4.1	0.7 ± 0.2	94.8 ± 13.4	258.6 ± 64.6
225 (16)	28.8 ± 5.0	0.7 ± 0.2	106.0 ± 22.0	213.9 ± 65.0
750 (17)	21.1 ± 2.4**	0.5 ± 0.1	112.6 ± 14.5	316.6 ± 41.7**

All the values are expressed as means + S.D. For other details please see Table 11.

* These values are significantly different from the corresponding values at 1 day of age ($P < 0.001$).

** These values are significantly different from the corresponding values at 225 days of age $P < 0.001$).

DNase may have an important role in aging cerebellum. These observations are once again in line with our results with chick brain (Shrivastaw and Subba Rao, 1975; Subba Rao and Shrivastaw, 1976).

The ratios of protein, RNA and the activities of DNases to DNA are presented in Table 13, since such an expression would indicate indirectly the increase in cell size and the enzyme activities per cell. There was actually a decrease in protein/DNA and RNA/DNA values between days 1 and 7 thereby indicating once again that the rate of DNA accumulation (hence cell proliferation) was higher than the rate of either protein or RNA accumulation (cell size increase) during this period. From the 7th day onwards both protein/DNA and RNA/DNA increased and these adult levels were maintained up to 225 days. Between 225 and 750 days there was a small but statistically significant decrease in protein/DNA values whereas the RNA/DNA ratio showed no change (although there was an apparent difference between the values, this was not statistically significant). The absence of any difference in RNA to DNA ratio between adult and old cerebellum was in agreement with the earlier findings with mouse cerebellum (Chaconas and Finch, 1973; Somarjski and Rolsten, 1973). However, the studies on RNA/DNA ratios in different regions (except corpus striatum) of rat brain by Shaskan (1977) showed an increase in this ratio between the adult and senescent rat brains. But, in this study the cerebellum was not analyzed while the present investigation deals exclusively with the changes in cerebellar region and it is therefore difficult to compare the two data. The decreased protein/DNA value with a simultaneous increase in total DNA content observed in the present studies (Tables 11 & 13) in aging cerebellum strongly suggest a rapid cell proliferating activity during this period.

Acid DNase activity per mg of DNA was highest at the earliest age period studied with a decrease of this value to a lower adult level subsequently. No change in this adult level activity could be noticed during old age (Table 13). However, alkaline DNase activity expressed per mg of DNA was highest in old age (750 days). There was a significant increase in this activity between 225 and 750 days, thereby indicating that this enzyme protein is probably synthesized in larger amounts in aging cerebellum. On the basis of these results, it is tempting to speculate that acid and alkaline DNases, in particular the latter may be playing a vital role in aging rat cerebellum.

CHAPTER IV

Effect of early postnatal nutritional deprivation on DNA, RNA protein and DNases in different regions of rat brain

CHAPTER IV

EFFECT OF EARLY POSTNATAL NUTRITIONAL DEPRIVATION ON DNA RNA, PROTEIN AND DNases IN DIFFERENT REGIONS OF RAT BRAIN

PART A

CEREBRUM (White and grey matter regions)

INTRODUCTION

A considerable body of evidence has accumulated in recent years to show that calorie/protein deprivation during early stages (critical growth period) of development leads to permanent biochemical deficits in brain in various species of animals (Culley and Mertz, 1965; Dobbing and Widowson, 1965; Benton, et al, 1966; Culley and Lineberger, 1968; Winick et al, 1968; Guthrie and Brown, 1968; Winick and Rosso, 1975; Cheek et al, 1976; Simsons and Johnston, 1976; Griffin et al, 1977 Subba Rao, 1979). However, these earlier studies, as well as some of the recent observations in this laboratory, also revealed that the timing and duration of both nutritional restriction and rehabilitation are important determining factors for the recovery of the brain to normalcy. Moreover, considerable plasticity or adaptive ability is exhibited by the brain with respect to the activities of some enzymes like DNases when exposed to nutritional stress, and this may vary from

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Part A of this chapter is written based on the paper entitled "Differential effects of early undernutrition in white and grey matter regions of rat brain", published in J. Neurosci. Res., 7 (1982) 279-287., and Part B based on the publication in J. Biosci., 4 (1982) 391-400, entitled "DNA, RNA, protein and DNases in developing rat cerebellum: Effects of early postnatal nutritional deprivation".

one region to the other depending upon the stage of development that the particular region is in (Subba Rao et al, 1978; Subba Rao, et al, 1980).

White matter region is composed of glial cells and myelinated axonal processes. The growth and development of this region occurs largely after birth in rat. On the other hand, grey matter composed of mostly neurons and some astroglial cells, is a region whose growth and development could be considered almost complete by birth in this species (Brizzee et al, 1964; Altman, 1969). We have therefore extended our earlier studies to examine the biochemical effects of early postnatal undernutrition and subsequent rehabilitation on white and grey matter regions separately. It is shown here that white matter is extremely vulnerable to nutritional stress during the weaning period but grey matter is largely resistant.

MATERIALS AND METHODS

Rats were fed on Hindustan Lever (New Delhi, India) "Rat and Mice Feed", which is complete in all nutritional aspects. On the day of birth, pups were assigned to mothers in predetermined numbers. The control group (A) had 6-8 pups with the mother and the undernourished (R) group had 18-20 pups with the mother. Undernutrition was imposed on rats from the day of birth to varying periods, viz., 10, 15 and 21 days. After this restricted period, the animals were rehabilitated to normal conditions by decreasing the litter size during the preweaning stage and by feeding ad lib. during the post weaning period. The rehabilitation was continued upto the 150th day. Such groups are designated as $R_{10}A_{150}$; $R_{15}A_{150}$,

etc., the first figure indicating the day up to which nutritional restriction was imposed and the second figure indicating the day upto which rehabilitation was carried out.

Animals were sacrificed by decapitation at various stages of life. Cerebral hemispheres were carefully removed from the brain (i.e., whole brain excluding brain stem, cerebellum and optic lobes). Starting from the dorsal side, the outer grey matter was gently scraped off with a scalpel, until no more grey matter could be visualized. Then the underlying white matter portion was collected separately. The whole separation was carried out at 0-4°C. After a few trial experiments the separation was achieved with consistency and reliability, as could be seen from the values reported in Table 14. During the early stages (10 days), the whole cerebrum was taken for the study, as white matter could not be separated effectively because of extremely low amounts. At 15 days, white matter from three or four brains was pooled in order to get sufficient material. The tissue was homogenized with a Potter-Elvehjem homogeniser in nine volumes of cold distilled water. Immediately a portion was taken for the extraction of DNA and RNA according to the procedure of Schmidt and Thannhauser (1945), except that the digestion was carried out at 37°C for 2 hr. DNA and RNA were estimated by measuring the UV absorption at 260 nm. Acid and alkaline DNases were assayed as described by McDonald (1955), by measurement of the increase in UV absorption at 260 nm of the acid soluble fraction following incubation. Protein was estimated by Lowry's method (1951). Phosphorus was measured by the procedure of Bartlett (1959).

Highly polymerized calf thymus DNA, yeast RNA, and bovine serum albumin were purchased from Sigma Chemical Company, St. Louis, MO, USA. All the reagents used were of analytical grade. All the results are subjected to statistical treatment according to the Student's 't' test.

RESULTS

Tables 14 and 15 show the effect of early postnatal undernutrition on wet weight and DNA content of white and grey matter regions. At 10 days, the whole cerebrum was used for analysis. Nutritional deprivation produced significant reduction in the weight even at 10 days, and with continued deprivation up to 15 and 21 days a clear-cut difference in the way the two regions are affected could be seen. Only the white matter content was reduced both at 15 and 21 days (30-50%) and no effect at all could be noticed in grey matter. Rehabilitation of the animals from the 10th, 15th and 21st days until 150 days resulted in recovery of the white matter to varying extents depending upon the time of initiation of rehabilitation. Maximum recovery was seen, as could be expected, in those animals rehabilitated from 10th day onwards ($R_{10}A_{150}$) (Table 14). The present results thus point out that the reduced brain weight in undernourished rats observed earlier in this laboratory as well as by other workers (Subba Rao *et al*, 1978, 1980; Winick *et al*, 1968; Culley and Lineberger, 1968; Altman *et al*, 1971) could actually be due to the reduced white matter content.

In line with the white matter content the DNA levels in white matter are also significantly reduced in undernourished animals at 15 and 21 days of age (Table 15). The reduction at 21 days is slightly more than

GROUP	WET WEIGHT OF THE REGION (gm)	
	CEREBRUM	
A_{10}**	0.58 ± 0.06	
R_{10}**	0.47 ± 0.03*	
	White	Grey
A_{15}	0.13 ± 0.015	0.55 ± 0.06
R_{15}	0.09 ± 0.010*	0.51 ± 0.04
A_{21}	0.18 ± 0.010	0.63 ± 0.09
R_{21}	0.09 ± 0.009*	0.61 ± 0.09
A_{150}	0.38 ± 0.050	0.80 ± 0.05
R_{10} A_{150}	0.34 ± 0.050	0.74 ± 0.08
R_{15} A_{150}	0.28 ± 0.025*	0.72 ± 0.05
R_{21} A_{150}	0.29 ± 0.030*	0.85 ± 0.05

A = Normal rats, R = Nutritionally restricted rats. The subscript indicates the postnatal day up to which the treatment is operative. For example, R_{15} A_{150} indicates that the animals are on restricted diet up to the 15th day, but from then onwards up to the 150th day food was allowed ad. lib. The number of animals in each group varied from 4 to 10. All the values are expressed as means ± S.D.

* These values are significantly different from the age-matched controls ($P < 0.005$).

** At 10 days, cerebrum as such was taken for the study as it was not possible to separate white and grey matter regions.

REHABILITATION ON DNA CONTENT OF WHITE AND GREY MATTER REGIONS IN RAT BRAIN

GROUP	CEREBRUM			
	DNA/ g.tissue		DNA/ region	
A_{10}	1.80 ± 0.20		1.00 ± 0.20	
R_{10}	2.00 ± 0.30		0.96 ± 0.15	
	White	Grey	White	Grey
A_{15}	0.94 ± 0.17	1.40 ± 0.16	0.12 ± 0.02	0.74 ± 0.09
R_{15}	0.93 ± 0.14	1.70 ± 0.24	0.08 ± 0.01*	0.75 ± 0.12
A_{21}	1.47 ± 0.04	1.45 ± 0.25	0.27 ± 0.02	0.85 ± 0.08
R_{21}	1.25 ± 0.04	1.40 ± 0.09	0.12 ± 0.01*	0.83 ± 0.05
A_{150}	1.44 ± 0.20	1.50 ± 0.14	0.54 ± 0.09	1.10 ± 0.16
R_{10} A_{150}	2.10 ± 0.20*	2.50 ± 0.20*	0.70 ± 0.07*	1.70 ± 0.12*
R_{15} A_{150}	1.50 ± 0.50	2.0 ± 0.19*	0.43 ± 0.04*	1.50 ± 0.20*
R_{21} A_{150}	1.50 ± 0.10	1.80 ± 0.20	0.40 ± 0.04*	1.50 ± 0.05*

All the values are expressed in mg and as means ± S.D. For other details please see Table 14.

* These values are significantly different from the corresponding age-matched controls ($P < 0.005$).

50%. However, rehabilitation started from the 10th day onwards and up to 150 days of age resulted in more than complete recovery ($R_{10}A_{150}$). It can also be seen that if the rehabilitation was initiated at a later date, i.e., either from 15th or 21st day, full recovery was not possible (groups $R_{15}A_{150}$, $R_{21}A_{150}$). Grey matter DNA content was not affected by postnatal undernutrition but here also, as in the case of white matter, rats undernourished up to the 10th day and rehabilitated subsequently showed higher amounts of DNA than did the control animals.

The effects of undernutrition on RNA and protein content of white and grey matter portions of rat brain are presented in Tables 16 & 17. Once again, only in white matter, a significant reduction of RNA and protein contents could be seen. Grey matter was unaffected. When the animals were rehabilitated either from the 10th or 15th day, the levels of RNA in white matter could be brought back to normal. However, if the rehabilitation started from weaning stage (21 days) the recovery of both RNA and protein contents was only partial and some 25-35% of the deficit still remained. Further, it is to be mentioned here that when animals were rehabilitated from the 10th day onwards the recovery of RNA level but not of protein was more than normal suggesting that as far as the RNA syntheis is concerned a more than necessary catch-up is operative but such a compensatory mechanism does not seem to operate for protein synthesis.

The concentration of DNA, RNA and protein per gram of both regions were also calculated and shown in Tables 15, 16 & 17. They were in general found to be unaltered as a result of undernutrition, since the

REHABILITATION ON RNA CONTENT OF WHITE AND GREY MATTER REGIONS IN RAT BRAIN

GROUP	CEREBRUM			
	RNA/ g. tissue		RNA/ region	
A_{10}	4.78 ± 0.37		2.73 ± 0.44	
R_{10}	4.61 ± 0.75		2.14 ± 0.26*	
	White	Grey	White	Grey
A_{15}	2.9 ± 0.18	3.9 ± 0.50	0.38 ± 0.05	2.10 ± 0.18
R_{15}	2.6 ± 0.30	4.1 ± 0.45	0.23 ± 0.03*	2.10 ± 0.28
A_{21}	3.5 ± 0.50	3.7 ± 0.20	0.56 ± 0.08	2.30 ± 0.10
R_{21}	3.6 ± 0.28	3.7 ± 0.22	0.40 ± 0.12*	2.20 ± 0.10
A_{150}	1.5 ± 0.12	2.0 ± 0.20	0.60 ± 0.08	1.60 ± 0.10
R_{10} A_{150}	2.4 ± 0.16*	3.0 ± 0.30*	0.82 ± 0.14*	2.10 ± 0.25*
R_{15} A_{150}	2.2 ± 0.20*	2.9 ± 0.20*	0.60 ± 0.06	2.00 ± 0.20*
R_{21} A_{150}	1.6 ± 0.07	2.1 ± 0.20	0.45 ± 0.03*	1.80 ± 0.25

All the values are expressed in mg and as means ± S.D. For other details please see under Table 14.

* These values are significantly different from the corresponding age-matched controls ($P < 0.001$).

REHABILITATION ON PROTEIN CONTENT OF WHITE AND GREY MATTER REGIONS IN RAT BRAIN

GROUP	CEREBRUM			
	Protein/g. tissue		Protein/ region	
A_{10}	92.5 ± 16.8		53.2 ± 11.6	
R_{10}	82.7 ± 17.8		39.0 ± 8.3*	
	White	Grey	White	Grey
A_{15}	75.3 ± 9.1	88.3 ± 7.2	9.70 ± 0.77	46.1 ± 3.4
R_{15}	55.0 ± 8.0*	97.5 ± 8.6	5.00 ± 0.15*	52.0 ± 4.0
A_{21}	116.0 ± 7.4	102.0 ± 15.0	21.00 ± 1.35	64.0 ± 8.8
R_{21}	109.0 ± 6.6	86.0 ± 9.0	9.90 ± 1.10*	52.0 ± 5.7
A_{150}	93.5 ± 11.0	109.8 ± 7.2	38.00 ± 5.30	85.0 ± 8.2
$R_{10}\ A_{150}$	96.3 ± 3.3	98.5 ± 5.2	33.00 ± 3.70	72.0 ± 9.3
$R_{15}\ A_{150}$	89.0 ± 9.3	102.8 ± 5.3	25.00 ± 3.70*	73.6 ± 5.0
$R_{21}\ A_{150}$	83.2 ± 6.6	99.0 ± 6.6	24.10 ± 2.90*	85.5 ± 6.6

All the values are expressed in mg and as means ± S.D. For other details please see Table 14.

* These values are significantly different from the corresponding age-matched controls ($P < 0.005$).

reduction in the wet weights and the levels of the nucleic acids and protein were parallel.

Since earlier studies from this laboratory have revealed a positive correlation between DNA content and the activities of two putative DNA degrading enzymes in chick and rat brain (Shrivastaw and Subba Rao, 1975; Subba Rao and Subba Rao, 1982 a), we have measured acid and alkaline DNase activities in the present group of rats. Tables 18 & 19 show the results obtained. The specific activity of acid DNase (Table 18) did not change as a result of postnatal undernutrition either in white matter or grey matter. The total activity, however, showed significant decrease in white matter of undernourished brains at 15 and 21 days of postnatal age. In the case of the grey matter region significant reduction in total acid DNase could be noticed only when the undernutrition was continued from birth to 21 days. In all these cases rehabilitation beginning from the 10th or 15th day up to the 150th day resulted in activities that are markedly higher than those noticed in corresponding age-matched controls.

The results concerning the alkaline DNase activity are similar to those for acid DNase in that the specific activity of this enzyme is unaffected by nutritional deprivation (Table 19). If anything, at 21 days the undernourished group exhibited higher specific activities than did the control animals. The trend was the same in both the regions studied. Even with respect to the total activity, only in white matter in the undernourished group at 15 days was a significant reduction observed. Interestingly, when the nutritional stress was continued up to 21 days the activities in the deprived

TABLE 18. EFFECT OF EARLY POSTNATAL UNDERNUTRITION AND SUBSEQUENT REHABILITATION ON THE ACTIVITY OF ACID DNase IN WHITE AND GREY MATTER REGIONS OF RAT BRAIN

GROUP	CEREBRUM			
	Specific Activity		Total Activity	
A_{10}	6.92 ± 1.90		338.12 ± 50.6	
R_{10}	7.80 ± 2.10		289.70 ± 31.0*	
	White	Grey	White	Grey
A_{15}	7.7 ± 1.3	7.2 ± 0.86	77.5 ± 16.2	310.8 ± 33.5
R_{15}	9.8 ± 1.7	8.6 ± 1.10	49.9 ± 7.4*	408.7 ± 40.8
A_{21}	7.4 ± 0.5	8.0 ± 1.30	155.2 ± 8.0*	499.3 ± 68.0
R_{21}	6.8 ± 0.5	6.9 ± 0.55	66.7 ± 7.8*	355.2 ± 23.5*
A_{150}	4.7 ± 0.5	4.9 ± 0.80	146.5 ± 14.4	388.0 ± 45.0
R_{10} A_{150}	11.0 ± 0.5*	10.5 ± 0.70*	356.0 ± 25.2*	692.0 ± 75.0*
R_{15} A_{150}	10.2 ± 0.9*	12.0 ± 1.60*	248.0 ± 155.0*	870.0 ± 155.0 *
R_{21} A_{150}	5.8 ± 0.8	5.8 ± 0.50	136.6 ± 17.7	479.5 ± 62.8

Specific activity is expressed as μg of acid-soluble DNA-P liberated/mg protein/ 2 hr. Total activity is obtained by multiplying the specific activity with the protein in mg in the region. All the values are expressed as means ± S.D. For other details please see Table 14.

*All these values are significantly different from the corresponding age-matched controls ($P < 0.005$).

TABLE 19. EFFECT OF EARLY POSTNATAL UNDERNUTRITION AND SUBSEQUENT REHABILITATION ON THE ACTIVITY OF ALKALINE DNase IN WHITE AND GREY MATTER REGIONS OF RAT BRAIN

GROUP	CEREBRUM			
	Specific Activity		Total Activity	
A_{10}	5.75 ± 1.4		292.70 ± 47.5	
R_{10}	8.46 ± 2.4		312.33 ± 76.6	
	White	Grey	White	Grey
A_{15}	14.0 ± 1.8	10.3 ± 1.7	129.0 ± 21.0	496.6 ± 60.2
R_{15}	16.8 ± 3.8	10.8 ± 2.0	83.0 ± 18.0*	417.0 ± 91.2
A_{21}	9.8 ± 1.0	14.0 ± 2.5	204.8 ± 12.9	796.5 ± 142.0
R_{21}	24.7 ± 1.1*	19.5 ± 2.4	244.7 ± 27.0	997.8 ± 8.0
A_{150}	5.3 ± 0.8	5.2 ± 0.9	212.3 ± 34.0	495.6 ± 37.4
R_{10} A_{150}	14.7 ± 2.1*	12.0 ± 0.8*	412.0 ± 44.0*	795.6 ± 32.2**
R_{15} A_{150}	14.1 ± 1.9*	12.6 ± 1.2*	353.8 ± 37.6*	910.0 ± 81.0**
R_{21} A_{150}	7.9 ± 0.7*	7.3 ± 0.7*	190.0 ± 24.2	632.0 ± 78.0**

Specific activity is expressed as µg of acid-soluble DNA-P liberated/mg protein/ 2 hr. Total activity is obtained by multiplying the specific activity with the protein in mg in the region. All the values are expressed as means ± S.D. For other details please see Table 14.

*All these values are significantly different from the corresponding age-matched controls ($P < 0.005$).

TABLE 20. EFFECT OF EARLY POSTNATAL UNDERNUTRITION AND SUBSEQUENT REHABILITATION ON THE ACTIVITIES OF ACID AND ALKALINE DNases EXPRESSED PER mg DNA IN WHITE AND GREY MATTER REGIONS OF RAT BRAIN

GROUP	CEREBRUM			
	Acid DNase		Alkaline DNase	
A_{10}	357.4 ± 38.0		297.6 ± 33.3	
R_{10}	294.9 ± 25.6		342.3 ± 89.7	
	White	Grey	White	Grey
A_{15}	725.6 ± 164.6	447.8 ± 57.2	1187.3 ± 144.8	642.0 ± 121.8
R_{15}	605.3 ± 55.3	478.2 ± 65.6	992.0 ± 55.3	576.6 ± 95.8
A_{21}	586.1 ± 56.1	556.1 ± 59.2	779.3 ± 113.6	899.0 ± 78.2
R_{21}	578.5 ± 34.5	429.5 ± 47.9	2157.5 ± 127.9*	1201.5 ± 77.3*
A_{150}	290.0 ± 24.5	415.8 ± 79.5	388.7 ± 51.6	458.1 ± 83.5
R_{10} A_{150}	513.3 ± 51.9*	416.8 ± 10.9	710.6 ± 103.4*	482.8 ± 67.5
R_{15} A_{150}	618.4 ± 102.4*	598.0 ± 96.2*	923.3 ± 152.4*	656.3 ± 106.5*
R_{21} A_{150}	345.2 ± 18.4	331.0 ± 30.0	446.6 ± 36.5	506.6 ± 38.2

All the values are the means ± S.D and expressed as μg of DNA-P liberated into acid soluble fraction/mg DNA/2 hr. For other details please see under Table 14.

*All these values are significantly different from the corresponding age-matched controls ($P < 0.005$).

group were significantly higher than in the normals. Rehabilitation of the deprived animals starting from 10th or 15th day through 150 days resulted in higher amounts of the enzyme.

Since early undernutrition followed by rehabilitation resulted in higher amounts of DNases, the activities of these enzymes are calculated per milligram of DNA and presented in Table 20. Such an expression expected to reveal whether each individual cell in the rehabilitated brains actually has higher amounts of these enzymes. As can be seen, the rehabilitated groups ($R_{10}A_{150}$; $R_{15}A_{150}$) particularly in white matter, show significantly higher activities of both acid and alkaline DNases. It is to be noted that the recovery in the $R_{15}A_{150}$ group is better than in the $R_{10}A_{150}$ group.

DISCUSSION

The main purpose of the present investigation was to examine whether postnatal undernutrition would affect both white and grey matter regions of rat brain to a similar extent. Two phenomena became apparent from the results obtained. First, there is a marked difference between the two regions in their susceptibility to nutritional deprivation. While grey matter is unaffected by postnatal calorie deprivation, white matter appears to be quite vulnerable under this condition. Thus, in respect to the various biochemical parameters studied, viz., wet weight, DNA, RNA, and protein content, it is in white matter that significant and marked reductions were noticed (Tables 15, 16 & 17). These effects, although apparent at 10 days, could be distinctly seen from the 15th day onwards. The present results also show that timely rehabilitation (from 10 days onwards) for a sufficient

period can correct these deficits. The differential behaviour of white and grey matter regions when exposed to postnatal nutritional stress could probably be explained on the basis of the cell types they are composed of and the replication schedule of such cells. White matter is largely composed of oligodendrocytes and astroglia along with the axonal processes, whereas grey matter can be considered a region largely made up of neurons and some astrocytes. Since neurons are believed to reach the postmitotic stage by birth in rat and glial cells particularly the oligodendrocytes, are known to proliferate postnatally in this species (Brizzee et al, 1964; Altman, 1969), the present results can be considered to be in full confirmity with the "vulnerable period" hypothesis of Dobbing (1972) as well as with the postulation of Winick and Coworkers (1968, 1970).

Since oligodendrocytes are the cells responsible for myelin synthesis, the present results also substantiate earlier findings on the decreased synthesis and content of myelin as a result of postnatal protein or calorie deficiency (Benton et al, 1966; Chase et al, 1967; Fishman et al, 1971; Nakhasi etal, 1975; Krigman and Hogan, 1976; Wiggins et al, 1976; Figlewicz et al, 1978; Kim and Pleasure, 1978). The present results are also in good agreement with the recent studies on different types of lipids in white and grey matter regions of undernourished rat brain (Reddy and Horrocks, 1982; Reddy et al, 1982). However, our results are in disagreement with those of Bass et al., (1970), who did not find any decrease in DNA content of the white matter region of rat brain in response to weaning undernutrition. The reasons for this discrepancy are not known.

The second phenomenon that was observed from these studies, probably of considerable importance, is the preferential way in which the acid and alkaline DNase activities are retained in both the regions of undernourished rat brain. In particular, the alkaline DNase activity seems to be conserved against the limited energy and protein available to the brain under experimental conditions. Thus the results shown in Tables 19 & 20 clearly indicate that the levels of this enzyme protein are maintained normally, and the adaptive or compensatory mechanism could be clearly seen when rats were exposed to undernutrition during the first 10 or 15 days after birth and then rehabilitated up to the 150th day ($R_{10}A_{150}$, $R_{15}A_{150}$). At this age both regions exhibited higher specific activities as well as total activities when compared to a normal brain of similar age. Although DNases are supposed to be primarily degradative in function, it is now suspected that these enzymes might be playing some important role either in the synthesis or repair of DNA (Laskowski, 1967; Lehman, 1967; Bernardi, 1971). The present results certainly adduce support for such an important role for these enzymes.

PART B

CEREBELLUM

INTRODUCTION

Considerable evidence has accumulated in recent years to show that calorie/protein deprivation during early stages of development ('critical growth period') would lead to permanent biochemical deficiencies in the brain of various species of animals (Subba Rao, 1979; Subba Rao et al, 1980). However, these studies were confined mostly to the changes in whole brain. It is becoming increasingly evident that different regions of brain have different schedules of development. Previous studies from this laboratory have shown developmental differences between grey and white matter regions (Subba Rao and Subba Rao, 1982 a) and also the differential effects on these two regions of early undernutrition and subsequent rehabilitation (Subba Rao and Subba Rao 1982 b).

The cerebellar region of the rat brain is known to develop during the early part of the postnatal period and hence represents another area of brain having a developmental time schedule different from both grey and white matter regions. We have therefore extended our earlier studies (Subba Rao and Subba Rao, 1982 b) to examine certain biochemical parameters in rat cerebellum exposed to early postnatal undernutrition and subsequent rehabilitation. It is shown here that early undernutrition decreases the DNA, RNA and protein contents significantly in the cerebellar region whereas the specific activities of acid and alkaline DNases were unaffected. Rehabilitation of the undernourished animals up to 150 days corrected the deficiencies.

MATERIALS AND METHODS

Materials and Methods are same as described in Part A.

RESULTS AND DISCUSSION

Table 21 shows the effect of early undernutrition on the wet weight and DNA contents of rat cerebellum. As can be seen, nutritional deprivation has significantly reduced the wet weight even at 10 days. Prolonged nutritional deprivation up to 21 days has no further effect on the wet weight. Rehabilitation of these undernourished groups, R_{10} and R_{15} up to 50 days has brought back the deficits to normalcy, whereas if the rehabilitation was initiated at later date i.e., from 21 days postnatal (R_{21}) full recovery was not possible at 50 days. However, longer rehabilitation up to 150 days did rectify the deficits in the wet weights.

In line with the wet weights, the DNA content is also significantly reduced by early postnatal undernutrition. At 21 days postnatal, the undernourished group has only 50% of DNA as that of age matched control. However, the concentration of DNA expressed per gram of tissue, was not effected by undernutrition at 10 and 15 days (Table 21), whereas significant reduction in the concentration of DNA could be observed at 21 days. These results are in agreement with the earlier studies (Culley and Lineberger, 1968; Winick, 1970; Balazs and Patel, 1973; Gopinath et al, 1976). As could be expected, the earlier the initiation of rehabilitation, the maximum is the recovery seen. Thus when the R_{10} group was rehabilitated up to 21 days postnatal, the DNA values recovered close to normal values whereas R_{15} and R_{21} groups have recovered to complete normalcy only after rehabilitating

TABLE 21. EFFECT OF EARLY [illegible]
ON WET WEIGHT AND DNA CONTENTS OF RAT CEREBELLUM

Group	Wet Weight	DNA (mg)	
		Per g.tissue	Per region
A_{10}	67 ± 10	6.4 ± 0.67	0.46 ± 0.13
R_{10}	41 ± 4*	8.6 ± 1.06	0.35 ± 0.05
A_{15}	90 ± 7	10.4 ± 2.9	0.97 ± 0.18
R_{15}	67 ± 12*	8.5 ± 1.0	0.60 ± 0.16*
R_{10} A_{15}	80 ± 7	8.9 ± 3.1	0.68 ± 0.20
A_{21}	135 ± 7	10.4 ± 2.8	1.35 ± 0.32
R_{21}	100 ± 12*	6.1 ± 0.62**	0.62 ± 0.101*
R_{10} A_{21}	120 ± 10	9.6 ± 2.4	1.20 ± 0.38
R_{15} A_{21}	100 ± 10	10.0 ± 0.8	1.10 ± 0.19
A_{50}	180 ± 10	6.9 ± 0.6	1.30 ± 0.15
R_{10} A_{50}	170 ± 10	9.7 ± 1.0**	1.70 ± 0.22*
R_{15} A_{50}	190 ± 10	7.0 ± 1.0	1.30 ± 0.10
R_{21} A_{50}	160 ± 8*	6.8 ± 1.0	1.10 ± 0.10
A_{150}	200 ± 30	5.1 ± 0.26	1.10 ± 0.1[illegible]
R_{10} A_{150}	180 ± 10	10.0 ± 1.6*	1.60 ± 0.1[illegible]
R_{15} A_{150}	190 ± 10	7.2 ± 1.0	1.25 ± 0.13
R_{21} A_{150}	170 ± 10	7.4 ± 0.5	1.25 ± 0.19

All the values are expressed as the means ± S.D. The number of samples analyzed at various ages varied from 6 to 10.

* All these values are significantly different from the corresponding age-matched controls (P 0.001).

** All these values are significantly different from the corresponding age-matched controls ($P < 0.005$).
A = Normal rats, R = Nutritionally restricted rats. The subscript indicates the postnatal day up to which the treatment is operative. For example R_{15} A_{150} indicates that the animals are on restricted diet up to the 15th day, but from then onwards up to the 150th day food was allowed ad. lib.

up to 50 days and 150 days respectively. It can also be noted that when the R_{10} group was rehabilitated either up to 50 days or 150 days both the concentrations as well as the total DNA contents showed remarkable recovery to values that were significantly higher as compared to the age matched controls.

Table 22 shows the effect of early postnatal undernutrition followed by rehabilitation on RNA and protein contents of rat cerebellum. It is clear, once again, that RNA and protein contents in undernourished rats have reduced significantly. However, the concentration of RNA was not affected by undernutrition whereas the protein concentration was significantly reduced in 21 days undernourished rats (about 30%). Rehabilitation of these undernourished groups R_{10}, R_{15} and R_{21} for different periods yielded varying extents of recovery of RNA and protein depending on the initiation and duration of nutritional deprivation, and of rehabilitation. It can also be noted that the protein contents were significantly low in $R_{21}A_{50}$ group however, rehabilitation up to 150 days ($R_{21}A_{150}$) resulted in full recovery. The RNA contents in rehabilitated groups ($R_{10}A_{150}$, $R_{15}A_{150}$) are significantly higher as compared to the age matched controls. The above results show that as far as the DNA and RNA synthesis is concerned more than necessary catch up is operative and such a compensatory mechanism does not seem to operate, however, in the case of protein.

Since earlier studies from this laboratory have revealed a positive correlation between DNA content and the activities of two putative DNA degrading enzymes, acid and alkaline DNases in chick and rat brain (Shrivastaw

TABLE 22. EFFECT OF EARLY POSTNATAL UNDERNUTRITION AND SUBSEQUENT REHABILITATION ON RNA AND PROTEIN CONTENTS OF RAT CEREBELLUM

GROUP	RNA (mg)		PROTEIN (mg)	
	Per g. tissue	Per region	Per g. tissue	Per region
A_{10}	7.44 ± 0.96	0.50 ± 0.08	117.0 ± 15.3	7.43 ± 1.0
R_{10}	6.13 ± 1.0	0.26 ± 0.06*	99.3 ± 10.3	4.14 ± 0.63*
A_{15}	5.82 ± 0.65	0.55 ± 0.07	116.0 ± 33.0	12.60 ± 2.80
R_{15}	6.20 ± 1.0	0.40 ± 0.07*	124.0 ± 30.0	7.60 ± 1.27*
R_{10} A_{15}	6.10 ± 1.0	0.48 ± 0.09	103.0 ± 10.8	8.20 ± 1.4
A_{21}	5.34 ± 0.38	0.70 ± 0.04	121.8 ± 23.3	15.53 ± 2.6
R_{21}	4.65 ± 0.57	0.46 ± 0.10	73.3 ± 7.5*	7.35 ± 1.45*
R_{10} A_{21}	5.50 ± 1.0	0.63 ± 0.10	108.0 ± 12.2	12.0 ± 2.2
R_{15} A_{21}	5.30 ± 0.80	0.60 ± 0.10	107.0 ± 11.2	13.7 ± 2.1
A_{50}	3.10 ± 0.30	0.55 ± 0.03	133.0 ± 3.3	23.7 ± 2.3
R_{10} A_{50}	4.00 ± 0.20*	0.70 ± 0.03*	148.0 ± 0.6	25.6 ± 0.7
R_{15} A_{50}	3.10 ± 0.20	0.59 ± 0.05	124.0 ± 4.6	23.4 ± 1.6
R_{21} A_{50}	3.10 ± 0.30	0.50 ± 0.06	76.0 ± 10.1*	12.0 ± 1.9*
A_{150}	2.50 ± 0.20	0.50 ± 0.04	126.0 ± 9.6	22.8 ± 1.6
R_{10} A_{150}	3.70 ± 0.40*	0.60 ± 0.06*	106.0 ± 8.6	19.1 ± 1.9
R_{15} A_{150}	3.50 ± 0.30*	0.64 ± 0.06*	140.0 ± 16.6	25.0 ± 3.1
R_{21} A_{150}	3.10 ± 0.20	0.53 ± 0.02	120.0 ± 15.1	20.1 ± 3.5

All the values are expressed as the means ± S.D. For other details please see Table 21.

*All these values are significantly different from the corresponding age-matched controls ($P < 0.00$

and Subba Rao, 1975; Subba Rao and Subba Rao, 1982 a), we have also measured the activities of these enzymes in the present group of rats. Tables 23 & 24 show the results obtained. The specific activity of acid DNase (Table 23) did not change as a result of postnatal undernutrition. The total activities, however, showed significant decrease in undernourished rat cerebellum at 15 and 21 days of postnatal age. In all these cases rehabilitation from 10th, 15th or 21st day up to 150 days resulted in activities (both specific as well as total) which are markedly higher than those noticed in corresponding age-matched controls. The results concerning the alkaline DNase activity (Table 24) are similar to that of acid DNase. However, in the case of alkaline DNase, the rehabilitation of groups R_{10} and R_{15} to 50 days ($R_{10}A_{50}$, $R_{15}A_{50}$) could itself bring significantly higher activities (both specific and total) as compared to the corresponding age-matched controls. These results confirm the earlier observation from this laboratory on white and grey matter regions of rat brain (Subba Rao and Subba Rao, 1982 b). It is of considerable importance to note the way in which both the acid and alkaline DNase activities have been retained in the undernourished rat cerebellum. In particular, the alkaline DNase activity seems to be conserved against the limited energy and protein available to the brain under experimental conditions. As can be seen from Table 24, this enzyme level is markedly higher in rehabilitated animals ($R_{10}A_{50}$) which clearly suggests preferential synthesis of this enzyme during rehabilitation. Although DNases are supposed to be primarily degradative nature in function, it is suspected now that these enzymes might be playing some important role either in the synthesis or repair of DNA. Thus earlier studies by Allfrey and Mirsky (1962), Gautier and Leonard (1962) have shown high levels of cellular DNases

TABLE 23. EFFECT OF EARLY POSTNATAL UNDERNUTRITION AND SUBSEQUENT REHABILITATION ON THE ACTIVITY OF ACID DNase IN RAT CEREBELLUM

GROUP	ACID DNase	
	Specific activity	Total activity
A_{10}	8.2 + 0.4	61.1 ± 10.8
R_{10}	10.4 + 2.2	42.6 ± 11.9
A_{15}	8.3 ± 2.2	103.8 ± 15.5
R_{15}	9.0 ± 2.3	68.1 ± 13.7**
R_{10} A_{15}	8.6 + 1.7	69.0 ± 7.4
A_{21}	8.6 ± 1.4	125.0 ± 19.2
R_{21}	10.6 ± 2.4	81.3 ± 21.8**
R_{10} A_{21}	8.0 ± 1.1	106.0 ± 29.0
R_{15} A_{21}	6.6. ± 1.0	85.7 ± 18.4**
A_{50}	7.2 ± 1.4	170.0 ± 22.4
R_{10} A_{50}	9.2 ± 0.5	234.0 ± 8.5
R_{15} A_{50}	6.8 + 0.6	156.0 ± 10.7
R_{21} A_{50}	11.5 ± 0.8*	136.0 ± 16.0
A_{150}	3.9 ± 0.5	118.0 ± 17.0
R_{10} A_{150}	10.4 ± 1.1*	177.0 ± 25.6*
R_{15} A_{150}	7.5 ± 1.9*	188.0 ± 28.7*
R_{21} A_{150}	7.1 - 1.2*	139.0 ± 10.5*

All the values are expressed as the means ± S.D. Specific Activity is defined as μg of DNA-P liberated/mg protein/2 hr. Total activity = Specific activity × Total protein in mg. For other details please see Table 21.

* All these values are significantly different from the corresponding age-matched controls ($P < 0.001$).

**All these values are significantly different from the corresponding age-matched controls $P < 0.005$).

TABLE 24. EFFECT OF EARLY POSTNATAL UNDERNUTRITION AND SUBSEQUENT REHABILITATION ON THE ACTIVITY OF ALKALINE DNase IN RAT CEREBELLUM

GROUP	ALKALINE DNase	
	Specific activity	Total activity
A_{10}	7.9 ± 1.4	50.6 ± 7.9
R_{10}	9.8 ± 3.0	39.0 ± 11.0
A_{15}	13.6 ± 3.6	179.1 ± 25.0
R_{15}	11.7 ± 2.8	87.2 ± 23.2*
$R_{10} A_{15}$	13.7 ± 3.2	104.0 ± 15.9*
A_{21}	12.8 ± 3.0	212.0 ± 37.5
R_{21}	16.5 ± 5.3	123.0 ± 31.0*
$R_{10} A_{21}$	12.8 ± 1.5	145.3 ± 25.4
$R_{15} A_{21}$	11.3 ± 1.8	146.5 ± 20.4
A_{50}	6.6 ± 1.0	147.0 ± 11.3
$R_{10} A_{50}$	23.6 ± 0.7*	588.0 ± 13.2*
$R_{15} A_{50}$	10.1 ± 0.8*	235.0 ± 14.7*
$R_{21} A_{50}$	14.3 ± 1.2*	157.8 ± 17.0
A_{150}	6.0 ± 0.6	143.0 ± 14.7
$R_{10} A_{150}$	16.8 ± 2.2*	298.0 ± 39.5*
$R_{15} A_{150}$	13.4 ± 2.8*	317.0 ± 34.6*
$R_{21} A_{150}$	11.7 ± 1.6*	227.0 ± 15.0*

All the values are expressed as the means ± S.D. Specific activity is defined as μg of DNA-P liberated/mg protein/2 hr. Total activity = Specific activity x Total protein in mg. For other details please see Table 21.

*All these values are significantly different from the corresponding age-matched controls ($P < 0.001$).

in wide variety of organisms during the interval in the growth cycle when DNA synthesis is proceeding at maximal rate. Studies with purified DNA polymerase Kornberg, 1964) revealed that nucleases can profoundly affect the template by providing required nicks, hence the rate of cell proliferation. It has been also shown by Yagi and Okamura (1965) that DNases serve for the excision of lesions introduced into the DNA as a result of exposure to UV irradiation or alkylating reagents thus permitting the repair of the impaired nucleic acid. On the basis of these experiments it has been proposed by Lehman (1967) that DNases might be playing a crucial role either in synthesis or repair of DNA. Later experiments by Bernardi (1971) and Slor et al.,(1973 support the above contention. Our present results also support such a concept.

We have shown earlier that grey matter is unaffected and white matter is most affected by early undernutrition. The present studies further point out the intermediary nature of the cerebellar region in the developmental schedule and its response to nutritional deprivation and subsequent rehabilitation. Thus the cerebellar region is affected by calorie restriction but proper rehabilitation could correct the defects.

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CHAPTER V

Increased DNA polymerase β-activity in different regions of aging rat brain

CHAPTER V

INCREASED DNA POLYMERASE β-ACTIVITY IN DIFFERENT REGIONS OF AGING RAT BRAIN

INTRODUCTION

Previous studies from this laboratory have shown that the DNA content of both rat (Subba Rao and Subba Rao, 1982 a) and chick (Shrivastaw and Subba Rao, 1975) brain, contrary to the earlier belief, goes up significantly during the later stages of the life span. The increase in DNA content in aging brain could be due to one or more of the following possibilities: (a) replication of glial cells, (b) repair of DNA in both neuronal as well as glial cells, and (c) an increase in the intracellular DNA not necessarily connected with the replication process. In any of these possibilities, a common biochemical feature that must be expressed in such cells is an increase in DNA polymerase activity. We have, therefore, examined the DNA polymerase activity in different regions of brain at various stages of the life span of the rat. Efforts were also made to distinguish between α and β polymerase activities making use of specific inhibitors. It is the purpose of this study to present evidence to show that after reaching a low adult level, DNA polymerase exhibits a second peak of activity around 1½ years of age. It is also shown that the increased activity is that of polymerase β-type.

MATERIALS AND METHODS

(Methyl-^{3}H)d TTP (Sp. Act. 46 Ci/m mole) was purchased from the Radiochemical Centre, Amersham, England. Unlabelled nucleotides dATP, dGTP, dCTP and dTTP were purchased from Sigma Chemical Company,

St. Louis, USA. 2', 3' dideoxy thymidine triphosphate (ddTTP) was purchased from P.L. Biochemicals. Aphidicolin was a gift from Dr. A.H. Todd of I.C.I. Ltd., U.K. All the other chemicals used were of analytical grade.

Tissue samples and preparation of homogenates

Rats were obtained from the animal house of the University and killed by decapitation. Cerebral hemispheres and cerebellum were removed and white and grey matter regions of cerebral hemispheres were separated. Cerebrum as such was taken for the study at 16th day prenatal and 7th day postnatal as it was not possible to separate white and grey matter regions at these ages. All the operations were carried out at 0 to 4°C. Ten percent homogenates of these regions were prepared by homogenizing the tissue in 0.02M Tris-HCl buffer pH 7.5 containing 0.1 mM β-mercapto-ethanol, 1 mM $MgCl_2$, 0.1 mM EDTA, 5% glycerol, 1% Triton X-100, and 0.5 M KCl. After the homogenization, the sample was kept at 0 to 4°C for 1 hr and centrifuged at 1,00,000 g for 1 hr. The clear supernatant thus obtained was used as the source of DNA polymerase. An aliquot of the supernatant was taken for the protein estimation according to Lowry's method (1951).

DNA polymerase assay

The reaction mixture contained in a total volume of 50 µl, 40 mM Tris-HCl pH 8.0, 1 mM BME, 7.5 mM $MgCl_2$, 4 mM ATP, 10 µg of 'activated DNA', 0.1 mM each of dATP, dGTP, dCTP and 20 µM of dTTP (1 µCi). Incubation was carried out at 37°C for 20 min. At the end of the incubation, 1 mg of DNA as a carrier was added and the reaction was stopped by adding

2 ml of cold 10% TCA. The samples were kept in ice for 10 min and centrifuged at 4,000 rpm. The precipitate thus obtained was washed thrice with 5% cold TCA and thrice with 95% ethanol. The precipitate after washings, was dissolved in 0.1 ml of 0.05 M NaOH and aliquots were taken into radioactive vials containing 10 ml of Bray's mixture and were counted in a Beckman LS-3133P liquid scintillation counter. The enzyme activity was linear upto 60 ug of protein. Specific activity was expressed as picomoles of TMP incorporated into DNA per mg protein per hour.

Studies on some of the properties of DNA polymerase in supernatants revealed that the activity of the enzyme was linear upto 150 µg in the case of young and up to 60 µg in the case of old. 20 µM TTP was found to be optimal for polymerase activity both in the case of young and old. Effect of aphidicolin on DNA polymerase has shown that 10 µM concentration (ratio of aphidicolin to dCTP, 2) reduces the DNA polymerase activity significantly which remains more or less same even at a concentration of 20 µM. 500 µM concentration of ddTTP (ratio of ddTTP to dTTP, 100) was shown to inhibit DNA polymerase to maximum extent, which remained at same level even at 1 mM concentration (Fig 11 to 18).

RESULTS AND DISCUSSION

The specific activity of DNA polymerase in different regions (white, grey and cerebellum) of developing and aging rat brain are presented in Table 25. As can be seen, the activity of DNA polymerase in cerebrum was highest at 16th day prenatal, the earliest time period studied, whereas the maximum activity in cerebellum was observed on 7th day postnatal.

Fig. 11. Effect of enzyme concentration on the DNA polymerase activity in young (7 day old) and old (540 days) rat cerebrum.

Fig.11

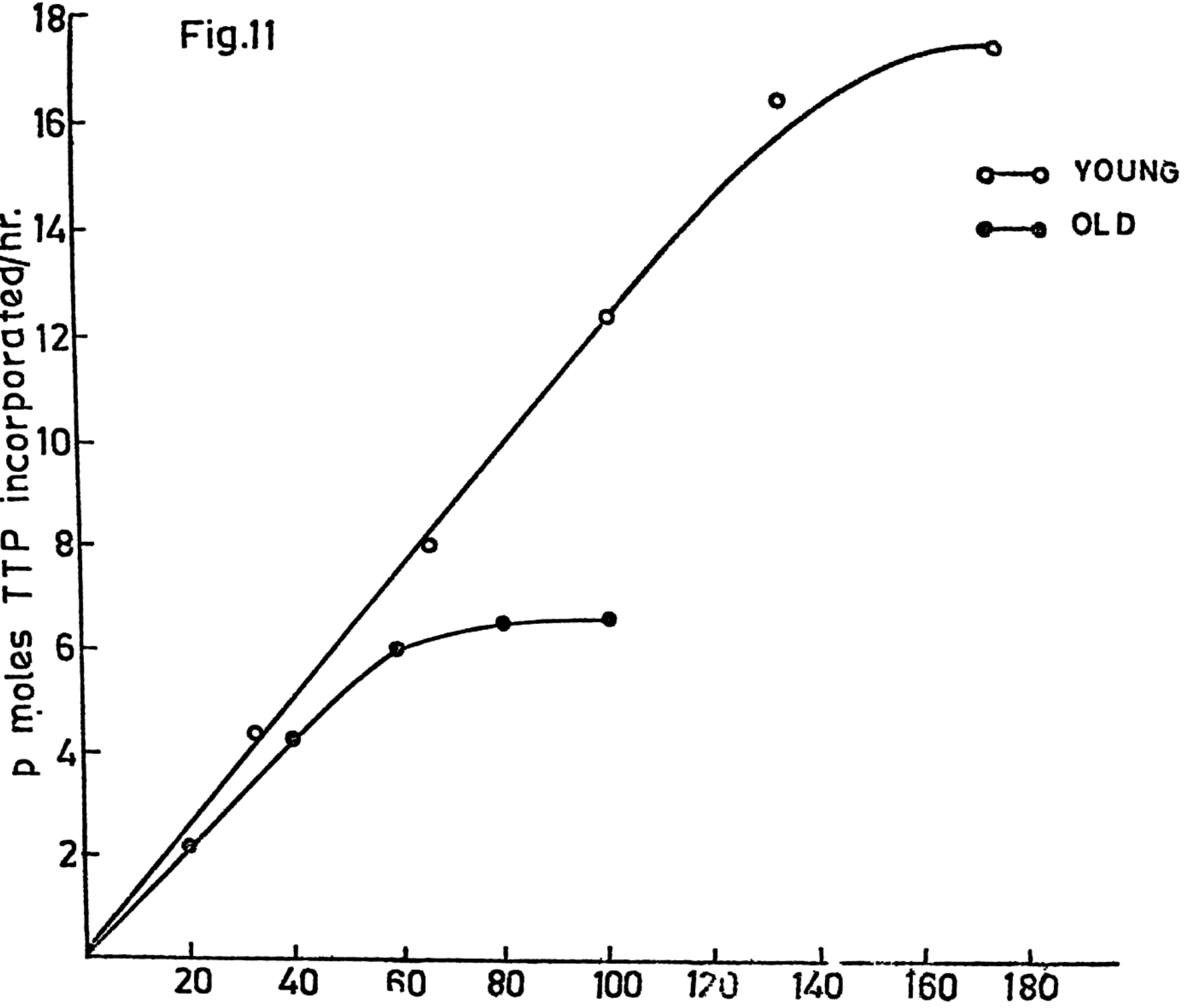
18
16
14
12
10
8
6
4
2
p moles TTP incorporated/hr.
20
40
60
80
100
120
140
160
180
YOUNG
OLD

Fig. 12. Effect of substrate concentration on the DNA polymerase activity in young (7 day old) and old (540 days) rat cerebrum. Each point represents the average of two experiments. Enzyme protein, 100 to 120 μg in the case of young and 50 to 60 μg in the case of old, was used.

Fig.12

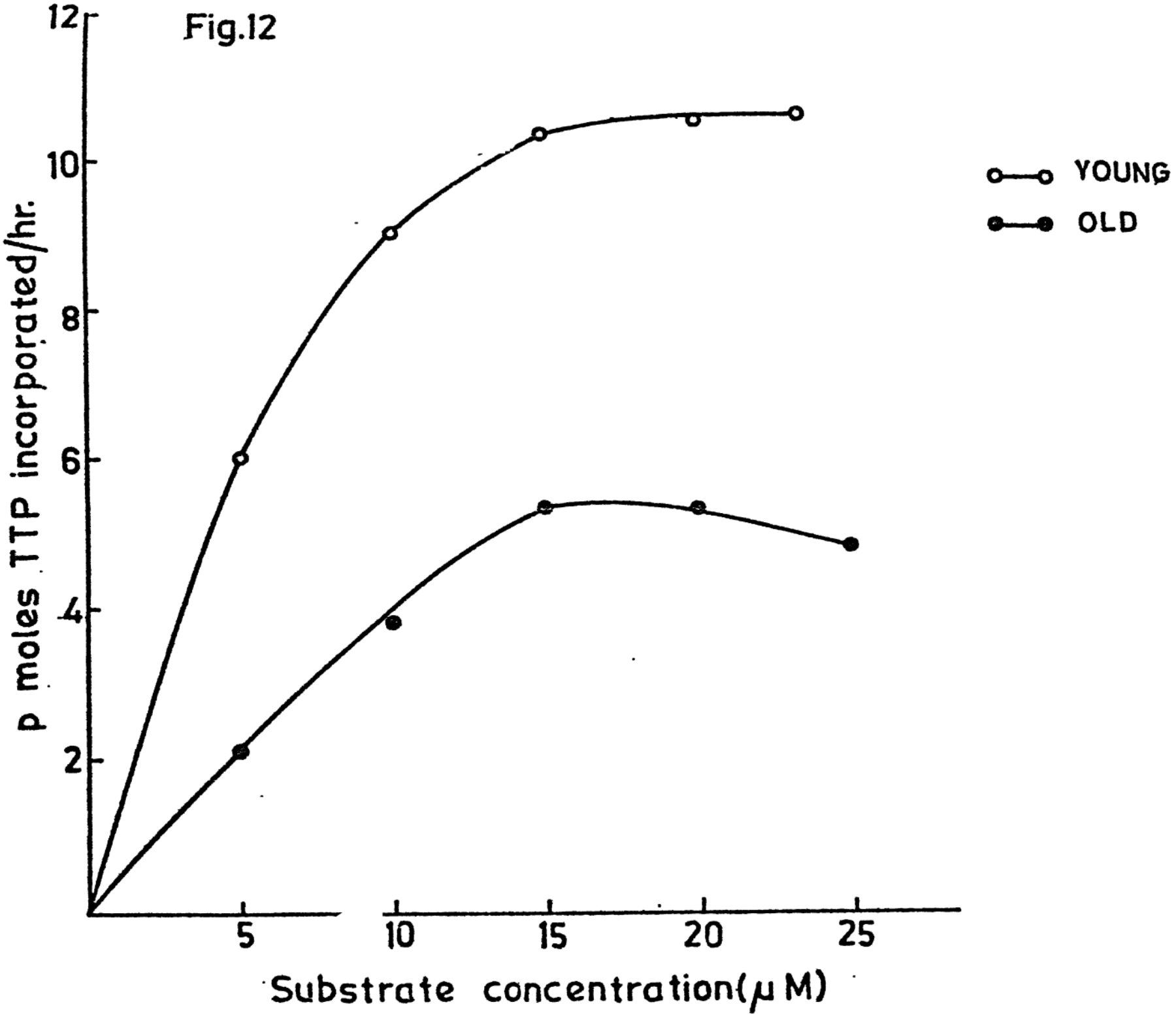
12
10
8
6
4
2
p moles TTP incorporated/hr.
5
10
15
20
25
Substrate concentration(μ M)
YOUNG
OLD

Fig. 13 Effect of substrate concentration on the DNA polymerase activity (Lineweaver-Burk plot, assuming the molecular weight of calf thymus to be 8.6×10^6).

Fig.13

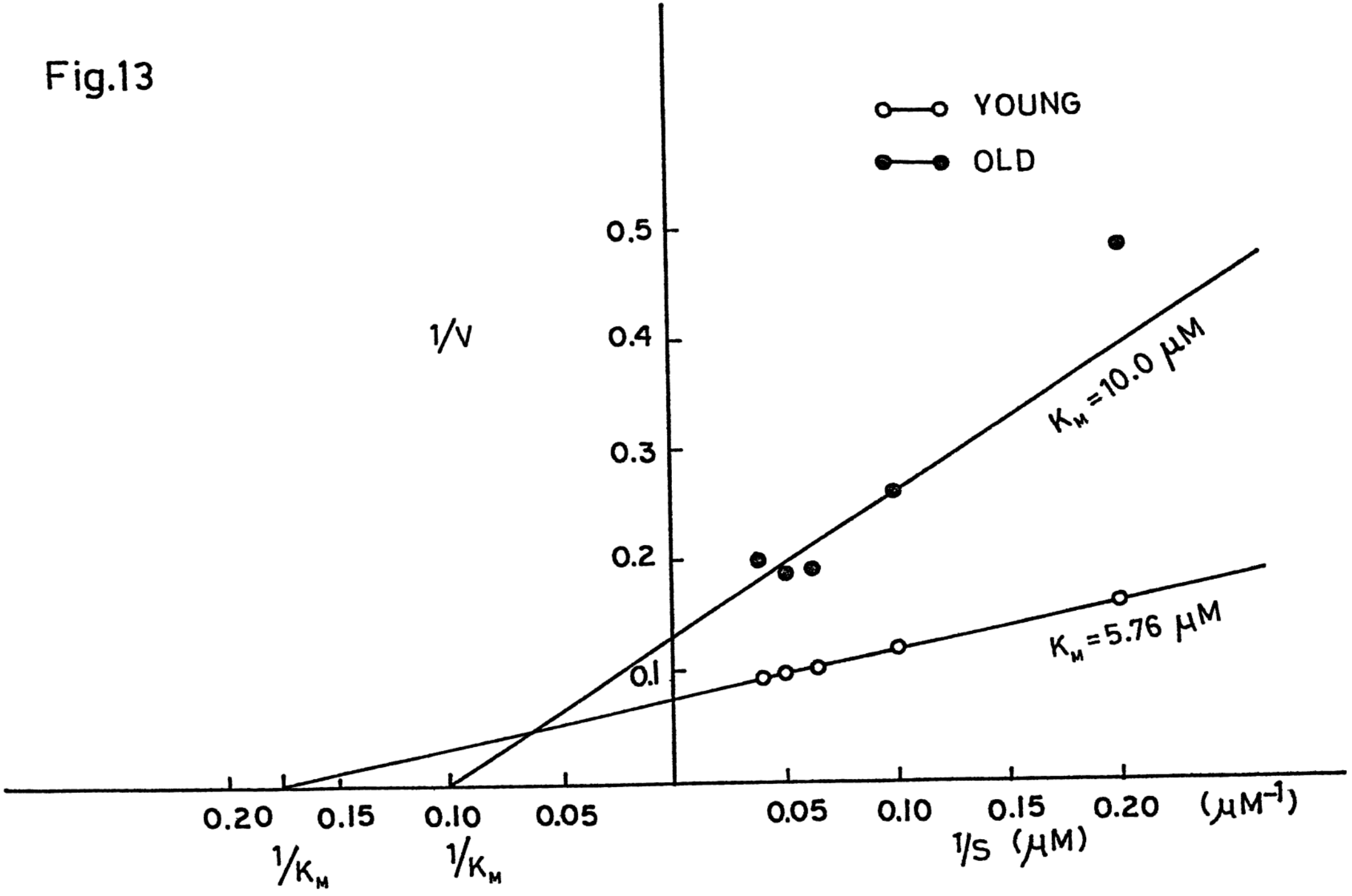
YOUNG
OLD
1/V
0.5
0.4
0.3
0.2
0.1
K_M=10.0 μM
K_M=5.76 μM
0.20
0.15
0.10
0.05
1/K_M
1/K_M
0.05
0.10
0.15
0.20
(μM^{-1})
1/S (μM)

Fig. 14. Effect of aphidicolin on the DNA polymerase activity in 7 day old rat cerebrum. Each point represents the average of two experiments. Enzyme protein used was 120-130 μg.

Fig.14

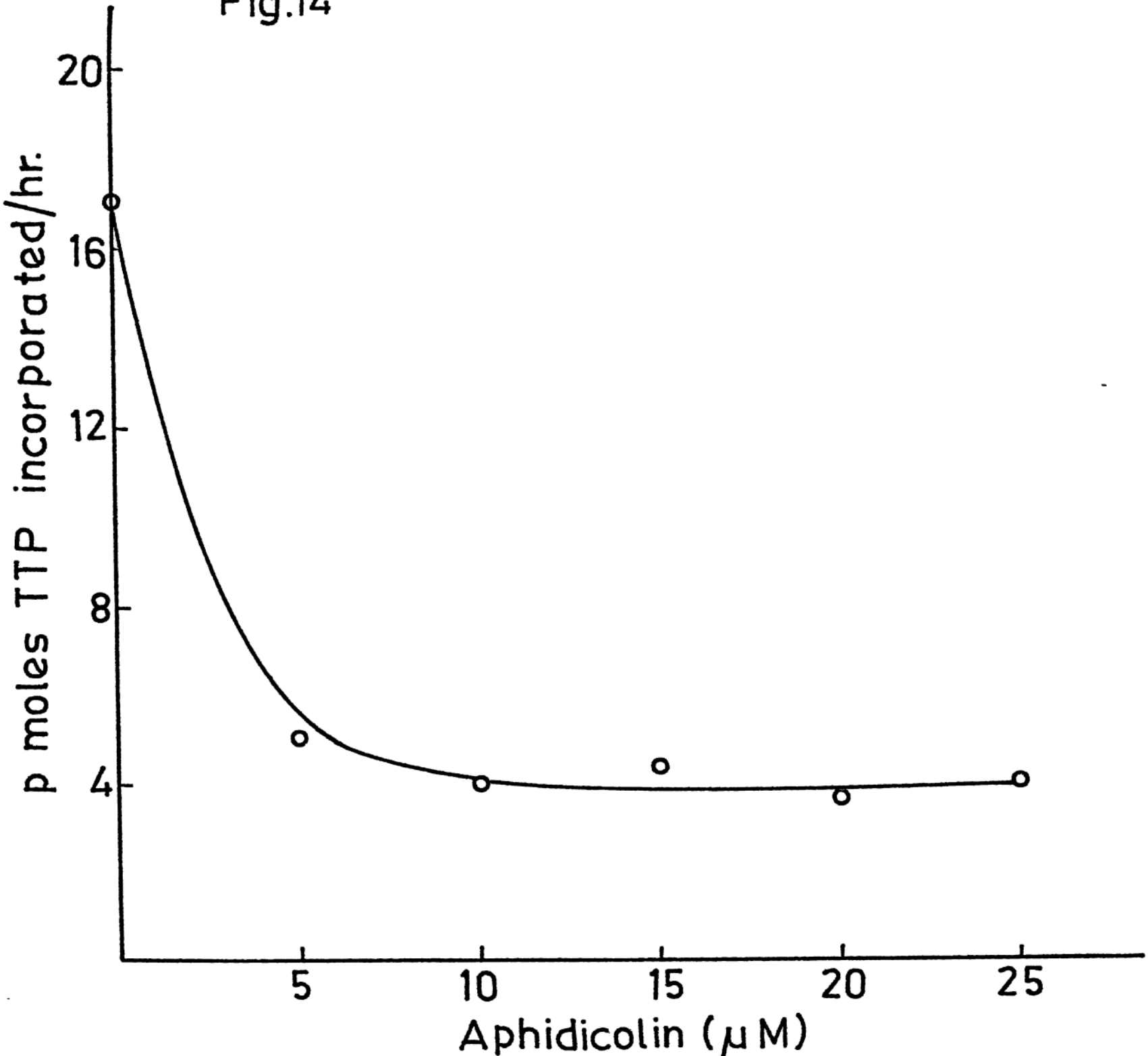
20
16
12
8
4
p moles TTP incorporated/hr.
5
10
15
20
25
Aphidicolin (μM)

Fig. 15. Effect of ddTTP on the DNA polymerase activity in 7 day old rat cerebrum. Each point represents the average of two experiments. Enzyme protein used was 120 to 130 μg.

Fig.15

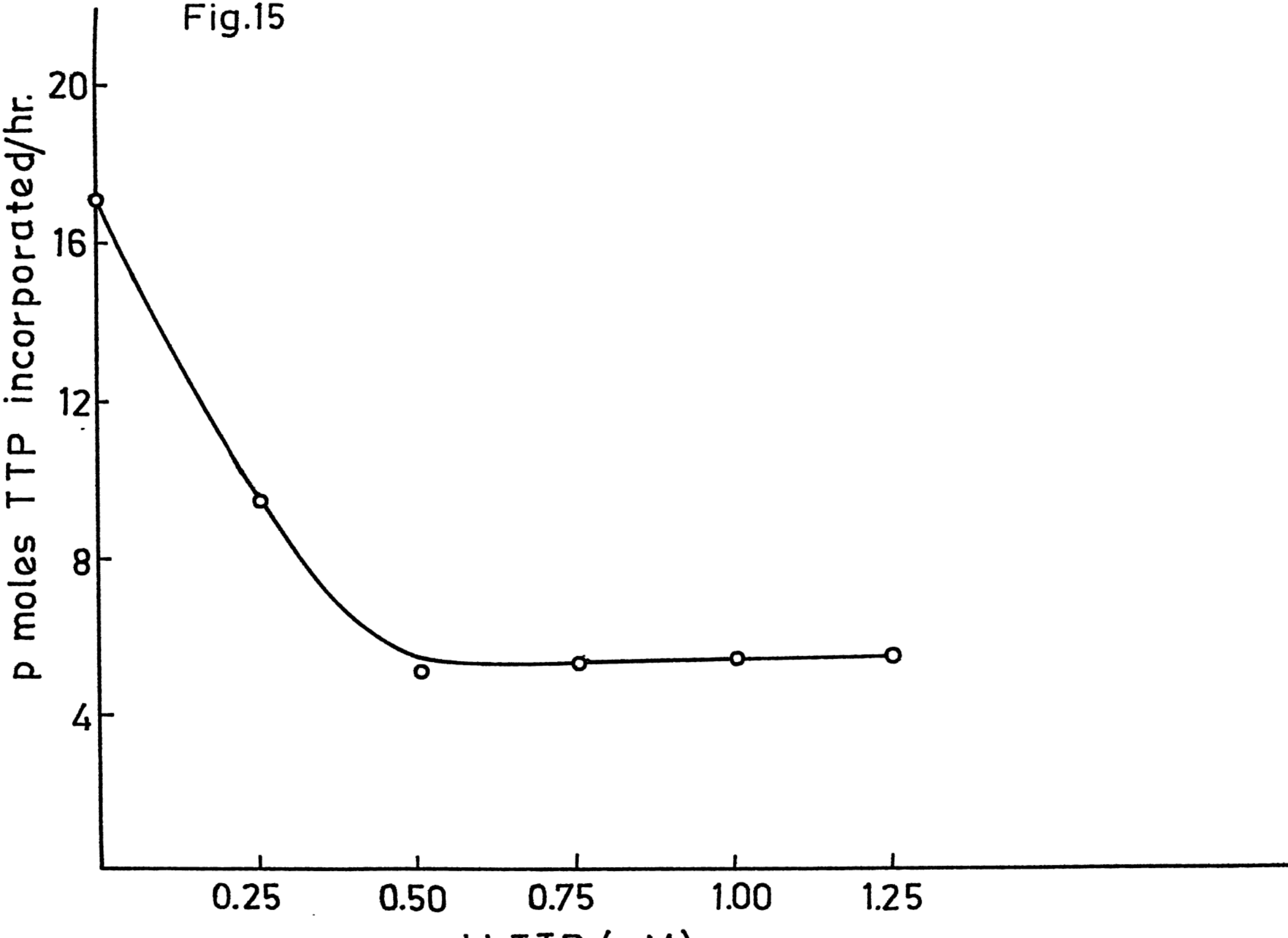

Fig. 16. Effect of susbtrate concentration on the DNA polymerase activity in 7 day old cerebrum in the presence of aphidicolin. Each point represents the average of two experiments. Enzyme protein used was 90 to 100 μg.

Fig.16

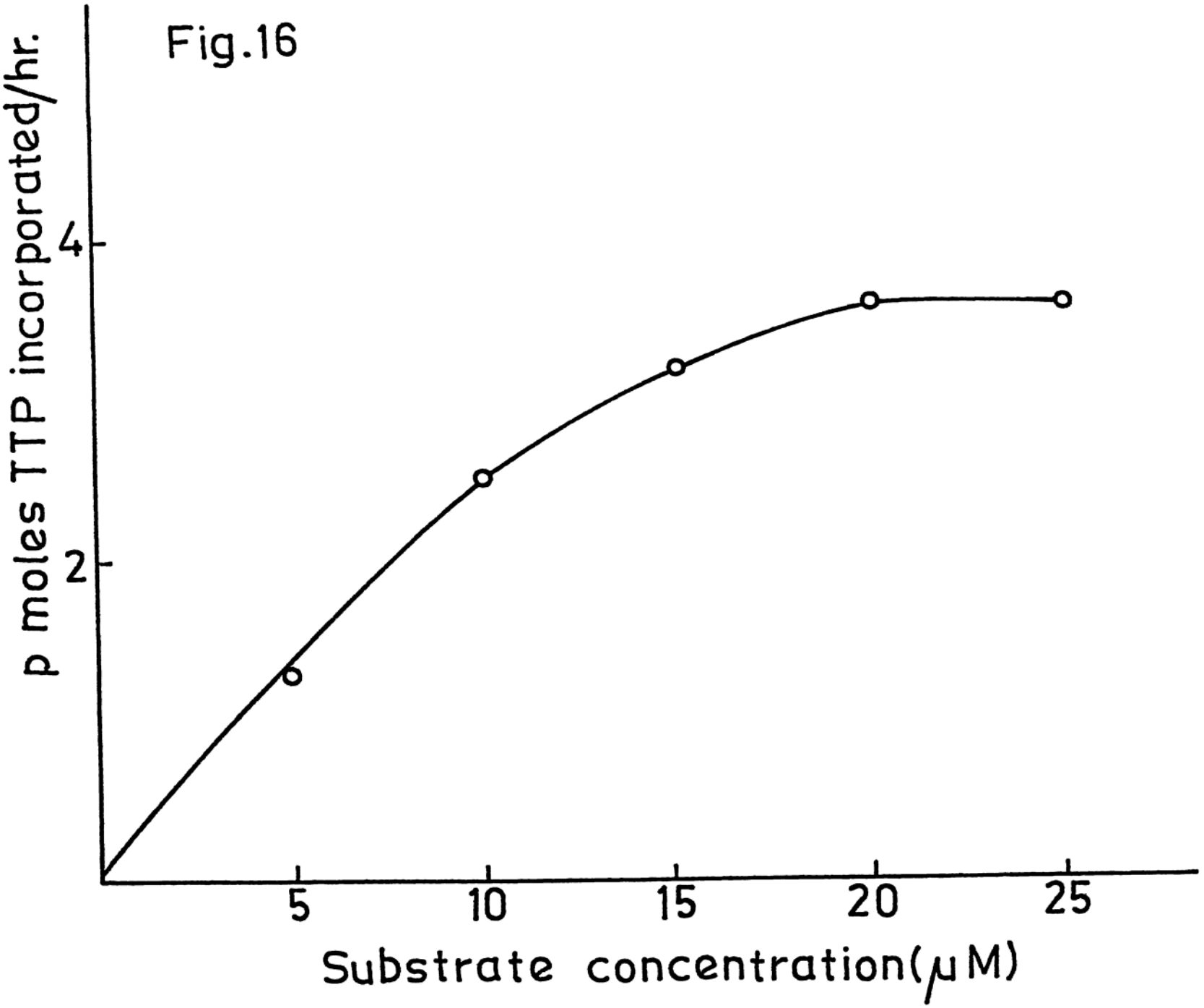
p moles TTP incorporated/hr.
4
2
5
10
15
20
25
Substrate concentration(μM)

Fig. 17. Effect of substrate concentration on DNA polymerase activity in 7 day old cerebrum in the presence of ddTTP. Each point represents the average of two experiments. Enzyme protein used was 90 to 100 μg.

Fig.17

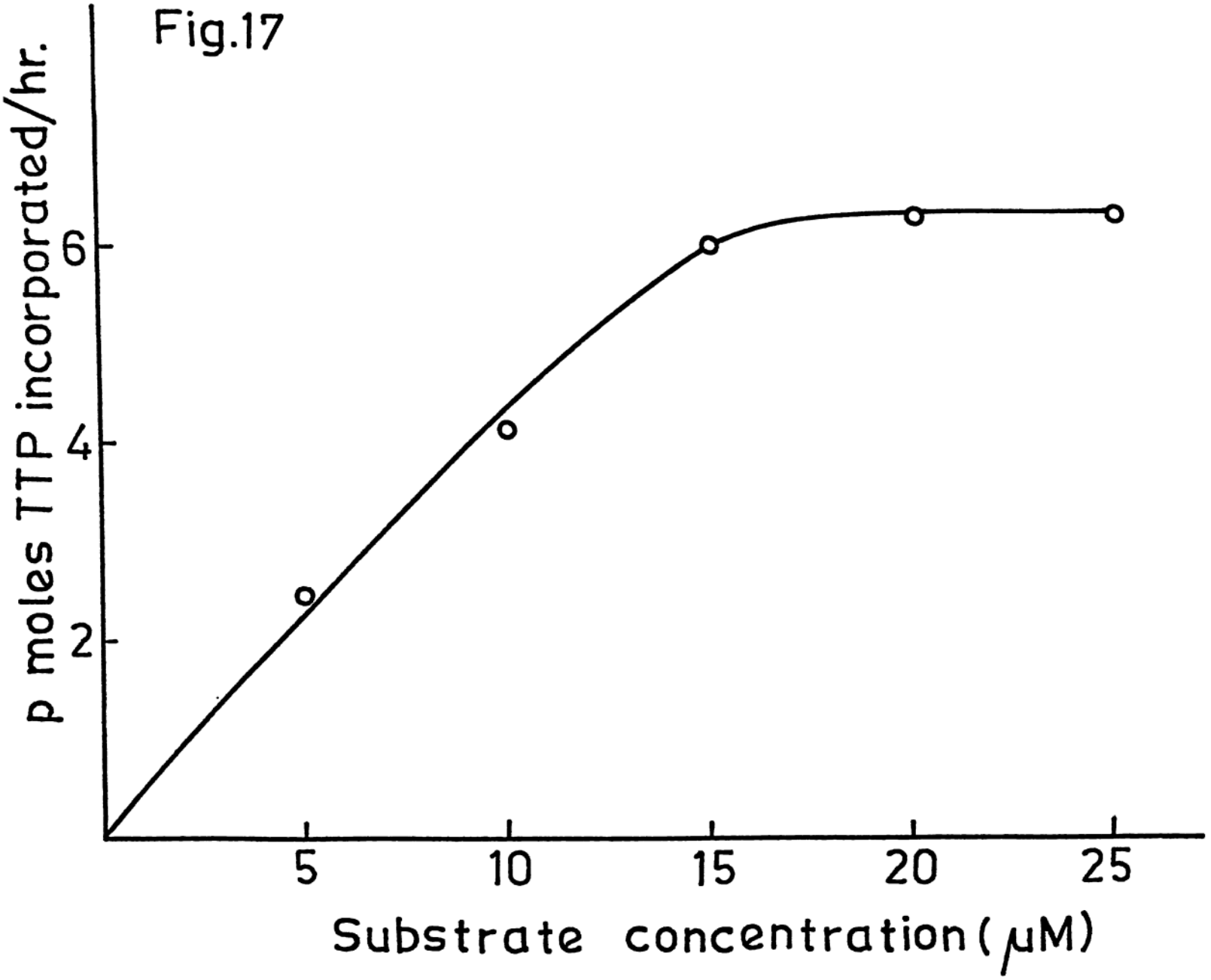
p moles TTP incorporated/hr.
2
4
6
5
10
15
20
25
Substrate concentration (μM)

Fig. 18. Lineweaver-Burk plot (assuming the molecular weight of calf thymus DNA to be 8.6×10^6) for DNA polymerase activity in 7 day old rat cerebrum (i) Control (ii) With aphidicolin (iii) With ddTTP.

Fig.18

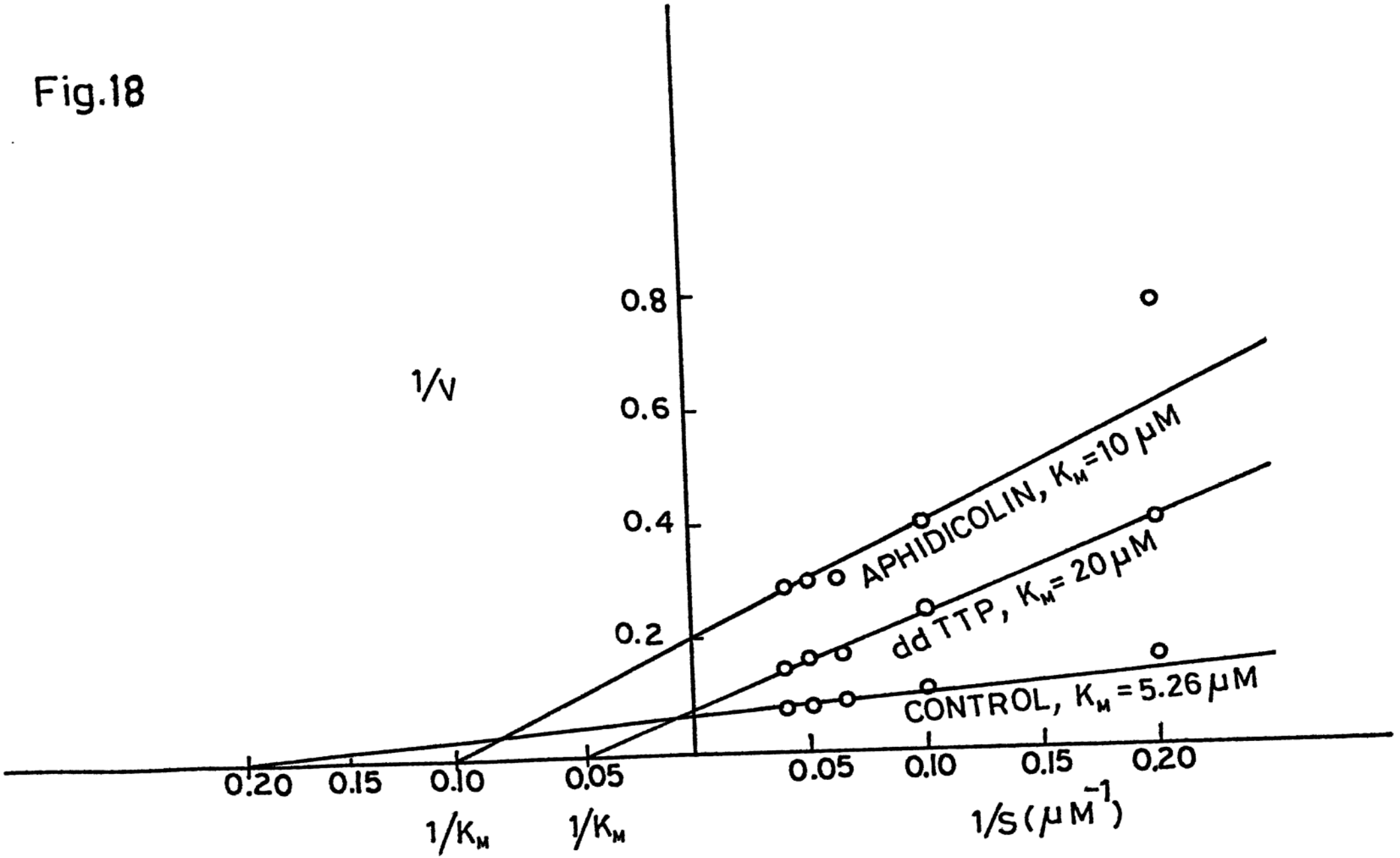
1/V
0.8
0.6
0.4
0.2
APHIDICOLIN, K_M=10 μM
dd TTP, K_M= 20 μM
CONTROL, K_M = 5.26 μM
0.20
0.15
0.10
0.05
0.05
0.10
0.15
0.20
1/K_M
1/K_M
1/S (μM^{-1})

TABLE 25. DNA POLYMERASE ACTIVITY IN DIFFERENT REGIONS OF DEVELOPING AND AGING RAT BRAIN

AGE IN DAYS	CEREBRUM		CEREBELLUM
*16th day embryonic (3)	510.4 ± 49.2		
*7 (12)	204.5 ± 51.9		577.0 ± 93.8
	White	Grey	
225 (12)	37.7 ± 5.7	49.5 ± 10.3	103.4 ± 14.6
365 (6)	86.1 ± 6.6	91.7 ± 11.2	126.9 ± 11.7
540 (12)	111.4 ± 17.2**	113.2 ± 17.9**	199.0 ± 23.9**
750 (6)	56.0 ± 5.7	59.8 ± 9.2	112.3 ± 18.7

Activity is expressed as picomoles of [^{3}H] TMP incorporated/mg protein/hour. Number of separate experiments at each age is given in the parentheses. For the DNA polymerase assay conditions please see text.

* Cerebrum as such as taken for the study at these ages, as it was not possible to separate white and grey matter regions.

** These values are signfiicantly different from those at 225 days of age ($P < 0.001$).

These results are in general agreement with the earlier findings of other workers ((Giuffrida et al, 1970; Brasel et al, 1970; Bakshi and Kumar, 1978). With the advancement of age, the activity decreased in all the regions studied, reaching a low adult value by 225 days. However, the activity once again increases significantly between 225 and 540 days exhibiting a peak value at 540 days. Thereafter, by 750 days of age the activity declines to a low level. To our knowledge, this is the first time that brain DNA polymerase activity was followed from early developmental stages to later part of the life span in a single study. It is, therefore, also the first time that a second peak of polymerase activity in aging brain could be noticed. Thus, these results substantiate our earlier findings regarding the increase in the DNA content in aging brain (Subba Rao and Subba Rao, 1982 a; Shrivastaw and Subba Rao, 1975).

It is known that there are at least three different DNA polymerases in mammalian cells; polymerase α, β and γ (Weissbach et al, 1975; Scovassi et al, 1980). In brain also similar type of diversity of DNA polymerases was noticed (Chiu and Sung, 1971). It is also believed that DNA polymerase α is the enzyme primarily involved in DNA-replication while β-polymerase is generally considered to be involved in repair process (Krokan et al, 1979; Loeb, 1974; Chang and Bollum, 1973; Waser et al, 1979; Weissbach, 1979). The precise physiological role of polymerase γ which is shown to be similar to mitochondrial DNA-polymerase (Hubscher et al, 1977) is not yet clear. Studies from different laboratories have shown that the activities of polymerase α and β could be distinguished by using specific inhibitors (Pedrali-Noy and Spadari, 1979; Ikegami et al, 1978; Ohahsi et al, 1978; Waqar et al,

1978; Edenberg et al., 1978; Van der Vliet and Kwant, 1978). Thus, α polymerase can be inhibited specifically by aphidicolin whereas 2', 3' dideoxythymidine triphosphate (dd TTP) has been shown to inhibit β-polymerase with only slight effect on α-polymerase.

It was therefore, of interest for us to see which type of polymerase is increased in the aging rat brain. We have made use of the two specific inhibitors, aphidicolin and dd TTP in order to distinguish α and β-polymerases. Our initial experiments with standard α-polymerase (Calf thymus, Sigma) and rat hepatoma β-polymerase (obtained from Dr. L.A. Loeb's laboratory, University of Washington, Seattle, U.S.A.) have confirmed that aphidicolin exerts marked inhibition (89-95%) on α-polymerase activity with little effect on β-polymerase activity. The reverse was true in the case of dd TTP as inhibitor. Table 26 shows the effect of aphidicolin and dd TTP independently on the cerebral grey and white matter DNA-polymerase activity at different stages of the life span of the rat. In embryonic brain (16 days of gestation) both aphidicolin and dd TTP inhibited the activity by about 50% indicating that at this early developmental stage both α and β-polymerases are probably present in equal proportions. However, at all other ages studied, only dd TTP exerted significant inhibition the range of inhibition being 45% (750 days) to 75% (540 days). Aphidicolin has no significant effect on the polymerase activity. The pattern is similar in both white and grey matter regions. These results are taken to indicate that from 7th day postnatal onwards the polymerase present in the cells of two regions is predominantly of β-type. From this it can also be inferred that the peak of polymerase activity found at 540 days is mainly due to an increase in β-polymerase.

TABLE 26. EFFECT OF APHIDICOLIN AND ddTTP ON DNA POLYMERASE ACTIVITY IN WHITE AND GREY MATTER REGIONS OF RAT BRAIN

Age in days	CEREBRUM		
	Control	Aphidicolin	ddTTP
16th day embryonic	510.4 ± 49.2	257.9 ± 17.03*	225.3 ± 12.3*
7	288.2 ± 27.9	226.8 ± 39.30	27.3 ± 8.9*

	WHITE MATTER			GREY MATTER		
	Control	Aphidicolin	ddTTP	Control	Aphidicolin	ddTTP
365	86.1 ± 6.6	87.8 ± 17.6	26.2 ± 6.7*	91.7 ± 11.2	83.8 ± 8.7	23.9 ± 4.5*
540	131.2 ± 15.9	125.8 ± 17.9	22.3 ± 2.71*	126.2 ± 10.4	133.8 ± 21.3	19.0 ± 2.3*
750	56.0 ± 5.7	41.1 ± 9.1	31.1 ± 4.4*	59.8 ± 9.2	51.0 ± 7.4	29.9 ± 4.4*

All the values are expressed in picomoles of [^{3}H]-TMP incorporated into DNA per mg protein/hr. The sample no. at each age is 6. For details regarding the assay condition please see text.

*All these values are significantly different from the corresponding control values ($P < 0.001$).

TABLE 27. EFFECT OF APHIDICCLIN AND ddTTP ON DNA POLY-MERASE ACTIVITY IN RAT CEREBELLUM

	AGE IN DAYS			
	7	365	540	750
Control	640.5 ± 16.3	126.9 ± 11.7	199.3 ± 10.8	112.3 ± 18.7
Aphidicolin	268.0 ± 13.9*	102.8 ± 6.8	219.4 ± 24.3	98.5 ± 12.2
ddTTP	274.8 ± 18.0*	44.5 ± 1.9*	33.0 ± 6.74*	64.8 ± 14.4*

All the values are expressed as picomoles of [^{3}H]-TMP incorporated into DNA per mg protein/hr. The sample no. at each age is 6. For details regarding the assay condition please see text.

*All these values are significantly different from the corresponding control values ($P < 0.001$).

Table 27 shows the effect of the two inhibitors on DNA polymerase in cerebellar region of the brain. It is known that cell replication in this region will be at a high level during the first week of postnatal period (Weichsel, 1974; Litteria, 1980; Subba Rao and Subba Rao, 1982 c). In line, the present results show that at 7 days the polymerase is inhibited (more than 50%) both by aphidicolin and ddTTP thus suggesting that both α and β-polymerases are present in equal amounts at this stage. In the case of adult (365 days) and aging cerebellum, however, only ddTTP inhibited the polymerase activity with aphidicolin having no significant effect. Thus, these results are similar in nature to those with cerebral white and grey matter regions (Table 26) thereby once again suggesting that at these adult and aging periods the predominant polymerase present is β-polymerase.

It, therefore, appears that the increased DNA polymerases activity (the second peak of activity) around 540 days of age is primarily due to an enhancement in polymerase β in all the regions studied. The question then, is whether this increase in β-polymerase can explain the accumulation of extra DNA found to be occurring at the same time of the life span of the rat as reported in earlier Chapters. Although β-polymerase is considered to be a repair enzyme, recent studies of Weissbach and others have shown increasing levels of DNA-polymerase β and γ during replication (Weissbach, 1977; de Recondo and Abadiedebat, 1976; Wang and Popenoe, 1977). Also, Butt et al., (1978) did find that DNA polymerase α was selectively extracted with 0.2M KCl in S phase L cells (where 20% of β-polymerase

is also extracted) the DNA synthesis was inhibited by only 40% in the isolated nuclei. Subsequent removal of 70% β-polymerase activity led to 80% inhibition of replication. These results are suggestive of the fact that both α and β-polymerases may be involved, in a concerted manner, in DNA replication. Even in the present studies, while the polymerase present in adult brain seem to be of β-type, the presence of some minimal amounts of α-polymerase cannot be ruled out. Thus it could be that some minimal levels of α-polymerase along with polymerase β may be carrying out both replicative and repair processes in aging brain.

It is possible that the increase in polymerase β during aging as noticed in the present studies may be occurring only in a particular type of brain cells e.g., the glial cells. This possibility can be checked in future studies with isolated cell fractions. Nevertheless, the present results along with the earlier data (Subba Rao and Subba Rao, 1982 a; Shrivastaw and Subba Rao, 1975) do reveal an interesting phenomenon namely an increase in DNA content as well as that of polymerase β in aging brain, which may be coming into operation as a compensatory or adaptive mechanism in the later stages of the life span.

CHAPTER VI

General Discussion

CHAPTER VI

GENERAL DISCUSSION

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 Slor 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; Nakashi et al, 1975; Krigman and Hogman, 1976; Wiggins et al, 1976; Kim 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 undernourished 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 β 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 Spadari, 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 Abadiedebat, 1976; Wang and Popenoe, 1977). Also, Butt et al.,(1978) found that when DNA polymerase α was selectively extracted out with 0.2 M KCl in S 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.

General Summary

GENERAL SUMMARY

1. The changes in DNA, RNA and protein contents and the activities of acid and alkaline DNases in different regions (White matter, Grey matter and Cerebellum) of developing and aging rat brain were studied.

2.. The wet weight of white matter increased steadily up to the last age point studied (750 days), whereas grey matter reaches a peak level at 30 days after birth and is maintained thereafter. However, cerebellum shows steady growth up to 60 days and was maintained constant up to 225 days with a significant increase in the wet weight between 225 and 750 days.

3. It appears that growth, apart from that during early stages, occurs again between ages 225 and 750 days in all the regions studied. The DNA content expressed either per gram or per whole region increased significantly during this period in all the regions, indicating cell proliferation even beyond the adult stages of life. Similarly considerable accumulation of RNA was also observed during this period. The marked decrease in protein/DNA ratio between these two stages of life (225 and 750 days) only in the case of white matter and cerebellum shows more pronounced cell proliferation in these regions as compared to grey matter.

4. The putative DNA degrading enzymes, acid and alkaline deoxyribonucleases showed a positive correlation with the rapid DNA accretion noticed during developmental stages and again between 225 and 750 days of

age. Thus, acid and alkaline DNases showed highest activities during the developmental stages (acid DNase in 1 day old and alkaline DNase at 30 days old) in both white and grey matter regions and decreased up to 225 days. However, a significant increase in the activity of these two enzymes was observed between 225 and 750 days in both the regions. Although acid DNase showed similar trend in cerebellum as in the case of white and grey matter regions, alkaline DNase remained constant from 1 day onwards to 225 days. However, a significant increase (100%) in the activity of alkaline DNase was also observed at 750 days even in this region. It is also clear from these studies that alkaline DNase exhibited maximum activity (both specific as well as total) in old age in all the regions studied.

5. Since, some differences were found in the regions studied, the effect of early postnatal undernutrition and subsequent rehabilitation on wet weight, DNA, RNA, protein and the activities of acid and alkaline DNases in all the regions (White, Grey and cerebellar regions) were studied.

6. In respect to the various parameters studied, white matter was found to be markedly vulnerable to undernutrition, but grey matter was unaffected. However, cerebellar region was moderately affected by undernutrition.

7. It has also been observed that the earlier the initiation of nutritional rehabilitation (10th day postnatal) the better was the recovery of the regions studied. In some cases early nutritional rehabilitation resulted in better than normal biochemical composition of the region.

8. The specific activity of acid and alkaline DNases were not affected by early undernutrition. However, the total activities of these enzymes were significantly low in undernourished rats. Rehabilitation of these deprived groups up to 150 days resulted in higher amounts of these enzymes as compared to those of age matched controls.

9. It appears that the two DNases are synthesized in a preferential manner during rehabilitation. It is also clear from these studies that the white matter region of the rat brain was the most affected region during undernutrition and grey matter was not affected. Cerebellar region, in terms of developmental schedule and response to the imposed caloric restriction, is intermediary between grey and white matter regions.

10. Since DNA content of rat brain goes up significantly during the later stages of the life span, the changes in DNA polymerase activity in different regions of rat brain at various stages of the life span were studied. Efforts were also made to distinguish between α and β polymerase activities by making use of specific inhibitors.

11. DNA polymerase exhibited highest activity during the early developmental stages with a decline to a low adult value by 225 days of age in all the regions (White matter, Grey matter and cerebellum) studied. However, the activity of this enzyme once again increased between 225 and 540 days thus showing a second peak in the later part of the life span. Studies with specific inhibitors like Aphidicolin and 2',3' dideoxy thymidine-5'

triphosphate on the enzyme activity have revealed that this rise in the DNA polymerase activity in various regions was mainly due to an increase in the polymerase β-type.

12. From these studies it is concluded that there occurs definitely a second bout of DNA accretion in various regions of aging rat brain studied. DNases (acid and alkaline) along with DNA polymerase β , showing a sympathetic relation to this DNA accumulation must be playing an important role in DNA synthesis and/or repair process.

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Appendix