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DNA, RNA, protein and DNases in developing rat cerebellum: Effects of early postnatal nutritional deprivation

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Abstract. The effect of early postnatal undernutrition and subsequent rehabilitation on wet weight, DNA, RNA, protein and the activities of acid and alkaline DNases in the cerebellar region of rat brain was studied. The cerebellar region was found to be affected significantly during early undernutrition. Further, earlier the initiation of nutritional rehabilitation the better was the recovery and in some cases timely nutritional rehabilitation resulted in better than normal biochemical composition of the brain. The specific activities of acid and alkaline DNases were not affected by early undernutrition. However, the total activities of these enzymes were significantly low in undernourished rats (R_{15} and R_{21}) Rehabilitation of these deprived groups upto 150 days resulted in higher amounts of these enzymes as compared to those of age-matched controls. It is concluded that the two DNases, are synthesized in a preferential manner during rehabilitation, It is further concluded that cerebellar region, in terms of development schedule and response to imposed calorie restriction, is intermediary between grey and white matter regions.

Keywords. DNA; DNases; undernutrition; rat 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, 1982a) and also the differential effects on these two regions of early undernutrition and subsequent rehabilitation (Subba Rao and Subba Rao, 1982b).

The cerebellar region of 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, 1982b) 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 upto 150 days corrected the deficiencies.

Materials and methods

Rats were fed 'rat feed' (Hindustan Lever, New Delhi) which is complete in all nutritional requirements. On the day of birth, the young rats were assigned to mothers in predetermined numbers. The control group (A) had 6 to 8 pups with the mother while the undernourished group (R) had 18 to 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 either by decreasing the litter size during the preweaning stage or by feeding *ad libitum* in the postweaning period. The rehabilitation was continued for varying periods. Such groups are designated as $R_{10}A_{15}$, $R_{15}A_{21}$, $R_{10}A_{50}$, etc., the first figure indicating the day upto which nutritional restriction was imposed and the second figure indicating the day upto which the rehabilitation was carried out, R and A representing restricted and adequate diets.

Animals were sacrificed by decapitation at various stages and the cerebellum was removed carefully. During the early stages *i. e.* 10, 1·5 and 21 days, 2 to 4 cerebella were pooled in order to obtain sufficient material. The tissue was homogenized with a Potter-Elvehjem type homogenizer in 9 volumes of cold distilled water. DNA and RNA were isolated according to the procedure of Schmidt and Thannhauser (1945) and were estimated by measuring the ultraviolet absorbance at 260 nm. The assay procedure for acid and alkaline DNases was described earlier (Shrivastaw and Subba Rao, 1975; Subba Rao and Subba Rao, 1982c). Protein was estimated by Lowry's method (Lowry *et al.,* 1951), while phosphorus was estimated 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, Missouri, USA. All the reagents used were of Analytical grade. All the results are subjected to statistical treatment according to Student's 't' test.

Results and discussion

The effect of early undernutrition on the wet weight of rat cerebellum is presented in figure 1. As can be seen, nutritional deprivation reduced the wet weight significantly even at 10 days. Prolonged nutritional deprivation upto 21 days postnatal had no further effect on the wet weight. Rehabilitation of the under-nourished groups, R_{10} and R_{15}, upto 50 days restored the deficits to normal values, 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 upto 150 days rectified the deficits in the wet weight.

In corollary with the changes in wet weights, the DNA content (figure 2a) is also significantly reduced by early postnatal undernutrition. At 21 days postnatal, the

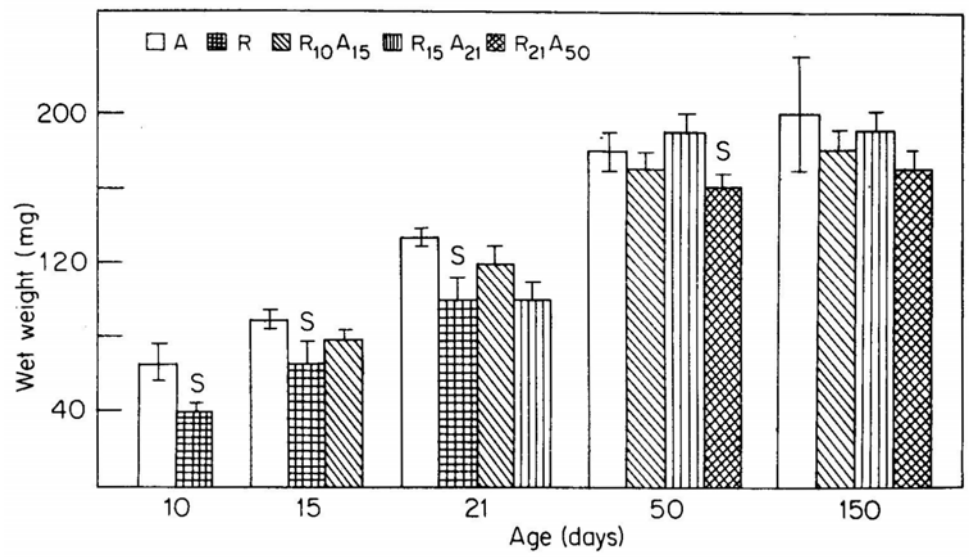

Figure 1. Effect of early postnatal undernutrition and subsequent rehabilitation on the wet weight of rat cerebellum.

A. Normal rats (viz. Normal 10 days old rat $A_{10;}$ normal 15 days old rat A_{15} etc.). R. Nutritionally restricted rats. (viz. Undernourished from 1st day to 10th day postnatal R_{10}, undernourished from 1st day to 15th day postnatal R_{15}, etc.). $R_{10}A_{15.}$ Rats undernourished from 1st day to 10th day postnatal and then rehabilitated for varying periods, (viz. Rats undernourished upto 10th day and then rehabilitated upto 15th day, R_{10} $A_{15;}$ Rats undernourished upto 10th day and then rehabilitated upto 21 day $R_{10}A_{21}$, etc). R_{15} $A_{21.}$ Rats undernourished from 1st day to 15th day postnatal and then rehabilitated for varying periods, (viz. Rats undernourished upto 15th day and then rehabilitated upto 21st day $R_{15}A_{21}$, etc). $R_{21}A_{50,}$ Rats undernourished from 1st day to 21st day postnatal and then rehabilitated for varying periods, (viz. Rats undernourished upto 21 st day and then rehabilita ted upto 50th day R_{21} A_{50}, etc.).

All the values are expressed in mg. ±S.D.

The number of animals in each group varied from 6 to 8. For other details please see materials and methods section of text.

'S' These values are sigificantly different from the corresponding age matched controls ($P<0.005$).

undernourished group has only 50% of DNA as that of age matched control. However, the concentration of DNA expressed per g of tissue (figure 2b), was not affected by undernutrition at 10 and 15 days, 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 greater is the recovery seen. Thus when the R_{10} group was rehabilitated upto 21 days postnatal, the DNA value reached almost to normal values whereas R_{15} and R_{21} groups recovered to complete normalcy only after rehabilitating upto 50 days and 150 days respectively (figure 2a). It can also be noted that when the R_{10} group was rehabilitated either upto 50 days or 150 days

both concentrations as well as the total DNA contents showed remarkable recovery to values that were significantly higher as compared to the age matched controls.

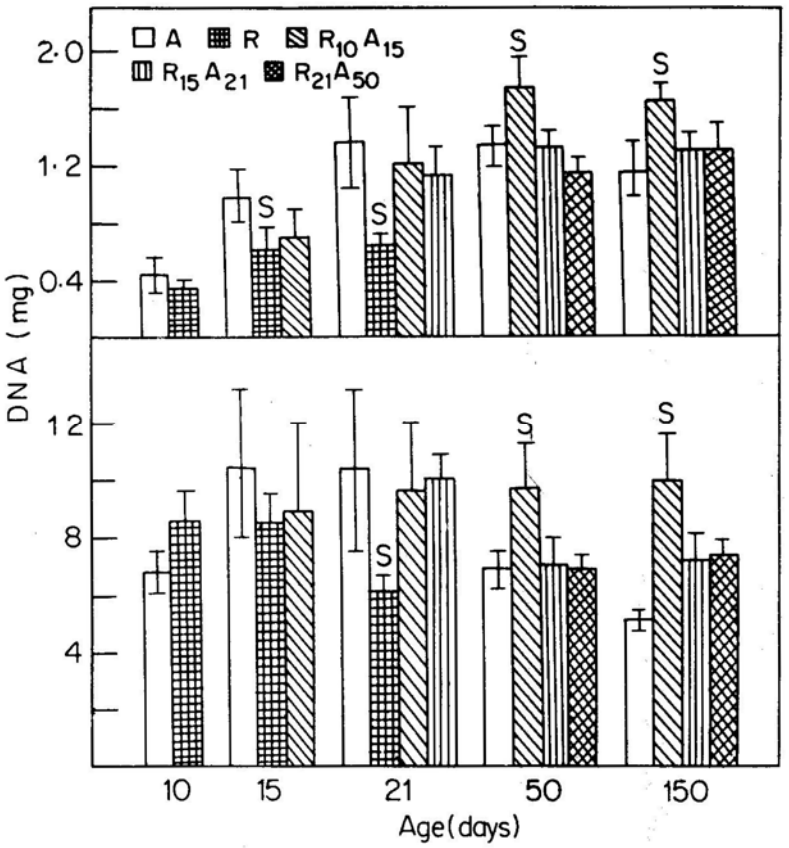

Figure 2. Effect of early postnatal undernutrition and subsequent rehabilitation on the DNA content of rat cerebellum.

(a) DNA content whole region.

(b) RNA content ,- expressed per gram of region.

A. Normal rats. R. Nutritionally restricted rats. $R_{10}A_{15,}$ Rats rehabilitated from 10th day postnatal. R_{15} $A_{21,}$ Rats rehabilitated from 15th day postnatal. R_{21} A_{50}, Rats rehabilitated from 21st day postnatal.

The number of animals in each group were the same as in figure 1. For other details please see under figure 1.

'S' These values are sigificantly different from the corresponding age matched controls ($P<0.005$).

The effect of early postnatal undernutrition followed by rehabilitation on RNA and protein consents of rat cerebellum are presented in figure 3 and 4. It is again clear that RNA and protein contents in undernourished rats reduced significantly (figures 3a and 4a). However, the concentration of RNA was not affected by undernutrition whereas the protein concentration was significantly reduced in 21 days undernourished rats (about 30%) (figures 3b and 4b). Rehabilitation of these

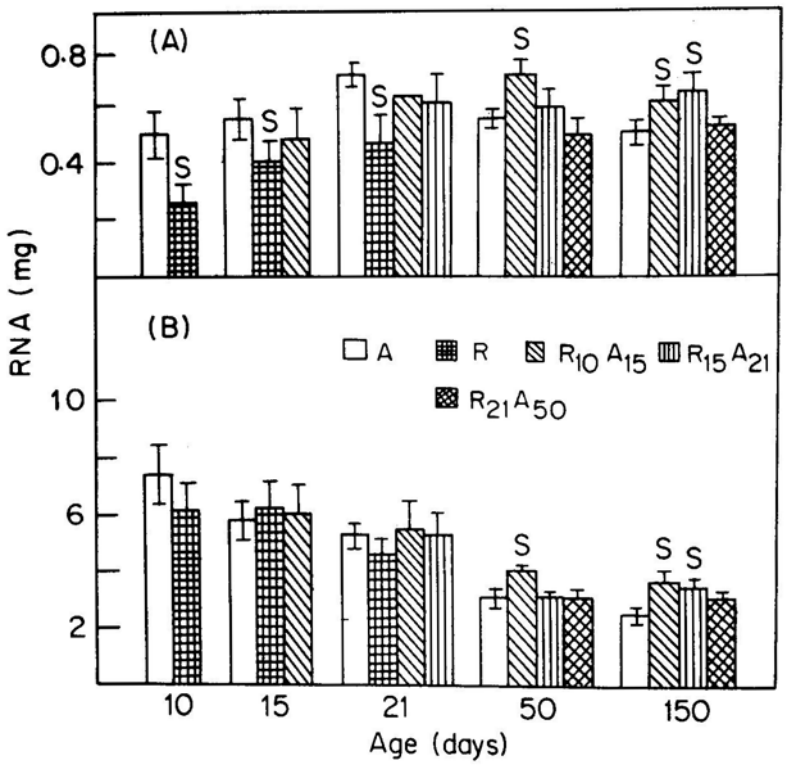

Figure 3. Effect of early postnatal undernutrition and subsequent rehabilitation on the RNA conent of rat cerebellum.

(A) RNA content per whole region.

(B) RNA content expressed per gram of region.

A. Normal rats. R, Nutritionally-restricted rats. R_{10} A_{15}, Rats rehabilitated from 10th day postnatal. $R_{15}A_{21}$, Rats rehabilitated from 15th day-postnatal. $R_{21}A_{50}$, Rats rehabilitated from 21st day postnatal.

The number of animals in each group were the same as in figure 1. For other details please see under figure 1.

'S' These values are sigificantly different from the corresponding age matched controls ($P < 0{\cdot}005$).

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 upto 150 days ($R_{21}A_{150}$) resulted in full recovery (figure 3a). The RNA contents in rehabilitated groups ($R_{10}A_{150}$, $R_{15}A_{150}$) are significantly higher as compared to the age-matched controls (figure 3a). The above results show that as far as the DNA and RNA synthesis is concerned complete recovery is operative and such a compensatory mechanism does not seem to operate, however, in the case of protein.

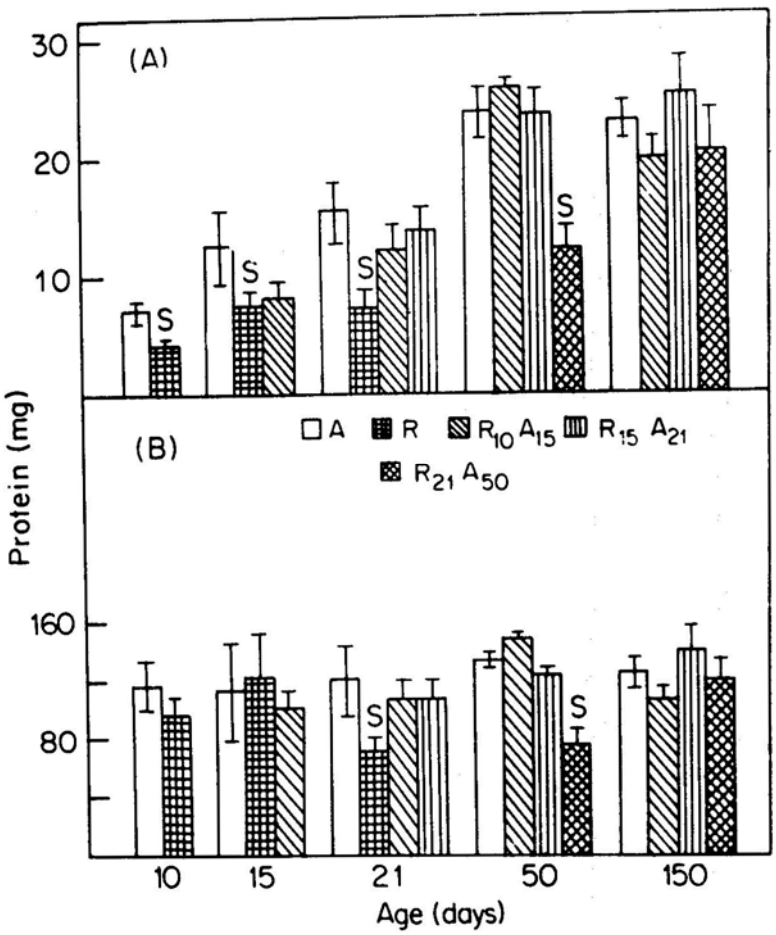

Figure 4. Effect of early postnatal undernutrition and subsequent rehabilitation on the Protein conent of rat cerebellum.

(A) Protein content per whole region.

(B) Protein content, expressed per gram of region.

A. Normal rats. R, Nutritionally restricted rats. R_{10} A_{15} Rats rehabilitated from 10th day postnatal. R_{15} A_{21} , Rats rehabilitated from 15th day postnatal. R_{21} A_{50}, Rats rehabilitated from 21st day postnatal.

The number of animals in each group were the same as in figure 1. For other details please see under figure 1.

'S' These values are sigificantly different from the corresponding age matched controls ($P<0.005$).

Since earlier studies from this laboratory 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 and Subba Rao, 1975; Subba Rao and Subba Rao, 1982a), we also measured the activities of these enzymes in

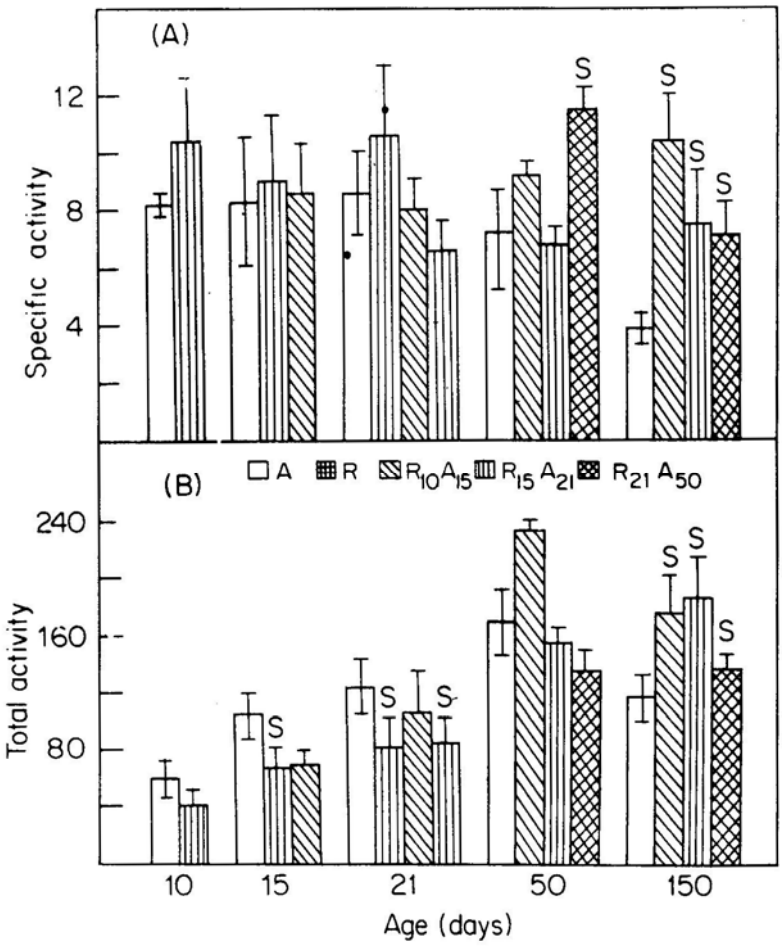

Figure 5. Effect of early postnatal undernutrition and subsequent rehabilitation on the activity of Acid DNase.

(A) Specific activity (µg-of DNA-P liberated per 2hr per mg of protein).

(B) Total activity , (Specific activity × Total Protein in mg)

A. Normal rats. R, Nutritionally restricted rats. $R_{10}A_{15}$, Rats rehabilitated from 10th day postnatal. $R_{10}A_{15}$, Rats rehabilitated from 15th day postnatal. $R_{21}A_{50}$, Rats rehabilitated from 21st day postnatal.

The number of animals in each group were the same as in figure 1 .For other details please see under figure 1.

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

the present group of rats. The results obtained are presented in figures 5 and 6. The specific activity of acid DNase (figure 5a) 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 (figure 5b). In all these cases rehabilitation beginning from 10th, 15th or 21st day upto 150 days resulted in activities (both specific as well as total), which were markedly higher than those noticed in corresponding age-matched controls. The results concerning the alkaline DNase activity (figure 6) are similar to that of acid DNase. However,

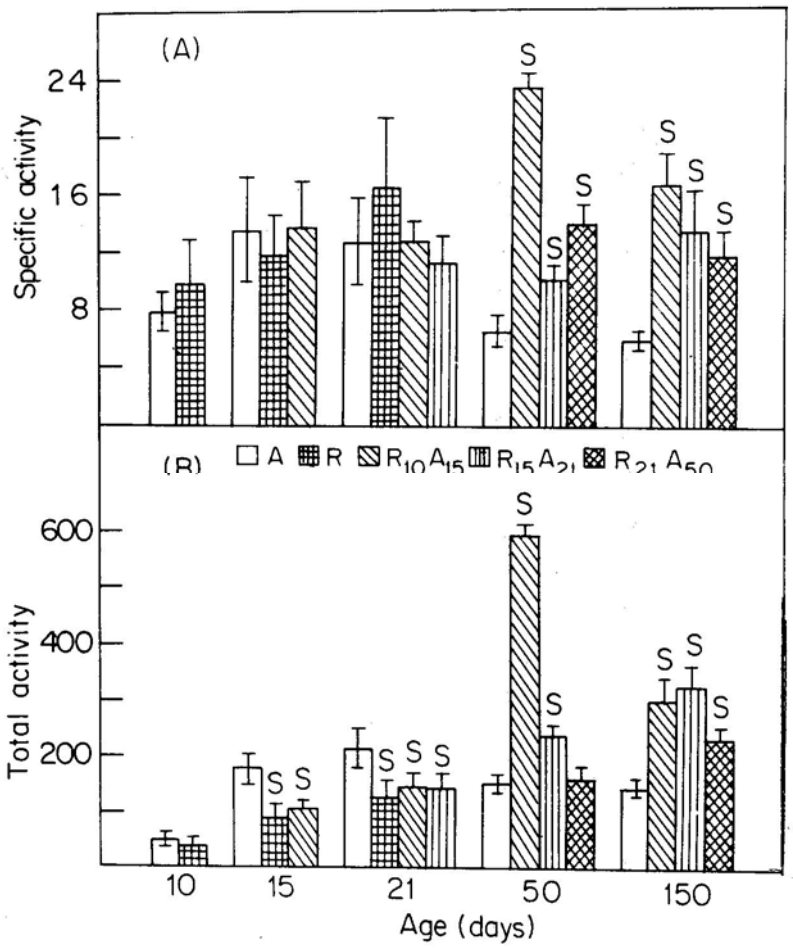

Figure 6. Effect of early postnatal undernutrition and subsequent rehabilitation on the activity of alkaline DNase.

(A) Specific activity (µg of DNA-P liberated per 2hr per mg of protein).

(B) Total activity (specific activity × total protein in mg).

A. Normal rats. R, Nutritionally restricted rats. $R_{10}A_{15,}$ Rats rehabilitated from 10th day postnatal. $R_{15}A_{21,}$ Rats rehabilitated from 15th day postnatal. R_{21} $A_{50,}$ Rats rehabilitated from 21st day postnatal.

The number of animals in each group were the same as in figure 1. For other details please see under figure 1.

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

in the case of alkaline DNase the rehabilitation of groups R_{10} 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 (figures 6a, 6b). These results confirm the earlier observation from this laboratory on white and grey matter regions of rat brain (Subba Rao and Subba Rao, 1982b). It is of considerable importance to note that both the acid and alkaline DNase activities were unchanged 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 figure 6 levels of this enzyme are markedly high in rehabilitated animals ($R_{10}A_{50}$, $R_{15}A_{150}$) and this clearly suggests preferential synthesis of this enzyme during rehabilitation. Although DNases are supposed to be primarily degradative in 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 in a 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 replication. It has also been 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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