UNDERNUTRITION AND THE DEVELOPING CEREBELLAR CORTEX IN THE RAT

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ABSTRACT

Undernutrition of the newborn rats, produced during the first 3 weeks by increasing the litter size and restricting the mother's diet, resulted in reduction of the body and brain weights of the experimental animals. One group of undernourished animals showed especially severe reduction of body and cerebellar weights. These animals, on the 10th postnatal day, had an immature cerebellar cortex corresponding to that of the 7th day postnatal control animals. The external granular layer persisted in the cerebellar cortex of the underweight animals until the 23rd day, while it disappeared by 20th day in the control animals. Mitotic activity was evident until the 21st postnatal day in these animals while it stopped in the normal animal by 16th postnatal day.

There was no marked difference in the fine structure of the various cell types in the control and undernourished animals. Midsagittal tracings of the cerebellar cortex showed a reduced surface area in the undernourished animals, while the thickness of the external granular layer and molecular layer did not show any significant difference when compared to that of the control animals, thus showing a reduction in total cell number, but not per unit area.

The normal morphological appearance of the cerebellar cortex in the underfed animals of higher weight probably indicates that these animals are adequately nourished in spite of the reduction in weight when compared to the control animals, which probably are overfed.

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INTRODUCTION

The effects of undernutrition in the early postnatal life of the rat are well documented in studies by various investigators. Significant reductions in the quantity of nucleic acids, protein content, and various other cellular constituents of the cerebellum in the undernourished rat during the first three postnatal weeks are recorded. There is a lot of controversy concerning these results among the various groups working on undernutrition. The literature is scant (12, 3) on brain morphology during neonatal undernutrition in the rat.

The controversial results on quantitation and the lack of enthusiasm among the investigators in carrying out the morphological studies during undernutrition can be only due to the inconsistent results obtained, probably as a result of variation in the individual growth of the experimental animals.

It was therefore decided to study the morphology of the developing cerebellar cortex of a large number of experimental rats during the first three weeks of postnatal undernutrition, and to correlate this study with the weight and the growth of the cerebellar cortex.

METHODS

Highly inbred rats (AS1), obtained originally from Otago Medical School, Dunedin, New Zealand, were undernourished by increasing the litter size to 16 during the first three weeks of postnatal life (15). The mothers were simultaneously fed a restricted (20 gms/day) protein diet. Normally fed animals of 6–8 litter size were utilized as controls. Animals from the 5th to 21st postnatal days were weighed and the individual weight was recorded. Ten animals of representative weight from each experimental group and an equal number of control animals were subjected to morphological studies. This procedure was repeated using random samples so that 20 animals were studied on each postnatal day. More undernourished animals were studied to confirm the changes on the 10th and 21st postnatal day so that 35 and 25 animals, respectively, were studied on these two days.

The animals were decapitated and the brain was carefully removed and fixed in buffered formalin for 24 hours. The cerebellum along with a portion of the brain stem extending from the midcollicular level to the lower limit of the 4th ventricle were excised, the surfaces blotted free of fluid and weighed on a Mettler analytical balance. The cerebrum was also weighed in the same fixative for five more days and then processed for light microscopy. Mostly, thionin stained sections were used for the study. Silver-stained sections were also used to study the 10th and 21st day specimens. All the observations were made from the cerebellar cortex of the vermis on either side of fissura prima sectioned at 6 micra.

The tracings (Figs. 1 & 2) were from the midsagittal sections using the projection head of an ultraphot. The combined thickness of the external granular and the molecular layers was measured using an ocular scale and calculated according to the micrometer scale.

Two animals each of control and experimental group II of the cerebellum were used for electron microscopy while the other half was weighed after one day’s fixation and then processed for light microscopy. The tissue for EM was washed with cacodylate buffer and fixed in glutaraldehyde for 2 hours and the cortex from the fissura prima region was cut, sliced and further fixed for two hours in 1% buffered osmium tetroxide. The pieces of tissue were then dehydrated in graded acetone and embedded in Durcupan. One micron thick sections were scanned to locate the cortex on either side of fissura prima. Ultrathin sections were then cut,
stained with uranyl acetate and lead citrate and scanned with a Philips 300 electron microscope.

RESULTS

The body, cerebral and cerebellar weights of the undernourished animals of all the age groups studied were considerably reduced. The weight of the cerebellum was directly related to the weight of the body, while the cerebral weight bore no such relationship. According to the recorded weights, the undernourished animals could be divided into two groups, the first group (I) possessing higher body and cerebellar weights than the second group (II). Tables 1 & 2 show the weights of two batches of control and undernourished animals from the 10th to the 15th postnatal days. Weights of only 5 control and 8 undernourished animals are reported as samples. The body and the cerebellar weights of the control and group II animals utilized for EM studies are given in Table 3. The animals belonging to the group II showed immature cerebellar morphology around the 10th postnatal day.

Tracings from the Midsagittal Plane

Fig. 1 shows the tracings of control and undernourished animals from both the groups on the 10th postnatal day. Fig. 2 shows those of the 16th and 21st postnatal days. The cerebellar cortex of the undernourished animals has less...
Fig. 2. Midsagittal tracings of the cerebellum from the 21 day old control (A) and undernourished rats (B) and 16 day old control (A') and undernourished (B'). Arrow indicates fissura prima.

surface area and immature folia and fissures; these findings were exaggerated in the animals belonging to group II.

Light Microscopy of the Cerebellar Cortex

Control rats: Developmental morphology in the cerebellar cortex of the normal rat has been extensively described (1). As has already been pointed out, our findings were restricted to the cortex of the vermis in the fissura prima. Cell proliferation in the external granular layer was very brisk during the 7th to 9th postnatal days, then gradually declined and became negligible by the 16th postnatal day. From then onwards mitotic activity was completely absent. The thickness of the external granular layer was directly related to the rate of cell proliferation. It reached a maximum during the 7th to 9th day and, as active migration of the cells started, this layer became depleted and completely disappeared by the 20th postnatal day. The thickness of the
### TABLE I

**Weights in Grammes of the 10th Postnatal Day Rats**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Serial no.</th>
<th>Body weight</th>
<th>Cerebral weight</th>
<th>Cerebellar weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>17.20</td>
<td>1.003</td>
<td>0.259</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18.00</td>
<td>0.946</td>
<td>0.288</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>17.45</td>
<td>1.000</td>
<td>0.268</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>17.00</td>
<td>0.955</td>
<td>0.259</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>17.30</td>
<td>0.955</td>
<td>0.264</td>
</tr>
<tr>
<td>Undernourished</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>1</td>
<td>8.50</td>
<td>0.676</td>
<td>0.171</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.00</td>
<td>0.684</td>
<td>0.169</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8.80</td>
<td>0.674</td>
<td>0.172</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>8.68</td>
<td>0.680</td>
<td>0.173</td>
</tr>
<tr>
<td>Group II</td>
<td>5</td>
<td>7.13</td>
<td>0.540</td>
<td>0.124</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7.16</td>
<td>0.560</td>
<td>0.128</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7.11</td>
<td>0.541</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7.23</td>
<td>0.580</td>
<td>0.139</td>
</tr>
</tbody>
</table>

### TABLE II

**Weights in Grammes of the 15th Postnatal Day Rats**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Serial no.</th>
<th>Body weight</th>
<th>Cerebral weight</th>
<th>Cerebellar weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>23.00</td>
<td>1.147</td>
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<tr>
<td></td>
<td>2</td>
<td>22.50</td>
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<td>0.293</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>22.45</td>
<td>1.060</td>
<td>0.299</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>21.80</td>
<td>1.058</td>
<td>0.262</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>22.80</td>
<td>1.033</td>
<td>0.315</td>
</tr>
<tr>
<td>Undernourished</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>1</td>
<td>15.65</td>
<td>0.852</td>
<td>0.268</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16.00</td>
<td>0.805</td>
<td>0.248</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15.80</td>
<td>0.891</td>
<td>0.248</td>
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<td></td>
<td>4</td>
<td>15.00</td>
<td>0.773</td>
<td>0.223</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>16.01</td>
<td>0.880</td>
<td>0.250</td>
</tr>
<tr>
<td>Group II</td>
<td>6</td>
<td>13.20</td>
<td>0.800</td>
<td>0.166</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>13.35</td>
<td>0.820</td>
<td>0.176</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>12.95</td>
<td>0.820</td>
<td>0.172</td>
</tr>
</tbody>
</table>

### TABLE III

**Weights in Grammes of the Rats of 10th Postnatal Day Used for Electron Microscopy**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Serial no.</th>
<th>Body weight</th>
<th>Cerebral weight</th>
<th>Cerebellar weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>16.00</td>
<td>0.942</td>
<td>0.216</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15.00</td>
<td>0.892</td>
<td>0.211</td>
</tr>
<tr>
<td>Undernourished</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>1</td>
<td>7.13</td>
<td>0.523</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.23</td>
<td>0.578</td>
<td>0.134</td>
</tr>
</tbody>
</table>
molecular layer was inversely proportional to that of the external granular layer and therefore appeared narrow by the 7th post-natal day; from then onwards it continued to get wider and attained mature appearance by the 20th day. This layer had the highest cell population during the 10th to 17th day, showing maximum cell migration during this period. The Purkinje cells of the 7th postnatal day were still immature, with lightly stained apical cytoplasm. By the 10th postnatal day these cells appeared mature, with basal and apical cytoplasm and processes developing from them. The cytoplasm appeared more condensed. Representative photomicrographs from 10th, 16th and 21st postnatal days are shown in Figs. 3, 4, & 5.

Group I rats: The morphological findings were the same as those of the control rats. The external granular layer in some animals appeared slightly thicker with a higher proliferative activity during the 10th to 13th day, as compared to the control cerebella of the same period (Fig. 3B).

Group II rats: Significant morphological differences were detected in the cerebellar cortex on the 10th to 12th days. The external granular layer attained maximum thickness only during this time, indicating the highest degree of proliferative activity. The proliferative activity was then seen to slow down, but persisted until the 21st postnatal day (Fig. 5). The external granular layer was present on the 21st day and was completely depleted only by the 23rd postnatal day. Active migration was noticed only from the 13th day and continued until the 21st postnatal day. Appreciable changes in the molecular layer were noticed only after the 10th postnatal day, when the active migration of cells from the external granular layer started. Purkinje cells were still immature, having lightly stained immature cytoplasm on the 10th postnatal day, and they only attained mature morphology by the 12th day.

From the 13th postnatal day onwards the light microscopic appearance of the cerebellar cortex was comparable to that of the control animals except for the persistence of the external granular layer and mitotic activity after the 20th postnatal day.

The Thickness of the External Granular and Molecular Layers

The widths of these two layers were measured together. There was no statistically significant variation among the three groups studied.

Fine Structure of the Cerebellar Cortex of the Control and Group II Animals of the 10th Postnatal Day

There was no difference in the fine structure of the various cell types in the control and group II animals. Purkinje cells of group II had lightly stained cytoplasm showing less RNP granules when compared to that of the control animals, confirming the immaturity of the cells. Detailed study of the morphology of the developing processes is being carried out.
DISCUSSION

Undernutrition during the immediate postnatal life of rats affects the body and brain weights of animals. The cerebellum is most affected (9, 2). In our...
study of the undernourished rats, there were two distinct weight groups: Group I, consisting of 10–12 animals having greater body and cerebellar weights, and group II, consisting of 4–6 animals having smaller body and cerebellar weights. It is obvious that the animals belonging to group II suffered in the competition for the 12 feeding stations in the rat (4), and as a result were severely undernourished. Since the limited nutrient in the milk of the rat is protein (11), these newborn animals can be considered to be suffering from protein-calorie malnutrition.

Undernutrition affects the growth rate of the cerebellum in the neonatal rats. The surface area in the midsagittal plane was reduced in all the undernourished rats during the first postnatal week. The folia and the fissures also appeared immature compared to those of the controls. These findings persisted until the 21st postnatal day and were more marked in the animals of group II. Though there was a reduction in the total volume of the cerebellum, the external granular and the molecular layers, when measured together, retained almost the same thickness as seen in the control animals. These findings show that there is a definite reduction in the total cell population, but not in the cell number per unit area. The reduction in total cell number has already been estimated with the DNA content as the index (9, 5, 13).

An obvious delay in morphological development was seen around the 10th postnatal day only in the cerebellar cortex of the animals belonging to group II. The thick granular layer, high mitotic activity, the narrow, poorly cellular molecular layer, and the immature Purkinje cells, comparable to those of the cerebellar cortex of the 7th to 8th postnatal day control rats, indicate a delay
in the morphological development of the cerebellar cortex during this period. Rapid cell acquisition during this period has been attributed (13) to less cell loss and not to delayed proliferative activity as we have observed. Migration seems to be in an active phase from the 13th day onwards. The cell proliferation becomes sluggish but persists till the 21st postnatal day and, as a result, migration is delayed, resulting in the persistence of the external granular layer till the 23rd postnatal day (3). The photomicrographs of the cerebellar cortex from the undernourished rats of the 20th postnatal day by Neville and Chase (12) also show the persistence of the external granular layer though it is not reported by them. Our findings indicate that undernutrition affects cell proliferation primarily but not cell migration and differentiation (5).

The morphological immaturity of the cerebellar cortex is evident only during the most vulnerable period (6) in the brain development of the rat. This could be due to delayed protein synthesis, the rate of which is high in the properly nourished rat of the 10th postnatal day (14), due to the insufficiency of dietary amino acids. It has already been reported that the DNA and total
protein contents are considerably reduced around this period in the under-
nourished rat cerebellum (17, 10).

The morphological development of the cerebellar cortex in the rats of group
I is comparable to that of the control animals. It shows that sufficient
nutrition is available to these animals for the normal morphological develop-
ment of the cerebellar cortex. It is likely that brain function depends on a
correct and orderly sequence of the neuronal migration and differen-
tiation (7) rather than on total cell population. If so, group I rats were getting enough
nutrition to bring about an orderly development of the cerebellar cortex,
resulting in normal functional development, in spite of the reduction in the
total cell population. This has to be investigated further. It may be worth-
while to suggest that the control animals were getting more than the required
amount of food, resulting in an accelerated growth of developing organs
during the immediate postnatal period (16). It is also known that normal
newborn rats receive an excess of calories during the suckling period (8).

Our findings show that results of investigations carried out in undernour-
ished animals vary according to the weights of the growing animals, which in
turn will be affected by the nutritional status of the animal. The maximum
nutritional requirement for normal morphological and functional develop-
ment in the experimental rat is still to be established. It still remains very
doubtful whether just undernutrition, causing delay in the maturity of de-
veloping brain, could be the actual cause of mental retardation in a child. The
environment and the environmental input to the mind play a very important
role in the elaboration of the faculties of the brain. Most of the investigations
carried out in human and experimental animals do not dissociate the two
factors.

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