# C H A P T E R III

# RESULTS AND DISCUSSION

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As stated earlier the present investigations were aimed at a study of the distribution of selected metabolites and enzymes in different regions of the rat brain and changes in the same with protein deficiency during the post-weaning period. The regions studied were cerebellum, medulla, pons, midbrain, olfactory lobes, visual cortex, basal ganglia,hypothalamus, corpus callosum and residual brain. The parameters measured were:

I. weight and moisture content
II. protein content
III. activities of the enzymes namely, glutamate dehydrogenase, glutamate decarboxylase, alanine aminotransferase, aspartate aminotransferase, glutamyl transferase and glutamine synthetase.
IV. oxygen consumption with glucose and glutamate as substrates
V. glutathione and ascorbic acid.

In addition, data were obtained on body weight and hemoglobin content of blood.

Section A

## BODY WEIGHT, BLOOD HEMOGLOBIN AND BRAIN WEIGHT

The weight changes of the LP and HP groups are shown graphically in Fig.1. The data on weight gain, hemoglobin and brain weights of rats fed LP and HP diets are given in Table 11.

As expected protein deficiency had a marked effect on growth. After 10 weeks of treatment body weights of the LP group were only 41% of those of the HP group. This was associated with reduction in brain weight (11%). Similar reductions in body weight and brain weight have been found both with protein deficiency and undernutrition as can be seen from Table 12.

A low hemoglobin content of the LP animals obtained in the present studies is consistent with other reports (e.g., Rajalakshmi, Malathy and Ramakrishnan, 1967).

## WET WEIGHT AND MOISTURE CONTENT

The wet weight and moisture content of different regions of the brain in LP and HP animals are given in Tables 13 and 14. There was about a 10% decrease in the

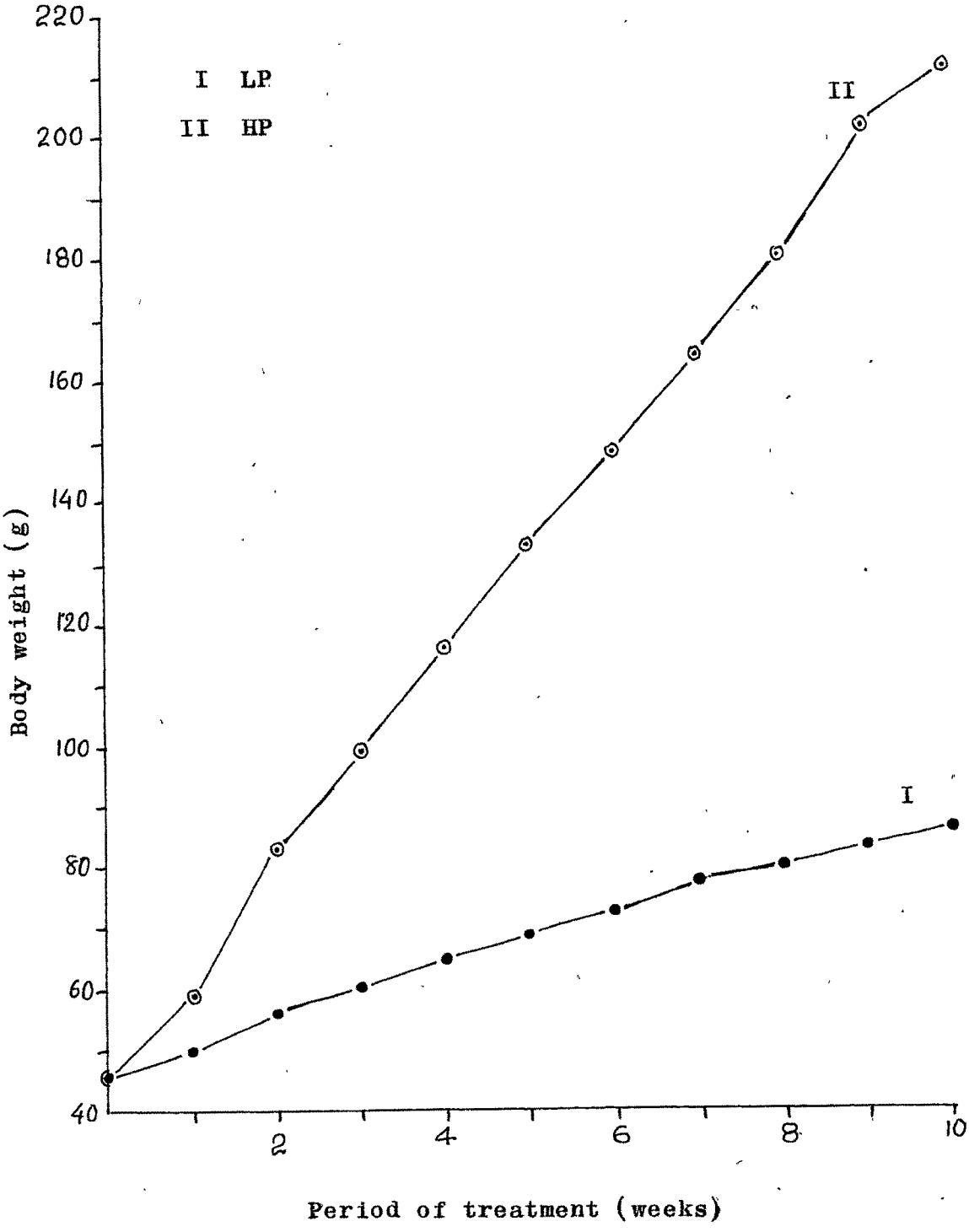


Fig.1. Growth rate of rats fed LP and HP diets.

Table 11: Data on weight gain and body composition of rats fed LP and HP diets

| | LP | HP | LP as % of HP |
|---|---|---|---|
| body weight (g) | | | |
| initial | 46±1.6 (20) | 46±2.2 (20) | 100 |
| final | 86±2.1 (20) | 211±16.9 (20) | 41 |
| gain in weight (g) | 40 | 165 | 24 |
| blood hemoglobin (g per 100 ml) | | | |
| initial | 12.2±0.35 (12) | 12.1±0.40 (8) | 101 |
| final | 13.4±0.22 (8) | 14.6±0.36 (8) | 92 |
| brain weight (g) | 1.54±0.4 (8) | 1.73±0.02 (8) | 89 |

The number of rats are shown in parenthesis.

Table 12: Body weight and brain weights of rats fed a low protein diet and of undernourished after weaning

| reference | treatment | values as per cent of control | |
|---|---|---|---|
| | | body weight | brain weight |
| present study | protein deficiency | 41 | 89 |
| Rajalakshmi et al.(1967) | " | 41 | 87 |
| Winick and Noble (1966) | under-nourished | 52 | 81 |
| Dobbing (1968) | " | 20 | 82 |
| Rajalakshmi and Ramakrishnan (1969) | " | 67 | 91 |

Table 13: Weight of different regions of the brain in rats fed LP and HP diets

| region | fresh weight (mg) | | LP as % of HP |
|---|---|---|---|
| | LP | HP | |
| cerebellum | 186±5.02 (169-199) | 204±4.77 (197-223) | 91 |
| medulla | 74±1.23 (70-77) | 83±3.65 (76-97) | 89 |
| pons | 82±1.30 (80-87) | 91±2.33 (87-97) | 90 |
| midbrain | 101±2.43 (94-108) | 113±3.31 (101-120) | 89 |
| olfactory lobes | 62±1.05 (60-65) | 77±3.86 (65-88) | 81 |
| visual cortex | 89±4.26 (77-98) | 107±4.75 (97-120) | 83 |
| hippocampus | 108±2.88 (98-113) | 120±3.36 (108-124) | 90 |
| basal ganglia | 85±2.78 (77-90) | 115±3.45 (105-125) | 74 |
| hypothalamus | 22±0.50 (20-23) | 24±0.67 (22-26) | 92 |
| corpus callosum | 30±1.28 (27-33) | 33±1.05 (30-35) | 91 |
| residual brain | 626±8.60 (605-650) | 699±16.1 (669-758) | 90 |
| whole brain weight(g) | 1.54 | 1.73 | 89 |
| " " " as calculated from the above data | 1.46 | 1.67 | 88 |

Five animals were used in each group.

Table 14: Moisture content of different regions of the brain in rats fed LP and HP diets

| region | moisture content(g per 100g) | | LP as % of HP |
|---|---|---|---|
| | LP | HP | |
| cerebellum | 78.0±0.1 (77.7-78.2) | 77.8±0.1 (77.5-78.0) | 100 |
| medulla | 73.3±0.2 (73.0-73.6) | 72.7±0.2 (72.4-73.1) | 101 |
| pons | 73.7±0.5 (73.2-74.6) | 72.8±0.3 (72.4-73.4) | 101 |
| midbrain | 76.9±0.2 (76.7-77.3) | 75.8±0.1 (75.6-76.0) | 101 |
| olfactory lobes | 81.6±0.2 (81.3-82.0) | 81.0±0.2 (80.7-81.3) | 101 |
| visual cortex | 80.3±0.1 (80.2-80.5) | 79.5±0.3 (79.1-80.1) | 101 |
| hippocampus | 79.9±0.1 (79.8-80.1) | 78.7±0.2 (78.4-79.1) | 102 |
| basal ganglia | 78.1±0.7 (76.8-79.3) | 77.0±0.4 (76.4-77.7) | 101 |
| hypothalamus | 81.1±0.1 (81.0-81.2) | 78.5±0.3 (77.9-79.1) | 103 |
| corpus callosum | 75.5±0.7 (74.7-76.8) | 74.2±0.6 (73.3-75.2) | 102 |
| residual brain | 79.0±0.4 (78.3-79.6) | 78.3±0.1 (77.9-78.8) | 101 |

Three animals were used in each group.

weight of the whole brain as well as different regions with protein deficiency. The basal ganglia, olfactory lobes and visual cortex however showed a greater reduction in weight.

The percentage weight of different regions compares with that in other studies (Table 15). However, the weight of the hypothalamus was less in these studies possibly because of differences in the extent of excision. Similarly the percentage weight of the visual cortex was slightly more.

Moisture content is slightly but consistently increased in all the regions in the LP animals. A similar increase in moisture content with protein deficiency has been found in rats by Prof. Nagchaudhury (personal discussion) and in pigs by Dickerson, Dobbing and McCance (1967). The difference in moisture content was evident at the time of dissection when it was found relatively more difficult to achieve a clear separation of the regions in the LP animals.

PROTEIN

The data on the protein content of different regions are given in Table 16. The same varied from 10.0-12.5% in the HP group and from 8.0-11.5% in the LP group. In general the values for protein content were less in the LP animals but the differences were statistically significant only

Table 15: Comparative data on the percentage weight of different regions in the rat brain

| region | present study | | Bennett et al. (1968) | Rajalakshmi and Patel (1968) |
|---|---|---|---|---|
| | LP | HP | | |
| cerebellum | 12.7 | 12.2 | 14.3 | 13.6 |
| brain stem | 10.6 | 10.4 | 12.4 | 11.6 |
| olfactory lobes | 4.2 | 4.6 | 3.2 | 4.0 |
| visual cortex | 6.1 | 6.4 | 3.4 | 5.0 |
| hippocampus | 7.4 | 7.2 | - | 6.2 |
| basal ganglia | 5.8 | 6.9 | 7.8* | 4.9 |
| hypothalamus | 1.5 | 1.4 | 2.3 | 2.7 |
| remaining brain | 51.7 | 50.7 | - | 52.5 |

*The corresponding region studied was caudate + putamen.

Table 16: Concentration of protein in different regions of the brain in rats fed LP and HP diets

| region | no. of[@] determi-nations | no. of[@] animals | protein (g per 100 g) LP | protein (g per 100 g) HP | LP as % of HP |
|---|---|---|---|---|---|
| cerebellum | 5 | 10 | 10.1±0.43 (9.2-11.7) | 11.7±3.1 (10.8-12.5) | 86* |
| medulla | 3 | 12 | 7.7±0.30 (7.1-8.0) | 10.0±0.23 (9.6-10.4) | 77** |
| pons | 3 | 12 | 8.0±0.88 (6.3-9.2) | 10.7±0.38 (10.0-11.3) | 75* |
| midbrain | 3 | 12 | 10.0±0.23 (9.6-10.4) | 11.0±0.62 (10.0-12.1) | 91 |
| olfactory lobes | 2 | 8 | 11.4±1.05 (10.4,12.5) | 12.3±0.20 (12.1,12.5) | 93 |
| visual cortex | 3 | 12 | 10.0±0.40 (9.2-10.4) | 11.5±0.56 (10.8-12.5) | 87 |
| hippocampus | 2 | 8 | 9.8±0.20 (9.6,10.0) | 10.4±0.85 (9.6,11.3) | 94 |
| basal ganglia | 2 | 8 | 10.6±0.20 (10.4,10.8) | 9.8±0.2 (9.6,10.0) | 108 |
| hypothalamus | 1 | 8 | 10.4 | 12.5 | 83 |
| corpus callosum | 1 | 8 | 8.6 | 11.7 | 74 |
| residual brain | 5 | 5 | 10.8±0.36 (10.0-11.7) | 12.6±0.58 (11.3-14.2) | 86* |

*Difference significant at 5% level.

**Difference significant at 1% level.

[@]In this and other tables this was the same for both groups except where specified otherwise.

in the case of the cerebellum, pons and residual brain. No decrease was found in the basal ganglia but the same showed a marked decrease in weight with deficiency so that the total amount of protein in this region would be less.

When the values were calculated on whole brain basis they were 10.2% in the case of the LP group and 11.7% in the case of the HP group so that the overall LP value was 87% of HP value. The values for the HP animals are in the range reported for 3 month old animals by other investigators (e.g., Maletta and Timiras, 1968). In both groups relatively high concentrations of protein were found in residual brain, hypothalamus and olfactory lobes whereas low concentrations were found in the medulla, pons and hippocampus. The basal ganglia and the corpus callosum showed respectively high and low values in the LP group, and the reverse, in the HP group.

The present data are compared in Table 17 with those reported for the rabbit brain (McCaman and Aprison, 1964), cat brain (Berl, 1966) and rat brain (Maletta and Timiras, 1968). In all the studies the medulla was found to have a lower concentration of protein than the visual cortex.

Table 17: Comparative data on the protein content of brain regions

| region | protein (g per 100 g) | | | | |
|---|---|---|---|---|---|
| | rat (present study) | | rat (Maletta and Timiras,1968) | rabbit (McCaman and Aprison,1964) | cat (Berl, 1966) |
| | LP | HP | | | |
| cerebellum | 10.1 | 11.7 | 13.1 | - | - |
| visualcortex | 10.0 | 11.5 | 11.9 | 9.8* | 9.3 |
| hypothalamus | 10.4 | 12.5 | 12.4 | - | 8.5 |
| medulla | 7.7 | 10.0 | - | 8.7 | 8.1 |
| midbrain | 10.0 | 11.0 | - | 10.2* | 8.2 |
| basal ganglia | 10.6 | 9.8 | - | 9.8* | 9.5* |
| pons | 8.0 | 10.7 | - | - | 8.2 |
| hippocampus | 9.8 | 10.4 | - | - | 9.6 |

*The corresponding regions studied were superior colliculus, cortex and caudate nucleus for midbrain, visual cortex and basal ganglia respectively.

This was also true of pons in rat and cat brains. In the case of rat and rabbit brains the midbrain also had a higher concentration but this was not true of the cat brain. Similarly, relatively high values were obtained in the rat for the hypothalamus and cerebellum. More extensive data are needed to confirm these differences as genuine species differences.

Section B

## ENZYME STUDIES

### GLUTAMATE DEHYDROGENASE (GDH)

The data on GDH are presented in Table 18. In both the groups the values were found to be high in the case of medulla, pons and midbrain and low in the case of the corpus callosum, hippocampus and cerebellum. To the latter list must be added the basal ganglia in the case of the HP group and olfactory lobes in that of the LP group. The values for different regions in the two groups were found to correlate highly with each other ($r=0.922$, $P < 0.01$).

The regions most affected by protein deficiency were the corpus callosum, cerebellum, olfactory lobes, midbrain and the residual brain containing the thalamus. The pons, basal ganglia and visual cortex were not affected by deficiencies. The remaining regions showed some decrease, but this was not statistically significant.

Table 18: Distribution of glutamate dehydrogenase in different regions of the brain in rats fed LP and HP diets

| region | | no. of determinations | no. of animals | glutamate dehydrogenase (enzyme units per g) LP | glutamate dehydrogenase (enzyme units per g) HP | LP as % of HP |
|---|---|---|---|---|---|---|
| cerebellum | LP | 8 | 16 | 1.6±0.12 (1.2-2.1) | 2.0±0.09 (1.8-2.6) | 80* |
| | HP | 9 | 18 | | | |
| medulla | | 5 | 20 | 2.8±0.19 (2.4-3.5) | 3.4±0.19 (3.0-4.1) | 82 |
| pons | LP | 4 | 16 | 2.8±0.18 (2.3-3.2) | 2.9-0.11 (2.7-3.2) | 97 |
| | HP | 5 | 20 | | | |
| midbrain | | 5 | 20 | 2.5±0.12 (2.2-2.9) | 3.1±0.11 (2.7-3.3) | 81** |
| olfactory lobes | | 4 | 16 | 1.7±0.10 (1.4-1.8) | 2.3±0.09 (2.1-2.4) | 74** |
| visual cortex | | 5 | 20 | 2.0±0.11 (1.8-2.4) | 2.2±0.14 (1.8-2.5) | 91 |
| hippocampus | | 4 | 16 | 1.5±0.16 (1.1-1.8) | 1.9±0.25 (1.4-2.3) | 79 |
| basal ganglia | | 4 | 16 | 1.9±0.17 (1.4-2.2) | 2.0±0.11 (1.8-2.3) | 95 |
| hypothalamus | | 2 | 16 | 2.1±0.04 (1.7,2.5) | 2.4±0.16 (2.2,2.5) | 88 |
| corpus callosum | | 2 | 16 | 1.3±0.02 (1.1,1.5) | 1.9±0.00 (1.9,1.9) | 68** |
| residual brain | | 9 | 9 | 2.0±0.07 (1.7-2.3) | 2.3±0.05 (2.2-2.6) | 87** |

*Difference significant at the 5% level.
**Difference significant at the 1% level.

GLUTAMATE DECARBOXYLASE (GAD)

The data on GAD are presented in Table 19. The highest concentration of the enzyme was found in the hypothalamus and midbrain and the lowest concentrations in the corpus callosum and pons. The low value for the corpus callosum is not surprising as it is composed of almost entirely white matter and the activities of GAD and other enzymes are less in white matter than in grey matter (e.g., Albers, 1960). The pattern of enzyme activity in different regions was found to compare generally with that in the monkey brain reported by Albers and Brady (1959) as can be seen from Table 20.

The values for the different regions in the two groups were found to be significantly correlated ($r=0.825$, $P < 0.01$).

All the regions except the olfactory lobes and hippocampus were affected by deficiency (Table 19). The LP values for the hypothalamus and basal ganglia were only about half the HP values with no overlap between the values for the two groups. The values for the medulla, visual cortex and the residual brain containing the thalamus are also markedly affected by the LP diet. However, the difference in the case of the hypothalamus was not statistically significant because of the small number of observations.

Table 19: Distribution of glutamate decarboxylase in different regions of the brain in rats fed LP and HP diets

| region | no.of determi-nations | no.of animals | glutamate decarboxylase (enzyme units per g) LP | glutamate decarboxylase (enzyme units per g) HP | LP as % of HP |
|---|---|---|---|---|---|
| cerebellum | 10 | 20 | 16±1.0 (11-20) | 22±1.1 (18-30) | 73** |
| medulla | 4 | 16 | 13±0.9 (11-15) | 22±2.4 (17-28) | 59** |
| pons | 5 | 20 | 12±1.5 (10-15) | 16±1.4 (13-20) | 75 |
| midbrain | 4 | 16 | 31±2.8 (24-36) | 41±2.4 (35-46) | 76* |
| olfactory lobes | 4 | 16 | 25±3.1 (18-31) | 25±3.6 (20-36) | 100 |
| visual cortex LP | 5 | 20 | 19±2.0 (14-23) | 30±3.0 (23-36) | 63* |
| HP | 4 | 16 | | | |
| hippocampus | 4 | 16 | 17±3.4 (14-28) | 20±1.9 (15-24) | 85 |
| basal ganglia LP | 3 | 12 | 16±2.9 (11-21) | 30±3.4 (23-36) | 53* |
| HP | 4 | 16 | | | |
| hypothalamus | 2 | 16 | 27±5.5 (21,32) | 53±3.2 (43,63) | 51 |
| corpus callosum | 1 | 8 | 5 | 8 | 63 |
| residual brain | 10 | 10 | 21±1.4 (16-28) | 32±2.3 (22-49) | 66** |

*Difference significant at 5% level.
**Difference significant at 1% level.

Table 20: Comparison of the distribution of glutamate decarboxylase in rat and monkey brains

| region | glutamate decarboxylase (per cent of midbrain value) | | |
|---|---|---|---|
| | rat present study | | monkey (Albers and Brady, 1959) |
| | LP | HP | |
| midbrain | 100 | 100 | 100 |
| hypothalamus | 96 | 114 | 96 |
| residual brain (thalamus) | 71 | 68 | 85 |
| visual cortex | 70 | 70 | 84 |
| cerebellum | 58 | 50 | 79 |
| pons + medulla | 56 | 49 | 34 |

ALANINE AMINOTRANSFERASE (GPT)

Data on the activity of GPT in different regions are shown in Table 21. In both groups the visual cortex, midbrain, olfactory lobes and cerebellum had relatively high values, whereas the hypothalamus and corpus callosum had relatively low values. The values for the two groups were found to be positively correlated ($r=0.695$, $P < 0.05$).

The LP values ranged from 11 to 33 whereas the HP values showed a greater variation from 13-50. The former were consistently less than the latter in all regions. The differences were statistically significant in the case of the cerebellum, medulla, pons, olfactory lobes, visual cortex and residual brain and were most marked in the medulla and pons. The results are in accord with previous studies carried out in this laboratory in which the enzyme in the whole brain is found to be affected in protein deficiency.

ASPARTATE AMINOTRANSFERASE (GOT)

The data on GOT are shown in Table 22. The values for the HP group varied for 113-153 whereas the corresponding range for the HP group was 89-122. In both groups the basal ganglia had relatively high values whereas the medulla had a low value. The values for the two groups were found to be positively correlated ($r=0.805$, $P < 0.01$).

Table 21: Distribution of alanine aminotransferase in different regions of the brain in rats fed LP and HP diets

| region | | no. of determinations | no. of animals | alanine aminotransferase (enzyme units per g) LP | alanine aminotransferase (enzyme units per g) HP | LP as % of HP |
|---|---|---|---|---|---|---|
| cerebellum | LP | 10 | 20 | 28±1.1 (20-31) | 44±3.0 (38-52) | 64** |
| | HP | 4 | | | | |
| medulla | | 4 | 16 | 15±1.9 (10-19) | 36±4.1 (28-43) | 42** |
| pons | | 5 | 20 | 18±1.4 (15-19) | 32±2.6 (26-40) | 56** |
| midbrain | | 4 | 16 | 32±3.2 (27-41) | 36±3.8 (30-47) | 89 |
| olfactory lobes | LP | 2 | 8 | 31±0.7 (30,31) | 36±0.9 (34-38) | 86* |
| | HP | 4 | 16 | | | |
| visual cortex | | 5 | 20 | 33±2.6 (23-38) | 50±4.8 (41-67) | 66* |
| hippocampus | | 4 | 16 | 28±1.0 (25-29) | 25±2.3 (21-31) | 112 |
| basal ganglia | LP | 3 | 12 | 22±2.6 (17-26) | 28±1.3 (25-31) | 79 |
| | HP | 4 | 16 | | | |
| hypothalamus | | 2 | 16 | 17±4.5 (12,21) | 16±4.0 (12,20) | 106 |
| corpus callosum | | 1 | 8 | 11 | 13 | 85 |
| residual brain | | 10 | 10 | 26±1.2 (18-29) | 33±1.9 (28-40) | 79** |

*Difference significant at 5% level.
**Difference significant at 1% level.

Table 22: Distribution of aspartate aminotransferase in different regions of the brain in rats fed LP and HP diets

| region | | no. of determinations | no. of animals | aspartate aminotransferase (enzyme units per g) LP | aspartate aminotransferase (enzyme units per g) HP | LP as % of HP |
|---|---|---|---|---|---|---|
| cerebellum | | 6 | 12 | 106±2.1 (97-111) | 142±2.0 (136-147) | 75** |
| medulla | | 2 | 8 | 89±3.0 (89,92) | 130±2.6 (128,133) | 68** |
| pons | | 3 | 12 | 98±4.9 (88-103) | 136±9.0 (122-153) | 72* |
| midbrain | | 2 | 8 | 91±2.0 (89,93) | 133±2.6 (131,136) | 68** |
| olfactory lobes | | 2 | 8 | 106±1.6 (105,108) | 136±0.0 (136,136) | 78** |
| visual cortex | | 3 | 12 | 104±5.4 (98-111) | 138±7.2 (125-150) | 75* |
| hippocampus | | 2 | 8 | 108±3.0 (105,111) | 143±5.0 (138,148) | 76* |
| basal ganglia | LP | 1 | 4 | 122 | 147±3.0 (144,150) | 83 |
| | HP | 2 | 8 | | | |
| hypothalamus | | 1 | 8 | 109 | 149 | 73 |
| residual brain | | 6 | 6 | 110±3.0 (103-122) | 153±6.8 (131-167) | 72** |

*Difference significant at 5% level.
**Difference significant at 1% level.

All the regions were affected by protein deficiency. although the values for basal ganglia and hypothalamus must be treated with reservations as they were based on only one determination. However a number of animals were used for each determination.

The LP values varied for 68% to about 83% of the HP values. This variation was much less than that in the case of other enzymes suggesting that protein deficiency has a more uniform effect on the activity of this enzyme in all the regions studied. The data confirm previous observations of the effects of protein deficiency on this enzyme.

GLUTAMYL TRANSFERASE

The data on glutamyl transferase are presented in Table 23. The lowest values were found in the corpus callosum, hypothalamus and basal ganglia whereas the highest concentration was found in olfactory lobes. The visual cortex, cerebellum, pons and midbrain also had relatively higher concentrations. The values for the two groups were found to be significantly correlated ($r=0.980$, $P < 0.01$).

Table 23: Distribution of glutamyl transferase in different regions of the brain in rats fed LP and HP diets

| region | | no. of determinations | no. of animals | glutamyl transferase (enzyme units per g) LP | glutamyl transferase (enzyme units per g) HP | LP as % of HP |
|---|---|---|---|---|---|---|
| cerebellum | | 6 | 12 | 752±21.6 (682-819) | 747±21.1 (676-830) | 101 |
| medulla | | 4 | 16 | 642±18.4 (594-682) | 687±9.3 (665-709) | 93 |
| pons | | 4 | 16 | 658±9.9 (632-687) | 727±15.2 (693-764) | 91** |
| midbrain | | 4 | 16 | 697±11.7 (682-731) | 713±6.0 (698-726) | 98 |
| olfactory lobes | | 4 | 16 | 880±13.1 (852-907) | 838±28.3 (759-880) | 106 |
| visual cortex | | 4 | 16 | 698±14.2 (665-731) | 752±14.8 (715-781) | 93* |
| hippocampus | | 4 | 16 | 524±9.5 (500-539) | 551±14.4 (511-599) | 95 |
| basal ganglia | | 4 | 16 | 407±21.0 (363-462) | 491±15.1 (451±517) | 83* |
| hypothalamus | LP | 1 | 8 | 407 | 473±5.5 | 84 |
| | HP | 2 | 16 | | (467,478) | |
| corpus callosum | | 2 | 16 | 394±26.0 (368,420) | 421±25.0 (369,446) | 94 |
| residual brain | | 6 | 6 | 561±11.6 (533-599) | 602±8.5 (567-627) | 93* |

*Difference significant at 5% level.
**Difference significant at 1% level.

The regions affected by protein deficiency were pons, visual cortex, basal ganglia and residual brain. A difference was also found in the case of the hypothalamus but this was not statistically significant presumably because of the small number of observations.

The pattern of distribution was found to resemble that found in the cat in some respects (Table 24). In both cases the corpus callosum was found to have a relatively low value whereas the midbrain and the visual cortex had relatively higher values. However, the basal ganglia and hypothalamus which had low values in the present studies had relatively high values in the cat brain. Further studies are needed to verify these observations.

GLUTAMINE SYNTHETASE

Data on the distribution of glutamine synthetase are presented in Table 25.

In both cases, the values for the corpus callosum and the basal ganglia were low whereas those for the cerebellum, olfactory lobes and visual cortex were high. The values for the two groups were found to be positively correlated ($r=0.892$, $P < 0.01$). The values for the LP group (8-19) tended to be somewhat less than those for

Table 24: Comparison of the regional distribution of glutamyl transferase in the rat and the cat brains

| region | values as per cent of medulla | |
|---|---|---|
| | rat (present study) | cat (Berl,1966) |
| medulla | 100 | 100 |
| cerebellum | 109 | 121* |
| pons | 106 | 104 |
| midbrain | 104 | 105* |
| visual cortex | 109 | 221 |
| hippocampus | 80 | 121 |
| basal ganglia | 71 | 152* |
| hypothalamus | 69 | 121 |
| corpus callosum | 61 | 84 |

*The corresponding regions studied for cerebellum, midbrain and basal ganglia were cerebellar hemispheres, midbrain tegmentum and caudate nucleus respectively.

Table 25: Distribution of glutamine synthetase in different regions of the brain in rats fed LP and HP diets

| region | no. of determinations | no. of animals | glutamine synthetase (enzyme units per g) LP | glutamine synthetase (enzyme units per g) HP | LP as % of HP |
|---|---|---|---|---|---|
| cerebellum | 6 | 12 | 14±0.86 (11-16) | 15±0.73 (13-18) | 93 |
| medulla | 4 | 16 | 9±0.50 (8-10) | 11±0.41 (10-12) | 82* |
| pons | 4 | 16 | 11±0.41 (10-12) | 12±0.41 (11-13) | 92 |
| midbrain | 4 | 16 | 12±0.91 (10-13) | 12±0.71 (10-13) | 100 |
| olfactory lobes | 4 | 16 | 16±0.96 (13-17) | 19±1.5 (16-23) | 84 |
| visual cortex | 4 | 16 | 13±0.65 (11-14) | 15±1.10 (12-17) | 87 |
| hippocampus | 4 | 16 | 9±1.10 (6-11) | 10±0.91 (8-11) | 90 |
| basal ganglia | 4 | 16 | 5±0.41 (4-6) | 9±1.40 (7-13) | 56* |
| hypothalamus | 2 | 16 | 11±1.6 (9,12) | 10±0.00 (10,10) | 110 |
| corpus callosum | 2 | 16 | 8±1.0 (7,9) | 9±0.71 (8,9) | 89 |
| residual brain | 6 | 6 | 11±0.76 (8-12) | 12±0.77 (10-13) | 92 |

*Difference significant at 5% level.

the HP group (9-19) but the differences were significant only in the case of basal ganglia and medulla. In previous studies also (Rajalakshmi et al., 1965) glutamine synthetase was found to be less affected than other enzymes.

The values in the present studies were found to be very much lower than those generally reported. This might be because, in the present studies, the blank consisted of the complete system without substrate whereas in other studies ATP was omitted from the system. This difference in assay system was noticed only at the time of writing up the thesis. The endogenous production of substrate might account for the low values obtained in the present studies.

The higher activity found in the cerebellum as compared to most regions is consistent with the difference between cerebellar and cerebral activity reported in rats (Wu, 1963) as can be seen from the following comparisons:

| | glutamine synthetase (enzyme units/g tissue) | |
|---|---|---|
| | present study | Wu (1963) |
| cerebrum | 12 | 66 |
| cerebellum | 15 | 90 |
| cerebral value as per cent of cerebellar value | 80 | 73 |

On the other hand, in other species such as ox, pig, rabbit, sheep and mink, cerebellar activity was found to be less (Wu, 1963). This was also true of the cat (Berl, 1966). This could account for the differences between the patterns of enzyme distribution in rat and cat brains (Table 26). However, in both cases the activity was found to be less in the corpus callosum.

Section C

OXYGEN UPTAKE BY BRAIN TISSUE SLICES AND HOMOGENATES

The absolute values for oxygen uptake under different conditions are presented in Tables 27 and 28. The values for the LP group are presented as per cent of HP values in Table 29. Table 30 compares oxygen uptake under different conditions.

Some interesting observations emerge from the data. First of all, both with slices and homogenates the increase in oxygen consumption over endogenous respiration was more with glucose as substrate than with glutamate as substrate. Similar observations have been made by Ghosh and Quastel (1954). However, Weil-Malherbe (1936) found an increased respiration with glutamate but this was when the addition was made to a basal medium containing glucose. The amount of glucose added and the concentration of electrolytes also differed in this study (Table 31).

Table 26: Comparison of the regional distribution of glutamine synthetase activity in the rat*

| region | values as per cent of cerebellum | |
|---|---|---|
| | rat (present study) | cat (Berl,1966) |
| cerebellum | 100 | 100* |
| medulla | 73 | 91 |
| pons | 80 | 100 |
| midbrain | 80 | 98* |
| visual cortex | 100 | 213 |
| hippocampus | 67 | 134 |
| basal ganglia | 60 | 139* |
| hypothalamus | 67 | 121 |
| corpus callosum | 60 | 66 |

*Corresponding regions studied for cerebellum, midbrain and basal ganglia were cerebellar hemispheres, midbrain tegmentum and caudate nucleus respectively.

Table 27: Oxygen uptake by brain tissue slices of different regions of the brain in rats fed LP and HP diets

| region | no.of determinations | no.of animals | microliters of oxygen uptake per 100 mg tissue per hour | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | none | | glucose | | glutamate | |
| | | | LP | HP | LP | HP | LP | HP |
| cerebellum | 3 | 9 | 32±4.8 (21-39) | 37±3.5 (31-43) | 91±2.7 (86-94) | 122±13.9 (108-150) | 57±2.7 (52-61) | 45±5.4 (39-56) |
| medulla | 3 | 18 | 33±0.6 (32-34) | 37±2.9 (32-42) | 58±3.8 (51-64) | 79±9.6 (64-97) | 46±4.1 (39-53) | 47±1.2 (45-59) |
| pons | 3 | 15 | 34±1.2 (32-36) | 31±1.9 (29-35) | 64±7.1 (53-77) | 70±2.4 (66-74) | 43±3.9 (38-51) | 39±1.9 (37-43) |
| midbrain | 4 | 20 | 31±4.7 (20-43) | 48±4.4 (39-59) | 110±3.9 (103-121) | 113±7.9 (99-132) | 60±1.5 (56-63) | 57±4.3 (49-69) |
| olfactory lobes | 3 | 18 | 39±1.9 (37-43) | 39±3.5 (32-44) | 83±2.2 (82-88) | 79±6.5 (67-89) | 62±2.3 (58-66) | 54±6.4 (42-63) |
| visual cortex | 3 | 15 | 46±3.4 (42-53) | 54±0.9 (52-55) | 111±7.5 (99-125) | 105±6.6 (97-118) | 70±6.5 (60-82) | 66±2.5 (63-71) |
| hippocampus | 3 | 15 | 44±3.8 (38-51) | 47±4.9 (39-56) | 111±5.5 (101-120) | 142±4.9 (133-150) | 82±2.0 (80-86) | 111±5.0 (102-119) |
| basal ganglia | 3 | 15 | 27±4.5 (18-33) | 54±5.2 (46-64) | 101±1.7 (98-104) | 133±4.9 (124-141) | 57±4.9 (48-65) | 93±4.0 (86-100) |
| hypothalamus | 1 | 15 | 49 | 61 | 90 | 109 | 74 | 81 |
| corpus callosum | 1 | 15 | 28 | 31 | 57 | 67 | 37 | 39 |
| residual brain | 3 | 3 | 49±2.4 (46-54) | 53±2.6 (49-58) | 87±5.3 (82-98) | 97±7.4 (82-105) | 67±0.9 (65-68) | 73±3.9 (67-80) |

Table 28: Oxygen uptake by brain tissue homogenates of different regions of the brain in rats fed LP and HP diets

| region | no.of animals | microliters of oxygen uptake per 100 mg tissue per hour | | | | | |
|---|---|---|---|---|---|---|---|
| | | none | | glucose | | glutamate | |
| | | LP | HP | LP | HP | LP | HP |
| cerebellum | 3 | 35 | 29 | 64 | 55 | 45 | 43 |
| medulla | 6 | 23 | 17 | 39 | 30 | 34 | 22 |
| pons | 5 | 22 | 18 | 41 | 31 | 23 | 21 |
| midbrain | 5 | 36 | 41 | 64 | 70 | 44 | 53 |
| olfactory lobes | 6 | 32 | 42 | 50 | 68 | 41 | 57 |
| hippocampus | 5 | 44 | 35 | 80 | 64 | 63 | 55 |
| hypothalamus | 15 | 37 | 41 | 62 | 70 | 54 | 53 |
| residual brain | 1 | 41 | 50 | 65 | 73 | 49 | 61 |

Values based on one determination.
It was not possible to get values for the visual cortex, basal ganglia and corpus callosum.

Table 29: Comparative effects of a low protein diet on oxygen uptake of brain tissue slices and homogenates

| region | LP values for oxygen uptake as per cent of HP values | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | without allowing for endogenous respiration | | | | | | after allowing for endogenous respiration | | | |
| | slices | | | homogenate | | | slices | | homogenate | |
| substrate added | none | glucose | glutamate | none | glucose | glutamate | glucose | glutamate | glucose | glutamate |
| cerebellum | 86 | 75 | 127 | 121 | 116 | 105 | 70 | 325 | 112 | 71 |
| medulla | 89 | 73 | 98 | 135 | 130 | 154 | 60 | 130 | 123 | 220 |
| pons | 110 | 91 | 110 | 122 | 132 | 110 | 77 | 113 | 146 | 33 |
| midbrain | 65 | 96 | 105 | 88 | 91 | 83 | 122 | 344 | 97 | 67 |
| olfactory lobes | 100 | 105 | 115 | 76 | 74 | 72 | 110 | 153 | 69 | 60 |
| visual cortex | 85 | 106 | 106 | - | - | - | 125 | 185 | - | - |
| hippocampus | 94 | 78 | 74 | 126 | 125 | 115 | 71 | 59 | 124 | 95 |
| basal ganglia | 50 | 76 | 61 | - | - | - | 97 | 77 | - | - |
| hypothalamus | 80 | 83 | 91 | 90 | 89 | 102 | 85 | 125 | 86 | 142 |
| corpus callosum | 90 | 85 | 95 | - | - | - | 81 | 129 | - | - |
| residual brain | 92 | 90 | 92 | 82 | 89 | 80 | 86 | 89 | 104 | 73 |

Table 30: Relative increase in oxygen uptake by brain tissue slices and homogenates with the addition of glucose and glutamate in LP and HP animals

| region | Oxygen uptake as per cent of endogenous uptake | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | slices | | | | homogenate | | | |
| | LP | | HP | | LP | | HP | |
| substrate added | glucose | glutamate | glucose | glutamate | glucose | glutamate | glucose | glutamate |
| cerebellum | 284 | 178 | 330 | 122 | 183 | 129 | 190 | 148 |
| medulla | 176 | 139 | 214 | 127 | 170 | 148 | 176 | 129 |
| pons | 188 | 126 | 226 | 126 | 186 | 105 | 172 | 117 |
| midbrain | 355 | 194 | 235 | 119 | 178 | 122 | 171 | 129 |
| olfactory lobes | 213 | 159 | 202 | 138 | 156 | 128 | 162 | 136 |
| visual cortex | 241 | 152 | 194 | 122 | - | - | - | - |
| hippocampus | 252 | 186 | 302 | 236 | 182 | 143 | 183 | 157 |
| basal ganglia | 374 | 211 | 246 | 172 | - | - | - | - |
| hypothalamus | 184 | 151 | 177 | 133 | 168 | 146 | 171 | 129 |
| corpus callosum | 204 | 132 | 216 | 126 | - | - | - | - |
| residual brain | 178 | 137 | 183 | 138 | 159 | 120 | 146 | 122 |

Table 31: Basal medium in the present studies compared with that of Weil-Malherbe (1936)

| component | millimoles per liter | |
|---|---|---|
| | Weil-Malherbe (1936) | present study |
| sodium | 151.0 | 165.0 |
| potassium | 4.8 | 7.7 |
| calcium | 2.6 | nil |
| magnesium | 1.2 | 1.5 |
| chloride | 132.0 | 134.0 |
| sulphate | 1.2 | 1.5 |
| phosphate | 16.0 | 19.5 |
| bicarbonate | nil | 4.6 |
| glucose | 11.0 | nil |

In the case of slices (Table 30) the per cent increase in oxygen consumption tends to be more in the HP group as compared to the LP group with the addition of glucose and less with the addition of glutamate although this is not true of all regions. In other words, the addition of glucose increases the difference between the two groups in the case of slices. This pattern is not observed with homogenates.

In many regions, the rate of endogenous respiration tends to be more in the HP group this difference being most evident in the case of the basal ganglia. This superiority is maintained with the addition of glucose as can be seen from percentage values presented in Table 29. However, when glutamate is used as substrate this difference either disappears or is reversed except in the case of hippocampus and basal ganglia. This trend is much more evident when the values are considered after allowing for endogenous respiration. Thus the data suggest the possibility of a greater accessibility of the brain tissue of LP animals to glutamic acid. It will be recalled in this connection that dietary supplementation with glutamic acid had differential effects on the two groups (Rajalakshmi et al., 1969).

Oxygen consumption tends to be decreased when homogenates are used in place of tissue slices except under

endogenous conditions in the LP group. This is clearly evident when the values are considered in terms of percentages (Table 32). The decrease in respiration when homogenates are used in place of tissue slices has been observed by other investigators (e.g., Reiner, 1947; Trojanova and Mourek, 1968). This is not surprising as ground tissue has to be supplied with many more nutrients than sliced tissue for efficient respiration (McIlwain, 1959).

The effects of homogenization are more evident in the HP group (Table 32) so that the differences between the two groups are decreased (Table 29). A similar phenomenon has been found in the dog brain in which age differences in oxygen consumption by brain tissue were less evident when homogenates were used in place of slices (Reiner, 1947).

The most striking observation is, however, the disappearance of the difference between the two groups with regard to oxygen consumption when glutamic acid is used as substrate as can be seen from the comparisons made in Table 29. In fact the difference would appear to be reversed in some cases if the values are considered after

Table 32: Oxygen uptake of brain tissue homogenates as compared to that of slices in LP and HP animals

| region | homogenate value for oxygen uptake as per cent of slice value | | | | | |
|---|---|---|---|---|---|---|
| | LP | | | HP | | |
| substrate added | none | glucose | glutamate | none | glucose | glutamate |
| cerebellum | 109 | 70 | 79 | 78 | 45 | 96 |
| medulla | 70 | 67 | 74 | 46 | 38 | 47 |
| pons | 65 | 64 | 53 | 58 | 44 | 54 |
| midbrain | 116 | 58 | 73 | 85 | 62 | 93 |
| olfactory lobes | 82 | 60 | 66 | 108 | 86 | 106 |
| hippocampus | 100 | 72 | 77 | 74 | 45 | 50 |
| hypothalamus | 76 | 69 | 73 | 67 | 64 | 65 |
| residual brain | 84 | 75 | 73 | 94 | 75 | 84 |

allowing for endogenous respiration. Further, when such allowance is made, the utilization of glutamate appears to be more efficient in the HP group when homogenates are used and less efficient when slices are used. The former observation was also made on homogenates prepared from whole brain (Table 33).

Table 33: Oxygen uptake by whole brain homogenate of rats fed LP and HP diets

| substrate added | oxygen uptake (microliters per 100 mg tissue per hour) | | per cent increase with substrate | |
|---|---|---|---|---|
| | LP | HP | LP | HP |
| none | 55±2.9 | 45±4.7 | - | - |
| glucose | 83±4.9 | 75±3.8 | 51 | 67 |
| glutamate | 63±1.5 | 60±3.0 | 14 | 33 |

Three animals were used in each group.

The reversal of the differences in the utilization of glutamate between the LP and HP groups when homogenates replace slices reinforces the suspicion that there are differences in cell permeability between the two groups. In this connection, as mentioned earlier, Platt and Stewart (1969) have reported changes in the appearance of the cell-membrane which appeared less distinct in the cerebellum of protein deficient dogs.

It should be pointed out however, that the results on homogenates of different areas do not include the data on the visual cortex, basal ganglia and corpus callosum and are based only on one determination in each case.

Both the amount of oxygen consumed and the effects on the same of the LP diet are found to vary from region to region. These variations, however, are not consistent. The corpus callosum, pons and medulla give relatively smaller values than other regions for oxygen consumption under all conditions (Tables 27 and 28). The values for the hypothalamus, visual cortex and the residual brain containing the thalamus tend to be generally higher. These differences are consistent with the observation of Tower (1959) who found the uptake of glutamic acid to be less in sub-cortical white matter than in cortical regions. The effects of the LP diet are most evident in the ganglia when slices are used and in the olfactory lobes when homogenates are used (Table 29). But the picture with regard to other regions varies under different conditions.

The relatively lower oxygen consumption of the medulla and the higher consumption of the cerebellum, basal ganglia and visual cortex with glucose as substrate have also been

reported in the dog brain (Himwich and Fazekas, 1941). Their results are compared in Table 34 with the present data.

The LP values for different regions were found to correlate with HP values for the corresponding condition (Table 35a).

Further, in either group, oxygen uptake under endogenous conditions was generally found to correlate with that when either glucose or glutamate was added (Table 35b) but no consistent correlations were found when values obtained with slices and homogenates were compared (Table 35c). This is consistent with the differential effects of homogenization in different groups and in different regions.

In summary, it may be said that in many brain regions in vitro oxygen uptake of brain tissue slices tends to be more in the HP animals. This difference tends to be either decreased or reversed with the use of glutamate as substrate. A similar phenomenon is observed when homogenates are used in place of slices. The differences found between the two groups with regard to the utilization of glutamate by tissue slices disappear in the case of most regions with the use

Table 34: Oxygen uptake by different parts of the brain

| region | | millilitres of oxygen uptake per g per hour | | |
|---|---|---|---|---|
| rat (present study) slice | dog (Himwich and Fazekas,1941) mince | present study | | Himwich and Fazekas, 1941 (mince) |
| | | LP | HP | |
| cerebellum | cerebellar cortex | 0.91 | 1.22 | 1.40 |
| medulla | medulla | 0.58 | 0.75 | 0.69 |
| midbrain | midbrain | 1.10 | 1.13 | 0.92 |
| visual cortex | cerebral cortex | 1.11 | 1.05 | 1.16 |
| basal ganglia | caudate nucleus | 1.01 | 1.33 | 1.36 |

Table 35a: Product moment correlations between oxygen uptakes under different conditions

| substrate added | 'r' between LP and HP values | |
|---|---|---|
| | slices | homogenate |
| none | 0.59 | 0.79* |
| glucose | 0.84** | 0.74* |
| glutamate | 0.80** | 0.78* |

Table 35b:

| | correlations with endogenous values | | | |
|---|---|---|---|---|
| | slices | | homogenate | |
| substrate | glucose | glutamate | glucose | glutamate |
| LP | 0.35 | 0.76** | 0.96** | 0.93** |
| HP | 0.61* | 0.73* | 0.97** | 0.96** |

Table 35c:

| substrate added | 'r' between slices and homogenate | |
|---|---|---|
| | LP | HP |
| none | 0.61 | 0.76* |
| glucose | 0.92** | 0.46 |
| glutamate | 0.96** | 0.60 |

*P < 0.05
**P < 0.01

of homogenates. These observations suggest the possibility of differences in cell-permeability to glutamic acid between the two groups.

Section D

## ASCORBIC ACID AND GLUTATHIONE

As mentioned earlier studies were conducted in order to confirm previous studies on ascorbic acid (Rajalakshmi et al., 1967) and to extend the same to the regional distribution of glutathione in the brain and its relation to that of ascorbic acid.

The distribution of ascorbic acid in different regions of the brain is shown in Table 36. The results confirm previous observations of a decrease in brain ascorbic acid with protein deficiency. No overlap was found between the values for the LP and HP groups except in the case of the basal ganglia. The regions most affected by the LP diet were the hypothalamus, pons, visual cortex and hippocampus. The first three of these regions were also found to be more affected in the previous studies. The basal ganglia were not affected either in the present or previous studies. However, the olfactory lobes which were found to be affected in the present studies were not affected in the previous studies.

Table 36: Distribution of ascorbic acid in different regions of the brain in rats fed LP and HP diets

| region | no. of determinations | no. of animals | ascorbic acid (mg per 100 g) LP | ascorbic acid (mg per 100 g) HP | LP as % of HP |
|---|---|---|---|---|---|
| cerebellum | 6 | 6 | 37.0±0.7 (31.5-39.6) | 42.4±0.7 (40.2-44.6) | 87** |
| medulla | 4 | 8 | 13.7±0.6 (12.6-15.1) | 17.7±0.7 (15.8-18.6) | 77** |
| pons | 4 | 8 | 11.5±0.2 (11.0-12.1) | 17.0±0.5 (16.3-18.3) | 68** |
| midbrain | 4 | 8 | 19.9±0.6 (18.2-21.0) | 26.4±1.2 (23.3-29.0) | 75** |
| olfactory lobes | 4 | 8 | 36.6±0.9 (34.2-38.7) | 42.7±0.6 (41.6-43.9) | 86** |
| visual cortex | 4 | 8 | 26.7±1.5 (24.1-30.2) | 38.6±0.5 (37.3-39.5) | 69** |
| hippocampus | 4 | 8 | 30.3±0.8 (27.9-31.6) | 42.1±0.6 (40.9-43.3) | 72** |
| basal ganglia | 4 | 8 | 31.0±0.6 (29.8-32.3) | 31.9±1.00 (30.1-34.1) | 97 |
| hypothalamus | 2 | 8 | 25.3±2.6 (22.6,27.9) | 40.2±1.9 (38.3,42.1) | 38* |
| corpus callosum | 2 | 8 | 19.8±1.5 (18.3,21.2) | 26.0±2.3 (23.7,28.3) | 76 |
| residual brain | 6 | 6 | 29.1±1.1 (26.8-33.8) | 34.3±0.7 (32.3-37.1) | 85** |

*Difference significant at 5% level
**Difference significant at 1% level.

In these as well as in other studies (Rajalakshmi et al., 1967; Rajalakshmi and Patel, 1968) the cerebellum, olfactory lobes, hypothalamus and visual cortex had higher concentrations of ascorbic acid than other regions such as the brain stem (pons, medulla and midbrain). In the present studies, the hippocampus was also found to have a higher concentration.

The data on glutathione are presented in Table 37. The range of values compares with that reported by other investigators for the whole brain (e.g., Isherwood,1959).

The values were found to be decreased with protein deficiency in all the regions. But the differences were not statistically significant in the case of some regions, namely, the midbrain, the basal ganglia and the corpus callosum. In the last case, however, there was no overlap in the values for the two groups. It will be recalled that the ascorbic acid concentration of the basal ganglia was also not affected. However, these results are based on a small number of observations.

The values for ascorbic acid in different regions were found to be significantly correlated with those for glutathione in both the LP and HP groups, the product-moment correlations being 0.76 in the former, and 0.77 in

Table 37: Distribution of glutathione in different regions of the brain in rats fed LP and HP diets

| region | no. of determinations | no. of animals | glutathione (mg per 100 g) LP | glutathione (mg per 100 g) HP | LP as % of HP |
|---|---|---|---|---|---|
| cerebellum | 5 | 15 | 28.1±1.2 (24.4-31.1) | 31.0±0.4 (30.2-31.9) | 91* |
| medulla | 3 | 18 | 20.0±0.5 (19.3-21.0) | 22.7±1.0 (21.0-24.4) | 88* |
| pons | 3 | 15 | 18.0±0.4 (17.6-18.9) | 19.5±0.6 (18.5-20.6) | 92 |
| midbrain | 3 | 15 | 24.1±0.6 (23.1-25.2) | 25.2±0.8 (23.9-26.5) | 96 |
| olfactory lobes | 3 | 18 | 26.0±1.1 (24.4-28.1) | 31.1±1.3 (28.6-33.2) | 84* |
| visual cortex | 3 | 15 | 27.9±0.8 (26.0-29.0) | 32.4±0.9 (30.7-33.6) | 86* |
| hippocampus | 3 | 15 | 28.4±0.3 (28.1-29.0) | 29.4±0.2 (29.0-29.8) | 97* |
| basal ganglia | 3 | 15 | 30.4±0.5 (29.4-31.1) | 32.9±1.2 (31.5-35.3) | 92 |
| hypothalamus | 2 | 30 | 25.6±0.4 (25.2,26.0) | 28.4±0.7 (27.7,29.0) | 90 |
| corpus callosum | 2 | 30 | 15.4±0.7 (14.7,16.0) | 18.3±0.7 (17.6,18.9) | 84 |
| residual brain | 5 | 5 | 25.3±0.5 (24.4-27.3) | 29.6±0.9 (26.5-31.1) | 95** |

*Difference significant at 5% level.
**Difference significant at 1% level.

the latter. In studies on women, similar correlations were obtained between the glutathione and ascorbic acid concentrations of placenta ($r=0.42$, $P < 0.01$) and blood ($r=0.36$, $P < 0.01$). However, the effects of protein deficiency on the two variables show some differences when the percentage values for the LP group are compared and the regions most affected in the case of ascorbic acid are not necessarily those most affected in the case of glutathione. It will be seen that the deficit of about 10% in brain glutathione with protein deficiency was much less than that of about 40% in liver glutathione found in other studies (Rajalakshmi and Ramakrishnan, 1969). This is consistent with the pattern for other substances.

In conclusion, the present studies mostly confirm previous observations on the effects of protein deficiency on the ascorbic acid content of different regions in the brain and show that the glutathione content of different regions in the brain is also similarly affected. The concentrations of the two substances in different regions were found to be correlated.

Section E

## A RESUME OF THE SECTIONS A-D

The data described in the preceding sections were reviewed in order to see if they suggest a general pattern

of metabolic activity in different regions and their susceptibility to protein deficiency.

The values for each region are expressed as percentage of the lowest value in Tables 38 and 39. It will be seen from the same that the extent of variation within brain regions differs with different parameters. The minimum variation is found in the case of GOT and protein. About a 100% variation is found in the case of glutathione, GDH, glutamyl transferase and respiration. It is much higher in the case of GPT (200%) and GAD (500%). In the case of vitamin C and glutamine synthetase the variation in the LP group is more than that of HP group. This is also true to some extent of protein, GAD and GOT. It can also be seen from these tables that some regions tended to have relatively high values for all the parameters whereas others had consistently smaller values. Generally low values were obtained in the case of the corpus callosum. This was particularly evident in the case of GAD and GPT. Medulla and pons also had generally low values as compared to other regions except in the case of GDH. On the other hand the values were found to be high or intermediate in the case of olfactory lobes, visual cortex and the residual brain containing the thalamus. This was also mostly true of the cerebellum, hypothalamus and midbrain. The hippocampus and basal ganglia had more

Table 38: Enzyme activities in different regions of HP animals expressed as per cent of lowest value

| region | GDH | GAD | GPT | GOT | glutamyl transferase | glutamine synthetase |
|---|---|---|---|---|---|---|
| cerebellum | 105 | 275 | 338 | 109 | 177 | 167 |
| medulla | 179 | 275 | 277 | 100 | 163 | 122 |
| pons | 153 | 200 | 246 | 105 | 173 | 133 |
| midbrain | 163 | 513 | 277 | 102 | 169 | 133 |
| olfactory lobes | 121 | 313 | 277 | 105 | 199 | 211 |
| visual cortex | 116 | 375 | 385 | 106 | 179 | 167 |
| hippocampus | 100 | 250 | 192 | 110 | 131 | 111 |
| basal ganglia | 105 | 375 | 215 | 113 | 117 | 100 |
| hypothalamus | 126 | 663 | 123 | 115 | 112 | 111 |
| corpus callosum | 100 | 100 | 100 | - | 100 | 100 |
| residual brain | 121 | 400 | 254 | 118 | 143 | 133 |
| highest / lowest | 1.8 | 6.6 | 3.9 | 1.2 | 2.0 | 2.1 |

Table 39: HP values for protein, glutathione, ascorbic acid and *in vitro* oxygen uptake expressed as per cent of the lowest value

| region | protein | glutathione | ascorbic acid | oxygen uptake by slices | | |
|---|---|---|---|---|---|---|
| | | | | none | glucose | glutamate |
| cerebellum | 119 | 169 | 250 | 119 | 182 | 115 |
| medulla | 182 | 124 | 104 | 119 | 118 | 121 |
| pons | 109 | 107 | 100 | 100 | 104 | 100 |
| midbrain | 112 | 138 | 155 | 155 | 169 | 146 |
| olfactory lobes | 126 | 170 | 251 | 126 | 118 | 138 |
| visual cortex | 117 | 177 | 227 | 174 | 157 | 169 |
| hippocampus | 106 | 161 | 248 | 152 | 212 | 285 |
| basal ganglia | 100 | 180 | 188 | 174 | 199 | 238 |
| hypothalamus | 128 | 155 | 236 | 197 | 163 | 208 |
| corpus callosum | 119 | 100 | 153 | 100 | 100 | 100 |
| remaining cortex | 129 | 162 | 202 | 171 | 145 | 187 |
| highest / lowest | 1.8 | 1.8 | 2.5 | 2.0 | 2.1 | 2.9 |

of intermediate values. The higher values generally obtained for the cerebellum, olfactory lobes, visual cortex, residual brain and hypothalamus are consistent with the greater proportion of grey matter in those regions. The high concentration of GDH in the medulla and pons in contrast to low values obtained in the case of most other parameters is indeed intriguing.

The effects of protein deficiency varied with the parameter measured and the regions studied. They are compared in Tables 40-41. It will be seen from the same that the enzymes GDH, GAD, GPT, GOT and vitamin C showed more change than the other parameters measured. The greater sensitivity of some regions as compared to others is strikingly evident in the case of vitamin C (hypothalamus), GAD (hypothalamus and basal ganglia), GPT (medulla and pons) and glutamine synthetase (basal ganglia). The significance of these differences is far from clear in the state of our present knowledge of the biochemistry of different regions.

Table 40: LP value for weight and enzyme activity expressed as per cent of HP value

| region | weight | GDH | GAD | GPT | GOT | glutamyl transferase | glutamine synthetase |
|---|---|---|---|---|---|---|---|
| cerebellum | 91 | 80 | 73 | 64 | 75 | 101 | 93 |
| medulla | 89 | 82 | 59 | 42 | 68 | 93 | 82 |
| pons | 90 | 97 | 75 | 56 | 72 | 91 | 92 |
| midbrain | 89 | 81 | 76 | 89 | 68 | 98 | 100 |
| olfactory lobes | 81 | 84 | 100 | 86 | 78 | 106 | 84 |
| visual cortex | 83 | 91 | 63 | 66 | 75 | 93 | 87 |
| hippocampus | 90 | 79 | 85 | 112 | 76 | 95 | 90 |
| basal ganglia | 74 | 95 | 53 | 79 | 83 | 83 | 56 |
| hypothalamus | 92 | 88 | 51 | 106 | 73 | 84 | 110 |
| corpus callosum | 91 | 68 | 63 | 85 | - | 94 | 89 |
| residual brain | 90 | 87 | 66 | 79 | 72 | 93 | 92 |
| range | 74-92 | 68-97 | 51-100 | 40-112 | 68-83 | 83-106 | 56-110 |

Table 41: LP value for protein, glutathione, ascorbic acid and *in vitro* oxygen uptake expressed as per cent of HP value

| region | protein | glutathione | ascorbic acid | oxygen uptake by slices | | |
|---|---|---|---|---|---|---|
| | | | | none | glucose | glutamate |
| cerebellum | 86 | 91 | 87 | 86 | 75 | 127 |
| medulla | 77 | 88 | 77 | 89 | 73 | 98 |
| pons | 75 | 92 | 68 | 110 | 91 | 110 |
| midbrain | 91 | 96 | 75 | 65 | 96 | 105 |
| olfactory lobes | 93 | 84 | 86 | 100 | 105 | 115 |
| visual cortex | 87 | 86 | 69 | 85 | 106 | 106 |
| hippocampus | 94 | 97 | 72 | 94 | 78 | 74 |
| basal ganglia | 108 | 92 | 97 | 50 | 76 | 61 |
| hypothalamus | 83 | 90 | 38 | 80 | 83 | 91 |
| corpus callosum | 74 | 84 | 76 | 90 | 85 | 95 |
| residual brain | 86 | 95 | 85 | 92 | 90 | 92 |
| range | 74-108 | 84-97 | 38-97 | 50-110 | 73-106 | 61-127 |