CHAPTER 3

AGE RELATED EFFECTS OF NUTRITIONAL VITAMIN B$_6$ DEFICIENCY ON BRAIN FREE AMINO ACIDS OF THE GLUTAMATE GROUP AND B$_6$-DEPENDENT ENZYMES OF GLUTAMATE AND 7-AMINOBUTYRATE SYSTEMS
The functioning of the mammalian brain is controlled by a number of factors like cell to cell interaction (transport of material between cells), the extra cellular environment within the CNS, nutrient supplies, electrolyte gradients, hormones and specific growth factors and factors external to the organ which affect learning and behaviour. Extensive reviews by Coursin (1965), Dobbing (1970), Rajalakshmi and Ramakrishnan (1972), Dodge et al. (1975b) and Dyson and Jones (1976) have provided considerable amount of information on nutrition and brain function. Dodge et al. (1975c) have reviewed the importance of vitamins on CNS functioning. Of all the vitamins, B₆ and related compounds have been shown to have the greatest spectrum of biochemical activities (Rev. Sturman and Rivlin, 1975) because of the multiplicity of reactions catalyzed by them and hence have a significant role in the maintenance of normal CNS function (Minard, 1967; Tapia et al., 1969; Snell, 1972; Schlesinger and Uphouse, 1972; Gey and Georgi, 1974). Vitamin B₆, an essential constituent of brain is not synthesized by mammalian tissues and has to be obtained from the diet (Thiele and Brin, 1968). Hence, a nutritional deficiency of vitamin B₆ affects the CNS and its functioning by causing multiple biochemical disturbances (Roberts et al. 1951b; Tower, 1956; McKhann et al. 1961; Dakshinamurthy and Stephens, 1969; Bayoumi and Smith, 1972; Bayoumi et al. 1972) and thereby changes in the...
metabolism of the brain. It has been suggested that the primary mechanism by which pyridoxine deficiency contributes to mental disturbances might be related to the disturbed amino acid pool of the brain and hence disturbed protein metabolism (Rev. Sturman and Rivlin, 1975). In this context, Tews and Lovell (1967) and Tews (1969) have studied mouse brain free amino acid changes as a function of pyridoxine deficiency while Roberts et al. (1951b), Bayoumi and Smith (1972) and Bayoumi et al. (1972) have studied the effects of pyridoxine deficiency on 7-ABA metabolism both during development and in the young adult rats and Brin and Thiele (1967) and Thiele and Brin (1968) on AsAT and ALAT activities.

All these reports have provided information concerning the effects of pyridoxine deficiency on amino acids and related enzyme systems either during developmental stages or in the young ones. The effects of the same during the later stages have not been reported. Gewacke et al. (1977) have examined the effects of B6 deficiency on the behaviour of old animals. But, to date data on metabolic and biochemical correlates of such a deficiency as a function of age is not reported.

The present study reports age related effects of nutritional pyridoxine deficiency on:

1. Brain free amino acids related to glutamic acid-7-aminobutyric acid, glutamine, aspartic acid and alanine, and
II. related B₆-dependent enzymes—glutamic acid
dercarboxylase, 7-ABA-transaminase, aspartate amino-
transferase, alanine aminotransferase and glutamo-
transferase.

Materials and Methods:

The animals were maintained on a special diet consisting
of 72% carbohydrate (double polished rice flour), 15%
casein—vitamin free, 7% groundnut oil, 4% Hawk—Oser mineral
mixture (Oser, 1965) and 2% vitamin mixture (Rajalakshmi et al.
1974a). The concentration of pyridoxine HCl however, was
increased to 50 μg/100 g. to ensure the adequacy of intake
(Beaton and Cheney, 1965). The experimental animals were
maintained on the above diet except that vitamin B₆ was
omitted.

Male rats aged 1 day, 21 days, 3, 12 and 24 months
were used for inducing deficiency. Neonatal pyridoxine
deficiency—from 1 day to 21 days (Group 1) was induced by
feeding the mothers with pyridoxine deficient diet (Bayoumi
and Smith, 1972). Rats aged 21 days (Group 2) and 3 months
(Group 3) were fed the deficient diet for 4 weeks (Tews and
Lovell, 1967; Bayoumi and Smith, 1973) while those aged
12 months (Group 4) and 24 months (Group 5) for 10 weeks
(Gewacke et al., 1977).
Table - 13

Body weight and brain weight as a function of age and pyridoxine deficiency in the rat

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Brain weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Experimental</td>
</tr>
<tr>
<td>1</td>
<td>37.4 ± 1.8</td>
<td>16.8 ± 0.9*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(44%)</td>
</tr>
<tr>
<td>2</td>
<td>80.2 ± 4.1</td>
<td>61.5 ± 4.2*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(77%)</td>
</tr>
<tr>
<td>3</td>
<td>151.8 ± 8.7</td>
<td>127.2 ± 7.7*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(84%)</td>
</tr>
<tr>
<td>4</td>
<td>249.3 ± 10.6</td>
<td>254.4 ± 10.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(102%) N.S.</td>
</tr>
<tr>
<td>5</td>
<td>289.9 ± 12.0</td>
<td>284.8 ± 15.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(98%) N.S.</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD of 12 observations.

Values in parenthesis indicate % of control values.

* p < 0.001  N.S. - Not significant
Biochemical studies:

The animals were decapitated at the end of the feeding period, and the biochemical assays were done. The details of the method are described in Chapter 1. For all the enzymes, the assays were performed with (50 µg/ml. of homogenizing medium) and without PLP to give an estimate of the degree of endogenous cofactor saturation of the enzyme.

Results:

Body weight and brain weight:

Table 13 shows the effect of pyridoxine deficiency at various ages on body weight and brain weight. It is observed that the deficiency during the neonatal period has resulted in a highly significant 56% (p<0.001) reduction in body weight. Groups 2 and 3 have also shown highly significant 23% (p<0.001) and 16% (p<0.001) reduction while in groups 4 and 5 the differences are very small and insignificant.

Brain weight is affected to a much lesser extent compared to the body weight. Groups 1 and 2 have shown 16% (p<0.001) and 11% (p<0.001) deficit while groups 3, 4 and 5 have shown no significant difference as compared to the control animals.

I. Glutamic acid, 7-ABA, glutamine, aspartic acid and alanine:

Results on the effect of pyridoxine deficiency induced at various ages on glutamic acid, 7-ABA, glutamine, aspartic acid and alanine:
acid and alanine are shown in Fig. 25 and per cent changes as compared to the controls of the same age in Fig. 26.

Deficiency in all the age groups studied showed significant decreases \((p<0.001)\) in the level of glutamic acid - 34% in Groups 1 and 5 and 29%, 27% and 30% respectively in groups 2, 3 and 4 (Fig. 26-a).

The level of 7-ABA is more affected by the deficiency at all ages compared to glutamic acid. Of all the 5 age groups, 2 and 3 appear to be more resistant to deficiency showing 49% and 43% deficits while maximal effect of 64% is observed in group 1 and 57% and 59% in groups 4 and 5 (Fig. 26-b). All the differences are highly significant \((p<0.001)\).

Level of glutamine shows a highly significant 37% \((p<0.001)\) decrease in group 1 and a small but significant \((p<0.05)\) 7% decrease in group 2. The other age groups are not affected significantly (Fig. 26-c).

In the case of aspartic acid, a deficit of 45% and 48% are observed in groups 1 and 2 while 23%, 33% and 30% in groups 3, 4 and 5 (Fig. 26-d).

No significant age-difference is observed for alanine—all age groups show significant increases of 23 to 28% (Fig. 26-e).

The effect of the deficiency on the ratio of excitatory/inhibitory transmitters \((\text{Glu} + \text{asp/7-ABA} + \text{ala})\) is shown in
Fig. 25(a–e)

Levels of glutamic acid, 7-aminobutyric acid, glutamine, aspartic acid and alanine as a function of age and pyridoxine deficiency in rat brain.

Values are represented as mean ± SD of 4 observations.

Gr. - Group       C - Control       E - Experimental
Per cent changes from the controls of brain glutamic acid, 7-aminobutyric acid, glutamine, aspartic acid and alanine in pyridoxine deficient rats of different age groups.

Gr. - Group  C - Control  E - Experimental
Fig 26

Glutamic acid  (a)  

\[-\text{aminobutyric acid} \quad (b)\]

- Glutamine  

Aspartic acid  (d)  

Alanine  (e)
Table - 14

Activity of glutamic acid decarboxylase as a function of age and pyridoxine deficiency in rat brain

<table>
<thead>
<tr>
<th>Group</th>
<th>Control + PLP</th>
<th>Control - PLP</th>
<th>Experimental + PLP</th>
<th>Experimental - PLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.26 ± 0.22</td>
<td>1.09 ± 0.11</td>
<td>3.81 ± 0.16</td>
<td>0.34 ± 0.07†</td>
</tr>
<tr>
<td>2</td>
<td>3.58 ± 0.24</td>
<td>2.20 ± 0.19</td>
<td>4.00 ± 0.19</td>
<td>1.36 ± 0.09†</td>
</tr>
<tr>
<td>3</td>
<td>4.23 ± 0.24</td>
<td>2.71 ± 0.15</td>
<td>4.10 ± 0.17</td>
<td>1.51 ± 0.10†</td>
</tr>
<tr>
<td>4</td>
<td>4.59 ± 0.21</td>
<td>3.24 ± 0.22</td>
<td>4.69 ± 0.15</td>
<td>1.37 ± 0.14†</td>
</tr>
<tr>
<td>5</td>
<td>5.10 ± 0.24</td>
<td>3.82 ± 0.24</td>
<td>5.15 ± 0.21</td>
<td>1.08 ± 0.13†</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD of 4 observations.

† p < 0.001
Figs. 27 and 28. The ratio has decreased by 14% in deficient animals of group 1, by 9% in groups 2 and 3 and by 4% and 7% respectively in groups 4 and 5.

II. PLP-dependent enzymes of glutamate and 7-ABA metabolism:

1. Glutamic acid decarboxylase:

Table 14 shows the activities of GAD in the presence and absence of exogenously added PLP in the brain of animals from the five age groups as a function of pyridoxine deficiency. % endogenous saturation of the enzyme with PLP calculated from values in Table 14 is shown in Fig. 29-a. Apoenzyme activity in terms of % of control value is shown in Fig. 30-a.

In the controls, the activity of GAD holoenzyme is 48% in group 1 and 61% and 64% in groups 2 and 3. However, at 12 months and 24 months there is a significant increase of 7% (p<0.01) and 11% (p<0.001) as compared to the adult.

In the deficient animals, there is a highly significant (p<0.001) decrease in the level of the holoenzyme at all ages as compared to the controls. Maximum effect is observed in group 1 showing only 15% activity while groups 2, 3, 4 and 5 show 38%, 36%, 30% and 21% respectively.

The GAD apoenzyme level has shown a highly significant 69% (p<0.001) increase in group 1 and a significant 12%
Fig. 27

Ratio of Glu. acid + asp. acid / 7-ABA + alanine as a function of pyridoxine deficiency in different age groups of rats.

Gr. - Group C - Control E - Experimental

Fig. 28

Per cent changes in the ratio of glu. acid + asp. acid / 7-ABA + alanine as a function of pyridoxine deficiency in different age groups of rats.

Gr. - Group C - Control E - Experimental
Fig. 27

Ratio

Glu. acid • Asp. acid / γ-ABA • Ala

C  E  Gr. 1
C  E  Gr. 2
C  E  Gr. 3
C  E  Gr. 4
C  E  Gr. 5

Fig. 28

%  100

C  E  Gr. 1
C  E  Gr. 2
C  E  Gr. 3
C  E  Gr. 4
C  E  Gr. 5
Table 15

Activity of 7-ABA-transaminase as a function of age and pyridoxine deficiency in rat brain.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ PLP</td>
<td>- PLP</td>
</tr>
<tr>
<td>1</td>
<td>1.08 ± 0.10</td>
<td>0.94 ± 0.07</td>
</tr>
<tr>
<td>2</td>
<td>1.30 ± 0.07</td>
<td>1.04 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>1.24 ± 0.05</td>
<td>1.03 ± 0.06</td>
</tr>
<tr>
<td>4</td>
<td>1.02 ± 0.14</td>
<td>0.74 ± 0.10</td>
</tr>
<tr>
<td>5</td>
<td>0.88 ± 0.14</td>
<td>0.52 ± 0.08</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD of 4 observations.

\[ p < 0.001 \]
(p<0.05) increase in group 2 in the deficient animals as compared to controls while the changes observed in groups 3, 4 and 5 are not significant.

2. \( \gamma \)-ABA-transaminase:

Table 15 shows the activities of \( \gamma \)-ABA-T in the presence and absence of exogenously added PLP in the brain of rats from 5 age groups as a function of pyridoxine deficiency. Fig.29-b shows \( % \) endogenous saturation of the enzyme with PLP and Fig.30-b, the apoenzyme activity in terms of \( % \) of control value.

In the normal rats, the activity of \( \gamma \)-ABA-T holoenzyme is 87%, 80% and 83% in groups 1, 2 and 3 while the same has decreased to 73% and 63% in groups 4 and 5.

In the deficient animals, there is a highly significant (p<0.001) decrease in the level of holoenzyme in all the age groups as compared to the controls. Maximum effect is observed in group 1 showing only 37% activity while groups 2, 3, 4 and 5 show respectively 62%, 75%, 55% and 49% of control values.

There is a decrease in the level of \( \gamma \)-ABA-T apoenzyme in pyridoxine deficient groups 1 and 2 showing 33% (p<0.001) and 22% (p<0.01) deficits respectively while in groups 3, 4 and 5 the differences are not significant.
Activity of aspartate aminotransferase as a function of age and pyridoxine deficiency in rat brain.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (+ PLP)</th>
<th>Control (- PLP)</th>
<th>Experimental (+ PLP)</th>
<th>Experimental (- PLP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34.9 ± 1.7</td>
<td>35.7 ± 2.40</td>
<td>36.2 ± 2.80</td>
<td>14.0 ± 1.40</td>
</tr>
<tr>
<td>2</td>
<td>35.2 ± 1.91</td>
<td>35.8 ± 2.90</td>
<td>34.5 ± 1.90</td>
<td>18.0 ± 2.60</td>
</tr>
<tr>
<td>3</td>
<td>38.1 ± 1.70</td>
<td>38.0 ± 3.00</td>
<td>35.4 ± 2.20</td>
<td>25.4 ± 1.00</td>
</tr>
<tr>
<td>4</td>
<td>41.4 ± 2.50</td>
<td>40.2 ± 2.80</td>
<td>32.2 ± 2.40</td>
<td>22.6 ± 2.10</td>
</tr>
<tr>
<td>5</td>
<td>40.2 ± 2.80</td>
<td>41.3 ± 2.80</td>
<td>26.0 ± 1.60</td>
<td>22.0 ± 1.60</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD of 4 observations.

\[ p < 0.001 \]
Activity of alanine aminotransferase as a function of age and pyridoxine deficiency in rat brain.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ PLP</td>
<td>- PLP</td>
</tr>
<tr>
<td>1</td>
<td>1.15 ± 0.14</td>
<td>1.03 ± 0.08</td>
</tr>
<tr>
<td>2</td>
<td>1.22 ± 0.14</td>
<td>1.18 ± 0.18</td>
</tr>
<tr>
<td>3</td>
<td>1.15 ± 0.15</td>
<td>1.07 ± 0.13</td>
</tr>
<tr>
<td>4</td>
<td>1.14 ± 0.09</td>
<td>1.05 ± 0.19</td>
</tr>
<tr>
<td>5</td>
<td>1.17 ± 0.06</td>
<td>1.15 ± 0.08</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD of 4 observations

1 p < 0.001
3. Aspartate aminotransferase:

Activities of AsAT in the presence and absence of exogenously added PLP in the brain as a function of age and pyridoxine deficiency are shown in Table 16. Endogenous saturation of the enzyme with PLP is shown in Fig. 29-c and apoenzyme activity in Fig. 30-c.

In normal rats, at all the ages studied, the enzyme is completely saturated with PLP since exogenous PLP has not resulted in significant changes in activity.

In the deficient rats, there is a highly significant (p<0.001) decrease in the level of the holoenzyme in all the age groups studied. Group 1 shows a 60% decrease while groups 2, 3, 4, and 5 show a decrease of 49%, 33%, 45% and 43% respectively.

No significant difference in the level of AsAT apoenzyme between the normal and deficient rats of groups 1, 2 and 3 are observed. However, a significant 22% (p<0.01) decrease in group 4 and a highly significant (p<0.001) 35% decrease in group 5 is observed.

4. Alanine aminotransferase:

Activities of AlAT in presence or absence of exogenously added PLP as a function of pyridoxine deficiency at different ages are shown in Table 17. As in AsAT, AlAT is also fully saturated with PLP at all ages in normal animals. But unlike
Activity of glutamotransferase as a function of age and pyridoxine deficiency in rat brain.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th>Experimental</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ PLP</td>
<td></td>
<td></td>
<td></td>
<td>+ PLP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.23 ± 0.14</td>
<td>1.16 ± 0.13</td>
<td>1.15 ± 0.12</td>
<td>0.52 ± 0.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.66 ± 0.14</td>
<td>1.70 ± 0.15</td>
<td>1.74 ± 0.19</td>
<td>1.59 ± 0.18</td>
<td>N.S.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.89 ± 0.16</td>
<td>1.85 ± 0.12</td>
<td>1.81 ± 0.14</td>
<td>1.88 ± 0.15</td>
<td>N.S.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.43 ± 0.14</td>
<td>2.58 ± 0.18</td>
<td>2.35 ± 0.17</td>
<td>2.48 ± 0.20</td>
<td>N.S.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.93 ± 0.15</td>
<td>2.98 ± 0.18</td>
<td>2.84 ± 0.24</td>
<td>2.93 ± 0.21</td>
<td>N.S.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD of 4 observations.

* p < 0.001

N.S. - Not significant
AsAT, deficiency has not resulted in significant changes in the apoenzyme level at any age (Fig. 30-d). However, the degree of decrease in the activity of AlAT holoenzyme at all ages is more when compared to that of the AsAT enzyme showing only 37%, 43%, 46%, 36% and 28% activity as compared to the controls (Fig. 29-d).

5. Glutamotransferase:

Activities of CT in the presence and absence of exogeneously added PLP in the brain as a function of pyridoxine deficiency at different ages are shown in Table 18. % endogenous saturation of the enzyme with PLP is shown in Fig. 29-e and apoenzyme activity in Fig. 30-e. In normal animals, CT compares well with AsAT and AlAT in the level of endogenous saturation with PLP. Pyridoxine deficiency has resulted in a highly significant 58% decrease in the level of holoenzyme in group 1 while the other groups show small differences which are not significant when compared to the controls. As in the enzyme AlAT, deficiency has not affected the level of apoenzyme significantly at any age.

Discussion:

Vitamin $B_6$, in the phosphorylated form is intimately involved in the amino acid metabolism (Meister, 1965). Hence, a nutritional pyridoxine deficiency results in disturbed amino acid metabolism (Revs. Sturman and Rivlin, 1975) mainly
Fig. 29(a-e)

Per cent endogenous saturation of brain GAD, \( \gamma \)-ABA-T, AsAT, ALAT and GT as a function of pyridoxine deficiency in different age groups of rats.

Gr. - Group  C - Control  E - Experimental
GAD, γ-ABA-T, AsAT, AlAT and GT apoenzyme activity as a function of pyridoxine deficiency in different age groups of rats.

Gr. - Group C - Control E - Experimental
Fig 30

(a) Glutamic acid decarboxylase
(b) L-ABA transaminase
(c) Aspartate aminotransferase
(d) Alanine aminotransferase
(e) Glutamotransferase
by its action on various PLP-dependent enzymes (Roberts et al., 1951a; Brin and Thiele, 1967; Thiele and Brin, 1968; Dakshinamurthy and Stephens, 1969; Stephens et al., 1971; Bayoumi and Smith, 1972; Bayoumi et al., 1972). To have a clear understanding of the differential sensitivity of various PLP-dependent enzymes to the deficiency, importance of the relative activities of the aminotransferases in regulating the concentration of amino acids with postulated neurophysiologic and metabolic functions and to emphasize the effects of age on the above functions, the results of age related effects of pyridoxine deficiency on the metabolism of the glutamate group of amino acids are discussed.

Body weight and brain weight:

It is clear from Table 13 that pyridoxine deficiency during the first 21 days of postnatal life results in a deficit in both body weight and brain weight. A highly significant 56% (p<0.001) deficit in body weight and 16% (p<0.001) deficit in brain weight is observed as a consequence of pyridoxine deficiency. These results are in accordance with that of Eberle and Eiduson (1968), Stephens et al. (1971) and Bayoumi and Smith (1972). Similar deficits in body weight and brain weight as a function of general undernutrition are reported by Adländ and Dobbing (1971), Rajalakshmi et al. (1974b) and Rajeswari and Radha (1980).
Deficient animals of group 2 also show highly significant 23% ($p<0.001$) reduction in body weight and 11% ($p<0.001$) reduction in brain weight. Bayoumi and Smith (1972) have also reported such deficits in body weight and brain weight in weanling rats subjected to pyridoxine deficiency. Further, when young adults were subjected to pyridoxine deficiency a 16% reduction in body weight was noted while the brain weight remained unaffected. So also, undernutrition in adult rats which resulted in a 40 - 45% reduction in body weight failed to show significant reduction in brain weight (Dobbing, 1970). Mature adults and old animals (groups 4 and 5), however, have shown no reduction either in body weight or brain weight.

Lack of effect of pyridoxine deficiency on body weight in older rats is also reported by Gewacke et al. (1977).

The present results on age related effects of pyridoxine deficiency on brain weight are well supported by the 'vulnerable period hypothesis' which states that 'the processes of development in the brain are likely to be more vulnerable to any stress at the time of their fastest growth rate' (Dobbing 1968). Concomittant with this is the maximum effect of pyridoxine deficiency during the first 21 days, the effect being much less in weanling rats while the other groups - young adult, mature adult and aged animals are not at all affected by the deficiency.

A highly significant decrease in the concentration of glutamic acid is observed as a function of pyridoxine
deficiency in all the groups studied. A maximum deficit of 34% is observed in group 1 (Fig.26-a). Thurston et al. (1971) have reported a similar decrease in glutamic acid level in developing brains of undernourished or protein deficient rats and this has been attributed either to a decreased synthesis of glutamate by brain or to an increased use of glutamate as an energy source (Thurston et al., 1971).

7-ABA is more affected by deficiency during the first three weeks compared to glutamic acid showing a 64% deficit (Fig.26-b). This can be explained by a very high decrease in the activity of GAD holoenzyme which is only 15% compared to the control animals (Fig.29-a).

Glutamine has shown a highly significant deficit of 37% (Fig.26-c) which is almost the same as its synthetic precursor glutamic acid. Whether this decrease in glutamic acid or glutamine can be explained by their synthesizing enzymes glutamic acid dehydrogenase and glutamine synthase is difficult to ascertain since they are not PLP-dependent. However, if the 'concept of vulnerability' (Dobbing, 1968) is applied, decreased GDH and GS activities might be a likely answer for decreased glutamic acid and glutamine levels.

The 45% decrease in the level of aspartic acid (Fig.26-d) observed can be attributed to decreased AsAT activity (Fig. 29-c).
Alanine has shown a 24% increase (Fig. 26-e) which may be explained on the basis of suppressed synthesis of glutamate because of the effects of deficiency on the transamination reaction catalysed by ALAT (Fig. 29-d).

Groups 2, 3 and 4 have shown a 29%, 27% and 30% decrease respectively in the level of glutamic acid. However, the aged brain (Group 5) compares well with the developing brain as far as the effect of deficiency on glutamic acid is concerned (Fig. 26-a).

The level of γ-ABA in groups 2, 3, 4 and 5 has decreased by 49%, 43%, 57% and 59% (Fig. 26-b). These correlate well with the activity of GAD holoenzyme (Fig. 29-a) associated with decreased availability of the precursor glutamic acid (Fig. 25-a) which has shown a pattern of change similar to γ-ABA under deficient conditions at all ages. Since the γ-ABA shunt represents an alternate pathway to the portion of the TCA cycle (Fig. 1) that leads from α-KG to succinate and since it is intimately related to the oxidative metabolism of carbohydrates in the CNS by means of this shunt, the observed results are very interesting and these clearly show that pyridoxine deficiency at any age interferes with the metabolic as well as neurophysiologic functions probably resulting in mental disturbances like seizures although such manifestations were not observed in the present study. Failure to observe seizures in the deficient rats has been reported by Lyon et al. (1958) and Tews and Lovell (1967). This has been
attributed to genetic variability in susceptibility to convulsions. However, other behavioural manifestations like abnormal movements, lethargic state of the animal, etc. confirmed the neurological involvement in deficient conditions at all ages.

Glutamine level in group 2 has shown a very small decrease of 7% and no significant change in the other 3 groups (Fig. 26-c). The result for weanling rat, however, compares with that of Tews and Lovell (1967). It appears that once the brain has matured, the enzyme synthesizing glutamine - glutamine synthase is not affected by pyridoxine deficiency and the effect may be only during the 'critical period' of brain development. Since the synthesis of glutamine is an energy requiring process catalyzed by the ATP dependent GS, glutamic acid might serve in dual functions - as a precursor for GS and probably as an energy source in undernourished brains as suggested by Thurston et al. (1971). However, these studies are restricted to developing animals and the present results give support to the fact that the same might apply to the adult as well as aged pyridoxine deficient animals.

Aspartic acid in group 2 is affected to the same degree as in group 1 (Fig. 26-d). This may be possible because adult levels are not reached by 21 days and there is a significant 20% increase between 21 days and 3 months (Chapter 1, Fig. 3-d). Groups 3, 4 and 5 show the same degree of change to
deficiency and this is again reflected in the activity of the enzyme AsAT (Figs. 29-c, and 30-c).

Alanine has shown almost the same degree of increase in all the later age groups studied (Fig. 26-e) which may be because of suppressed activity of AlAT directed towards glutamate formation thereby causing increased accumulation of alanine.

Results on the effects of deficiency on the ratio of excitatory/inhibitory transmitters (Glu + asp/γ-ABA + ala) are very interesting (Figs. 27 and 28). The ratio is affected only by deficiency during developmental stages (group 1) and other four groups show a small decrease in the ratio. Since the balance between excitatory/inhibitory transmitters rather than the absolute amounts is important in the regulation of neuronal activity (Timiras et al., 1973) small changes observed reflect on maintenance of normal neurophysiologic function. This probably provides an excellent biochemical explanation for the absence of convulsions observed in the present study as well as in the studies of Lyon et al. (1958) and Tews and Lovell (1967). However, large decrease in the absolute amount of each amino acid clearly reflects on the decrease in the degree of efficacy of signals as well as brain energy metabolism since all these amino acids are involved in neural metabolic functions in addition to neurophysiologic functions.
Free amino acid levels in the brain are controlled by various factors other than the synthesizing and degrading enzymes investigated - like the passage of amino acids between plasma and brain across the blood-brain-barrier and entry of these substances into or exit from the tissue itself by means of cellular transport (Tews, 1969). Further, Heindel and Riggs (1968) have suggested that basic failure in amino acid transport may be related to the depletion of growth hormone seen in pyridoxine deficient animals. But how far this applies to the brain tissue has to be investigated.

The activity of GAD (Table 14, Figs. 29-a and 30-a) assayed without exogenous PLP which is indicative of the endogenous saturation of the enzyme with PLP is only 48% at 21 days and adult levels are reached only around 3 months. This again may be attributed to the availability of cofactor which has also reached the adult level around 3 months (Chapter 2, Fig. 21). These factors would account for the maximum rate of increase of GAD activity between 21 d and 3 months observed (Chapter 1, Fig. 11-a), which correlates well with the increase of its product 7-ABA (Chapter 1, Fig. 2-a). The results clearly show that at normal physiological levels of tissue B6, only 64% of the apoenzyme is in the holoenzyme form as observed in group 3 i.e. in the brain of young adult. However, a significant 7% increase at 12 months (p<0.01) and a highly significant 11% increase at 24 months (p<0.001)
in the level of the GAD holoenzyme is observed as compared to the adult. This would again reflect on the increased PLP levels (Chapter 2, Fig. 21) during aging.

Pyridoxine deficiency has resulted in a highly significant (p<0.001) decrease in the holoenzyme in all the age groups studied. However, maximum effect is observed in group 1 in which only 15% of activity is observed. Aged brain (Group 5) almost compares with the developing brain in the level of the holoenzyme. Comparatively, groups 2 and 3 appear to be more resistant to deficiency and aging effect has begun even at 12 months (Fig. 29-a).

An increase of 69% in the level of GAD holoenzyme is noticed in pyridoxine deficient developing brain (group 1). Similar increase has been reported by Stephens et al. (1971) and Bayoumi and Smith (1972). In group 2 also, there is a significant 12% increase in the apoenzyme level. Bayoumi and Smith (1972) have suggested that the increase of GAD apoenzyme in response to B₆ deficiency is a property of the developing and not of the matured brain. One possible explanation for the present observation would be that the enzyme GAD has reached only 50% of adult level at 21 days and the adult level being attained around 3 months (Chapter 1, Fig. 11-a). The other may be the contribution of the cerebellum since this is the only region of adult brain in which the GAD apoenzyme increased in response to pyridoxine deficiency (Bayoumi and Smith, 1973). Presumably, this is a compensatory
mechanism to allow any available pyridoxal phosphate to be captured by this enzyme to maintain as much \( \gamma \)-ABA synthesis as possible (Barker and Bender, 1980). This enzyme induction during development has been attributed to various factors - changes in the levels of cofactor, substrate or product (Bayoumi and Smith, 1973). It is suggested that a hormonal factor may be involved in the induction process (Bayoumi and Smith, 1973) since it is known that vitamin B\(_6\) deficiency produces hormonal alterations (Hsu, 1963). It is clearly seen in the present study that the maximum potential activity (or apoenzyme content) of the brain is not changed by pyridoxine deficiency in the 3, 12 and 24 months groups and it is only the degree of saturation of the enzyme with the coenzyme that is notably decreased resulting in lowered activities.

The degrading enzyme of \( \gamma \)-ABA-\( \gamma \)-ABA-T (Table 15, Figs. 29-b, 30-b) is affected to a lesser extent than GAD at all the ages and this is reflected in the levels of \( \gamma \)-ABA (Fig. 25-b). The activity of \( \gamma \)-ABA-T holoenzyme is 87\%, 80\% and 83\% in groups 1, 2 and 3 and aging has resulted in decreases of the same to 73\% and 63\%. Such a decrease in the endogenous cofactor saturation would account for decreased \( \gamma \)-ABA-T activities observed at 12 and 24 months (Chapter 1, Fig.11-b).

Deficiency has resulted in a significant decrease in the level of holoenzyme in all the age groups compared to
controls. Maximum effect is seen during the developmental period. Decreases in enzyme activity as a consequence of pyridoxine deficiency during development is also reported by Bayoumi and Smith (1972). Group 3 appears to be least affected by the deficiency and the resistance to deficiency decreases gradually as the animal ages (Groups 4 and 5).

There is a 33% decrease (p<0.001) in group 1 and a 22% decrease (p<0.01) in group 2 in the level of apoenzyme which means that deficiency has resulted in loss of apoenzyme as well as holoenzyme ultimately resulting in decreased 7-ABA-T activity. However, like GAD in groups 3, 4 and 5, no significant difference in the level of apoenzyme as compared to controls is observed and the decrease in activity observed is only due to decrease in the endogenous saturation with the coenzyme. The enzyme in the aged brain is as vulnerable to deficiency as in the developing brain although affected to a lesser degree.

AsAT (Table 16, Figs. 29-c and 30-c) in the control animals is fully saturated with PLP at all ages. This fact may be of functional significance since this enzyme (Balazs and Haslam, 1963, 65; Haslam and Krebs, 1963) is important for the oxidation of amino acids through the TCA cycle.

In the deficient animals, there is a decrease in coenzyme saturation of the apoenzyme at all ages. Maximum effect is seen in the developing brain (group 1) and the
adult brain (group 3) appears to be the most resistant to deficiency.

The level of apoenzyme has remained the same in groups 1, 2 and 3 while deficiency at older ages - at 12 and 24 months has resulted in a significant depletion of 22% (p<0.01) and 35% (p<0.001) in the level of the apoenzyme. It appears that the degraded apoenzyme causes a redistribution of vitamin for more essential reactions - probably a compensatory mechanism under deficient conditions.

Since AsAT is an enzyme involved in both metabolic and neurophysiologic functions, decreased apoenzyme and holoenzyme levels in older ages would reflect an impairment of both these functions. Since no significant changes as compared to adults were seen at 12 and 24 months in the activity of AsAT (Chapter 1, Fig.15-a), the observed results would imply that the effect has wholly resulted as a consequence of pyridoxine deficiency.

AlAT (Table 17, Figs.29-d and 30-d) in controls, like AsAT is fully saturated with the coenzyme at all the ages studied. In the deficient animals, there is a highly significant decrease in the holoenzyme levels. The degree of decrease in the aged brain (group 5) exceeds even that of the developing brain (group 1). At all the ages studied the effect of deficiency is more severe on the alanine enzyme than the aspartate enzyme. This agrees with the results of
Brin and Thiele (1967) which is in support of other studies in which ALAT has shown to be more sensitive to physiologic changes, whether vitamin B₆ deficiency or other (Awapara, 1953; Brin and McKee, 1956; Brin et al., 1960) showing that the rate of turnover of the apoenzyme-coenzyme may be greater for the alanine than for the aspartic enzyme (Brin et al., 1960). Similarly, a greater effect on ALAT due to pyridoxine deficiency is reported in developing chick brain (Jourdikian and Daghir, 1977).

The enzyme GT (Table 18, Fig. 29-e and 30-e) like AsAT and ALAT is fully saturated with the coenzyme in the control brains at all the ages studied.

Of the 5 groups studied, deficiency has affected only group 1 i.e. the developing brain in which the holoenzyme level is only 42% that of the control. This observed deficit might probably be due to immature binding or saturation of the coenzyme with the apoenzyme. Once bound, this enzyme appears to be the most resistant to deficiency since deficiency did not have any effect at later ages. This has support in the studies of Cooper and Meister (1972) on GT who showed that GT contains PLP which is bound in an unusually tight form.

These results are very interesting since GT has been shown to supply α-KG which serves as an important energy substrate in the aged brain (Chapter 1). Even under deficient
conditions, it appears that \( \alpha \)-KG serves as the major substrate for oxidation which is replenished by the activity of GT at the expense of glutamine which again has shown no significant change under deficient conditions. Thus, it is the binding property of the enzyme with the cofactor that really serves as a compensatory mechanism to maintain homeostasis in the aged brain.

It is observed that B\(_6\) deprivation which leads to decrease in the coenzyme (Stephens et al., 1971; Kurtz et al., 1972) has resulted in significant depression of the activity of most of the B\(_6\)-dependent enzymes each one being affected to different degrees at different ages. This difference may be due to any of the following phenomena: affinity of binding of the coenzyme to apoenzyme, degree of saturation of the apoenzyme by the coenzyme, stabilization of the apoenzyme and dependence of the synthesis of apoenzyme on the coenzyme. Further, at all ages, the transaminases are less susceptible to deficiency than the decarboxylase. This differential susceptibility would reflect weaker a PLP-apoenzyme binding in the decarboxylase system (Scriver and Hutchinson, 1963).

It is clear from the results that all the parameters studied are maximally affected by deficiency during the first 21 days. This would agree with the 'vulnerable period hypothesis' (Dobbing, 1968). However, other age groups are no exception to the effects of deficiency. Ingram and
McDaniel (1980) failed to find significant differences in passive avoidance learning task between young, middle aged and aged B6 deficient rats. But Gewacke et al. (1977) showed that B6 deficiency induces behavioural alteration in fully matured, middle aged and retired breeder rats. Gewacke's retired breeders were only about 12 months old and probably the effects would be more if older rats were used. From the above results, it is clear that neurochemical changes can be produced with B6 deficiency induced at any stage in the life span of an animal. Most of the nutritional studies on brain have concentrated only in the developmental stages based on the fact that structural development of the brain is completed in early life, but these studies have ignored the high metabolic activities of the adult tissue as well as the problems of maintenance of homeostasis by the old brain for which various compensatory mechanisms should function together. The present study clearly reveals the importance of age in nutritional studies and paves the way for future studies of the interaction between age and diet in neurological performance of the animal.