SOME REGIONAL PATHOLOGIC AND METABOLIC CONSEQUENCES IN MOUSE BRAIN OF PYRITHIAMINE-INDUCED THIAMINE DEFICIENCY* †

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A prominent feature of the neuropathological lesions resulting from thiamine deficiency is their specific localization. The areas affected are somewhat variable from species to species, but quite reproducible within a species. At least one structure, the vestibular nucleus, seems to be affected in all of the species so far studied. Studies of regional changes in total tissue thiamine levels (1) and pyruvate decarboxylase activity in deficient rat brain (2) showed no recognizable correlation between the severity of the biochemical changes and the location of the pathological lesions. A similar study of transketolase activity did show such a correlation, but the differences reported were not great (3). In another study, changes in both pyruvate decarboxylase and transketolase activities were shown to exhibit some regional specificity (4).

Recently, changes in the levels of certain metabolites have been found in the brains of mice made thiamine deficient by pyrithiamine treatment (5). It was hoped that a regional study of metabolite changes in pyrithiamine-treated mouse brain would help to clarify the problem of regional susceptibility to thiamine lack and perhaps to assist in the identification of the pathogenetic process itself. An anatomical study of the brains of these animals was undertaken first, since the location of the lesions had to be accurately known.

METHODS

Preparation of Animals: Weanling Swiss Webster mice were given a basal diet supplemented with a fortification mixture free of thiamine (Nutritional Biochemical Corporation). Control mice received daily 64 µg thiamine-HCl intraperitoneally. Pyrithiamine-treated animals received daily intraperitoneal injections of 50 µg pyrithiamine (neopyrithiamine bromide, California Corporation for Biochemical Research) plus

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1 µg of thiamine-HCl (5). In the first 8 days, control mice steadily gained weight, whereas pyrithiamine-treated mice stopped gaining weight on the 5th day and started to lose on the 8th day (fig. 1). At 7 days, pyrithiamine-treated animals exhibited weakness, ataxia, and exaggerated jerking and spinning movements when held or gently spun by the tail ("Woolley-White sign" (6)).

From this time on, animals for the 2 portions of the experiment, anatomical and biochemical, were treated differently: The animals used for the biochemical assays were killed by freezing in CCl₄ at -150° on the 8th day. Some of the pyrithiamine-treated animals were reanimated with 1 mg thiamine given intraperitoneally 5 hours before freezing. The 12 animals used for the anatomical portion of the study were given 1 mg of thiamine-HCl on the 8th day and after 1 day of rest pyrithiamine injections were resumed. This cycled treatment was continued for 2 to 6 weeks, and the clinical appearance of the animals, rather than a fixed time schedule, was used to terminate each course of pyrithiamine injections with an injection of thiamine. This prolonged period of treatment was necessary because no anatomical lesion was found at the end of the first 8 days.

Tissue Preparation: Anatomical: In 9 mice the brain was fixed by perfusion of neutral buffered formalin through the heart at a pressure of 70 cm water (three animals died unexpectedly and were fixed as soon as possible by immersion in formalin). The head was excised and hardened overnight in fresh formalin. The brain and cervical spinal cord were dissected and cut in coronal section. Paraffin-embedded blocks were cut at 5 or 10 µ and stained with hematoxylin and eosin or luxol fast blue-Nissl stains.
Tissue Preparation: Biochemical: Five areas of brain were selected for analysis on the basis of the anatomical study. The frozen mouse was firmly clamped on a piece of dry ice, and the dissection was performed under a dissecting microscope in a −20°C cold room. A squared-off 18G hypodermic needle fitted with a plunger was used to sample the specific areas. Each piece was a small cylinder of tissue 2 to 3 mm long and 0.5 mm in diameter. The samples were weighed at −20°C on a quartz-fiber balance (7) with a sensitivity of 0.006 mg/mm. The average weight was 1 mg (0.5 to 2 mg). The posterior aspects of the cerebellum and medulla were exposed first, and a combined sample from the right and left cerebellar hemispheres was taken. Next, sequential cross sections were made rostrally up the medulla until the 8th nerve was seen entering the ventral cochlear nucleus, and the vestibular nuclear convexities were identified on the floor of the 4th ventricle. A combined bilateral sample was taken of the vestibular nuclei, followed by a similar sampling of the cochlear area lateral and ventral to the vestibular sample. The cochlear area included tissue from the dorsal and ventral cochlear nuclei, the trapezoid body, and the spinal tract and spinal tract nucleus of the trigeminal nerve. A bilateral combined sample was next taken from the parietal cortex. Then the dorsal thalamus was exposed with successive horizontal cuts which clearly revealed the fornix and stria medullaris. A bilateral combined sample was taken from the anterior dorsal aspects of the thalamus.

In the preparation of extracts, each tissue sample was homogenized in 10 ml of 3.0 M HClO4 at −12°C in small tubes specially fitted with sanded glass rods. This homogenate was then diluted with 90 ml of deionized water, centrifuged to remove the protein, and the supernatant liquid was neutralized with 2.0 M K2HCO3 containing 5 mM EDTA. The mixture was centrifuged to remove the KClO4 precipitate, and the clear extract (pH 6.5 to 7.0) was stored at −70°C until the time of assay.

Assay Method for Metabolites: Enzymatic assays based on the fluorescence of pyridine nucleotides were used for all substrates. Enzymatic cycling (8) was employed so that small aliquots of each extract could be used, ensuring that all 5 metabolites could be measured on each tissue sample from each mouse. The steps of the enzymatic assays are listed in tables 1 and 2. Except for the pyruvate and α-ketoglutarate assays, and the cycle for DPNH, they have been reported before (9, 10).

The assay for α-ketoglutarate is a modification of a published method (11) in that TPNH has been substituted for DPNH with a subsequent cycling of TPNH* and ascorbate has been added to reduce the tissue blank*. Since the analysis is based on TPNH* formation, the TPNH present in the original brain extract contributes to the apparent α-ketoglutarate level. Therefore, values obtained were corrected by subtracting the levels of TPNH*, measured separately by direct enzymatic cycling.

The assay for pyruvate depends upon its stoichiometric oxidation with DPNH* and CoA by pyruvate dehydrogenase to yield acetyl CoA, CO2, and DPNH. For this assay pyruvate dehydrogenase was isolated from pig heart muscle essentially according to the method of Hayakawa et al. (12). Stopping after the high speed centrifugation step with an (NH4)2SO4 fractionation to remove contaminating lactate dehydrogenase, the final preparation was free of LDH activity but contained α-ketoglutarate dehydrogenase activity at a level of 20 per cent of that of pyruvate dehydrogenase. This contaminant was further reduced to less than 1 per cent by incubating the amount of enzyme preparation needed for the assay in 0.1 M PO4 buffer, pH 7.0, containing 5 mM MgCl2, for 5 minutes at 3° just before use. The amount of enzyme added to each tube was adjusted to give a half time of 5 minutes. The assay yielded 85 to 90 per cent recovery of standards when used in conjunction with cycling. The 10 to 15 per cent loss was due in part to a small amount of residual DPNH oxidase in the enzyme preparation, and in part to the oxidation of DPNH in the alkaline destruction of DPNH prior to cycling.

* F. Matschinsky, personal communication
TABLE 1

Primary Reaction Conditions Prior to Cycling

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Buffer</th>
<th>Reaction Reagent</th>
<th>Vol. of Extract</th>
<th>Incuba-</th>
<th>time</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>Tris-HCl, 50 mM, pH 8.6; 50 μl</td>
<td>Hexokinase 2.5 μmol/ml; glucose-6-PDH 0.4 μmol/ml</td>
<td>2 μl</td>
<td>20 mins</td>
<td></td>
</tr>
<tr>
<td>P-creatine†</td>
<td>Tris-HCl, 60 mM, pH 8.0; 45 μl</td>
<td>Hexokinase 2.5 μmol/ml; glucose-6-PDH 0.4 μmol/ml; creatine kinase 0.1 mg/ml</td>
<td>1.5 μl</td>
<td>20 mins</td>
<td></td>
</tr>
<tr>
<td>6-P-glucose</td>
<td>Tris HCl, 40 mM, pH 8.0; 29 μl</td>
<td>6-P-glucose DH 7 μmol/ml</td>
<td>10 μl</td>
<td>30 mins</td>
<td></td>
</tr>
<tr>
<td>α-ketoglutarate</td>
<td>Phosphate, 50 mM, pH 7.6; 15 μl</td>
<td>Glutamate DH 90 μmol/ml; TPNH 10 μmol; NAD 0.3 M</td>
<td>5 μl</td>
<td>30 mins</td>
<td></td>
</tr>
<tr>
<td>Pyruvate</td>
<td>Phosphate, 60 mM, pH 7.6; 15 μl</td>
<td>Pyruvate DH Coenzyme A 60 μmol; DPN 40 μmol; Thiamine pyrophosphate 30 μmol</td>
<td>5 μl</td>
<td>30 mins</td>
<td></td>
</tr>
</tbody>
</table>

* All reactions were carried out at 24 to 26° in tubes whose internal dimensions were 8 x 70 mm.
† Abbreviations used: Tris, tris (hydroxymethyl) aminomethane; DH, dehydrogenase; EDTA, ethylene diamine tetra-acetic acid.
‡ The sample for P-creatine was a 25 μl portion taken from the ATP incubation mixture at the end of the incubation. The TPNH in that portion was destroyed by adding 10 μl 0.3 N HCl. After 10 minutes at 0°, 5 μl of 0.15 M Tris base was added, and 5 μl of a reagent containing Tris-HCl, 30 mM, pH 8.6; TPN, 100 μM; MgCl₂, 20 mM; dithiothreitol, 0.4 mM; ADP, 155 μM; hexokinase, 22.5 μg/ml; glucose-6-P dehydrogenase, 3.6 μg/ml; and creatine kinase 0.9 mg/ml, to give a reagent whose composition during incubation is given above in the table.

TABLE 2

Conditions for Cycling

Cycling reactions were carried out for 1 hour, all at 38° except that for pyruvate, 25°. The cycling steps for ATP, P-creatine, and 6-P-glucose were performed in fluorometer tubes, the other two were in the same tubes in which the primary reaction had been conducted. The cycling steps for ATP, P-creatine, 6-P-glucose and α-ketoglutarate were stopped by heating 2 min at 100°. The product of cycling, 6-P-glucose, was measured in a reagent containing Tris-HCl, 20 mM, pH 8.6; TPNH, 0.1 mM; EDTA, 0.1 mM; 6-P-glucose dehydrogenase, 5 μg/ml. In the case of ATP, P-creatine and 6-P-glucose (from brain), 1 μl of this reagent was added to the tubes in which cycling was performed. In the case of α-ketoglutarate, 50 μl of the cycling mixture was added to 1 ml of reagent in a fluorometer tube. The reaction was complete in 20 min at 25°.

The conditions for cycling of the pyruvate assay are given in the text (Methods).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Preparation for Cycling</th>
<th>Cycle Conditions</th>
<th>Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>3 μl 1.3 N NaOH, 20 min, at 25°</td>
<td>3 μl alkaline step to 100 μl cycle reagent</td>
<td>GAPDH 4.7 μg/ml; GDH 9.4 μg/ml, 1,000 cycles/hr</td>
</tr>
<tr>
<td>P-creatine†</td>
<td>3 μl 3 N NaOH, 20 min, at 75°</td>
<td>6 μl alkaline step to 100 μl cycle reagent</td>
<td>Same as for ATP</td>
</tr>
<tr>
<td>6-P-glucose</td>
<td>3 μl 0.9 N NaOH, 20 min, at 25°</td>
<td>20 μl alkaline step to 50 μl cycle reagent</td>
<td>GAPDH 30 μg/ml; GDH 80 μg/ml, 5,000 cycles/hr</td>
</tr>
<tr>
<td>α-ketoglutarate</td>
<td>3 μl 0.6 N HCl, 20 min, room temp.</td>
<td>40 μl cycle reagent to total volume of acid step</td>
<td>GAPDH 16 μg/ml; GDH 22 μg/ml, 2,000 cycles/hr</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>3 μl 1.0 N NaOH, 20 min, at 25°</td>
<td>75 μl cycle reagent to total volume of alkaline step</td>
<td>GAPDH 20 μg/ml; MDH 5 μg/ml, 1,000 cycles/hr</td>
</tr>
</tbody>
</table>

* Enzyme abbreviations used are: GAPDH, glucose-6-P dehydrogenase; GDH, glutamate dehydrogenase, GAPDH, glyceraldehyde-3-P dehydrogenase; MDH, malate dehydrogenase.
The enzymatic cycle for DPNH employed the sequential oxidation and reduction of DPNH by malate dehydrogenase (MDH) and yeast glyceraldehyde-3-P dehydrogenase (GAPDH):

\[
\text{oxalacetate} \xrightarrow{\text{MDH}} \text{malate} \xleftarrow{\text{GAPDH}} \text{DPNH} \xrightarrow{\text{AsO}_4^\text{-}} \text{glyceraldehyde-3-P} \xleftarrow{\text{GAPDH}} \text{DPN}^+ \]

This represents a modification of the system of Matschinsky* who employed glutamate dehydrogenase instead of malate dehydrogenase.

The cycling reagent contained 0.1 M arsenite buffer, pH 7.0, 7 mM EDTA, 3 mM mercaptoethanol, 1 mM glyceraldehyde-3-P, 1 mM oxalacetate. The small amount of phosphate (4 mM) carried into the cycling mixture from the primary reaction mixture for the pyruvate assay did not interfere. The levels of the 2 enzymes were adjusted to give an appropriate cycling rate (table 2). The cycle was stopped and the malate that had accumulated was measured in a single step, by adding sample to a fluorometer tube containing 1 ml of a reagent consisting of hydrazine-HCl, pH 10, 1.0 M; EDTA, 0.1 mM; DPNH, 0.1 mM; and malate dehydrogenase, 10 μg/ml. The reaction was complete in 30 minutes at 25°.

The conditions for cycling TPNH and TPNH are taken from Lowry et al. (8) with some modification of enzyme levels to give appropriate rates (table 2).

Assays were done in triplicate, and where the variability was greater than 10 per cent they were repeated.

RESULTS

Anatomical Study

No neuropathological lesions were seen in 2 animals killed at the height of symptoms at the end of the first week of treatment. It was for this reason that animals were treated with pyridoxine much longer for the anatomical than for the biochemical study.

In the chronically treated animals, the vestibular nuclei were most severely involved (fig. 2) followed by the thalamus and hippocampus. Occasional lesions were encountered in the mammillary body, inferior colliculus, amygdaloid nucleus and globus pallidus. No lesions were seen in other areas of the cerebral hemispheres or in the cerebellum. Viscera: heart, lung, liver, kidney, spleen, pancreas and adrenal gland were examined in 4 animals; no lesions were identified.

The lesions in brains adequately fixed by perfusion were marked by a striking vacuolization of the neuropil (fig. 3). Glial cells and neurons showed cosinophilia of the cytoplasm with some condensation of the nuclear chromatin. Larger and more advanced lesions contained hemorrhages about the capillaries and necrosis with polymorphonuclear leukocyte infiltration.

* F. M. Matschinsky, to be published.
Fig. 2. Medulla oblongata in a mouse killed after 23 days of pyrithiamine treatment interspersed with occasional injections of thiamine (see Methods). The animal had neurological symptoms for 3 days before it was killed. The vestibular nuclei show vacuolization. Hematoxylin and eosin stain; 35 x.

Fig. 3. Thalamus in a mouse killed after 21 days of pyrithiamine treatment with occasional injections of thiamine. At the end of the first week the animal displayed neurological symptoms for 4 days, and again for 5 days just before death. Petechial hemorrhages are present. The area on the left shows vacuolization of glial cells and neuropil as well as polymorphonuclear leukocyte infiltration. Hematoxylin and eosin stain; 500 x.
Biochemical Study

Metabolite Levels in Controls: The deeper brain areas, vestibular nucleus, cochlear region and thalamus, tend to be lower in P-creatine and α-ketoglutarate, a little lower in ATP, and a little higher in pyruvate than the more superficially located cerebellum and parietal cortex (table 3). The high level of P-creatine in normal cerebellum has been seen before (13).

The differences in control levels of ATP, P-creatine, α-ketoglutarate, and
TABLE 3
Levels of Metabolites in Control Mice

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Cerebellar Cortex</th>
<th>Vestibular Nuclei</th>
<th>Cochlear Area</th>
<th>Parietal Cortex</th>
<th>Dorsal Thalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/kg wet weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-ketoglutarate</td>
<td>90 ± 7</td>
<td>55 ± 9</td>
<td>68 ± 4</td>
<td>93 ± 20</td>
<td>59 ± 25</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>131 ± 20</td>
<td>335 ± 50</td>
<td>152 ± 33</td>
<td>154 ± 27</td>
<td>171 ± 23</td>
</tr>
<tr>
<td>6-P-gluconate</td>
<td>11.8 ± 1.6</td>
<td>11.7 ± 2</td>
<td>12.5 ± 1.4</td>
<td>9.4 ± 0.9</td>
<td>9.6 ± 1.0</td>
</tr>
</tbody>
</table>

|          | µmol/kg wet weight |                   |               |                |                |
|----------|-------------------|                   |               |                |                |
| ATP      | 3.51 ± 0.25       | 2.55 ± 0.16       | 2.79 ± 0.17   | 3.23 ± 0.14    | 2.91 ± 0.11    |
| P-creatin| 6.45 ± 1.52       | 2.57 ± 0.42       | 3.14 ± 0.23   | 1.25 ± 0.27    | 4.13 ± 0.22    |

Fig. 5. α-Ketoglutarate, pyruvate and 6-P-gluconate levels in various brain regions in individual pyrithamine-treated animals. The 3 animals represented by the closed symbols had neurological signs: all 3 had the Woolley-White sign (see Methods); 2 could not keep their balance on a horizontal stick and the third had "convulsions". The 2 animals represented by the open symbols had none of these findings. Note that the scale for 6-P-gluconate is more than 20 times that for pyruvate and α-ketoglutarate. Control levels are indicated by horizontal dotted lines.

pyruvate in the deeper brain regions, as compared to the more superficial areas, are probably attributable in part to the fact that the deeper regions are slower to freeze. Anoxic changes can progress a little farther in the deeper areas. Each of the differences noted is in the appropriate direction (9, 11). Whether other factors also contribute to the differences seen is not known.
Changes Following Pyrithiamine Treatment: In the pyrithiamine-treated animals, 6-P-gluconate, pyruvate and α-ketoglutarate all become elevated (fig. 4). The metabolite undergoing the greatest relative rise in each region is α-ketoglutarate, which in one region increased 17-fold. The region showing the greatest relative rise for each of the 3 metabolites is the thalamus. The next most severely affected region is the vestibular nucleus for 6-P-gluconate and α-ketoglutarate, and it also showed the highest absolute pyruvate level. The cerebellum and parietal cortex were least affected.

No significant changes in ATP or P-creatine were observed in any area as a result of pyrithiamine treatment or realimentation (fig. 4). This is in contrast to the relatively small, but significant, increases in these substances found in the study of whole brain (5). The difference is probably due to the fact that the animals used in the present study were killed at an earlier stage in the disease than those of the previous one. In the present study, some animals showed symptoms, others did not. In the previous study, the animals were not killed until symptoms were severe.

With realimentation, 6-P-gluconate, α-ketoglutarate and pyruvate all returned toward control levels (fig. 4). At the end of the 5-hour realimentation period, 6-P-gluconate was within 50 per cent of control in each area, α-ketoglutarate and pyruvate were back to within 100 per cent of control values. (In parietal cortex, in which pyruvate increased very little with pyrithiamine, no fall was seen with realimentation.) The rapid reversal was true even for the areas susceptible to later permanent damage, indicating the reversible nature of the biochemical lesion, in the early stages.

The levels of pyruvate, and more especially of α-ketoglutarate, were very variable in pyrithiamine-treated animals, particularly in regions where the mean increases were large. When the data for individual animals are examined, a striking correlation between neurological signs and biochemical changes is observed (fig. 5). In general, levels of all 3 metabolites were higher in the symptomatic mice than in those showing no neurological signs. The differences between symptomatic and asymptomatic mice were greater in the regions destined to show pathological changes, and in those regions the differences were greatest in α-ketoglutarate, least in 6-P-gluconate levels.

DISCUSSION

Anatomical Changes: The distribution of lesions in mice treated chronically with pyrithiamine, as described here, is quite similar to that seen in thiamine deficient monkey (14) and in Wernicke's disease in man (15). Symmetrical lesions in anterior thalamus, mammillary bodies, inferior colliculus, vestibular nucleus, hippocampus and globus pallidus are seen in all 3 species. In thiamine deficient rats, the lateral pontine tegmentum is most severely affected. Other brain stem nuclei are also involved, but lesions rostral to the mesencephalon are not described (1). Whether the anatomical differences between thiamine deficient rats and pyrithiamine-treated mice are the result of differences in species, treatment, or both is not known.
There has been much debate as to the specific cellular target in thiamine deficiency, both in man and animals. Early work by Alexander (16) emphasized vascular changes as the primary pathogenic event. Collins et al. (15) emphasized neuronal damage in the dorsal thalamus in cases of Wernicke's disease. Recent work from several laboratories (3, 17) has focused on changes seen in the oligodendrocyte, especially in the lateral vestibular nucleus of the rat. Looking at this same area, however, Tellez and Terry (18) described hypertrophic and degenerative changes in synaptic endings and axons as the first characteristic change. Ule and Kolkmann (19) found hydropic changes in processes of both neurons and glia. Necrosis and hemorrhage were later events. The present study was not designed to reveal the earliest histopathological changes. There were prominent changes in glial cells in the region of the lesions, but neurons were also affected in these areas. The presence of petechial hemorrhages suggested lesions of considerable age.

**Biochemical Findings:** The changes in 6-P-gluconate, pyruvate and α-ketoglutarate levels are in the same direction as those described earlier for whole brain (5). In that study it was found that 6-P-gluconate and xylulose-5-P were elevated 3 to 5 fold, pyruvate increased 4 fold and α-ketoglutarate rose to 7 times the control level.

The present results demonstrate that in comparison to whole brain the metabolite changes are greater in areas susceptible to microscopic lesions and smaller in areas in which no lesions are found. The cochlear area is an exception to this statement. No lesions were seen in the area in chronically treated mice, but marked biochemical changes did occur.

In the whole brain a substantial drop (50 to 75 per cent) was observed in the *in vitro* activities of 3 thiamine-dependent enzymes, transketolase, pyruvate dehydrogenase, and α-ketoglutarate dehydrogenase (5). The effective *in vivo* activities are perhaps better assessed from the levels of the substrates for these enzymes. Provisionally, the levels of 6-P-gluconate, pyruvate and α-ketoglutarate respectively may be considered indicators of the degree of inhibition of these enzymes*. If so, the local changes in these metabolites observed here indicate that each of the 3 enzymes suffers to about the same extent from the primary event, which is presumably thiamine pyrophosphate depletion (20). Thus, each enzyme appeared to be less inhibited in the cerebellum and cerebral cortex and more inhibited in the vestibular nucleus and dorsal thalamus. In general, the response in each area was greatest for α-ketoglutarate, least for 6-P-gluconate.

Since each of the 3 thiamine dependent systems appears to be affected more severely in structures destined to show lesions, the problem of thiamine deficiency raises 2 separate questions. The first is: why does thiamine deficiency affect the thiamine dependent systems more severely in some areas of brain

* Xylulose-5-P might have been better than 6-P-gluconate as an indicator for transketolase, since it is a substrate for the enzyme. However, the assay for 6-P-gluconate is simpler, and changes in levels of the 2 metabolites approximately parallel each other in pyrithiamine poisoning (5).
than in others? The answer to this question may well be sought in some aspect of the metabolism of thiamine itself, or perhaps in regional differences in metabolic rate. The lack of regional differences in thiamine levels (1) suggests the latter alternative, but it may be that thiamine pyrophosphate would show greater regional differences than total thiamine.

The second question is: how does the derangement of metabolism by thiamine lack lead to functional (and finally anatomical) disorder? The data on metabolite levels imply that impairment of α-ketoglutarate dehydrogenase activity is greater than that of pyruvate dehydrogenase. This is in contrast to direct in vitro measurements of cerebral enzyme activities which show that inhibition of α-ketoglutarate dehydrogenase is the same (21) or less (5) than that of pyruvate dehydrogenase in pyriphamnine-treated animals, and usually much less in thiamine deficient animals (21, 22). It may be that α-ketoglutarate dehydrogenase has a smaller functional margin of safety. Both dehydrogenases seem to be functionally more disabled than transketolase. Thus, the tentative conclusion would be that α-ketoglutarate dehydrogenase is the weakest link. This does not tell why function is affected, since ATP and P-creatine levels are unchanged even in the most severely affected regions, showing that metabolic generation of energy has not been compromised. In any event, in view of the wide-spread metabolic disturbance much more information must be accumulated before a pathogenetic role can be assigned to any one of the systems in which thiamine participates.

The results of the present study suggest that metabolite levels may be much more sensitive indicators of enzyme deficit than the activities, as measured in vitro, of the enzymes themselves. On the one hand a large change in activity of an enzyme present in great excess may have little functional effect, whereas even a slight reduction in the activity of another enzyme operating on a narrow margin may result in large changes in concentration of its substrate or product. The existence of regional inequalities in the margin of safety would help to explain the simultaneous occurrence of bold regional patterns of metabolite changes and anatomical alterations along with a much more subtle pattern of changes in enzymatic activity.

SUMMARY

An anatomical and regional biochemical study has been made of the brains of mice made thiamine-deficient by treatment with pyriphamnine. Since no lesions were found in 2 mice after 8 days of treatment, although neurological symptoms had appeared, the anatomical study was performed on 12 chronically deficient animals, after 2 to 6 weeks of pyriphamnine treatment. The biochemical study was done on acutely deficient animals after 8 days of pyriphamnine treatment.

Anatomically, lesions were found in vestibular nuclei, thalamus, hippocampus, mammillary body, inferior colliculus, and basal ganglia. No lesions were seen in cerebral hemispheres or cerebellum. Microscopically, there was condensation of nuclear chromatin and cytoplasmic condensation and eosino-
philia in oligodendroglioma. There were similar cytoplasmic changes in neurons. Petechial hemorrhages and overt necrosis appeared to be later stages in the development of the lesions.

Biochemically, 6-P-gluconate, pyruvate and α-ketoglutarate levels were elevated in all areas studied (vestibular nuclei, cochlear area, cerebellum, thalamus and parietal cortex). These elevations ranged in magnitude from about 30 per cent for 6-P-gluconate in cerebellum to almost 1000 per cent for α-ketoglutarate in thalamus. The elevations of each of the metabolites were consistently greatest in structures where lesions were found eventually. In any given locale, increases were greatest for α-ketoglutarate and usually least for 6-P-gluconate. Changes were greater in animals exhibiting neurologic symptoms than in those without them. Realimentation with thiamine largely reversed the changes seen, even in those structures destined otherwise to show lesions.

No changes were found in P-creatine or ATP levels in any region as a result of pyrithiamine treatment.

REFERENCES