NEUROCHEMICAL CHANGES IN LEIGH'S DISEASE

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Summary A series of children with Leigh's disease had normal hepatic pyruvate carboxylase activity, increased cerebral thiamine diphosphate, and decreased cerebral thiamine triphosphate. These thiamine esters were normal in liver. The author suggests that the histologic changes of Leigh's disease, as well as the similar changes of Wernicke's disease, could be due to a deficiency of cerebral thiamine triphosphate.

Leigh's disease (LD), or subacute necrotizing encephalomyelopathy, usually presents as loss of skills and hypotonia in an infant (1). Unusual presentations in our patients have included acquired blindness, Ondine's curse and an episodic movement disorder. Although death usually occurs in the first few years of life one patient with low normal intelligence died when she was 32 years old (2).

The heterogeneity of symptoms and clinical course suggests that LD is due to any of several genetic disorders. The constancy of the neuropathologic findings is more consistent with a single disease process. The brains of LD patients demonstrate symmetrical and punctate areas of capillary infiltration and necrosis in the brain stem and basal ganglia, which are almost indistinguishable from that seen in thiamine deficiency states (3).

Investigations into the biochemical etiology of LD have followed either of two theories. Several authors, impressed with the frequency of lactic acidosis in LD, have studied the activity of enzymes which convert pyruvate into Kreb's cycle intermediates (4-6). Others, more impressed with the pathological similarities of Leigh's and Wernicke's diseases, have studied the cerebral metabolism of thiamine by LD brains (7). This report will review our investigations into both theories.

Pyruvate carboxylase activity
Stimulated by the reports of Clayton et al. (4), Tang et al. (5), and De Groot and Hommes (6), the activity of hepatic pyruvate carboxylase was determined on autopsy specimens from two LD patients, and on material obtained by percutaneous biopsy of 6 patients with the clinical diagnosis of LD. (In one patient material was obtained both during life and at autopsy.) All patients had a neurodegeneration, lactic acidosis and inhibitor (see below) in their urine, and in the urine of their parents (8). Control specimens were obtained from 6 patients with other conditions. These specimens were obtained either at autopsy, or as part of the evaluation of elevated concentrations of lactate in blood. None of the second group of patients had the inhibitor in their urine. The pyruvate carboxylase activities were determined by Doctors Utter, Isohashi, Leiter and Swack (9).

The range of pyruvate carboxylase activity in the control series was 6.1-13.2, with a mean of 10.6 (±2.59) units. In the patients with LD the range was 8.1-16.1, and the mean was 10.4 (±2.97). In no LD patient in this series were we able to document a deficiency or absence of pyruvate carboxylase. Careful scrutiny of prior reports of LD patients with deficient pyruvate carboxylase activity (4-6) indicates that either the enzyme activity was not ac-
Fig. 1. The cerebral reaction which is inhibited by the body fluids of untreated patients with LD.

Cerebral thiamine metabolism

All patients with LD who have been properly tested have in their body fluids a factor which inhibits the synthesis of thiamine triphosphate (TTP) by beef brain. The inhibited reaction is illustrated in Fig. 1. Several characteristics of the inhibitor lead us to believe that it is a single and specific compound, probably a protein. These characteristics are: 1) the inhibiting factor is specific for the brain thiamine diphosphate phosphokinase, and does not affect the synthesis of TTP by other tissues; 2) when stored frozen the activity of the inhibitor cannot be detected after 2 weeks, but the inhibiting activity can be preserved indefinitely if the inhibitor is stored frozen in 50 mM 2-mercaptoethanol; 3) utilizing a Sephadex G-200 column, the inhibitor is eluted at a molecular weight of about 37,000; 4) brief incubation with trypsin destroys the inhibitor, but incubation with either neuraminidase or phospholipase has no effect on its activity. Acetone extraction similarly destroys the activity of the inhibitor (II).

This inhibiting compound could either be a byproduct of LD, or it could be etiologic in the pathogenesis of LD. If this inhibiting compound is etiologic in LD the following biochemical conditions should be observed; 1) to be effective the inhibitor must be in the central nervous system; 2) the inhibited enzyme must be normally active in the brains of LD patients once the inhibitor is removed; 3) there should be an increased concentration of the substrate of the inhibited enzyme (TDP) in the brain; 4) the end-product, TTP, should be reduced; 5) as the inhibitor is specific for the brain enzyme, the concentrations of TDP and TTP should be normal in other tissues, e.g., liver; and 6) the inhibitor should not stimulate thiamine triphosphatase. In order to define the role of the inhibitor in LD, these conditions were evaluated in several LD patients.

A. Inhibitor in the central nervous system.

That the inhibitor is present in the cerebrospinal fluid (CSF) of patients with LD has been demonstrated in at least one patient (7). The CSF from two untreated patients with LD inhibited the cerebral thiamine diphosphate phosphokinase 32% and 38% respectively, whereas the CSF of three patients with other conditions inhibited 0%. The inhibition of the LD patients' CSF was essentially the same as their urine. Considering that the inhibitor is present in CSF, and considering its molecular weight (v.s.) we assume that it gets into the brain.

B. Inhibited enzyme in LD brains. In order to learn if the inhibited enzyme is present and otherwise active in brains of patients with LD, acetone extracts were made of generous
Table 1. Thiamine diphosphate phosphokinase activity in acetone extracts of Leigh’s disease and normal brains.

<table>
<thead>
<tr>
<th>Source of brain</th>
<th>Thiamine phosphokinase activity (μg TTP synthesized/mg protein/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leigh’s disease</td>
<td>1.50</td>
</tr>
<tr>
<td>Leigh’s disease</td>
<td>1.38</td>
</tr>
<tr>
<td>Control (leukemia)</td>
<td>1.55</td>
</tr>
<tr>
<td>Bovine</td>
<td>1.48</td>
</tr>
</tbody>
</table>

portions of two such brains, obtained at autopsy (8). (The exposure to acetone inactivates the inhibitor (v.s.) and is the standard preparation for the cerebral thiamine diphosphate phosphokinase.) Table 1 shows the activity of thiamine pyrophosphate phosphokinase in the acetone extracts of two LD brains, the brain from a leukemic patient, and a bovine brain. Although the numbers of specimens are small, the activity of this thiamine phosphokinase are the same in the LD brain as in the control brain. (Other parameters of enzyme activity were not examined).

C. Thiamine phosphate esters in LD brains. Impressed that the inhibitor and the inhibited enzyme were present in the central nervous system, we examined a frontal pole of the brain from each of 5 patients with documented LD for the concentrations of thiamine and its phosphate esters (7,11). The frontal pole was chosen for analysis as it is histologically normal, and therefore any changes should be related to the underlying disease, rather than being secondary to artefacts of tissue destruction. The concentrations of thiamine and its 3 phosphate in 5 LD brains and 6 control brains are shown in Fig. 2. The concentrations of thiamine and thiamine monophosphate are the same in LD and controls. The concentration of TDP is significantly increased ($P<0.05$), in the LD brains. The difference in the TTP values is even more striking ($P<0.002$), and this reduction in TTP in LD brains has been previously reported (7,10). These abnormalities of TDP and TTP are consistent with the effect of an inhibitor on the brain phosphokinase.

D. Thiamine phosphate esters in LD livers. TDP and TTP concentrations were also measured in the livers of several LD and control patients (Fig. 3). Apparently the changes in concentrations of the TDP and TTP are specific for brains.

E. Effect of inhibitor on thiamine triphosphatase. Although the above findings are consistent with the effect of an inhibitor on the cerebral thiamine diphosphate phospho-

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Fig. 2. Concentrations of thiamine and its phosphate esters in brain. Concentrations of thiamine (T), thiamine monophosphate (TMP), thiamine diphosphate (TDP) and thiamine triphosphate (TTP) were measured in the frontal pole of the brains from 5 Leigh’s disease (LD) patients, and 6 control patients (C). The differences between the LD and the C values are significant for TDP ($P<0.05$) and TTP ($P<0.002$).
kinase, such effects would also be observed if the inhibitor were stimulating a thiamine triphosphatase, i.e., the reverse reaction of Fig. 1, rather than inhibiting the synthesis of TTP. To study this possibility "inhibitor" was incubated with TTP (gift of Dr. Yusa, Sankyo Co., Tokyo) and a beef brain extract (Table 2). (Save for the replacement of TDP by TTP the conditions were identical to those used in the inhibitor assay.) The addition of beef brain extract to the TTP resulted in the reduction of the TTP, indicating that the extract contains a thiamine triphosphatase. However the addition of "inhibitor" to the thiamine triphosphatase did not increase the breakdown of TTP. This observation indicates that the "inhibitor" is not stimulating thiamine triphosphatase under these conditions.

Speculation

Could the excess of TDP or the deficiency of TTP produce the neuropathologic changes which are so typical of LD? Support for the role of TTP deficiency in producing the changes comes from consideration of Wernicke's disease, with its almost identical neuropathology (3). The lesions of Wernicke's disease are associated with a deficiency of thiamine and, I assume, all thiamine phosphate esters. If this is true, then the one chemical change common to Leigh's and Wernicke's diseases is the deficiency of cerebral TTP. That TTP has a role in the central nervous system is suggested by the reports of Itokawa and Cooper (12,13), and Berman and Fishman (14). Its exact function is still unclear.

CONCLUSIONS

1) Pyruvate carboxylase activity is normal in the liver of all the LD patients whom we've studied.
2) The abnormal concentrations of TDP and TTP in the brain are consistent with the effect of an inhibitor on cerebral thiamine diphosphate phosphokinase.
3) The inhibitor appears to work by inhibiting the above enzymes rather than stimulating a thiamine triphosphatase.
4) Consideration of Leigh's and Wernicke's disease suggest that TTP has an important and ill-defined function in the central nervous system.

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REFERENCES

2) Sipe, J. C., Neurology (Minneapolis), 23, 1030 (1973).
5) Tang, T. T., Good, T. A., Dyken, P. R., Johnsen,


9) To be published.


