Impairment of pyridoxal phosphate dependent metabolic reactions in a child with subacute necrotizing encephalopathy

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Infantile subacute necrotizing encephalopathy (ISNE), which has been described by Leigh (1951), is a disorder which shows an excessive autosomal inheritance pattern and is considered to be the result of an inborn error of metabolism (Richter, 1957). Among the possible biochemical abnormalities, thiamine deficiency (Leigh, 1951; Richter, 1957; Feigin and Woolf, 1954); impairments in thiamine utilization (Greenhouse and Schneck, 1968); chronic lactic acidosis (Namiki, 1965); elevated pyruvate level (Worsley, Brookfield, Elwood, Noble, and Taylor, 1965); and the presence of endogenously produced 'toxin' (Crome, 1964) have been reported. In this paper subnormalities in the activity of the enzymes decarboxylating 5-hydroxytryptophan, glutamic acid, and taurine, as well as impairment in utilization of vitamin B6 derivatives, are reported.

CASE REPORT

L.E. was a 6-month-old white female admitted to the Pediatric Department at the University of Missouri Medical Center with the chief complaint of progressive irritability, loss of interest in surroundings, general decrease in activity, and loss of appetite of one month's duration.

The patient was described as normal at birth. Pregnancy, delivery, and the neonatal period were said to be uncomplicated. Birth weight was 6 lb. 3 oz. (3·6 kg). She developed normally relative to motor, social, and adaptive milestones until she was 5 months old when the above symptoms began to manifest themselves. The patient would no longer reach out for objects. Her social responses were noted to have decreased progressively with decrease in laughing and crying relative to environmental and interpersonal changes. There was a gradual and progressive loss of head control as well as progressive feeding difficulty.

There are four siblings. Both parents and the first girl born to the family are healthy. The second girl showed a 'central nervous system disorder' from birth, associated with measles encephalitis at the age of 2 years and at the present time is institutionalized. A third child, another girl, died at the age of 6 months from infantile subacute necrotizing encephalopathy. Post-mortem examination revealed pathology similar to this case. It is the fourth girl who is being reported here. Pregnancies and deliveries of all four children were uneventful.

On admission, weight, height, and head circumference were 'significantly lower than normal'. Lethargy and little or no spontaneous movements were noted; however, the infant responded to light and auditory stimuli. Temperature was normal and there were no signs of neck rigidity or palpable abnormalities of the fontanelle. There was poor head control as well as poor grasp, rooting, and sucking reflexes and the Moro reflex was present and complete. Generalized muscle hypotonicity with brisk and symmetrical deep tendon reflexes were noted.

LABORATORY STUDIES

Electroencephalogram showed diffuse slowing more marked on the left side. Electrocardiogram, electromyogram, and radiographs of the chest and skull were normal. The examination of the urine revealed a negative ferric chloride test and no evidence of aminoaciduria, metachromatic substances, or cytomegalic inclusion bodies. CBC, serum electrolytes, bicarbonate, BUN, blood glucose, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), and Guthrie test revealed no abnormalities. Cerebrospinal fluid contained a slightly elevated lactic acid dehydrogenase of 47·5 BB units (normal 5 to 40 units), but was otherwise normal. Chromosome study revealed no abnormalities. Other studies will be discussed later in this paper.

The disease progressed rapidly. Nystagmus, pale optic discs, and maculae were observed. Reactions to light and sound disappeared. Difficulties in swallowing with accumulation of mucus in the pharynx and episodic choking were problems at this point. Lingual fascicu-
lations with deviation of the tongue to the right were noted. The muscle hypotonia and deep tendon reflexes increased and periodically bilateral ankle clonus was reported. Babinski reflex was present on the right and there was a normal plantar response on the left.

Death occurred 22 days after admission with progressive apnoea and finally cardiac arrest.

NECROPSY At necropsy, multiple circumscribed areas of necrosis (similar to recent infarcts) were noticed bilaterally in putamen, globus pallidus, and the caudate nucleus. Along the walls of the aqueduct and the third ventricle a continuous dark pinkish area was bilaterally present. Histopathological examination revealed numerous newly formed and proliferating capillaries with varicos dilatation in the focal lesions of the basal ganglia. Astrocytes and microglia heavily infiltrated the area around the third ventricle. No lesions were observed in the optic tracts, cerebellum, spinal cord, or mammary bodies. No deposition of lipids could be detected.

The necropsy report on the patient’s sister, together with preserved brain slides, were reviewed and compared. Both localization and type of lesions seemed strikingly similar in the two cases. Therefore, the diagnosis of infantile subacute necrotizing encephalopathy (ISNE) was confirmed by the necropsy reports.

RATIONALE FOR STUDYING AMINES AND COENZYME B6
In normal children, the serotonin level (Hazra, Benson, and Sandler, 1965; Mitchell, and Cass, 1959) and the urinary excretion of 5-hydroxyindoleacetic acid (Serra, 1958; Rappallini and Murtagh, 1962) approach adult values between 6 weeks and 4 months of age. The complete absence of urinary 5-hydroxyindoleacetic acid (adult normal value 2 to 9 mg/24 hr) suggested a possible abnormality in the amine metabolism in this patient. Since the common denominator of this disorder is a necrotizing process in the brain-stem, it was decided to study the biogenic amines which play a decisive role in the maintenance of integrity of the extrapyramidal motor system in the brain. Any other biochemical studies conducted were consequential and explorative in nature and were carried out to delineate further the abnormalities found.

When a lack of 5-HIAA was discovered in the randomly collected urine, an attempt was made to study this metabolite more carefully. Therefore, two 24-hour urine samples were collected from the patient and a child of similar age and weight, who was hospitalized for a routine non-neurological condition, served as her control. The urine was frozen at —20°C, without addition of any preservative, immediately after it was voided. The negligible concentration of 5-HIAA in the 24-hour urine sample confirmed the previous findings of the clinical laboratory.

Since the enzymes which synthesize serotonin, dopamine, and gamma-aminobutyric acid are B6 dependent (Holtz and Palm, 1964), it was decided to examine the two systems extensively. At necropsy, a portion of the basal ganglia was removed for histopathological examination as well as for the estimation of the concentrations of the amines and determination of the activities of certain key enzyme systems. The basal ganglia of two children who died from bacterial endocarditis and Wilm’s tumour respectively served as control. The necropsy material, as well as histological examination of the brain tissue, revealed no detectable pathology in the latter children. Because of our previous knowledge that the patient’s sister was affected with ISNE as well as the fact that brain amine concentration will decrease upon storage, the brain was removed within 30 minutes after death.

MATERIALS AND METHODS

Urinary 4-pyridoxic acid, xanthurenic acid, taurine, dopamine, and 5-hydroxyindoleacetic acid were determined according to the methods of Reddy, Reynolds, and Price (1958); Satoh and Price (1958); Hope (1957); Carlsson and Waldeck (1958); and Udenfriend, Weissbach, and Brodie (1958) respectively. The brain dopamine, noradrenaline, and serotonin were determined according to the method of Fleming, Clark, Fenster, and Towne (1965), while the concentration of tryptamine was determined utilizing the method of Eccleston, Ashcroft, Crawford, and Loose (1966). The activity of brain glutamic acid decarboxylase and pyridoxal phosphokinase were estimated according to the methods of Lowe, Robins, and Eyerman (1958), and McCormick, Gregory, and Snell (1961) respectively. The concentration of the amine and the activity of the enzymes were calculated on the basis of dry brain weight.

RESULTS

The biochemical abnormalities which were found in the urine as well as in the basal ganglia are summarized in Tables I and II respectively. There occurred an increase in the 24-hour excretion of 4-pyridoxic acid, as well as xanthurenic acid, while the concentration of taurine became depressed. Among the biogenic amine metabolites, the concentration of 5-hydroxyindoleacetic acid was almost undetectable, while a copious quantity of dopamine was discovered in the urine. Among the biogenic amines and enzymes measured in the basal ganglia, the concentrations of dopamine, noradrenaline, and serotonin and the activity of glumatic acid

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>ABNORMAL EXCRETION OF BIOGENIC AMINES AND VITAMIN B6 METABOLITES IN THE URINE OF THE PATIENT WITH ISNE</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Pyridoxic acid (μM/24 hr)</td>
<td>Patient with ISNE 12:80 Normal child 1:85</td>
</tr>
<tr>
<td>Xanthurenic acid (μM/24 hr)*</td>
<td>0:20 0:750</td>
</tr>
<tr>
<td>Taurine (mg/24 hr)</td>
<td>0:70 12:8</td>
</tr>
<tr>
<td>Dopamine (mg/24 hr)</td>
<td>20:8 0:15</td>
</tr>
<tr>
<td>5-Hydroxyindoleacetic acid (5-HIAA) (mg/24 hr)</td>
<td>&lt;0:1 4:0</td>
</tr>
</tbody>
</table>

*No-tryptophan load was given.
TABLE II

DERANGEMENT IN THE CONCENTRATION OF BIOGENIC AMINES AND IN THE ACTIVITY OF CERTAIN B6 RELATED ENZYMES IN THE BASAL GANGLIA OF THE PATIENT WITH ISNE*

<table>
<thead>
<tr>
<th>Amines (µg/g)</th>
<th>ISNE</th>
<th>Wilms' tumour</th>
<th>bacterial endocarditis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>&lt;0.05</td>
<td>0.25</td>
<td>0.32</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>&lt;0.02</td>
<td>0.32</td>
<td>0.40</td>
</tr>
<tr>
<td>Serotonin</td>
<td>&lt;0.01</td>
<td>0.40</td>
<td>0.52</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>0.40</td>
<td>undetectable</td>
<td>undetectable</td>
</tr>
<tr>
<td>Glutamic acid decarboxylase µ mole GABA/g weight brain/hr</td>
<td>0.70</td>
<td>10.8</td>
<td>12.0</td>
</tr>
<tr>
<td>Pyridoxal kinase (µ-mole/mg protein/hr)</td>
<td>0.85</td>
<td>0.15</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*The concentration of amines and the activity of the enzymes are expressed on the basis of dry weight of the brain.

decarboxylase were depressed, while the concentration of tryptamine and the activity of pyridoxal kinase were significantly increased.

DISCUSSION

The experimental evidence indicates that in this patient the metabolism of vitamin B6 and biogenic amines were altered. The elevated level of xanthuronic acid along with the reduced level of taurine are suggestive of a generalized state of vitamin B6 deficiency. This suggestion is strengthened by the fact that the activities of cysteine sulfenic acid decarboxylase (as evidenced by low taurine excretion) and glutamic acid decarboxylase (as evidenced by a direct measurement in the basal ganglia), which require pyridoxal phosphate (PLP) as their coenzyme, were depressed. Although the biochemical data are indicative of tissue vitamin B6 deficiency, the excretion of 4-pyridoxic acid, the end-product of vitamin B6, became elevated several-fold. It is also of interest that the activities of SGOT and SGPT, the B6-requiring enzymes, were normal. Cox, Murray, and Boone (1962), while studying tritium-labelled pyridoxine, found that the major portion of pyridoxine is excreted unchanged, 17% is stored in the tissue to be utilized for future coenzyymatic activity, and less than 2% is enzymatically metabolized to 4-pyridoxic acid which is excreted in the urine. The elevated 4-pyridoxic acid excretion in the urine could result from (1) pyridoxine injection or (2) lack of storage of the compound in the tissue which is subsequently subjected to catabolism. In the case of our patient, no pyridoxine was administered. The presence of depressed activity of cysteine sulfenic acid decarboxylase, which is found in most peripheral organs (Blaschko, 1942; and Blaschko, Carter, O'Brien, and Stanley, 1948); and of glutamic acid decarboxylase, which is predominantly in the central nervous system (Vates, Agranoff, and Sokoloff, 1959), suggests that a state of impairment in B6 utilization existed in the presence of an adequate intake of vitamin in the diet. The evidence for a tissue depletion of vitamin B6 was strengthened by the observation that the activity of pyridoxal kinase, an enzyme which converts pyridoxal, pyridoxamine, and pyridoxine to their phosphorylated forms, was elevated several-fold in the basal ganglia. This postulate is in keeping with the observation of Ebadi and McCoy (1966) and Ebadi, Russell, and McCoy (1968) that the tissue level of vitamin B6 and biogenic amines influence the activity of pyridoxal kinase in brain.

In mammalian tissues the available dopamine is formed from dopa by the aid of a PLP-dependent enzyme, dopa decarboxylase (Lovenberg, Weissbach, and Udenfriend, 1962). The dopamine is then hydroxylated by dopamine \( \beta \)-hydroxylase to yield noradrenaline (Udenfriend and Creveling, 1959) and is oxidatively deaminated to form 3,4-dihydroxyphenylacetic acid, and the latter compound becomes o-methylated to produce homovanillic acid (Carlsson and Hillarp, 1962; Andén, Roos, and Werdinius, 1963) (Fig. 1). In this patient the concentration of dopamine and noradrenaline in the basal ganglia were extremely low (Table II). These low concentrations of the two amines could be caused by the depressed activity of dopa decarboxylase (which is a PLP-dependent enzyme) resulting in inadequate formation of dopamine and of course, indirectly, noradrenaline. Although the basal ganglia contained very little dopamine, the urinary excretion of dopamine was high. These inconsistencies are very difficult to explain. Assuming that the low concentration of dopamine in the basal ganglia resulted from a depression in the activity of dopa decarboxylase, then why were similar enzymes not inhibited in the peripheral systems?

The findings can be explained only if one postulates that the activity of tyrosine hydroxylase and/or dopa decarboxylase in the brain and monoamine oxidase in the peripheral tissues were selectively inhibited (Fig. 1). Because of an insufficient amount of experimental evidence, further description of this complex mechanism will not be undertaken.

The serotonin content of the basal ganglia was quantitatively negligible. This low concentration of serotonin in the brain is, however, in keeping with the extremely low excretion of 5-hydroxyindoleacetic acid in the urine (Table I).

The existing serotonin in the body is formed from decarboxylation of 5-hydroxytryptophan by
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tyrosine hydroxylase* dopa decarboxylase* dopamine β hydroxylase

TYROSINE → DOPA → DOPAMINE → NORADRENALINE

Monoamine oxidase

3,4 dihydroxyphenylacetic acid

COMT

Homovanillic acid

FIG. 1. Biochemical pathways leading to formation and degradation of dopamine. The possible enzymatic inactivation in the patient’s brain is designated in two reactions by single asterisks.

the aid of a PLP-dependent 5-hydroxytryptophan decarboxylase (Lovenberg et al., 1962). The 5-hydroxytryptophan is produced by direct hydroxylation of tryptophan under the catalytic action of tryptophan hydroxylase. Tryptophan can also be directly decarboxylated, by tryptophan decarboxylase, to yield tryptamine (Werle and Mennicken, 1937). Serotonin and tryptamine are oxidatively deaminated to yield 5-hydroxyindoleacetic acid which is excreted in concentrations of 2 to 9 mg/24 hr, and indoleacetic acid which is excreted in concentrations of 5 to 18 mg/24 hr (Jackson and Chandler, 1939; and Weissbach, King, Sjoerdsmma, and Udenfriend, 1959) (Fig. 2). Although the concentration of serotonin in the basal ganglia is approximately 0.5 μg/g wet brain tissue, the concentration of tryptamine is negligible, since it is very rapidly oxidized to indoleacetic acid which is excreted in the urine. It is rather interesting that in this patient, in the absence of serotonin, the concentration of tryptamine became elevated (Table II). There is little doubt that the build-up of this

FIG. 2. Biochemical pathways leading to formation and degradation of serotonin and tryptamine. The postulated enzymatic inactivation which led to decreased concentration of serotonin and increased concentration of tryptamine in the patient with ISNE are designated by a single asterisk and a double asterisk respectively.
amine is due to inhibition of the activity of mono-
amin oxidase. The depressed concentration of
serotonin in the brain, along with decreased excretion of
5-hydroxyindoleacetic acid in the urine, is clearly
suggestive of inadequate synthesis of serotonin, which
results from an inhibition of tryptophan hydroxylase and/or
5-hydroxytryptophan decar-
boxylase.

It is extremely difficult to understand the relation-
ship between the disease subacute necrotizing ence-
phalopathy and the biochemical abnormalities
detected. For example, it is not known to what
extent, if any, the discussed biochemical abnor-
malities contribute to the onset and pathological
characteristic of the disease, nor is it clear to what
degree these derangements are manifestations of the
disease state with a far more complicated cause and
etiology. However, it is unlikely that the degenera-
tive processes in the brain-stem would have been
arrested, even if one had the capability of biochemi-
cally correcting the reported B6 and amin aberr-
nalities. Furthermore, it is conceivable that these
multiple enzymatic blockades, as reported in this
paper and other studies (Greenhouse and Schneck
1968; Namiki, 1965; and Worsley et al., 1965), are
the results of non-specific action of a substance or
substances elaborated by the disease, capable of
creating a massive breakdown in decarboxylating
mechanisms everywhere. The evidence presented in
this report indicates that the inhibition in de-
carboxylating systems might be due to impairment in
catalytic utilization of vitamin B6 derivatives.

SUMMARY

This paper reports the biochemical abnormalities
found in the basal ganglia and the urine of a child
with subacute necrotizing encephalopathy. The
urinary levels of 4-pyridoxic acid, xanthurenic acid,
and dopamine were elevated, whereas the levels of
taurine and 5-hydroxyindoleacetic acid were
depressed. The basal ganglia levels of dopamine, nor-
adrenaline, serotonin, and the activity of the glutamic
acid decarboxylase were depressed, whereas the level of
tryptamine and the activity of pyridoxal kinase
were elevated. These data are interpreted to indicate
that in this child there occurred an impairment in
utilization of vitamin B6 and in amino acid
decarboxylating systems.

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crypsis materials as well as histopathological
information about the brains of the children studied.

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