Effect of Treatment on the Metabolism of Tryptophan in Childhood Epilepsy

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In a previous paper (Hughes, Bower, Raine, and Syed, 1966) it was shown that about one-third of patients with childhood epilepsy showed, on admission, an abnormality of tryptophan metabolism characterized by a high excretion of xanthurenic acid in the urine following an oral load of tryptophan. This is usually accompanied by high excretion of certain other metabolites of tryptophan having in common that their metabolism is dependent upon an enzyme for which pyridoxine is an essential co-factor.

The present findings extend those of an earlier study (Bower, 1961; Bower and Hughes, 1961) in which it was also shown that treatment of patients with infantile spasms with ACTH or corticosteroids (the form of therapy still favoured for this condition) resulted in a reduction in the excretion of xanthurenic acid in all cases. At that time, the effect of pyridoxine, a deficiency of which most conveniently accounted for the biochemical abnormality, had been examined in only 3 patients with somewhat equivocal results.

We present further studies of the biochemical and clinical effects of pyridoxine and of corticotrophin and steroids both singly and in combination. In addition to xanthurenic acid, several other pyridoxine-dependent metabolites have been studied, and the effect on these of withdrawal of treatment for various periods has been examined.

This study also gives consideration to the value of the initial xanthurenic acid excretion in response to a tryptophan load, in predicting the clinical response to the two alternative forms of therapy, pyridoxine and corticosteroids. In a small number of patients an attempt has been made to determine the smallest dose of pyridoxine or ACTH required to restore the xanthurenic acid excretion to normal.

It is found that all forms of therapy reduce the excretion of xanthurenic acid and, where it was abnormal initially, restore it to normal levels. It is also shown that pyridoxine does this much more promptly than does corticosteroid therapy. Withdrawal of the latter type of therapy results in an increase in xanthurenic acid excretion after a further tryptophan load, whereas in those patients treated with pyridoxine, the xanthurenic acid excretion has remained normal for many months after cessation of treatment.

Material and Methods

The subjects are divided into clinical groups as defined in the previous paper (Hughes et al., 1966). Group 1 consists of 14 healthy control children; Group 2 of 20 patients with neurological disorders unaccompanied by convulsions. The effect of treatment with pyridoxine has been studied in 9 patients with epilepsy (Group 3). The effects of pyridoxine, steroids, and ACTH have been studied in patients with infantile spasms (9 in Group 4, the cryptogenic group, and 5 in Group 5, the symptomatic group). In addition 2 patients with neurological disease (Group 2), who had a high excretion of xanthurenic acid, were tested with pyridoxine and the results of similar studies are reported in these too. Details of the performance of tryptophan load tests and subsequent analyses, EEG scoring, and assessment of mental status have been described earlier (Hughes et al., 1966).

Results

The results of tryptophan load tests performed in patients in the different clinical groups, before and while receiving the several individual or combined forms of treatment, are given in Table I. This Table also records any observed improvement either in the clinical state or in the EEG. All but 4 of the patients (who will be discussed later) were treated for periods believed to be therapeutically significant.

The effect of treatment on the excretion of xanthurenic acid by patients in the several groups is summarized in Table II. This shows that in nearly all subjects the xanthurenic acid excretion was reduced to below 3 mg./24 hr., and in the
TABLE I

Biochemical and Clinical Changes in Response to Treatment

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Metabolite Excretion (mg./24 hr.)</th>
<th>Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Treatment</td>
<td>During Treatment</td>
</tr>
<tr>
<td></td>
<td>XA*</td>
<td>XA</td>
</tr>
<tr>
<td>Pyridoxine Alone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>11</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>16.0</td>
</tr>
<tr>
<td>Group 3</td>
<td>14</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>24.6</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>26.4</td>
</tr>
<tr>
<td>Group 4</td>
<td>34</td>
<td>20.3</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>19.3</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>29.1</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>9.9</td>
</tr>
<tr>
<td>Group 5</td>
<td>34</td>
<td>65.3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

XA* = xanthurenic acid as determined by the ferric alum method; the remainder of the metabolites were determined after chromatographic separation. XA = xanthurenic acid, K = kynurene, KA = kynurenic acid, 3-OHK = 3-hydroxykynurenine, 3-OHAA = 3-hydroxyanthranilic acid, Sl. Imp. = slight improvement, O = no change.

TABLE II

Effect of Treatment on Xanthurenic Acid (XA) Excretion

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Clinical Group</th>
<th>No. of Cases</th>
<th>No. of Cases Excreting &gt; 3 mg. XA Before Treatment</th>
<th>No. of Cases in Previous Col. Excreting 3 mg. XA or Less After Treatment</th>
<th>Change in XA Excretion in Remaining Cases (mg./24 hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridoxine</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>16.5 → 3.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>10</td>
<td>7</td>
<td>(26.4 → 3.1)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>(9.9 → 4.3)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>(65.3 → 3.6)</td>
</tr>
<tr>
<td></td>
<td>(4 + 5)</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Pyridoxine + steroids</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ACTH</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>ACTH + steroids</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Steroids</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>All ACTH and/or steroids</td>
<td>(4 + 5)</td>
<td>11</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>
remainder the excretion was very considerably reduced, almost to these same levels. It is interesting to note that though variations in xanthurenic acid excretion within the range of 0 to 3 mg./24 hr. are probably not significant, where the excretion was within this range before treatment, in nearly every case the excretion was further reduced. There were no significant differences in the results in the different clinical groups or with the different forms of treatment. The effects of the various forms of treatment on the clinical and EEG states of the patients were the same as those described in a larger series (not all of which were studied biochemically) and reported elsewhere (Jeavons and Bower, 1964). Briefly, ACTH and steroids produced a greater improvement than pyridoxine both in spasms and in EEG abnormality; no definite difference between the effects of ACTH and steroids could be detected.

All the patients considered so far have received treatment for one week or more with the exception of four (Group 2: Cases 11 and 12; and Group 3: Cases 42 and 46) who formed part of a special study to determine the minimal dose of pyridoxine required to reduce the excretion of xanthurenic acid to less than 3 mg./24 hr. Patients other than these four had received pyridoxine doses of 50-100 mg./day for at least one week. In these four patients single doses of pyridoxine were given after an initial tryptophan load test, and this was followed immediately by a further test. The results following different amounts of pyridoxine are shown in Table III. Thus 100 mg. and 25 mg. of pyridoxine reduced the xanthurenic acid excretion in 3 patients

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose of Pyridoxine (mg.)</th>
<th>Xanthurenic Acid Excretion (mg./24 hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>2-11</td>
<td>25</td>
<td>13·2</td>
</tr>
<tr>
<td>2-12</td>
<td>10</td>
<td>16·5</td>
</tr>
<tr>
<td>3-42</td>
<td>100</td>
<td>29·1</td>
</tr>
<tr>
<td>3-46</td>
<td>25</td>
<td>65·3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

(Groups 3: Case 42; and Group 2: Case 11 to within the normal range) and in one patient (Group 2: Case 12) as little as 10 mg., almost corrected the abnormality. Another patient (Group 3: Case 46), however, had a much higher initial xanthurenic acid excretion and required first 25 mg. and then a further 50 mg. to bring the value nearly to normal. Thus, while comparatively small amounts of pyridoxine may correct the biochemical defect, the amount required may be related to the severity of the initial abnormality.

An attempt has been made to study the effects of single days of treatment with ACTH (given 8 hourly) in a similar manner, but so far this has been frustrated in various ways. Results on 3 patients, however, suggest that the effect of ACTH is less immediate and dramatic than pyridoxine. After treatment of one patient with ACTH for 4 weeks, the xanthurenic acid excretion was still 3·9 mg.; another patient whose xanthurenic acid excretion was 14·1 mg. before treatment, still excreted 20·5 mg. after one week of ACTH. In a third patient the xanthurenic acid excretion before and after treatment for 3 weeks with ACTH was 22·7 mg. and 11·2 mg., respectively, whereas after a single intramuscular injection of pyridoxine this fell to 3·8 mg. This last patient is unusually refractory, since in all other patients treated for this length of time, the xanthurenic acid excretion had become normal.

Where the excretion of the metabolites of tryptophan, other than xanthurenic acid, has been determined both before and after treatment with pyridoxine, and in some cases after withdrawal of this therapy, the results are shown in Fig. 1.

Two patients (Group 3: Case 11; Group 5: Case 8) have data before treatment, during treatment which had been given for several days, and after withdrawal of treatment. The others had single doses of pyridoxine: 2 patients (Group 2: Cases 11 and 12) were restored to normal or near normal levels in one step, whereas one (Group 3: Case 46) required two doses to achieve this (see Table III).

Fig. 1 shows that in every case treatment lowered the excretion of each of the metabolites, and where 3-hydroxyanthranilic acid was absent before, this substance was detected after treatment. The effect of withdrawal of treatment, however, was less predictable: for each metabolite, one patient excreted more and the other less than when on full treatment. Unfortunately, however, a particular subject did not respond in the same way with respect to each metabolite. It is, therefore, not possible to draw any general conclusions concerning the effect of treatment on the excretion of these several metabolites.

**Discussion**

Although in nearly all the cases we have studied any biochemical abnormalities that have been detected have been restored to normal, there has been a disappointing clinical response to pyridoxine, and, in the long term, to ACTH and steroids. Furthermore, where some clinical improvement has
For further details a-e 1959; Leriche, in metabolism upper limit Few B6 vitamin seen, has led test has biochemical Bower, discussed is it is response especially in dose of this has studied 140-100 -120-50 40 60- 284 3-OHK KA 3-OHAA

FIG. 1.—Metabolite excretion in relation to treatment. Patients a-e are respectively 3-11, 2-11, 2-12, 5-8, 3-46. For further details see text. The horizontal solid and dotted lines represent the lower limit of results in patients with an abnormal excretion of xanthurenic acid, and the upper limit of those with a normal excretion of this metabolite (see Fig. 1 Hughes, Bower, Raine and Syed, 1968). The metabolites are denoted by the abbreviations in the footnote to Table 1.

been seen, this has not correlated with the initial biochemical abnormality, while the tryptophan load test has been of little value in predicting either the value or the outcome of treatment.

The fact that treatment with ACTH or steroids has led to improvement in some patients with infantile spasms justifies a trial of these drugs, especially in those in the cryptogenic group. If the response is poor or not sustained after withdrawal, it is probably worth while trying pyridoxine in the dose of 50 mg. daily for 2 or 3 weeks. This subject is discussed in greater detail elsewhere (Jeavons and Bower, 1964).

Since Cochrane's report (1959) several workers in different centres have studied aspects of tryptophan metabolism in patients with infantile spasms and the effect on this of various forms of treatment, notably vitamin B6 (Jeune, Cotte, Hermier, Yasse, and Leriche, 1959; Segni and Gandullia, 1962; Careddu, 1963; Hagberg, Hamfelt, and Hansson, 1964, 1965). Few authors have studied more than 2 or 3 patients and rarely have the diagnostic criteria been explicitly stated. With the exception of Careddu (1963), however, most reports agree that pyridoxine lowers the excretion of xanthurenic acid where this was initially raised.

Careddu (1963) finds with Jeune et al. (1959) that ACTH restores the xanthurenic acid excretion to normal levels, though the effect of this drug is less dramatic than that of pyridoxine.

Children with other varieties of epilepsy have been studied biochemically by Hagberg et al. (1964) and Hottinger, Berger, and Krauthammer (1964). Both groups found that a proportion of these excreted an abnormal amount of xanthurenic acid which in some cases was corrected by pyridoxine.

Hottinger et al. (1964) studied some patients with neurological disorders unaccompanied by convulsions, but it is not possible to determine from their report which of these excreted increased amounts of xanthurenic acid.

Our own findings conflict only with those of Careddu (1963), as we have not yet failed to correct the biochemical abnormality by giving pyridoxine in moderate doses.

Tryptophan metabolites other than xanthurenic acid have been studied only to a variable extent, and no clear statement has so far been made of the effect on the excretion of these of different forms of treatment. In general, the response has been that which would have been predicted from the correction of a defect of pyridoxine utilization, and our results too are in accordance with this interpretation.

The study of Careddu (1963) of the effect of ACTH on the excretion of xanthurenic acid and several other metabolites is of special interest. He showed that ACTH continued to improve the biochemical abnormality after 15 days, in that when treatment was prolonged to 30 days, the xanthurenic acid excretion fell still further. Kynurenine and acetyl kynurenine were hardly affected by this treatment, but an increase was demonstrated in the excretion of methylincotinamide and a much greater increase in N-methyl-2-pyridone-5-carboxamide (pyridone). The effect on these last two substances strongly suggests that the ACTH is affecting the pyridoxine-dependent enzyme kynureninase which catalyses the conversion of 3-hydroxykynurenine to 3-hydroxanthranilic acid.

Effect of minimal doses. Nearly all previous studies of the effect in human subjects of pyridoxine on abnormalities of tryptophan metabolism have involved pharmacological as distinct from physiological doses of the vitamin. For example, Hagberg et al. (1964, 1965) used 100-160 mg./day; Segni and
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Gandullia (1962) 50-80 mg./day, Cochrane (1959) 25-260 mg./day, French, Grueter, Druckman, and O'Brien (1965) 50 mg./day, in each instance for several days. Careddu (1963), who found pyridoxine ineffective, gave an unspecified dose for 'a few days'.

In a valuable study of the amounts of pyridoxine necessary to relieve convulsions and to correct the biochemical abnormality in infants who were rendered pyridoxine deficient by an artificial milk preparation, Bessey, Adam, and Hansen (1957) showed that after supplementation of the deficient diet by only 0.26 mg./day the convulsions ceased, and this only needed to be increased to 1.2 mg./day in order to restore the excretion of xanthurenic acid completely to normal.

Even those abnormal children classified as 'pyridoxine dependent' required less than 3 mg./day to maintain a normal excretion of xanthurenic acid, though in this condition up to 15 mg./day were necessary to keep the children free of convulsions (Scriver, 1960; Scriver and Hutchison, 1963).

Our own study, in which single doses of pyridoxine have been given, shows that as little as 10 mg. has almost corrected the biochemical abnormality, 25 mg. has corrected this when initially it was of moderate severity but, in a more severe abnormality, a further single dose was required to restore the excretion of xanthurenic acid to normal. While these results with single doses of moderate size cannot easily be compared with the effects of much smaller doses given over several days, it is clear that whatever the biochemical abnormality may be in these patients with neurological disorders, it appears to be corrected by quite small supplements of pyridoxine and, as will be discussed later, this 'cure' appears to be long lasting.

The effect of pyridoxine on the excretion of metabolites other than xanthurenic acid has been shown by French et al. (1965) to bring this nearer to normal values. Our own results agree with this, though Careddu's (1963) study again proved exceptional. Studies by Heeley and Roberts (1965) in psychotic children and by Heeley (1965) in untreated patients with phenylketonuria also show that pyridoxine corrects the excretion of kynurenine and 3-hydroxykynurenine when this was initially abnormal.

The effect of small doses of ACTH and corticosteroids does not appear to have been studied, either with respect to clinical or biochemical abnormalities. Jeune et al. (1959) found that doses of ACTH of 25 units/day for 12 days, the shortest period they studied, always restored the excretion of xanthurenic acid to normal. Careddu (1963) gave doses of 5 units/kg. day which would usually be of the same order as those used by Jeune et al. (1959) and ourselves, and studied their effect after 15 and 30 days. There was a notable fall in xanthurenic acid after the shorter period and this fell still further after 15 more days of treatment.

Our own attempt to study the effect of single days of treatment with ACTH has unfortunately not provided any useful information.

The permanence or otherwise of the effects of pyridoxine and steroids has been examined by a few authors. Hagberg et al. (1964) showed that after withdrawing pyridoxine their three patients all excreted abnormal amounts of xanthurenic acid within 7 days, and this increased further in two patients during the following week. Jeune et al. (1959) found that two patients excreted abnormal amounts of xanthurenic acid 5 and 17 days after discontinuing treatment with ACTH. The second of these was studied 2 weeks later and found then to excrete normal amounts of xanthurenic acid, even though no further treatment had been given. Our own results show that in two patients 1 month and 2 years after withdrawing pyridoxine, the xanthurenic acid excretion remained normal. After withdrawal of ACTH and/or steroid for at least one week, however, 6 of 7 cases showed an increase in xanthurenic acid excretion.

**Metabolic significance of biochemical changes.** The possibility that the changes in the several tryptophan metabolites before and after treatment may be interpreted in terms of pyridoxine deficiency requires further examination. Reference to the metabolic scheme (Fig. 2) shows that increases in the excretion of kynurenine, kynurenic acid, xanthurenic acid, and 3-hydroxykynurenine, and lowered excretion of 3-hydroxanthranilic acid observed in the present patients, are just those changes that would be predicted when pyridoxine is
deficient. Furthermore, these changes are reversed when pyridoxine is given, while in a few studies in which human subjects have been deprived of pyridoxine the same changes are described for kynurenic, 3-hydroxykynurenic, kynurenic acid, xanthurenic acid, and acetylkynurenic (Yess, Price, Brown, Swan, and Linkswiler, 1964). Changes in xanthurenic acid have also been recorded by Greenberg, Bohr, McGrath, and Rinehart (1949), Bessey et al. (1957), Wachstein and McCully (1960), Babcock, Brush, and Sostman (1960), Hodges, Bean, Ohlson, and Bleier (1962), and Baker, Canham, Nunes, Sauerlich, and McDowell (1964) who also studied 3-hydroxykynurenic and showed that the excretion of free pyridoxine also fell during deprivation.

The consistency of these changes with the known role of pyridoxine in tryptophan metabolism is all the more significant because of the fact that in a number of other states, where there are similar disturbances in tryptophan metabolism, not all of these changes are those expected in a state of pyridoxine deficiency. For example, in rheumatoid arthritis, increased excretion of 3-hydroxykynurenic acid, kynurenic acid, and kynurenic acid is associated with increased instead of lowered 3-hydroxynanthranilic acid and a normal excretion of xanthurenic acid (Bett, 1962).

In pregnancy, increased excretion of xanthurenic acid (Vandelli, 1951; Sprince, Lowry, Folsome, and Behrman, 1951; Wachstein and Gudaitis, 1952) or of xanthurenic acid, kynurenic acid, 3-hydroxykynurenic acid, and acetyl-kynurenic contrasts with the associated decreased excretion of kynurenic acid and an increase in nicotinamide and its metabolites (Brown, Thornton, and Price, 1961; Wertz, Lojkin, Bouchard, and Derby, 1958): the changes in diabetes (Wiseman, Kalant, and Hoffman, 1958) and scleroderma (Price, Brown, Rukavina, Mendelson, and Johnson, 1957) too are not readily explained in terms of pyridoxine deficiency.

The similarity between the effects of ACTH and pyridoxine on these metabolic changes is not easy to explain. The study of Caredu (1963) already referred to, in which it was demonstrated that treatment with ACTH led to an increased excretion of pyridone, suggests very strongly that this part of its effect is mediated through its action on some aspect of pyridoxine utilization.

Possible nature of biochemical defect. Attempts to demonstrate the kynurenine pathway of tryptophan metabolism in brain have so far failed. Increased excretion of xanthurenic acid may therefore not reflect cerebral metabolism at all. Con-
At present there is no reason to prefer one of these hypotheses to the others. In this connexion, however, it has recently been reported that in the human brain an increase in the level of pyridoxine and related compounds begins during the second month after birth (Tursky, Pechán, Zelinková, and Sedláč, 1965). It is at this period that both myelination and growth and differentiation of neurones are occurring, and it is possible that if this increased demand for pyridoxine is not met, permanent brain damage will result. It is also interesting that the onset of infantile spasms is within the succeeding few months.

Summary

The tryptophan load test has been applied to 31 children at various times in relation to treatment. The clinical groups studied comprise children with chronic cerebral disorders unassociated with epilepsy; children with epilepsy other than infantile spasms; and children with infantile spasms, subdivided into cryptogenic and symptomatic groups. Treatment was with pyridoxine, ACTH, and steroids, singly or in combination. The excretion of xanthurenic acid and, in some cases, four other metabolites has been determined.

All forms of therapy corrected the biochemical abnormality when it was initially abnormal; the effect of pyridoxine appeared to last longer than that of ACTH or steroids. The dose of pyridoxine required to correct the biochemical abnormality was often very small. Unfortunately biochemical improvement is not accompanied by a comparable degree of clinical improvement. The nature of the biochemical abnormality has been discussed.

We are grateful to Professor D. V. Hubble for his interest and help; to the physicians of the Children’s Hospital and the paediatricians of the Birmingham Region for allowing us to investigate their patients; to the nursing staff for the care they have taken with the tests; and to Dr. P. M. Jevons for his analysis of the EEG’s. This study was generously supported by the Endowment Fund of the United Birmingham Hospitals and the League of Friends of the Children’s Hospital.

REFERENCES

Hughes, Bower, Raine, and Syed

Addendum

Since our two papers were written the results of similar studies by Hagberg, Hamfelt, and Hansson (1966a, b) have been published. Their findings are similar to ours with one major exception: in their patients the abnormalities after tryptophan loading were found almost exclusively in the group with cryptogenic epilepsy, whereas in our patients such findings were equally frequent in patients with neurological disease, with or without epilepsy. We agree with them in finding no relation between such biochemical abnormalities and age, coexistence of mental subnormality, or the type of epilepsy, and we too were disappointed in the use of the tryptophan load test as a predictor of therapeutic response to pyridoxine.

REFERENCES
