THE EFFECT OF ANTICONVULSANT THERAPY UPON THE ABSORPTION OF FOLATES

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SUMMARY

1. The absorption of orally administered pteroylmonoglutamate and pteroyltetraglutamate, measured as peak rise in serum folate levels, was examined in normal volunteers and in epileptic patients on anticonvulsant drugs.

2. Although the fasting serum folate levels were low in the patients, they absorbed the two folate forms as well as the volunteers did and there was no difference between the absorption of pteroylmonoglutamate and pteroyltetraglutamate.

3. The findings indicate that the folate deficiency, caused by anticonvulsant drugs is not due to interference with the intestinal conjugase or the mechanism of absorption in itself.

Key words: anticonvulsant drugs, intestinal absorption, synthetic pteroylmonoglutamate, synthetic pteroyltetraglutamate

It has been generally accepted that diphenylhydantoin, the barbiturates and primidone can affect the folate status of individuals receiving long-term therapy with one or more of these drugs. The associated folate deficiency can lead to a megaloblastic anaemia, and possibly to neurological disturbances (Reynolds, 1968). Thus, it may be of considerable value to supplement such patients with folic acid at an early stage of the deficiency or even prophylactically. Furthermore, studies of the way in which these drugs interfere with the absorption or metabolism of the folates may shed light on the pathophysiology of other diseases in which folate deficiency plays a part.

Because a substantial amount of the dietary folate is in the polyglutamate form and the peptide chain has to be broken down to mono- or at least di-glutamate before the substance enters the blood, it has been suggested that the anticonvulsant drugs interfere with folate absorption by inhibiting the hydrolysis of pteroylpolyglutamates. This reaction is catalysed by the enzyme $\gamma$-glutamyl carboxypeptidase (folic acid conjugase). The object of the present study was to investigate this hypothesis.

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study was to test this hypothesis as well as the possibility of a disturbance in the mechanism of absorption itself. Consequently, we have compared absorption of pteroylmonoglutamate with that of synthetic pteroyltetraglutamate in normal volunteers and in patients on long-term anticonvulsant therapy.

MATERIALS AND METHODS

Volunteers and patients
Six normal male students volunteered for the study. Their ages ranged from 22 to 37 years. Six male epileptic patients, who were permanently hospitalized because of mental retardation, therapy-resistant epilepsy or social difficulties, were chosen for the study. Their ages ranged from 37 to 69 years. Special care was taken to avoid subjects with any digestive trouble or a history of gastrointestinal dysfunction. Five patients were on phenytoin and phenobarbitone and one was on primidone and phenobarbitone. In addition, two of the patients were taking carbamazepine and three ethosuccimide. Diazepam and chloropromazine were taken by four and two of the patients, respectively.

Experimental design
The experiments were performed on two consecutive days without previous preloading with folic acid. The volunteers and the patients were each divided randomly into two groups with three subjects in each. The groups that received pteroylmonoglutamate the first day got pteroylpolyglutamate the second day, and vice versa. Each substance (1 µmol) was given with a standardized light breakfast together with the usual medication at 08.00 hours after an overnight fast. Blood for analysis of the serum folate concentration was obtained by venepuncture immediately before breakfast and 1, 3 and 4 h after the folate intake. No food, drink or smoking were permitted during the 4 h of the test. The blood was allowed to clot in a dark room at 22°C. The serum was then transferred to tubes, prepared with ascorbic acid and stored at −20°C until analysed.

Assay of folic acid
Folic acid was estimated microbiologically with Lactobacillus casei (A.T.C.C. 7469). The assay was performed as described by Hansen (1964) with the following modifications: the incubation time at 37°C was shortened to 20 h, a larger initial inoculum was used and the medium was Dano Folic Acid Casein (Ferrosan Pharmaceuticals AB, Copenhagen). Growth was estimated turbidimetrically on a Beckman B spectrophotometer at 675 nm and compared with values obtained from a standard curve made up from known amounts of synthetic pteroylmonoglutamate. All samples were assayed in duplicate.

Preparation of pteroyltetraglutamate
Pteroyltetraglutamate was synthesized as described by Krumdieck & Baugh (1969) with the modifications recommended by Baugh, Stevens & Krumdieck (1970). Pteroyltetraglutamate was further purified by Sephadex G-15 (2.4 cm x 80 cm) chromatography. The eluting buffer was 0.05 M-ammonium carbonate with 0.2% mercaptoethanol. This technique makes it possible to separate pteroyltetra-, tri-, di- and mono-glutamate and pteroic acid with a high degree of specificity. The purity of the tetraglutamate was checked in two ways. (1) Thin layer
chromatography on cellulose MN 300 G (Macherey, Nagel & Co., W. Germany) [solvent 15% (w/v) \( \text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O} \) and 0.5% (w/v) mercaptoethanol in water], showed only one visible spot.

(2) *Streptococcus faecalis* responds only to the di- and mono-glutamate but not to pteroyl-glutamates of higher order. Our pteroyltetraglutamate was assayed with this micro-organism and no growth was detectable. Our strain of *Lactobacillus casei* responds quantitatively to pteroyltetraglutamate and shows no increase in the activity after conjugase treatment.

**RESULTS**

'Peak rise' values were calculated by subtracting the fasting values from the peak value of each absorption test. The normal volunteers showed no difference between the absorption of pteroyltetraglutamate and pteroylmonoglutamate as demonstrated by serum concentration curves and statistics applied to the peak rise values in serum folate (Table 1). There was no

<table>
<thead>
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<th>Subject no.</th>
<th>Pteroylmonoglutamate</th>
<th>Pteroyltetraglutamate</th>
<th>Difference</th>
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Mean difference = +0.82, SEM = ±1.127, \( t = 0.73, P > 0.4 \).

<table>
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<tr>
<td>6</td>
<td>7.8</td>
<td>10.2</td>
<td>-2.4</td>
</tr>
</tbody>
</table>

Mean difference = -3.77, SEM = ±0.943, \( t = 3.99, P < 0.01 \).
in all patients according to the standards of this laboratory. (2) There was a significantly greater peak rise the second day than the first day (Table 2). (3) There was no demonstrable difference between the serum values after folate monoglutamate and folate tetraglutamate, respectively (Fig. 1).

![Fig. 1. Serum folate in six subjects on chronic anticonvulsant therapy after the ingestion of 1 μmol of pteroylmonoglutamate (▲) or pteroyltetraglutamate (○). Each point is the mean of six determinations. Vertical lines represent 2 SD.]

**DISCUSSION**

Folate deficiency in patients receiving anticonvulsant drugs is well documented in the literature (for references see Reynolds, 1968). All six of our patients had initial serum folate levels below 5 ng/ml, the lower limit for normals in our laboratory. The slower plasma clearance of folate on the second day, when the patients had been preloaded with the test dose of the first day strongly suggests that the tissues, too, were relatively depleted of folate (Chanarin, Laidlaw, Loughridge & Mollin, 1960). Because the normal subjects could be assumed to have a normal folate status, the lack of effect from the first dose on the plasma clearance on the second day was expected.

We were unable to show any difference in the absorption of pteroylmonoglutamate and pteroyltetraglutamate. This was true for the patients on chronic anticonvulsant therapy as well as for the volunteers, indicating that neither the conjugase nor the absorptive process is significantly affected by phenytoin in vivo. Small differences may, however, have been overlooked because of the small number of patients studied—a reservation that also applies to several of the studies referred to below.
Anticonvulsants and folate absorption

Hoffbrand & Necheles (1968) and Rosenberg, Streff, Godwin & Castle (1968) have published observations suggesting malabsorption of pteroylpolyglutamate due to an inhibitory effect of phenytoin on conjugase. Several investigators have failed to confirm their results, *in vitro* as well as *in vivo* (Baugh & Krumdieck, 1969; Bernstein, Gutstein & Weiner, 1970; Houlihan, Scott, Boyle & Weir, 1972; Perry & Chanarin, 1972). However, the *in vivo* work of the latter authors is not quite relevant to the patients on anticonvulsant therapy because the test subjects were not taking phenytoin chronically. We are unable to explain the difference between our results and those published in 1968, unless it can be ascribed to the use of a partly purified yeast extract in the earlier experiments, in contrast to our synthetic pteroyltetra-glutamate.

Gerson, Hepner, Brown, Cohen, Herbert & Janowitz (1972) showed that phenytoin infused into the proximal jejunum inhibits the absorption of pteroylmonoglutamate in humans. In our experiments, where both substances entered the upper gastrointestinal tract in the physiological way, there was no indication that the patients absorbed less of the pteroylmonoglutamate than the normal volunteers.

If the absorption of folates is not impaired substantially in treated epileptics, other ways in which folate deficiency might come about in these patients will have to be considered more seriously. Maxwell, Hunter, Stewart, Ardeman & Williams (1972) have published results that indicate a correlation between hepatic microsomal enzyme activity and the degree of folate deficiency in red cells of chronically treated epileptic children. They have formulated a hypothesis suggesting that the increased hepatic enzyme activity would lead to an increased demand for and metabolism of folate coenzymes. However, Spray & Burns (1972) were unable to demonstrate any decrease in rat liver folate in spite of a definite increase in hepatic enzymatic activity caused by phenytoin and/or phenobarbitone.

One possible reason why anticonvulsant drugs deplete the tissues of folate might be that folate excretion is promoted by the drugs. Little has been done to clarify this aspect of drug–folate interaction. Perry & Chanarin (1972) have investigated the 6 h urinary excretion of folate after an oral load of pteroylmonoglutamate with and without phenytoin. Though no difference in excretion could be demonstrated statistically, the values suggest an increased folate excretion when phenytoin was given. This kind of experiment should be extended and repeated on larger numbers of patients on chronic anticonvulsant therapy before the urinary excretion hypothesis can be discarded.

ACKNOWLEDGMENTS

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