Malabsorption of Folic Acid due to Diphenylhydantoin

By Miriam B. Dahlke and Elizabeth Mertens-Roesler

Subnormal serum folate levels are common in patients receiving anticonvulsant drugs, and overt megaloblastic anemias occur in a few of such patients. In a recent study of 60 adults on antiepileptic medication, Klipstein1 reported that 58 per cent had serum folate levels of less than 5.0 mg per ml. The mechanism whereby these drugs depress the serum folate level is unknown. Proposed explanations for the phenomenon include competition for enzyme sites concerned with the reduction and further activation of folic acid, inhibition of gastrointestinal conjugase, and increased urinary excretion. Interference with the storage of folates must also be considered. The absorption of pharmacologic doses of folic acid has been reported as normal.

The purpose of this report is to present the results of a survey of 59 children receiving diphenylhydantoin and observations made over a period of 1 year on an adult epileptic with a severe folate deficiency. The results of in vitro experiments designed to study the effect of diphenylhydantoin on the utilization of folates by L. casei and on conjugase activity are included.

Methods and Patient Material

Serum folate activity was determined by the "standard method" of microbiologic assay described by Herbert,13 and whole blood folate activity by the method of Cooper and Lowenstein.16 The "free" folate content of washed erythrocytes was measured using 1 ml. of packed thrice saline-washed erythrocytes. Urine folate activity was assayed using 1 ml. of a 24-hour urine specimen collected in a brown bottle containing several drops of volatile preservative. Hospital diets were assayed for "free" and "total" folate content by the method of Herbert17 using desiccated chicken pancreas as the source of conjugase. Pancreatic secretion, assayed for conjugase activity, were obtained from fasting patients before and after stimulation with secretin, 1 unit per Kg. of body weight. Serum B12 activity was determined by the method of Spray.18

Fifty-nine children from the pediatric neurology clinic at the Philadelphia General Hospital were evaluated. Diphenylhydantoin was prescribed in a dosage of greater than 2.0 mg per lb per day for 21 of these children. The remaining 38 received a lower dosage schedule.

Case Report

E. F., a 54-year-old epileptic, was admitted with a megaloblastic anemia. She had

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Bacto-Chicken Pancreas, obtained from Difco Labs.
Secretin, obtained from Vitrum Labs., Stockholm.

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been taking 400 mg. phenobarbital daily for 40 years and 400 mg. diphenylhydantoin daily for 20 years. There had been a marked decrease in food intake and a weight loss of 30 pounds during the 4 months prior to admission. The hemoglobin was 4.7 Gm. per 100 ml., the reticulocytes 1.0 per cent, and the white cell count 6300; the platelet count was 116,000. The serum B₁₂ level was 400 μg. per ml. (normal, 150 μg. per ml. or greater) and the serum folate level was less than 1.0 μg. per ml.

Diphenylhydantoin and phenobarbital were continued during the entire study. There was no hematologic response to 14 days of hospital diet, containing 50-60 μg. of free folate and 100-150 μg. of total folate daily. Desiccated chicken pancreas, 750 mg. per day, was then administered in divided doses with meals. The folate content of the daily conjugase supplement was 1.8 μg. A reticulocyte peak was observed 12 days after the institution of the conjugase preparation and was followed by a rise in hemoglobin to 8 Gm. per cent. The conjugase supplement was discontinued for 21 days; during this time the hemoglobin remained stable. Readministration of the conjugase preparation was followed by a secondary reticulocyte peak, and a hemoglobin level of 15 Gm. per cent was reached after a total of 65 days of conjugase administration.

However, the serum and whole blood folate levels remained low after an additional 2 months of conjugase therapy. The latter was discontinued and folic (pteroylglutamic) acid was administered by mouth in "physiologic" quantities, starting with 25 μg. daily. The dosage schedule was adjusted upward every 10 or 20 days. Normal serum and whole blood folate levels were reached only after the administration of 600 μg. of folic acid daily. Therefore, subject E.F. (1) had a hematologic response to oral supplements of conjugase containing negligible amounts of folic acid and, (2) had subnormal serum and erythrocyte folate levels after 90 days of supplemental folic acid, in doses ranging up to 300 μg. daily. A Schilling test and a D-xylose absorption study performed 1 year after admission were normal.

Results

Survey of Pediatric Patients

Of the 59 children studied, 54 per cent had serum folate levels of less than 5.0 μg. per ml. (Table 1). The occurrence of subnormal serum folate levels in relation to dosage and duration of therapy with diphenylhydantoin was evaluated. The incidence of subnormal serum folate levels in the group receiving greater than 2.0 mg. of drug per lb. per day was 59 per cent, compared to 47 per cent in the group receiving the lower dosage schedule. Hence, subnormal serum folate levels were commonly observed in the lower dosage schedule group and the per cent incidence was only slightly less than in the group of children on the higher dosage schedule. Subnormal folate levels were observed during the first 6 months of drug therapy and the incidence did not increase progressively with time thereafter. The range of time the children were on diphenylhydantoin prior to obtaining the serum for folate levels was 2 months to 5 years. Of interest was one subject in whom the serum folate level fell from a pretreatment value of 10.9 to 2.6 μg. per ml. after 2 months of therapy. This lack of correlation of subnormal serum folate levels with dosage and prolonged duration of therapy was observed by Klipstein in adult patients.

Since the erythrocyte folate content has been interpreted as an index of tissue stores, serum and whole blood folate levels were measured in 15 of the children. Of the 11 having subnormal serum levels, all had low erythrocyte folate levels, indicating that folate stores decrease progressively with the serum levels.
<table>
<thead>
<tr>
<th>HOSPITAL DIET</th>
<th>HOME DIET</th>
</tr>
</thead>
<tbody>
<tr>
<td>1750 MG D D</td>
<td>1500 MG D D</td>
</tr>
</tbody>
</table>

Fig. 1.—Hematologic course of subject E. F.

Fig. 2.—Effect of incremental doses of folic acid on serum and whole blood folate levels in subject E. F.

**In Vitro Studies**

The Effect of Diphenylhydantoin on Conjugase Activity. Duodenal aspirates, collected before and after stimulation with secretin, and sera were compared with commercial chick pancreas for ability to release *L. casei* supporting folates.
Table 1.—Serum Folate Levels and Incidence of Subnormal Values in 59 Pediatric Subjects Receiving Diphenylhydantoin

<table>
<thead>
<tr>
<th>Serum Folate Level (mg./ml.)</th>
<th>No. Patients</th>
<th>Per Cent Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥7</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td>5-6.9</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>3-4.9</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>Subnormal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-2.9</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>1-1.9</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>&lt;1</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>59</td>
</tr>
</tbody>
</table>

*The range of time the children were on diphenylhydantoin prior to obtaining the serum for folate levels was 2 months to 5 years. Thirty had received medication for less than 2 years, 29 for more than 2 years.

—i.e. tri-, di-, and monoglutamates from the polyglutamates contained in a standard diet. The sera and duodenal aspirates were obtained from patients receiving diphenylhydantoin and from control subjects. In addition, diphenylhydantoin was added to commercial chick pancreas prior to overnight incubation with a standard diet.

All duodenal aspirants, obtained after secretin stimulation, and all sera showed conjugase activity equal to or greater than the commercial chick pancreas (Table 2). Body fluids from subjects receiving diphenylhydantoin demonstrated conjugase activity equal to that observed in body fluids from control subjects. Furthermore, diphenylhydantoin added to the commercial pancreas prior to overnight incubation with the standard diet did not inhibit the conjugase activity of the chick pancreas.

The Effect of Diphenylhydantoin on the Growth Response of *L. casei* to Folates. The folates studied were pteroylglutamic acid, standard diets and sera. Figure 3 shows the effect of replacing 1.0 ml. of ascorbate phosphate buffer in each assay flask of a standard folic acid growth curve with 1 ml. of ascorbate phosphate buffer containing 5.0 μg. of diphenylhydantoin. The growth response of *L. casei* to increments of folic acid, in the presence of diphenylhydantoin, was not depressed. The apparent enhancement of growth observed here was also noted by Klipstein and may be due to the additive buffering effect of the alkaline diphenylhydantoin. Similar negative results were obtained when diphenylhydantoin was added to sera and standard diets.

Sera from subjects receiving diphenylhydantoin were studied for ability to inhibit the growth response of *L. casei* to pteroylglutamic acid. The per cent recovery of 5.0 mg. of folic acid added to such sera ranged from 92 to 110 per cent. The per cent recovery of folic acid added to control sera ranged from 90 to 109 per cent.

Studies on Subject E.F.

Ability to Store Folates. The accumulation of conjugated folates in the erythrocyte was evaluated by comparing the *L. casei* activity of saline-washed
Table 2.—Effect of Diphenylhydantoin on Conjugase Activity

<table>
<thead>
<tr>
<th>Source of Conjugase</th>
<th>&quot;Total&quot; Folate Content of Diet* (μg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum, Subject 1</td>
<td>179</td>
</tr>
<tr>
<td>Serum, Subject 2</td>
<td>146</td>
</tr>
<tr>
<td>Serum, Subject 3</td>
<td>161</td>
</tr>
<tr>
<td>Serum, Subject 4</td>
<td>197</td>
</tr>
<tr>
<td>Serum, Subject EF</td>
<td>164</td>
</tr>
<tr>
<td>Serum, Control 1</td>
<td>152</td>
</tr>
<tr>
<td>Serum, Control 2</td>
<td>200</td>
</tr>
<tr>
<td>Duodenal aspirate, Subject 1</td>
<td>77,158t</td>
</tr>
<tr>
<td>Duodenal aspirate, Subject 2</td>
<td>193,210t</td>
</tr>
<tr>
<td>Duodenal aspirate, Control</td>
<td>98,180t</td>
</tr>
<tr>
<td>Desiccated chick pancreas</td>
<td>148</td>
</tr>
<tr>
<td>Desiccated chick pancreas plus diphenylhydantoin</td>
<td>158</td>
</tr>
</tbody>
</table>

*"Free" folate content, 57 μg.
*Before and after secretin stimulation.
*0.5 mg. diphenylhydantoin per ml. conjugase solution.

Fig. 3.—L. casei growth response to folic acid (-----), and to folic acid plus 5.0 μg. diphenylhydantoin (——).

lysed erythrocyte with the L. casei activity of lysed erythrocytes incubated in native plasma. The polyglutamates of the erythrocyte by the latter method are deconjugated, or made available to L. casei, during incubation with plasma conjugase. The difference between the 2 values represents conjugated folates—i.e., polyglutamates. In addition, the washed erythrocytes were treated with desiccated chick pancreas to assure that the lower values obtained were not due to loss of folates through washing. The rise in total erythrocyte folate activity paralleled the rise in serum levels as the oral supplements of folic acid were increased, and the ratio of conjugated folates to total folates increased.
## Table 3.—Erythrocyte Content of Conjugated Folates

<table>
<thead>
<tr>
<th>Dosage Folic Acid (ugm./day)</th>
<th>Serum</th>
<th>Erythrocytes in Plasma (Total)</th>
<th>Washed Erythrocytes (Free)</th>
<th>Washed Erythrocytes plus Chick Pancreas (Total)</th>
<th>Ratio of Conjugated* to total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;1.0</td>
<td>30</td>
<td>21</td>
<td>29</td>
<td>1:3</td>
</tr>
<tr>
<td>75</td>
<td>1.1</td>
<td>62</td>
<td>38</td>
<td>—</td>
<td>1:2</td>
</tr>
<tr>
<td>150</td>
<td>1.3</td>
<td>58</td>
<td>38</td>
<td>67</td>
<td>1:2</td>
</tr>
<tr>
<td>450</td>
<td>3.1</td>
<td>76</td>
<td>35</td>
<td>72</td>
<td>1:2</td>
</tr>
<tr>
<td>600</td>
<td>8.5</td>
<td>250</td>
<td>145</td>
<td>230</td>
<td>1:2</td>
</tr>
</tbody>
</table>

*Conjugated folates = total folates minus free folates.

from 1 to 3, to 1 to 2 (Table 3). Therefore, during continued diphenylhydantoin therapy, the patient was able to conjugate folates in the erythrocyte as the supplements of folic acid were increased.

### Oral Absorption of Folic Acid
Five oral tolerance curves after the administration of 600 μg. of folic acid are shown in Figure 4. The uppermost curve, showing highest serum levels, was observed when diphenylhydantoin was withheld for 20 hours prior to the folic acid. The bottommost curve, showing lowest serum levels, was obtained when diphenylhydantoin and folic acid were administered simultaneously. The 3 intermediate curves resulted when the anticonvulsant was withheld for 4, 12, and 16 hours prior to the folic acid. The abnormal curves observed at 0, 4, 12 and 16 hours could reflect (1) impaired absorption, (2) more rapid tissue clearance, or (3) increased renal excretion of folic acid. Therefore, the following studies were performed.

1. **Intravenous Clearance of Folic Acid.** Folic acid, 15 μg. per Kg., was administered intravenously on 2 separate days. On the first day, 100 mg. diphenylhydantoin was given orally 30 minutes before the intravenous folic acid, on the second day, no diphenylhydantoin was given for 20 hours prior to the intravenous folic acid. No appreciable difference was observed between the 2 intravenous clearance curves (Fig. 5), indicating that the flat oral tolerance curve at 0 hours was not due to more rapid tissue clearance of folic acid.

2. **Renal Excretion of Folic Acid.** The amount of folate excreted in the urine during the first 12 hours of the several oral tolerance tests was determined (Table 4). The folate excreted during each of these periods increased as diphenylhydantoin was withheld for longer intervals prior to the test dose of folic acid. These results indicate that the flat oral tolerance curve observed at 0 hours was not due to increased renal excretion of folates induced by the simultaneous dose of diphenylhydantoin, but was due to decreased absorption of folic acid.

3. **Absorption of Folic Acid after Intravenous Diphenylhydantoin.** The effect of the intravenous administration of diphenylhydantoin on the absorption of folic acid is shown in Figure 4. Oral diphenylhydantoin was withheld for 20 hours, 600 μg. of folic acid was given by mouth at 0 hours, and 100 mg. of diphenylhydantoin was administered intravenously at plus 15 minutes. The observed serum levels were threefold greater than those of the comparable
Fig. 4.—Oral tolerance curves after 600 µg folic acid. Diphenylhydantoin was withheld for 0, 4, 12, 16 and 20 hours prior to the folic acid (---). Diphenylhydantoin was withheld for 20 hours. At plus 15 minutes diphenylhydantoin was given by intravenous route (—).

Fig. 5.—IV clearance of folic acid, one-half hour and 20 hours after diphenylhydantoin.
Table 4.—Renal Excretion of Folates after the Oral Administration of Folic Acid

<table>
<thead>
<tr>
<th>Period Diphenylhydantoin withheld prior to Folic Acid (hrs.)</th>
<th>Dosage of Folic Acid (µg)</th>
<th>Urine Folate Content (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 hours</td>
<td>2nd hour</td>
</tr>
<tr>
<td>0</td>
<td>600</td>
<td>39</td>
</tr>
<tr>
<td>4</td>
<td>600</td>
<td>57</td>
</tr>
<tr>
<td>12</td>
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<td>65</td>
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<td>20</td>
<td>600</td>
<td>150</td>
</tr>
<tr>
<td>20</td>
<td>600</td>
<td>155</td>
</tr>
<tr>
<td>20</td>
<td>600</td>
<td>160</td>
</tr>
</tbody>
</table>

standard tolerance test of 0 hours indicating that orally administered, not parenterally administered, diphenylhydantoin significantly decreases folic acid absorption. However, the observed serum levels are not as great as those attained in the standard oral tolerance test at 20 hours. This small difference may be the effect of that portion of the intravenously administered drug excreted in the bile.21

DISCUSSION

Subnormal serum folate levels, observed in 54 per cent of 59 children receiving anticonvulsant medication, were not related to dosage of drug and were observed early in the course of therapy. Studies on an adult, with replete folate stores, demonstrated decreased absorption of “physiologic” quantities of folic acid during the 16 hours following diphenylhydantoin administration. The effect was greatest at 0 hours and was not observed at 20 hours. The gradual rise of this patient’s serum folate level in response to increasing amounts of folic acid, despite continued diphenylhydantoin therapy, suggests the observed inhibitory effect of diphenylhydantoin on folate absorption can be reversed by increasing amounts of folic acid. Earlier studies reporting normal folic acid absorption in patients receiving anticonvulsant drugs employed “pharmacologic” test doses, 2.2 mg.,11 3 mg.,11,13 and 5 mg.2,3,6-9 In several reports, the anticonvulsant was discontinued prior to the absorption study.6,8,9,11,13 In a recent report, impaired absorption of B₁₂ and D-xylene was described in folate-depleted subjects receiving anticonvulsant drugs.22 Absorption of both substances returned to normal after treatment with folic acid, despite continued anticonvulsant therapy. Presumably the defective absorption of both B₁₂ and D-xylene was secondary to the effect of the folate deficiency on the intestinal mucosa. The subject of this report demonstrated, at a time of replete body stores, normal D-xylene absorption when the sugar was administered with diphenylhydantoin and when diphenylhydantoin was withheld for 20 hours. Under these two conditions, the absorption of 600 µg. of folic acid differed greatly.

The nature of the folate compound or compounds optimally absorbed is unknown. Baker, Frank and Sobotka23 compared serum folate levels (L. casei) in 10 normal persons after the oral administration of folic acid, a diglutamate and a triglutamate. The diglutamate and triglutamate were given in an
amount equivalent to 5.0 mg. of folic acid. Highest serum levels were found after the triglutamate, lowest levels after folic acid. In patients with non-tropical sprue an almost flat tolerance curve was observed after folic acid but a definite peak occurred after the triglutamate. It was postulated that the mucosal cells in the patient group were deficient in a glutamate conjugating system needed to convert unconjugated folic acid into an absorbable form. It is possible that diphenylhydantoin interferes with an intracellular conjugating system for monoglutamates. Folic acid, a monoglutamate, was the only folate employed in the absorption studies of this report. A comparable study of the absorption of a triglutamate would be of interest.

Membrane transport of folic acid has been considered by Johns, Sperti and Burgen. They noted that despite a similar high affinity for dihydrofolate reductase, methotrexate is cleared from the plasma much slower than folic acid and they suggested that the remarkable tissue accumulation of folic acid depends not only upon specific binding within the cell, but also upon a selective uptake process mediated by transport across the cell membrane. Such a carried transport system for folic acid was subsequently demonstrated from renal tubular lumen to renal tubular cell in the dog.

A similar carrier transport mechanism for folic acid in the intestinal mucosa must be considered as an alternate possible site at which diphenylhydantoin interferes with folate absorption.

Conjugase activity has been demonstrated in the intestinal secretions of normal subjects and subjects with tropical sprue. In this study we demonstrated conjugase activity in the intestinal secretions and sera of subjects receiving anticonvulsant drugs, and were unable to demonstrate inhibition of conjugase activity by diphenylhydantoin in vitro. However, the subject of the case report showed a hematologic response to oral supplements of conjugase, in the form of desiccated chick pancreas, despite the prior demonstration of conjugase activity in her duodenal aspirate. The possible explanations for these conflicting observations are several. Quantitatively more absorbable folate may be released from polyglutamates in the diet by increasing amounts of conjugase. A second possibility is that endogenous conjugase may not be active in the alkaline secretions of the small intestine since the optimal pH for conjugases from various sources has been reported as 4.5 to 7.26 A third possibility is that human intestinal conjugase may release only monoglutamates from the diet, whereas the primary folate released by chick pancreas is a diglutamate. It appears that the latter is more readily absorbed in the human than is the monoglutamate. Furthermore, if diphenylhydantoin blocks an intracellular conjugating system needed to convert monoglutamates into an absorbable form, the administration of chick pancreas with dietary polyglutamates could bypass this block.

The failure to reach normal serum and erythrocyte folate levels during the period of conjugase therapy indicates that the quantity of folate absorbed was still suboptimal. The minimal daily folate requirement, or the amount of folate required to replenish the daily loss of stored folates and required for daily cellular metabolism, has been estimated as 50 µg. The additional
amount required to replenish depleted body stores in a patient with a normal hemoglobin level has not been established. The subject of this report did not attain normal serum or erythrocyte folate levels after supplemental folic acid, given in incremental doses from 25 to 300 μg for 90 days. This folate deficiency, apparently due to decreased folate absorption induced by diphenylhydantoin, was corrected by the administration of 600 μg of folic acid daily.

SUMMARY AND CONCLUSIONS

A high incidence of subnormal serum folate levels in pediatric subjects receiving diphenylhydantoin is reported. The effect is observed shortly after the onset of therapy and is not related to dosage of drug. An adult epileptic, who presented with a megaloblastic anemia secondary to a diphenylhydantoin-induced folate deficiency, demonstrated normal serum and erythrocyte folate levels only after folic acid was administered orally in amounts of 600 μg per day. The folate deficiency was due to malabsorption of folic acid induced by diphenylhydantoin. Folic acid tolerance tests performed at 0, 4, 12, 16 and 20 hours after diphenylhydantoin showed a progressive rise in serum levels as diphenylhydantoin was withheld for longer periods prior to the test dose of folic acid. Further evidence of improved absorption was an associated rise in urinary folates.

In addition, the patient demonstrated a convincing hematologic response to ingested conjugase, in the form of chick pancreas. The hematologic response was observed, despite prior demonstration of conjugase activity in the patient's intestinal secretions. Attempts to show inhibition of chick pancreas conjugase activity by diphenylhydantoin in vitro were unsuccessful. Several explanations for these conflicting observations are offered.

SUMMARIO IN INTERLINGUA

Es reportate un alte incidentia de subnormal nivellos seral de folato in subjectos pediatric sub tractamento con diphenylhydantoina. Le effecto es observate brevemente post le initiation del therapia e non es correlacionate con le nivello de dosage. Un epileptico adulte, presentate con anemia megaloblastic secundari a un carentia de folato iniuite per diphenylhydantoina, monstrava normal nivellos seral e erythrocytic de folato solo post que acido folic habeva essite administrate per via oral in quantitates de 600 μg per die. Le carentia de folato esseva le effecto de malabsorption de de acido folic iniuite per diphenylhydantoina. Tests de tolerantia pro acido folic effectuate 0, 4, 12, 16, e 20 horas post le administration de diphenylhydantoina revelava un augmento del nivellos seral in correlation progressive con le tempore del suspension de diphenylhydantoina ante le administration del dose de testage de acido folic. Evidentia additional de un meliorate absorption esseva le associate augmento de folatos urinari.

In plus, le patiente manifestava prova convincente de un responsa hematologic al ingestion de conjugase (in le forma de pancreas de gallina). Le responsa hematologic esseva notate in despecto de un demonstration precedente de activitate de conjugase in le secretiones intestinal del patiente mesme. Essayos de demonstrar in vitro un inhibition de activitate de conjugase de pancreas de gallina per le influentia de diphenylhydantoina non esseva successose. Plure possibile explicationes de iste observationes contradictori es presentate.

REFERENCES

MALABSORPTION OF FOLIC ACID DUE TO DIPHENYLHYDANTOIN

Malabsorption of Folic Acid due to Diphenylhydantoin

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