Certain Aspects of Glycine and Histidine Metabolism in Patients with Sprue

By C. E. BUTTERWORTH, JR., JOSE SOLER, RAFAEL SANTINI, JR., AND ENRIQUE PEREZ-SANTIAGO

It has been believed that tropical sprue is caused by a primary folic acid deficiency, although direct evidence in support of this concept is inadequate. While the administration of folic acid to patients with sprue in relapse will lead to clinical improvement, the remissions are often incomplete and malabsorption persists. Sprue may be conspicuously absent in the presence of gross vitamin and protein deficiencies, and on the other hand may appear among prosperous, well-fed persons. The irregular distribution of endemic sprue about the world has been the subject of comment.

In a previous study it was found that tissue levels of folic acid appear to be only moderately reduced in Puerto Rican subjects with sprue in relapse. The concentration of folic acid in leukocytes was within the normal range in certain of the severely ill patients. In another study it was observed that such patients are capable of converting glycine to serine, a reaction which requires folic acid. It would appear then that the primary defect in the disease may be a disturbance in only certain of the several folic acid derivatives or in the enzymes for which they are the co-factors, rather than in the over-all quantity of folic acid. The investigations to be described represent attempts to confirm previous studies and to localize further metabolic abnormalities in patients with sprue in relapse.

The first approach was a clinical study concerning glycine-serine interrelationships which have been extensively studied from a biochemical point of view. According to Peters and Greenberg N10-hydroxymethyl-THFA is the essential one-carbon donor involved in the conversion of glycine to serine. Hydroxymethyl-THFA is derived from N-formyl THFA by the reducing action of TPNH. Albrecht and Broquist have shown that HLCF occurs normally in human urine. The reactions may be summarized by the scheme shown in figure 1.

In view of previous evidence that patients with sprue in relapse are capable of converting glycine to serine, it seemed theoretically possible to reverse...
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Fig. 1.—Diagram illustrating some of the transformations which occur in folic acid during its participation in associated reactions. The shaded portion of the molecule does not change and for the sake of clarity it has been omitted after the first representation.

this reaction by providing an excess quantity of serine. This might lead to a net synthesis of CF, which has been shown to be about ten times as potent as FA in the treatment of sprue, or it could exert a sparing action on the natural stores of this factor.

The second approach concerned the urinary excretion of formimino glutamic acid (FIGLU), a compound which has been found in the urine of folic acid-deficient animals and of humans receiving folic acid antagonists. Luhby et al. have observed excessive urinary excretion of FIGLU in cases of sprue, and in megaloblastic anemia of pregnancy, presumably on the basis of folic acid deficiency. It has been shown that FIGLU is an intermediate in the catabolism of histidine, and acts as a formylating agent according
to the scheme shown in figure 2. It seemed worthwhile to determine whether or not this system is impaired in patients with sprue.

MATERIAL AND METHODS

The diagnosis of tropical sprue was based on the clinical findings of diarrhea, glossitis, weight loss and demonstration in the laboratory of megaloblastic bone marrow together with intestinal malabsorption. Seven such patients received DL-serine administered orally in doses of 5.0 grams twice daily, dissolved in water. This dose was continued for 7 days in two subjects, for 10 days in four subjects and for 13 days in another. During the period of therapy with serine, patients were maintained on a traditional native diet which is low in proteins and vitamins and consists mainly of rice and beans. Reticulocyte counts were made daily as an index of response. Routine blood counts were also obtained, but since all subjects received folic acid after the period of serine therapy it was not possible to make observations on the long-term effects of serine alone.

The urinary excretion of folic acid during serine therapy was determined in 24 hour urine specimens from two subjects. The assay was performed as described previously with the use of S. fecalis, which measures citrovorum factor as well as folic acid.

Fig. 2.—Scheme of formylating action of FIGLU in catabolism of histidine.
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Fig. 3.—Hemoglobin and reticulocyte values of a 37 year old male with untreated tropical sprue, illustrating modest elevations of the reticulocyte count following the administration of D-serine (10 Gm. daily), folic acid (5 mg. daily) and ferrous sulphate (0.3 Gm. daily). The patient was discharged from the hospital on the day indicated by the vertical dashed lines. Prior to administration of ferrous sulphate the serum iron concentration was 39 μg. per 100 ml.

Chromatographic analysis of components was also carried out, with the use of a bacteriologic bioautographic technic, on specimens which had been concentrated by absorption on activated charcoal (Norit-A).

The determinations of FIGLU in urine samples were made through the kindness of Miss Rita C. Gardiner at the National Institutes of Health with the use of an enzymatic and microbiologic method. Twenty-four hour urine samples were collected from three patients without special preparation, and from a fourth patient following the oral ingestion of a 1 gram dose of L-histidine. Specimens were acidified and refrigerated for preservation prior to the assay procedure.

RESULTS

Four of the seven sprue patients who received serine showed definite elevations of the reticulocyte count temporally related to serine administration. The two best responses are illustrated in figures 3 and 4. There was only one patient in the series who failed to exhibit some increase in the reticulocyte count. In two others the results were classified as equivocal because of inadequate response or complicating factors.

Three patients were given serine only after a prolonged period (three weeks) of baseline observations while consuming a low-protein, vitamin-poor diet. During this period they also received antibiotics in unsuccessful attempts to produce hematologic improvement by altering intestinal bacterial flora. None showed a reticulocyte count in excess of 2.0 per cent during this
period. Two of these three showed a reticulocyte peak on the third day after beginning serine dosage, the peaks being 4.0 per cent and 6.8 per cent, respectively. The other did not show a reticulocytosis until folic acid was given.

Several patients reported an improvement in diarrhea during serine therapy, but there was no other remarkable clinical benefit. The hemoglobin rose slightly in three and fell in two. One of the subjects (fig. 3) who did not exhibit an increase in hemoglobin concentration after serine therapy likewise did not respond to folic acid. He was found to have a serum iron concentration of 39 μg per 100 ml., and subsequently responded to supplementary iron therapy.

In one subject a reticulocyte peak of 10.2 per cent occurred on the third day of serine therapy, but this patient had also received a single oral dose of 1,000 μg of vitamin B12 10 days earlier. He was included in the study when there had been no apparent response one week after receiving vitamin B12. While there is evidence that vitamin B12 may be poorly absorbed from the intestine in patients with sprue, even in the presence of intrinsic factor,28

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Fig. 4.—Hemoglobin and reticulocyte values of a 30 year old female with untreated tropical sprue illustrating reticulocyte peaks following the administration of dL-serine (10 Gm. daily) and later the administration of folic acid (5 mg. daily). The numerals indicate courses of oral antibacterial therapy which were without effect clinically or hematologically. This patient excreted 26.1 micromolar equivalents of FIGLU prior to serine therapy.
TABLE 1.—Values for 24 Hour Urinary Excretion of FIGLU

<table>
<thead>
<tr>
<th>Subject</th>
<th>Urinary FIGLU in Micromolar Equivalents per 24 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.0</td>
</tr>
<tr>
<td>2</td>
<td>26.1</td>
</tr>
<tr>
<td>3</td>
<td>10.2</td>
</tr>
<tr>
<td>4 (after histidine)</td>
<td>485.0</td>
</tr>
</tbody>
</table>

it cannot be stated with certainty whether serine or vitamin B₁₂ produced the reticulocytosis in this case. The response was therefore considered equivocal although it is possible that serine could have caused the reticulocytosis or contributed to it. In spite of the reticulocytosis and elevation of hemoglobin, this man continued to have diarrhea. He was given folic acid orally and later parenterally. One week after the end of serine administration, intractable vomiting developed. The serum potassium was low and the electrocardiogram showed changes consistent with hypokalemia. He was given glucose, saline and potassium chloride intravenously, but failed to respond and died six days after the beginning of folic acid and 13 days after the end of serine therapy.

Bacteriologic assay of urine collected on the sixth, seventh and eighth days of serine therapy from the man whose course is shown in figure 3 revealed 1.7, 0.4, and 1.9 µg., respectively, per day of folic acid-like compounds. Chromatograms on two of the specimens revealed that citrovorum factor was present. It was observed earlier that some patients with untreated sprue excrete citrovorum factor in the urine. The other patient, whose reticulocyte peak was 6.1 per cent, excreted 6.8 micrograms of folic acid in the 24 hour urine specimen collected during serine administration. Chromatography was not performed.

The values for the 24 hour urinary excretion of FIGLU are given in table 1. By this method excretion of more than 10 micromolar equivalents is considered excessive, although other investigators have found that normal persons excrete no FIGLU even after a loading dose of histidine. It is significant that three subjects in the present study excreted appreciable amounts of FIGLU even without a loading dose of histidine. It is of further interest that the subject who excreted 26.1 micromolar equivalents of FIGLU was capable of responding to the administration of serine (fig. 4).

DISCUSSION

Evidence is available that at least 10 different folic acid-like compounds are present in human cells, and that folic acid participates in the transfer of formyl, hydroxymethyl and formimino groups. It also appears that a folic acid derivative is involved in the hydroxylation of phenylalanine to produce tyrosine. In view of these multiple actions it would be unreasonable to expect an optimal reticulocyte response in sprue patients by stimulating or shifting the natural equilibrium of only one system. However, the demonstration of a definite but suboptimal reticulocyte response such as that described above is corroborative evidence that the folic acid compounds and the enzymes...
involved in the interconversion of glycine and serine are capable of function.
It is believed that serine administration leads ultimately to the formylation
of available THFA, producing citrovorum factor. Although the utilization
of serine as such has not been excluded, this seems unlikely since there is
evidence that serine is not deficient in the plasma of these patients. It is of
interest that a requirement has been shown for ATP in converting N^5-formyl
THFA to N^10-formyl THFA. On the other hand, it has been shown that
the reverse reaction which might occur with an excess of serine will proceed
spontaneously. If these findings are applicable to man, it is apparent that
the conversion of serine of glycine plus CF does not require an energy input.

The bacteriologic assays performed on urine specimens from two subjects
during serine therapy revealed folic acid activity which was within the nor-
mal range as reported by Girdwood, Condit and Grob and Jandl and
Lear. Bioautographs of paper strip chromatograms demonstrated qualita-
tively that CF was present in each of the two specimens tested. Although the urine
from this subject was not assayed for folic acid activity prior to the ad-
ministration of serine, urinary assays from the other subjects have revealed
continued excretion of folic acid, CF and related compounds even during
relapse. It would appear that such patients are either not deficient in these
compounds or else that they have an obligatory loss which does not change
in the face of systemic deficiency or increased demands. It is of considerable
interest that Jandl and Lear found normal urinary excretion of folic acid in
two of four patients with megaloblastic anemia associated with cirrhosis.
These authors suggested that an abnormally high requirment for folic acid
may exist in such subjects. Condit and Grob have shown that folic acid is
readily diffusible, and Register and Sarett have shown that fasting does not
reduce the urinary loss of folic acid in normal subjects. These observations
suggest that renal excretion of folic acid may constitute an important problem
in certain persons with increased requirements or inadequate supplies of the
vitamin. The present study suggests that in patients having megaloblastic
anemia associated with sprue, sufficient quantities of folic acid remain to
permit formation of derivatives which are active in promoting erythrocyte
maturation. It is conceivable that serine might act by accelerating the turn-
over of a small amount of THFA involved in the transport of single carbon
units. However, the demonstration of normal amounts of urinary folic acid
activity in these subjects tends to make this explanation unnecessary.

In connection with the patient who died in a state of hypopotassemia,
it is pertinent to consider possible renal damage resulting from serine toxicity.
Wachstein found that L-serine produced renal tubular necrosis in rats
when given in a dose of 1 gram per kilogram of body weight. The minimal
toxic amount was 0.1 gram per kilogram, at which level mild, focal, reversible
lesions are demonstrable. This is comparable with the level used in the human
subjects of this study, that is, approximately 0.2 gram per kilogram. It was
found that protein deficiency enhanced the toxicity, and that the damage
resulted from the D-isomer, since the L-isomer is not toxic. In the present case,
the maintenance of urine output, the delayed onset and the presence of low
rather than high potassium levels argue against serine toxicity as the cause
of the patient's demise.
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It may be seen that all four patients tested for the urinary excretion of FIGLU presented evidence of an abnormality in the metabolism of histidine. The patient who excreted 26.1 micromolar equivalents of FIGLU per day was later shown to be capable of responding to serine administration (fig. 4). The accumulation of FIGLU could be explained by (1) deficiency of folic acid co-factors which act as receptors for the formimino group, (2) the presence of folic acid competitors, (3) metabolic blocks at subsequent reaction sites leading to retrograde accumulation of metabolites, or (4) deficiency of the enzyme which has been termed “FIGLU transferase.” The first possibility seems unlikely since the same co-factors are concerned in glycine-serine metabolism and appear to be adequate. The second possibility also seems unlikely, since no folic acid antagonists could be found in the urine of eight sprue patients in a previous study. The third possibility has not been entirely excluded because the FIGLU technic does not detect minor increases in the urinary excretion of formimino-THFA. However, the failure to observe a high output of compounds with folic acid activity in the S. fecalis assay in two subjects is evidence against this point. The evidence suggests that persons with tropical sprue in relapse may be deficient in FIGLU transferase, while on the other hand both the enzymes and co-factors concerned with glycine-serine interconversions are essentially normal.

It is of interest that Chanarin et al. have recently described a case of megaloblastic anemia due to primidone, in which there was no evidence of folic acid depletion. However, the administration of folic acid corrected the anemia. This may be somewhat analogous to the situation in the megaloblastic anemia of cirrhosis or sprue. Rough calculations based on the reported concentration of folic acid in human whole blood, human leukocytes and in animal tissue indicate that the total body folic acid of an average-sized adult is about 10 mg. Chang suggests that the values may be somewhat higher. At any rate the daily administration of 5 to 10 mg. of folic acid undoubtedly constitutes more than replacement therapy. By the same criteria tests of absorption and excretion with the 5 mg. dose of folic acid are probably too gross to detect differences in one, or a few, of the components in the folic acid pool. Comment has recently been made on the growing recognition that folic acid may at times act “pharmacologically,” rather than merely to abolish a deficiency. Certainly a therapeutic response to the administration of an agent does not necessarily prove that it had been previously deficient. It is our tentative belief that altered folic acid metabolism in sprue probably concerns only one or a few derivatives which are not detected as such in measurements of the folic acid pool, and that the quantity of these components is related to the function of associated enzyme systems. We agree with French et al. that the entire concept of sprue as a dietary deficiency disease is open to question.

SUMMARY

1. The administration of serine to seven patients with megaloblastic anemia due to sprue produced a suboptimal but definite reticulocytosis in four, and an equivocal reticulocyte elevation in two. It is believed that serine served as a formylating agent, leading to the formation of CF from THFA.
Two subjects excreted normal quantities of folic acid in the urine during serine therapy.

2. The administration of histidine to one patient led to an excessive urinary excretion of FIGLU. Three other subjects with sprue excreted increased amounts of FIGLU in the urine without a prior loading dose of histidine.

3. Since the folic acid co-factors concerned with glycine and serine metabolism appear to function adequately in sprue patients, and since similar co-factors are concerned with histidine metabolism, the urinary excretion of FIGLU by these patients suggests an abnormality of the enzyme which converts FIGLU to glutamic acid (FIGLU transferase). Further studies will be necessary to clarify this inference.

4. The findings are discussed, and the view that folic acid may have a “pharmacologic” effect in certain forms of megaloblastic anemia is considered.

**SUMMARIO IN INTERLINGUA**

1. Le administration de serina a septe I)atielltes con anemia megalobastic causate per sprue produceva un un definite (ben que suboptimal) reticulocytosis in quatro e un elevaticn equivoc del numeration reticulocytic in duo alteres. Es opinate que serina serviva como agente de formylation, resultante in le formation de factor citrovorum ex acido tetrahydrofolic. Duo del patientes excerneva quantitates normal de acido folic in le urina durante le periodo del therapia a serina.

2. Le administration de histidina a un patiente resultava in un excessive excretion urinari de acido formimino-glutamic. Tres altere subjectos con sprue excerneva augmentate quantitates de acido formimino-glutamic sin previe administration de histidina.

3. Viste que le co-factores folico-acitlic que es concernite con le metabolismo de glycina e serina pare functionar adequaremente in patientes con sprue e viste que simile co-factores es concernite con le metabolismo de histidina, le excretion urinari de acido formimino-glutamic in iste patientes pare reflecter un anormalitate del enzyma que converte acido formimino-glutamic in acido glutamic. Studios additional es necessari pro clarificar iste disturbation.

4. Le constatationes es discutite. Es prendite in consideration le possibilitate que acido folic exerce un effecto “pharmacologic” in certe formas de anemia megaloblastic.

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