Absorption of Physiologic Doses of Folic Acid in Subjects with Tropical Sprue Responding to Tetracycline Therapy

By Frederick A. Klipstein

The megaloblastic anemia which is characteristically present in persons with advanced tropical sprue is usually due to a combined deficiency of folate and vitamin B₁₂. Deficiency of vitamin B₁₂ in this condition has been clearly demonstrated to be secondary to malabsorption of this vitamin. The factors responsible for folate deficiency are less well-defined. Impaired absorption of pharmacologic doses of crystalline folic acid has been found present in from 44 to 100 per cent of groups of patients investigated and reduced absorption of smaller, 200 μg, doses detected in from 35 to 47 per cent. In the few studies in which serum folate determinations have been correlated with the results of absorption studies of crystalline folic acid, reduced absorption has been found present in some, but not all, subjects with folate deficiency.

In 1961, Sheehy and his associates in Puerto Rico reported that treatment with physiologic doses of 25 μg. crystalline folic acid resulted in a hematologic response in some individuals with tropical sprue who had had no previous response to dietary folate. This observation led these investigators to propose that the absorption of a physiologic dosage of crystalline folic acid is unimpaired in some patients with tropical sprue and that folate deficiency in this circumstance is secondary to malabsorption of dietary polyglutamate forms of folate. Subsequent work has established the fact that therapy with oral tetracycline results in improvement in the intestinal lesion and in a remission of the megaloblastic anemia in some persons with tropical sprue. The hematologic response in this circumstance appears to be related to folate repletion, since serum folate concentrations have been found to rise to within the normal range during tetracycline therapy in some persons, whereas serum vitamin B₁₂ concentrations remain consistently depressed.

The physiologic mechanisms responsible for folate repletion during tetracycline therapy are unknown. Two alternatives suggest themselves: 1) That tetracycline directly improves the mucosal lesion with resultant improvement in the absorptive capacity for crystalline folic acid. Such has been shown...
to be the case for xylose\textsuperscript{2,12-16} and tetracycline therapy was found in one study to improve the absorption of pharmacologic doses of crystalline folic acid in 7 of 13 subjects who had residual malabsorption of this vitamin following previous treatment with folic acid.\textsuperscript{2} That tetracycline increases the intestinal concentration of the enzyme folate deconjugase, which is responsible for converting dietary polyglutamate forms of folate to more absorbable monoglutamate forms, such as crystalline folic acid. Folate deconjugase has been identified within the mucosa of the small intestine in normal subjects\textsuperscript{17} and recent evidence suggests that in certain conditions reduced activity of this enzyme is associated with impaired absorption of polyglutamate forms of folate.\textsuperscript{18,19} The absorption of polyglutamate folate, in the form of yeast, has been found to be subnormal in some patients with tropical sprue who have normal absorption of a pharmacologic dose of crystalline folic acid.\textsuperscript{20} Folate deconjugase has been demonstrated in the succus entericus of patients with tropical sprue,\textsuperscript{21} but its concentration within the intestinal mucosa has not been reported to date. It is perhaps relevant, however, that the activity of those mucosal enzymes which have been measured by histochemical or biochemical technics in tropical sprue, have been found to be decreased prior to treatment and increased to normal after therapy with tetracycline.\textsuperscript{15}

Investigative approaches concerning the factors responsible for folate repletion during tetracycline therapy have been hampered by the fact that the usual technics employed to test the absorption of crystalline folic acid require preliminary saturation with pharmacologic doses of folic acid in order to prevent spuriously low levels in the serum or urine in the presence of folate or vitamin $B_{12}$ deficiency\textsuperscript{22,23} and such doses would be therapeutic by themselves in tropical sprue. Anderson and her colleagues, on the other hand, have shown that determination of folic acid absorption by assaying the fecal excretion of radioactivity after an oral test dose of tritium-labeled folic acid ($^{3}$H-FA) does not require preliminary saturation since it is unaffected by the presence of folate deficiency and that with this technic, small, physiologic test doses are equally sensitive in detecting malabsorption as the larger pharmacologic doses employed in the other test.\textsuperscript{8} Thus this technic, the validity of which has now been attested to by reports from several other laboratories,\textsuperscript{8,10,24} permits evaluation of folic acid absorption without a resultant therapeutic effect in subjects with untreated tropical sprue.

In the present study, the response to oral tetracycline has been ascertained in three patients with tropical sprue, all of whom presented with a megaloblastic anemia due to combined folate and vitamin $B_{12}$ deficiency, and all of whom were found to have normal absorption of a physiologic dose of crystalline folic acid, as tested by the fecal excretion of a test dose of $^{3}$H-FA, prior to treatment.

**Patient Material**

All 3 patients were women, ages 14 to 69 years. Case 1 came from Guatemala, case 2 from Puerto Rico, and case 3 from the Dominican Republic. Case 1 became symptomatic while in the tropics; cases 2 and 3 became symptomatic in New York City. Case 1 noted
the sudden onset of diarrhea 4 months prior to admission; this was followed by anorexia, a 40 pound weight loss, and weakness of progressive severity. Case 2 noted the onset of anorexia 9 months prior to admission and subsequently lost 30 pounds in weight. She became progressively weaker but at no time had diarrhea. Case 3 presented with a 6 months history of diarrhea, anorexia, weight loss of 40 pounds, and weakness.

METHODS

Serum folate concentrations were assayed with *Lactobacillus casei*;25 in this laboratory, normal subjects are found to have values ranging between 7 to 20 μg./ml., values between 5 and 7 μg./ml. are indeterminant, and values of less than 5 μg./ml. are considered indicative of folate deficiency. The absorption of crystalline folic acid was measured by 2 technics: 1) the absorption of physiologic doses was determined by administring an oral dose of 25 μg. of folic acid labeled at the 3', 5'-position with tritium* (specific activity 48,500 mc./mM), and determining the percentage of radioactivity present in the subsequent 72 hr. stool collection using the method originated by Dr. Selwyn Baker and described by Kremenchuzky et al.;24 2) the absorption of pharmacologic doses of folic acid was measured by assay with *Streptococcus faecalis* of hourly serum samples taken after an oral test dose of 40μg of folic acid per Kg. of body weight, administered 36 hrs. after completion of saturation with parenteral folic acid, 15 mg. daily for 3 days. Normal subjects have peak serum levels greater than 40 μg./ml. at 1 to 3 hrs. following the oral test dose.26

Serum vitamin B₁₂ concentrations were assayed with *Lactobacillus leichmannii*;27 normal values in this laboratory range from 150 to 900 μg./ml. Vitamin B₁₂ absorption was measured by the Schilling test;28 values reported are for a 24 hr. urine collection following an oral test dose of 1 μg. of ⁶⁰CoB₁₂ given with intrinsic factor. Normal values in this laboratory range from 8 to 20 per cent of the oral test dose.

Determinations of the 5 hr. urinary excretion and serum concentration at 1 and 2 hrs. after an oral test dose of 25 g. of d-xylose were performed by the technic of Roe and Rice.29 Serial observations of vitamin concentrations, urine and serum xylose concentrations, and fecal radioactivity were determined collectively at the termination of the study period in order to minimize variation due to technic. Fecal fat determinations were performed on 72 hr. stool collections, obtained while the patient was maintained on an 80 g. fat diet, by the method of van de Kamer.30 Intestinal biopsy specimens were obtained with a Crosby capsule which was positioned under radiographic control in the region of the ligament of Treitz.

RESULTS

Pretreatment Observations

**Hematologic studies** (Table 1). All 3 patients were anemic, and 2 (cases 2 and 3) had leukopenia and thrombocytopenia. All 3 had florid megaloblastic changes in the bone marrow and subnormal serum concentrations of folate and vitamin B₁₂. The serum iron concentration was normal in each patient.

**Intestinal function and morphology** (Table 2). All 3 patients had steatorrhea and impaired absorption of xylose. Examination of the stool was negative for blood, ova, and parasites in each patient. Jejunal biopsies showed marked abnormalities of villous structure in all 3 subjects. Villi were reduced in height and thickened in cases 1 and 2; no villi were apparent in the biopsy obtained from case 3. In all 3 cases, the surface epithelial cells were flattened and pseudostratified, and there was a dense inflammatory infiltrate within the lamina propria.

*Obtained from Nuclear-Chicago Corp.
<table>
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<tr>
<th>Case</th>
<th>Hemo-</th>
<th>Reticu-</th>
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<th>Serum</th>
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<td>µg./ml.</td>
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*a* Pretreatment observations.

*b* Observations conducted at the termination of the study period.

*c* Observations conducted after subsequent antibiotic therapy for 6 months in cases 1 and 2, and 2 months in case 3. Patients also received folic acid, 15 mg., and vitamin B₁₂, 1000 µg., during absorption studies at the termination of period *b*.
Table 2.—Gastrointestinal Symptoms, Function and Morphology Before and After Therapy

<table>
<thead>
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<th>Case</th>
<th>Diarrhea</th>
<th>Weight (lbs)</th>
<th>Fecal fat (g/24 hr)</th>
<th>Xylose absorption</th>
<th>Folic Acid absorption</th>
<th>14C-FA absorption</th>
<th>Jejunal morphology</th>
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a Pretreatment observations.
b Observations conducted at the termination of the study period.
c Observations conducted after subsequent antibiotic therapy for 6 months in cases 1 and 2, and 2 months in case 3. Patients also received folic acid, 35 mg., and vitamin B₁₂, 1000 μg., during absorption studies at the termination of period b.

* Serum peak at 1 to 3 hr. after oral test dose.
† Percentage of oral dose excreted in 24 hr.
**Observations During Treatment**

**Case 1 (Fig. 1).** The patient was maintained on a regular hospital diet for 14 days during which time there was no change in her diarrhea, appetite, weight, or urinary excretion of xylose. Serum folate and vitamin B₁₂ concentrations remained subnormal, there was no increase in the reticulocyte count, and bone marrow examination at the termination of this period showed persistence of florid megaloblastic changes. The absorption of ³H-FA prior to treatment was 64.2 per cent of the oral test dose.

She was then placed on oral tetracycline, 1 Gm. daily, for a 21 days period. By the end of the first week, her diarrhea had decreased slightly, jejunal biopsy showed some morphologic improvement, and the fecal fat excretion between days 10 and 14 had returned to within normal limits. Serum folate concentration rose on day 9 to within the normal limit, and on day 10 there was an initial increase in the reticulocyte count which reached a peak of 8 per cent on day 12. By the end of the 3-week period of treatment, diarrhea had ceased, she had gained 8 pounds, the serum folate concentration was normal, the serum vitamin B₁₂ concentration was unchanged from pretreatment values, the serum iron concentration had fallen to the subnormal range, and bone marrow aspiration showed normoblastic erythropoiesis with persistence of giant metamyelocytes. At this time, values of the urinary excretion and serum concentration of xylose were increased but not normal. Jejunal biopsy, obtained on day 20, showed further improvement in villous structure, normalization of the superficial epithelium, and reduced infiltration of the lamina propria. Determinations of ³H-FA absorption during the period...

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**Fig. 1.—Response to therapy in case 1.**
Fig. 2.—Response to therapy in case 2.

of tetracycline therapy, obtained on days 5–8, 10–13, and 17–20, showed no significant change from the pretreatment value. Folic acid absorption, determined by microbiologic assay following parenteral saturation with this vitamin at the termination of the study period, showed a peak serum value of 82 μg./ml. The absorption of $^{60}$CoB$_{12}$ at this time was 0.9 per cent of the oral test dose.

At the end of an additional 6 month period of therapy with oral tetracycline, 250 mg. twice daily, she had gained an additional 18 pounds, her hemoglobin concentration was 14.1 Gm./100 ml., and serum folate, iron, and vitamin B$_{12}$ concentrations were normal. The absorption of $^{3}$H-FA was 78 per cent and the microbiologic determination of folic acid absorption was normal. The serum xylose was normal but the urinary excretion, although increased, remained subnormal. The absorption of $^{60}$CoB$_{12}$ was now within the normal limits. Jejunal biopsy showed persistent mild abnormalities.

Case 2 (Fig. 2). During a one week period on regular diet alone there was no change in the serum folate or vitamin B$_{12}$ concentrations, reticulocyte count, or xylose absorption. The absorption of $^{3}$H-FA was 83.2 per cent. She was then placed on oral tetracycline, 1 Gm. daily, for a 20 days period. By day 6, the reticulocyte count began to rise, reaching a peak of 11.5 per cent on day 17. Neither the serum folate nor vitamin B$_{12}$ concentration increased significantly during the study period. By the end of the first week, her appetite had improved and she commenced to gain weight. Fecal fat excretion on days 3–6 had returned to within normal limits; the urinary excretion of xylose on day 7 was increased. Jejunal biopsy obtained on day 6 showed slight but definite improvement in villous architecture; subsequent
jejunal biopsies obtained on days 10 and 16 showed no further improvement. Determination of $^3$H-FA absorption obtained on days 3–6, 10–13, and 17–20 showed only slight variation from the pretreatment value. Folic acid absorption determined microbiologically after folic acid saturation at the termination of the study period was normal. $^{60}$CoB$_{12}$ absorption was 5.8 per cent of the oral test dose.

Following tetracycline therapy for an additional 6 months, the patient gained a further 23 pounds. The hemoglobin concentration and serum folate, vitamin B$_{12}$, and iron values were normal at this time. Xylose absorption was now normal, but $^{60}$CoB$_{12}$ absorption remained subnormal. Jejunal morphology appeared normal.

Case 3 (Fig. 3). The patient was maintained on a regular diet for a period of 21 days. During the first week of this period, her appetite improved markedly, her caloric intake increased from an estimated 185 calories per day prior to hospitalization to 2000 calories per day, and within the next 2 weeks she gained 5 pounds. Her diarrhea persisted, but fecal fat excretion fell from 36.3 Gm. per 24 hours. on admission to 17.3 Gm. per 24 hrs. on days 9–12, and was 21.6 Gm. on days 17–20. Xylose determinations obtained on days 14 and 20 showed increased urinary excretion but no appreciable rise in peak serum values. The absorption of $^3$H-FA, determined on days 9–12 and 17–20, ranged from 75.9 per cent to 78.6 per cent. Jejunal biopsy obtained on day 12 showed definite morphologic improvement; villi were now apparent and the superficial epithelium appeared cuboidal. Repeat biopsy on day 21 showed no appreciable change. The reticulocyte count rose to 2 per cent on day 13 and remained in this range; serum concentrations of folate and vitamin
B₁₂ did not increase, and the bone marrow remained floridly megaloblastic at the end of this period.

The patient next received tetracycline for a period of 17 days. This was associated with decrease in her diarrhea and continued weight gain. The reticulocyte count rose on day 5, reaching a peak of 12 per cent on day 8. The serum concentrations of folate and vitamin B₁₂ remained subnormal. Fecal fat excretion was not appreciably changed when tested on days 6–9, but had returned to normal when ascertained on days 13–16. Urinary and serum values of xylose were increased when tested on day 10; these values were not appreciably changed on day 16. Jejunal biopsy obtained on day 13 showed further improvement in villous architecture; villi were longer and less blunted; the superficial epithelium was now columnar, and the inflammatory infiltrate within the lamina propria was decreased. The absorption of ³⁵⁵H-FA, tested on days 6–9 and 13–16, was 70.5 per cent and 83.9 per cent. Microbiologic determination of folic acid, conducted following saturation with folic acid at the termination of the study period, was normal. ⁶⁰⁰CoB₁₂ absorption was 4.9 per cent of the oral test dose.

Following tetracycline therapy for an additional 2 months, the patient continued to have mild diarrhea, but she gained an additional 11 pounds. The hemoglobin concentration was 12.6 Gm./l00 ml., the serum folate and iron concentrations were normal, but the serum vitamin B₁₂ concentration remained depressed. The absorption of xylose, folic acid, and vitamin B₁₂ were all now normal, and jejunal biopsy showed only mild abnormalities.

**Discussion**

Sheehy and his co-workers observed a hematologic response to treatment with a daily oral dose of 25 μg. of crystalline folic acid in 7 of 26 Puerto Ricans with tropical sprue, who had not experienced a hematologic response to the previous administration of a regular hospital diet, which contains approximately 700 μg. of total folate activity. This dosage of crystalline folic acid approximates the daily physiologic requirement. It has been found to be effective not only in persons with a megaloblastic anemia due to nutritional folate deficiency, but also in individuals with folate deficiency induced by diphenylhydantoin therapy, a condition in which the absorption of pharmacologic doses of crystalline folic acid is usually normal but that of polyglutamate folate appears to be impaired. Sheehy and his colleagues interpreted the results of their therapeutic studies to indicate that the absorption of physiologic doses of crystalline folic acid was normal in those subjects who had a hematologic response to this dosage, but technics to confirm this were not available to these investigators at the time of their study.

The absorption of crystalline folic acid has subsequently been tested in only a relatively few subjects with tropical sprue. Those studies which have been reported are summarized in Table 3; the results indicate that the absorption of a pharmacologic dose of folic acid is impaired in over one-half of subjects, whereas the absorption of a smaller, 200 μg. dose is impaired less...
Table 3.—Studies of the Absorption of Crystalline Folic Acid in Tropical Sprue

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<tr>
<th>Location (Ref.)</th>
<th>Subjects studied</th>
<th>Subjects with malabsorption</th>
<th>Location (Ref.)</th>
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* Determined by assay of peak serum folate concentrations following an oral test dose of 40 μg./Kg. body weight.42
† Determined by assay of fecal radioactivity following an oral test dose of 3H-FA.24

frequently. With a few exceptions,4-7 the majority of subjects found to have malabsorption of pharmacologic doses of folic acid have had folate deficiency.4-7 The absorption of a smaller, 200 μg. dose, on the other hand, was subnormal in only 6 of 17 subjects with folate deficiency due to tropical sprue who were studied by Paterson and his co-workers.1

In the present study, the absorption of the same dosage of crystalline folic acid, 25 μg., found by Sheehy et al. to be effective when administered on a daily basis in some patients with tropical sprue, has been tested in 3 subjects prior to treatment with tetracycline. The absorption was found to be within the normal range for this and other laboratories8-10,24 in two cases and to be low-normal in the third. These values, which were reduplicated on several occasions throughout the study, were in accord with the normal values for the absorption of a pharmacologic dose of crystalline folic acid which were ascertained by microbiologic technics at the termination of the initial 20 day period of treatment with tetracycline.

The administration of a regular hospital diet for from one to three weeks was not associated with a hematologic response in any of the three patients, although it was accompanied, for unknown reasons, with intestinal improvement in one. The subsequent administration of oral tetracycline was associated with a hematologic response and intestinal improvement in all three. The cause of the hematologic response was documented to be folate repletion by a rise in the serum folate concentration to normal in Case 1. It is probable that the study period was insufficiently long to observe a rise in the serum folate concentration in the other two cases. Serum folate concentrations have been found to return to normal in the majority of persons with tropical sprue who have had a hematologic response to tetracycline.14,15 however, this response has been delayed in some subjects until after the reticulocyte response. O'Brien and England, for example, found that the increase in serum folate concentration postdated the reticulocyte response in their patients with tropical sprue during antibiotic therapy and that the serum level did not return to normal until after a month of treatment.14 a period which is in excess of the initial 20 day treatment period of the present study. An increase in the serum folate concentration has also been found to occur only after the hematologic response in most subjects receiving treatment with phys-
Physiologic doses of folic acid in tropical sprue as well as in other conditions. Vitamin B₁₂, on the other hand, does not appear to be relevant to the hematologic response secondary to tetracycline therapy since the serum concentration and the absorption of this vitamin have been found to remain consistently subnormal, as was the case of the three subjects in the present study, during and after the hematologic response.

The positive therapeutic response to oral tetracycline therapy in these 3 subjects with tropical sprue who had a normal capacity to absorb physiologic doses of crystalline folic acid prior to treatment, favors the concept that antibiotic therapy improves the absorption of polyglutamate forms of folate in this condition. The validity of this hypothesis, however, awaits testing by direct assay of folate deconjugase activity of the mucosa prior to and during therapy. Moreover, it would appear to be premature, and probably incorrect, to consider that reduced activity of this enzyme is the exclusive cause of folate deficiency in tropical sprue. The results of the present study are applicable only to subjects who have normal pretreatment absorption of a physiologic dose of crystalline folic acid. It would seem probable that in those instances where malabsorption of physiologic or pharmacologic doses of crystalline folic acid are demonstrable, impaired monoglutamate absorption is also of relevance in the etiology of folate deficiency.

Summary

The response to therapy with oral tetracycline has been assessed in three subjects with tropical sprue, all of whom presented with a megaloblastic anemia due to combined folate and vitamin B₁₂ deficiency, and all of whom were shown to have normal absorption prior to treatment of a physiologic dose of 25 μg of folic acid, as tested by assay of the fecal excretion of a tritium-labeled test dose.

Treatment was associated with clinical and intestinal improvement and a hematologic response in all three subjects. The serum folate concentration rose to normal in one subject and the absorption of a pharmacologic dose of folic acid was normal when tested by microbiologic assay at the termination of the 20 day treatment period in all three. In contrast, both the absorption and serum concentrations of vitamin B₁₂ remained subnormal.

These observations confirm the fact that folate deficiency can be present in some subjects with tropical sprue in whom the absorption of a physiologic dose of folic acid is normal. They suggest that in this circumstance folate repletion and the hematologic response secondary to tetracycline therapy in tropical sprue is mediated by a factor other than the absorption of crystalline folic acid; it is likely that this factor is increased absorption of dietary polyglutamate forms of folate.

Summario in Interlingua

Le responsa al therapia con tetracyclina oral esseva evaluate in tres subjectos con sprue tropic, omnes vidite initialmente con anemia megaloblastic in consequentia de un combinate carentia de folato e vitamina B₁₂ e omnes recognoscite subsequentemente (ante le tractamento) como characterisate per un absorption normal de doses physiologic de
μg acido folic secundo le essayage del excretion fecal de un dose experimental marcate con tritium.

In omne le tres subjectos, le tractamento esseva associate con melioration clinic e intestinal e con un responsa hematologic. Le concentration de folato in le sero montava a un nivello normal in un del subjectos, e in omne le tres le absorption de un dose pharmacologic de acido folic esseva normal secundo tests microbiologic effectuate a termination del tractamento que habeva essite continuate durante 20 dies. Per contrasto, tanto le absorption de vitamina B₁₂ como le concentration seral de illo remaneva subnormal.

Iste observationes confirna le facto que carentia de folato pote esser presente in certe subjectos con sprue tropic in qui le absorption de un dose physiologic de acido folic es normal. Illos suggestiona que sub iste circumstantias le repletion de folato e le responsa hematologic a! therapia con tetracyclina in sprue tropic es mediate per un factor altere que le absorption de crystallin acido folic. Il es probable que iste factor es le augmentate absorption de dietari formas polyglutamatic de folato.

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