Jejunal Perfusion of Simple and Conjugated Folates in Tropical Sprue

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Research Article

Absorption of labeled simple 3′,5′,9′-^{3}H pteroylmonoglutamate, ([^{3}H]PG-1) and conjugated pteroyl-μ[^{14}C]glutamyl-γ-hexaglutamate, ([^{14}C]PG-7) folates was assessed in six patients with tropical sprue, before and after 6 mo of treatment, utilizing jejunal perfusion and urinary recovery techniques. Degradation products of [^{14}C]PG-7 which were produced during perfusion were identified by DEAE-cellulose column chromatography. Jejunal mucosal activities of folate conjugase, lactase, sucrase, and maltase were measured in every patient. Malabsorption of both [^{3}H]PG-1 and [^{14}C]PG-7 was found in every untreated patient, with significant improvement after therapy. The urinary excretion of ^{3}H and ^{14}C paralleled the luminal disappearance of both isotopes. The chromatographic patterns of intraluminal degradation products of [^{14}C]PG-7 obtained during perfusion did not differ from those previously found in normal subjects and were similar in studies performed before and after treatment. The activity of folate conjugase was increased in the mucosa of the untreated patients when compared to the post-treatment levels while the activities of mucosal lactase, sucrase, and maltase were originally low and increased significantly after therapy. These observations suggest that folate conjugase originates at a different mucosal locus than the brush border disaccharidases, and are consistent with previous evidence that folate conjugase is an intracellular enzyme. The present studies have demonstrated unequivocal malabsorption of both simple and conjugated folates in tropical sprue. In tropical sprue, folate malabsorption is the reflection of impaired […]

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unequivocal malabsorption of both simple and conjugated
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sorption is the reflection of impaired folate transport
and not of impaired hydrolysis.

INTRODUCTION

Folate deficiency is almost always present in Puerto
Rican patients with tropical sprue (1) and is probably
a reflection of the generalized intestinal malabsorption
which characterizes this syndrome (2). Although more
than 85% of the dietary folates are present as conju-
gated pteroylpolyglutamates (3), scant attention has
been paid to the assessment of the absorption of these
folates in tropical sprue. The intestinal mucosa is a po-
tent source of the γ-carboxypeptidase known as folate
conjugase which is required for the cleavage of the
γ-peptide linkages present in conjugated folates (4, 5).
In vivo studies in the dog and the human have shown
that cleavage of pteroylpolyglutamates to pteroylmono-
glutamate (simple folate) occurs during or soon after
intestinal absorption (4–6). Thus, the malabsorption of
conjugated folates may result from decreased mucosal
hydrolysis, decreased intestinal transport of pteroyl-
monoglutamate, or a combination of these factors. The
recent development of more refined techniques to deter-
mine folate conjugase activity in intestinal mucosa (7)
and to measure intestinal luminal disappearance, as well
as luminal degradation products of pure synthetic con-
jugated folate (8), prompted us to study the simultane-
ous absorption of separately labeled simple and conju-
gated folates in six patients with tropical sprue.

TABLE I
Laboratory Investigations before and after Treatment*

Case	Hemoglobin (>13)	Serum Folate (>5)	Serum Vitamin B₁₂ (>150)	Whole blood folate (>100)	Xylose Urine (>5)	Xylose Serum (>30)	Fecal fat excretion (<6)	⁵⁷CoB₁₂ absorption (>35.0)	Jejunal morphology (0)
	g/100 ml	ng/ml	pg/ml	ng/ml	g/5 h	mg/100 ml	%	%	
1	9.5	3.0	100	30	2.8	20	41.4	6.4	4⁺
1‡	12.7	8.5	550	240	7.1	48	4.1	49.6	2⁺
2	5.0	1.0	85	35	2.7	26	1.9	1.1	3⁺
2‡	12.2	11.0	550	160	8.3	45	3.2	42.7	1⁺
3	5.9	4.0	45	40	1.3	13	10.4	17.6	3⁺
3‡	16.0	>20.0	>1,000	540	4.9	46	5.5	38.5	2⁺
4	4.3	2.5	55	20	2.6	13	9.8	22.7	4⁺
4‡	14.4	>20.0	550	385	5.2	54	2.2	35	2⁺
5	5.7	1.0	85	35	1.5	25	25.6	8.1	4⁺
5‡	15.3	18.5	700	330	5.4	32	3.0	60.2	2⁺
6	4.8	0.1	40	10	1.0	29	27.1	10.6	4⁺
6‡	12.3	>20.0	480	350	4.6	49	4.4	65.8	2⁺

* Normal values are in parentheses at tops of columns.

‡ After treatment with oxytetracycline, folic acid, and vitamin B₁₂ for 6 mo.

METHODS

Patients. Six Puerto Rican patients with untreated tropical sprue, aged 20–55 yr, with a history of diarrhea for at least 6 wk before evaluation, and a mean weight loss of 18±2.4 pounds (mean±SEM) were investigated. Informed oral and written consents were obtained before experimental procedure. All procedures were reviewed and approved by the Committees of Human Rights of the University of Puerto Rico and the University of California, Davis. Upon admission to the General Clinical Research Center of the University of Puerto Rico School of Medicine, patients were placed on a 80 g fat diet. Since the patients were markedly anorectic, a precise assessment of the patient's fat intake was performed by a dietician during subsequent days to estimate the amount of fat ingested. After completion of all studies, patients were treated with folic acid (1 mg daily), vitamin B₁₂ (1 mg intramuscularly monthly), and oxytetracycline (250 mg twice daily) for a 6-mo period.

Biochemical and absorption studies (Table I). Hemoglobin determinations were performed by the cyanomethemoglobin method, serum and whole blood folate were assayed with *Lactobacillus casei* (9, 10), and serum vitamin B₁₂ levels with *Lactobacillus leichmannii* (11). Bone marrow aspirations were performed the day of admission. Xylose excretion was measured in 5-h urine samples and serum was obtained 1 and 2 h after a 25-g oral dose by the method of Roe and Rice (12). Multiple jejunal biopsies were obtained with a Rubin hydraulic tube (Quinton Instruments, Seattle, Wash.) positioned under fluoroscopic control in the tion period on a diet containing 80 g fat/day, stools were fixed in 10% neutral buffered formalin before staining with hematoxylin-eosin for light microscopy. Biopsy sections were coded and read without knowledge of the clinical or laboratory status of the individual. The changes were graded according to the severity of abnormalities in the villus architecture and cellularity of the lamina propria: normal represents the villus architecture seen in biopsy specimens from normal North Americans; 1⁺ indicates mild changes restricted to increased chronic inflammatory cells within the lamina propria; 2⁺ indicates more severe infiltration of the lamina propria, blunting of villus architecture, and a decrease in the villus: crypt ratio; 3⁺ indicates more severe changes with a villus: crypt ratio in the range of 2: 1; and 4⁺ indicates a completely flat mucosa. These designations are similar to those described by Schenk of Klipstein (13). Five other jejunal biopsy specimens were immediately frozen using dry ice-acetone and stored at −70°C for subsequent enzymatic analyses. After a 3-day dietary equilibration period on a diet containing 80 g fat/day, stools were collected for 5 days. Fecal fat determinations were performed by the method of van de Kamer et al. (14). The results were expressed as percent of dietary fat excreted in the feces per day. Vitamin B₁₂ absorption was assessed with a whole body counter technique (15) by measuring the radioactivity retained 7 days after an oral test dose of 0.5 µg of ⁵⁷CoB₁₂ administered concomitantly with intrinsic factor. On completion of the above studies, folate perfusion was performed. All procedures were repeated in each patient after full clinical recovery.

Infusion solution. 3′,5′,9′[³H]pteroylmonoglutamate ([³H]-PG-1)[1] was obtained commercially (Amersham/Searle Corp., Arlington Heights, Ill.) and was repurified chromatographically as described below. Pteroyl-μ[¹⁴C]glutamyl-γ-hexaglutamate ([¹⁴C]PG-7) was synthesized by the solid phase method (16) and provided by the Nutrition Program of the University of Alabama. In synthesis, the ¹⁴C label was attached to the first glutamyl unit next to the pteroyl moiety

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[1] *Abbreviations used in this paper:* L, lactase; M, maltase; PEG, polyethylene glycol; [³H]PG-1, 3′,5′,9′[³H]pteryl-monoglutamate; [¹⁴C]PG-7, pteroyl-μ[¹⁴C]glutamyl-γ-hexaglutamate; S, sucrase.

and was thus retained as part of the folate molecule after hydrolysis. Before use, the folates were purified by column chromatography and quantitated spectrophotometrically as described. The sp act of each folate was approximately 1.8 $\times 10^6$ cpm/μmol. The perfusion solution was prepared as 1 liter of isotonic sodium chloride containing 0.75 μmol each of [³H] PG-1 and [¹⁴C]PG-7 and 10 g of polyethylene glycol 4,000 (PEG). The pH was adjusted to 7.0 before use.

Procedure. The small bowel was intubated after an overnight fast with three fused polyvinyl tubes which were weighed by a mercury bag and positioned under fluoroscopic control so that the infusion port lay at least 10 cm beyond the ligament of Treitz. The aspiration ports were located 15 and 30 cm downstream from the infusion port. The test solution was infused into the jejunum with a peristaltic infusion pump (Model 1023, Harvard Apparatus Co., Millis, Mass.) at a constant rate of 9.2 ml/min. A 40-min equilibration period was allowed after which samples of intestinal contents were collected by siphonage, over ice, at the two distal openings at a rate of 1 ml/min for 60 min. After removal of a 7-ml aliquot from each aspirate, the contents were brought to pH 2.8 by the addition of 10% trichloroacetic acid and stored at $-70°C$ until subjected to column chromatography.

Each subject received an intramuscular injection of 15 mg of folic acid (Lederle Laboratories, Pearl River, N. Y.) immediately and 24 h after perfusion to flush the labeled folates from the tissues. Urine was collected for 48 h from the start of the infusion, its volume measured, and a 1-ml sample counted in the manner described below. The percent of urinary recovery of each labeled folate was calculated, taking into account that after intestinal aspiration each patient retained 800 ml of the infused solution containing 0.6 μmol of each labeled folate. Creatinine clearances were performed simultaneously.

Assays. Aliquots of each solution and each intestinal aspirate were used to determined the concentration of PEG, the radioactivity of each label, and concentrations of sodium and chloride. A modified turbidometric method was used to determine the concentrations of PEG (17). After decolorization with 37% H_2O_2, 1 ml of each sample was prepared for counting by adding 10 ml of scintillation fluid (Scintisol, Isolab, Inc., Akron, Ohio). Radioactivity was assessed in a Beckman LS-230 liquid scintillation counter (Beckman Instruments Inc., Fullerton, Calif.) set for double isotope counting so that there was no drift of ³H to the ¹⁴C counting channel and a correctable spillover of 0.330 of ¹⁴C counts to the ³H channel. The counting efficiencies were 65% for ¹⁴C and 25% for ³H. Disappearance of each label from the 30-cm perfused segment was calculated as previously described (8). Transintestinal movement of water, sodium, and chloride was calculated for the distal 15-cm segment using conventional formulas (18). The frozen and acidified remaining aliquot was thawed, neutralized, and then subjected to column chromatography on DEAE cellulose chloride with a linear sodium gradient to separate degradation products of [¹⁴C]PG-7, as previously described (8).

In vitro studies. 25 ml of intestinal juice, obtained by siphonage from the jejunum of an untreated patient and frozen at $-70°C$ was thawed and diluted three times in saline to approximate intraluminal concentrations during perfusion. One-half was adjusted to pH 4.5 and the other half to pH 6.5; [¹⁴C]PG-7 was added to each portion in the same concentrations, 0.75 μM, as employed in the in vivo perfusion studies. After incubation at 37°C for 15 min, the reaction was stopped by acidification with 10% trichloro-

acetic acid to pH 2.8 and the mixture frozen at $-70°C$. The reactant mixtures were subsequently subjected to column chromatography.

Column chromatography. Analytical ion exchange chromatography using DEAE cellulose chloride was employed to identify degradation products of [¹⁴C]PG-7 in aspirates obtained from the proximal and distal ports during the perfusion procedures and from the in vitro incubation mixture. The chromatographic procedures were identical to those previously described (8), except that the final analytical column measured 17×0.9 cm and 6-ml fractions were collected. This modification was necessary because of the use of a smaller dose of administered folate than that previously employed and permitted adequate separation and identification of radioactive peaks. Synthetic, spectrally pure, nonradioactive markers for pteroylmono- (PG-1), pteroyldi- (PG-2), and pteroyltriglutamate (PG-3) were added to each sample before column application.

Enzymatic assays. Folate conjugase in the mucosal biopsies was determined by the charcoal precipitation method of Krumdieck and Baugh (7) utilizing pteroylglutamyl-γglutamyl[¹⁴C]glutamic acid (synthesized and provided by Dr. Carlos L. Krumdieck) as a substrate. Based on preliminary experiments showing saturation kinetics at a substrate concentration between 10 and 30 nmol/1.5 ml reaction tube, a concentration of 20 nmol per tube, a pH of 4.5, and incubation time of 15 min was employed in each assay. Activities of lactase, sucrase, and maltase were determined by the method of Dahlqvist (19). Mucosal protein was analyzed by the method of Lowry et al. (20). Results were expressed as nmol/mg protein per 15 min for folate conjugase and as U/g protein per h for lactase (L), sucrase (S), and maltase (M).

In carrying out these studies, the clinical and laboratory evaluation of the patients as well as the assays for PEG and electrolytes were performed at the University of Puerto Rico, whereas after shipment of the frozen materials on dry ice, the isotope counting, column chromatography and enzymatic assays were performed at the University of California, Davis. All data were analyzed using the paired *t* test. Thus, each patient served as his own control, before and after treatment.

RESULTS

Biochemical and absorption studies (Table I)

Before treatment, all six patients were anemic with low levels of serum and red cell folate and serum B₁₂. The values for absorption of D-xylose and vitamin B₁₂ were markedly low in all six patients; steatorrhea was significant in five out of the six. The jejunal mucosal abnormality was graded as 4⁺ in four patients and 3⁺ in two. After 6 mo of treatment, each patient had regained his normal weight and bowel habits. The post-treatment hemoglobin levels were greater than 12 g/100 ml and the levels of serum and red cell folate and serum B₁₂ were within or greater than the normal range. Values of serum folate greater than 20 ng/ml and of serum B₁₂ greater than 1,000 pg/ml in the treated patients reflect the recent administration of either vitamin. The intestinal absorption of D-xylose, dietary fat, and vitamin B₁₂ were normal after treatment. The jejunal morphol-
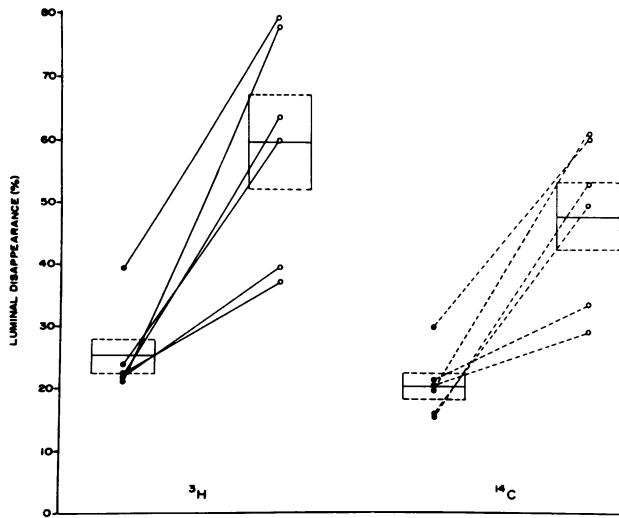
FIGURE 1 Luminal disappearance of labeled [³H]pteroyl-monoglutamate and [¹⁴C]pteroylheptaglutamate from the perfused jejunum before and after 6-mo treatment, expressed as percent of infused compound per 30-cm jejunal segment. The closed circles (●) represent original values and the open circles (○) the values obtained after treatment. Bar and dotted lines represent mean±SEM.

ogy improved by two grades in all but one patient. Although none of the post-treatment biopsies were normal by North American standards, the histology was consistent with the previously reported jejunal mucosal abnormalities present in asymptomatic Puerto Ricans (21).
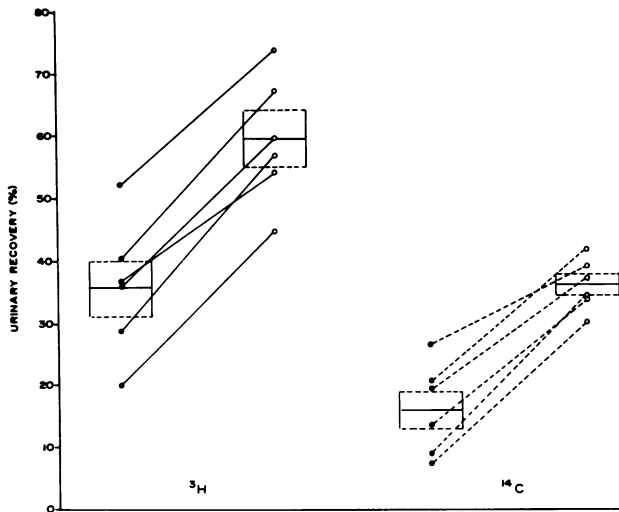

FIGURE 2 Recovery of each label in the urine collected for 48 h, expressed as percent of administered radioactivity after administration by jejunal perfusion of [³H]pteroylmono-glutamate and [¹⁴C]pteroylheptaglumate. The closed circles (●)represent original values and the open (○) the values obtained after treatment. Bar and dotted lines represent mean±SEM.
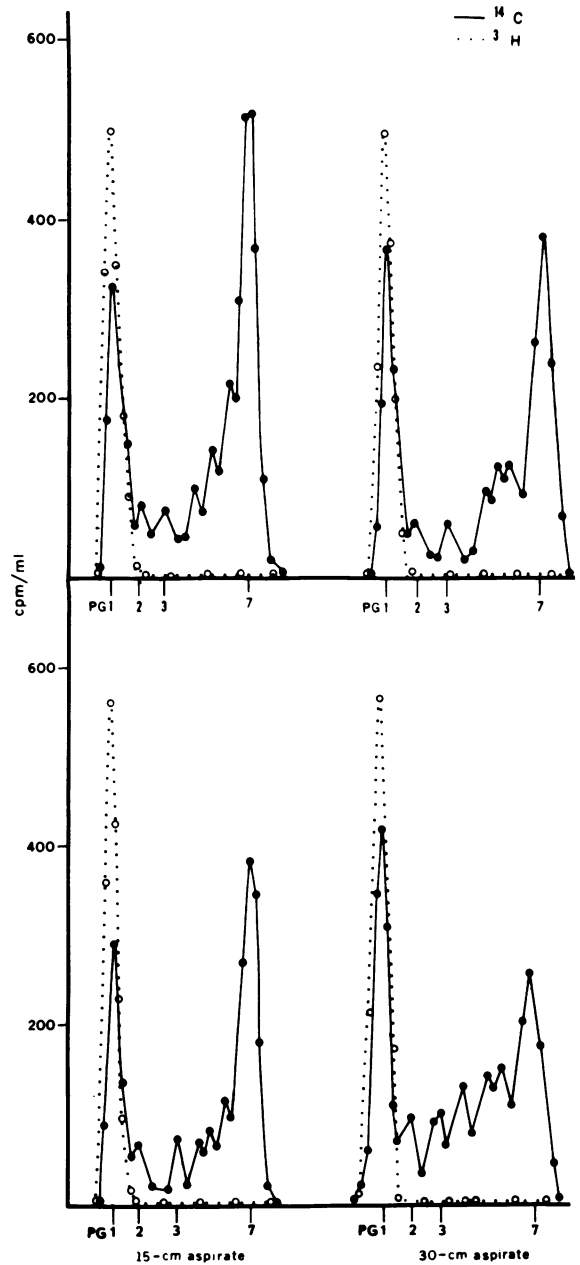

FIGURE 3 Chromatographs of intestinal aspirates obtained 15 and 30 cm downstream from the infusion port from patient 3 before (above) and after (below) treatment. The infusate contained equimolar (0.75 μM) concentrations of [³H]PG-1 and [¹⁴C]PG-7. Unlabeled markers added to the aspirates identify pteroylhepta, -tri, -di, and monoglutamate spectrophotometrically. Both before and after treatment, perfusion resulted in degradation of [¹⁴C]PG-7. Between the 15-cm and 30-cm ports there was a simultaneous decrease in the fraction of the surface area of the chromatographs represented by [¹⁴C]PG-7 and an increase in the fraction represented by [¹⁴C]PG-1.
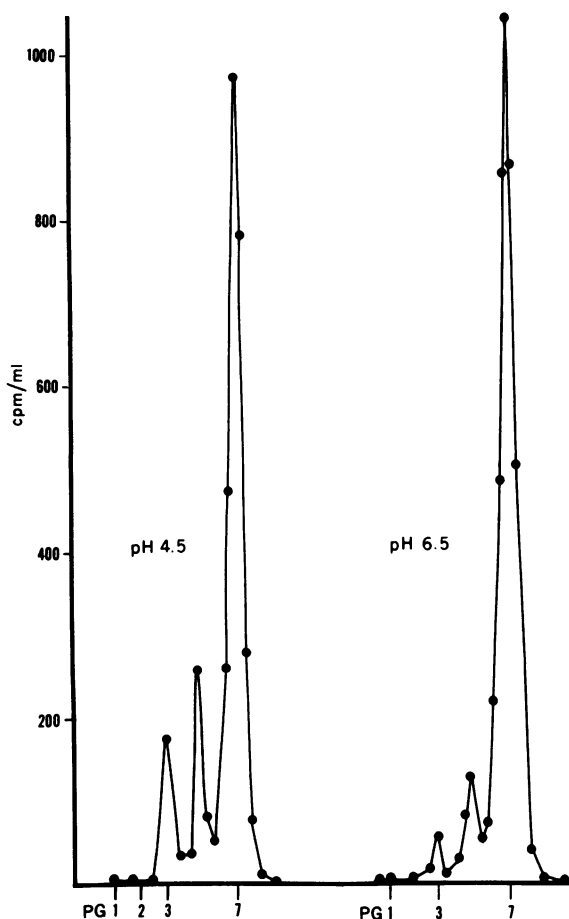
FIGURE 4 Chromatograph of incubated mixture (15 min, 37°C, pH 4.5 or pH 6.5 of [^{14}C]PG-7 in intestinal aspirate obtained by siphonage from an untreated patient before perfusion. Minimal degradation of [^{14}C]PG-7 was found at either pH, in contrast to the results of in vivo perfusion shown in Fig. 3.

Luminal disappearance of ^{3}H and ^{14}C labels (Fig. 1)

The initial luminal disappearance of [^{3}H]pteroyl-monoglutamate and [^{14}C]pteroylheptaglutamate was 25.4±2.9% and 20.7±2.2% (mean±SEM), respectively. After treatment, both values increased significantly to 59.7±7.3% and 47.9±5.6% ($P < 0.005$, < 0.005, respectively). The luminal disappearance of [^{3}H]pteroyl-monoglutamate was greater than that of [^{14}C]pteroyl-heptaglutamate before ($P = 0.05$) and after ($P < 0.005$) recovery.

Urinary recovery of ^{3}H and ^{14}C labels (Fig. 2)

The pre- and post-treatment recoveries of each label paralleled the luminal disappearances. Initially, recovery of ^{3}H was 35.7±4.5% and that of ^{14}C was 16.3±3.0% (mean±SEM). After treatment, urinary recovery of

^{3}H rose significantly to 59.4±4.2% and that of ^{14}C to 36.0±1.7% (mean±SEM) ($P < 0.001$, < 0.001, respectively). The urinary recovery of ^{3}H exceeded that of ^{14}C both before ($P < 0.005$) and after treatment ($P < 0.005$). Creatinine clearances performed simultaneously were normal in all patients, ranging from 72.27 to 116.36 ml/min per M$_2$.

Column chromatography

In vivo studies. A spectrum of ^{14}C-labeled pteroyl-polyglutamates was recovered from the proximal and distal aspirates during jejunal perfusion of each patient. Passage of the infusion solution resulted in the simultaneous decrease of the fraction of each chromatogram pair represented by [^{14}C]PG-7 and increase of the fraction represented by [^{14}C]PG-1. Similar patterns were obtained during folate perfusion performed before and after treatment. A representative set of patterns obtained from two studies in the same patient is illustrated in Fig. 3.

In vitro studies. After incubation at either pH 4.5 or pH 6.5 of [^{14}C]PG-7 with intestinal aspirate obtained by siphonage, incomplete degradation was observed with no detectable [^{14}C]PG-1 (Fig. 4).

Movement of water and electrolyte (Fig. 5)

Before treatment, there was net secretion into the distal 15 cm of the perfused lumen of water, sodium, and chloride in all but two patients. After recovery, there was a shift of the mean values to lesser secretion or net absorption, but the change was not significant.

Mucosal enzyme activity (Fig. 6)

Initially, the mean activity of folate conjugase was 6.9±0.6 nmol/mg protein per 15 min which was significantly greater ($P < 0.05$) than the activity of 4.8±0.4 nmol/mg protein per 15 min after recovery. On the
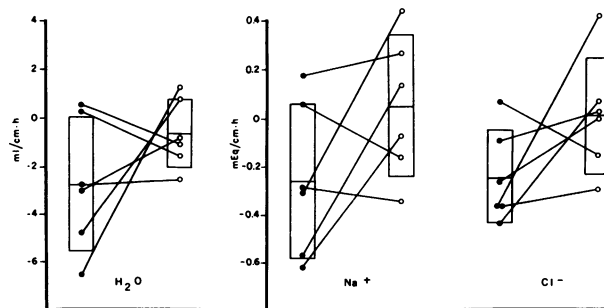

FIGURE 5 Trans-intestinal movement of water, sodium and chloride in the distal 15 cm during perfusion studies before (●) and after (○) recovery. Initial net secretion (−) of water and sodium was observed in four patients and of chloride in five patients. The changes after recovery were not significant.

other hand, the activities of mucosal L, S, and M were markedly depressed before treatment as compared to the activities found after recovery.

DISCUSSION

The development of methods for the synthesis of labeled pteroylpolyglutamates has permitted a greater definition of the process involved in the intestinal absorption of conjugated folates (16, 22). In several patients this process has been approached by measuring levels of serum radioactivty and(or) excretion of the radioactive label in the urine or expired air (4–6, 23, 24). Administering selectively labeled pteroylpolyglutamates to patient volunteers, Butterworth et al. showed that serum radioactivity and folate activity rose in parallel when the compound was synthesized with the ^{14}C label on the pteroyl moiety or the first glutamyl unit, whereas $^{14}CO_2$ was recovered in the expired air when the label was placed on any glutamyl unit beyond the first (4). Subsequent experiments in the dog demonstrated that ^{14}C-labeled pteroylmonoglutamate was the principal folate recoverable in the portal blood after placement of various [^{14}C]pteroylglutamates, labeled on the first glutamyl unit, within in vivo intestinal loops (6). These two studies provided compelling evidence that pteroylpoly-glutamates are hydrolyzed to pteroylmonoglutamate during transport across the small intestinal mucosa. In recent related studies, Godwin and Rosenberg compared the urinary recovery of tritium after the oral administration of [^3H]pteroylmonoglutamate and synthetic [^3H]-pteroylheptaglutamate on successive tests in human volunteers "tissue saturated" with parenteral folic acid. In a 48-h urine collection the mean recovery of the label after administration of 0.60 μmol of [^3H]pteroylhepta-glutamate was 56.1% while the mean recovery of tritium was 70.8% after the subsequent oral administration of a similar dose of [^3H]pteroylmonoglutamate (24).

Recently the technique of jejunal perfusion was used to measure the simultaneous disappearance of [^{14}C]PG-7 and [^3H]PG-1 in normal human volunteers (8). As compared to oral tolerance methods, this approach does not depend upon variable effects of hepatic and renal metabolism and does not require saturation of body tissues with folic acid. Since both folate forms are administered simultaneously, this method obviates the potential problem of variation when comparisons are made of results from more than one test in the same individual. By chromatography of intestinal contents obtained during perfusion, this method also permits the in vivo assesment of hydrolysis of [^{14}C]PG-7. In the present studies, this method was complemented by the measurement of urinary recovery of each label and by the direct assay of mucosal folate conjugase using a pure synthetic substrate.
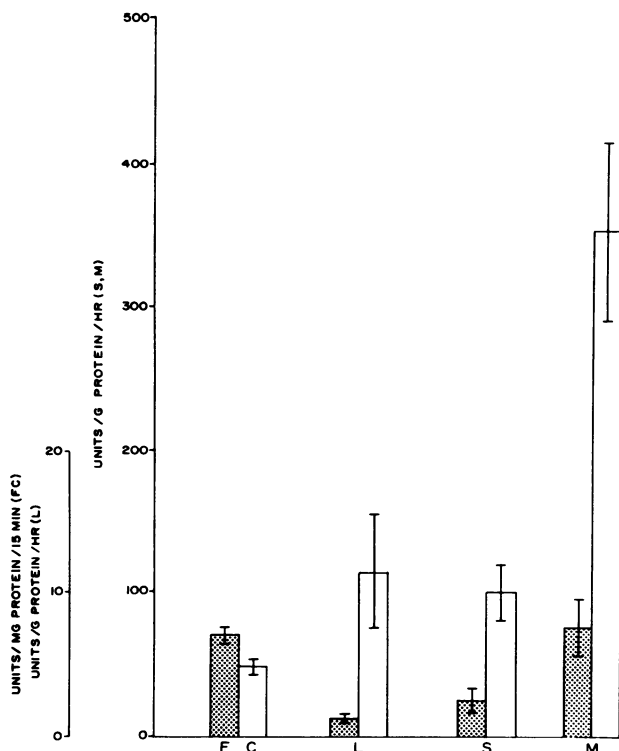

FIGURE 6 Enzyme activities in jejunal mucosa before (solid bars) and after treatment (open bars). FC, folate conjugase; L, lactase; S, sucrase; M, maltase. Treatment resulted in a significant decrease in activity of FC ($P < 0.05$) and significant increases in L ($P < 0.01$), S ($P < 0.01$), and M ($P < 0.005$).

With the combined techniques of luminal disappearance and subsequent urinary recovery the present studies have shown marked malabsorption of both folate forms in untreated tropical sprue. With each patient as his own control, each measure of absorption was significantly improved with treatment. Relative differences in results from the two techniques may reflect the fact that jejunal perfusion measures events from a finite segment while urinary recovery preceded by tissue saturation measures absorption from the entire intestine. Both before and after treatment, the absorption of pteroyl-monoglutamate was greater than that of pteroylhepta-glutamate, an observation previously made in normal subjects (8, 24). The pretreatment urinary recovery values of each folate were less than one-half and the post-treatment values about the same as those found in a study of pteroylmono- and pteroylheptaglutamate absorption in normal North Americans (24). The present data imply that folate and water transport by the jejunum are unrelated, since the change in water transport which followed treatment did not parallel that of improved folate absorption. These findings may be consistent with those of Gerson et al. who described glucose

enhancement of water and folic acid uptake from the perfused jejunum, but no apparent relationship of water and folic acid uptake from a saline solution (25). The results confirm the findings of Hoffbrand et al. (26) who found malabsorption of equimolar amounts of unlabeled pteroylmonoglutamate and pteroylheptaglutamate with successive oral tolerance tests and measurement of serum levels in eight patients with tropical sprue. The present data are more conclusive since the method used in the latter study required prior treatment of the patients with a tissue-saturating dose of folic acid.

The data obtained by chromatographic analysis of the intestinal aspirate and by enzymatic assays of the mucosal biopsies imply that folate malabsorption in tropical sprue is not caused by deficient hydrolysis of pteroylpolyglutamate but reflects decreased intestinal transport of its principal degradation product. In vivo hydrolysis of [^{14}C]PG-7 was shown by the identification of a chromatographic spectrum of degradation products in the intestinal aspirates obtained during perfusion which was similar to those previously described in normal subjects (8), and which was similar in studies performed both before and after treatment (Fig. 3). As in the previous study of normals (8), the present data suggest that hydrolysis of[^{14}C]PG-7 is a consequence of its contact with the intestinal mucosa. Insignificant degradation of [^{14}C]PG-7 was found in vitro at two different pH levels and under conditions which were designed to mimic the folate and intestinal juice concentrations obtained during in vivo perfusion (Fig. 4). Direct enzymatic assays of jejunal mucosa obtained by biopsy showed that folate conjugase is actually increased in untreated tropical sprue compared to values obtained after treatment, the reverse of the pattern of pre- and post-treatment activities of jejunal disaccharidases (Fig. 6). Folate conjugase activities in the post-treatment biopsies were similar to a series of North American controls (27). Conceivably, increased folate conjugase in the mucosa of untreated tropical sprue could reflect the greater cellular infiltration of the lamina propria. The cell type responsible for mucosal folate conjugase has not been precisely defined. However, by measurement of folate conjugase activity in peripheral lymphocytes, Jägerstad et al. have shown indirectly that the cells of the lamina propria are an unlikely enzyme source (28). Halsted et al. have recently shown deconjugation of [^{14}C]PG-7 by isolated mucosal epithelial cells (29).

Present evidence from three different in vitro studies suggests that mammalian intestinal mucosal folate conjugase is intracellular and not a surface brush border enzyme (29–31). The present studies indirectly and partially support this concept by showing that the mucosal activity of folate conjugase was not affected by tropical sprue in parallel to known surface disaccharidase en-

zyme activity. If folate conjugase is indeed an intracellular enzyme, the finding of intraluminal hydrolytic products of [^{14}C]PG-7 during its jejunal perfusion must represent a process of back diffusion after the parent molecule has been transported intact into the mucosal cell, as suggested by recent in vitro studies (29). On the other hand, the possibility that products of hydrolysis of [^{14}C]PG-7 appear in the lumen as a result of reaction with a surface-active folate conjugase before mucosal transport has not been totally excluded by the available data.

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