

Pteroylglutamic Acid Malabsorption in Tropical Sprue

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Pteroylglutamic acid (PGA) absorption was assessed in ten untreated tropical sprue (TS) and eight control subjects utilizing a marker perfusion technique. Physiologic concentrations of the vitamin (25 ng/ml) dissolved in iso-osmotic solutions containing either mannitol or glucose at a concentration of 55.6 mM were perfused on each subject on two consecutive

days. A statistically significant difference in PGA absorption between TS and control subjects was obtained only when glucose was present in the perfusate. Thus, unequivocal malabsorption of PGA is demonstrable in all subjects with TS when more refined techniques than the ones applied heretofore are utilized.

FOLATE DEFICIENCY IS almost invariably present in subjects with tropical sprue (TS) studied in Puerto Rico. Although the most important single factor responsible for such deficiency is probably folate malabsorption, the results obtained utilizing the conventional techniques available for the assessment of absorption of this vitamin have yielded erratic results.¹

The development of more sensitive marker perfusion techniques² have allowed us to study the transport of pteroylglutamic acid (PGA) across the jejunum of subjects with this syndrome. The results reported herein demonstrate PGA malabsorption in all the TS subjects studied when physiologic concentrations of the vitamin³ were perfused with a glucose-containing solution.

MATERIALS AND METHODS

Ten untreated TS and eight control subjects, aged 20–62 yr, were studied after obtaining informed oral and written consent. All TS subjects had xylose and B₁₂ malabsorption; seven of them had steatorrhea, and all had jejunal mucosal abnormalities. They also had megaloblastic bone marrows and low serum and red cell folate and B₁₂ levels. Most of them were anemic, with a hemoglobin level of 6.4 ± 2.5 g/100 ml (mean \pm SEM). The control subjects were asymptomatic and had normal xylose and B₁₂ absorption as well as normal hemoglobin and serum and red cell folate and B₁₂ levels.

After an overnight fast, the subjects were intubated with a triple lumen tube whose proximal aperture was located fluoroscopically adjacent to the ligament of Treitz. The tube had a mixing segment of 15 cm and a study segment of 30 cm.⁴ Solutions were perfused at a rate of 9.2 ml/min using a peristaltic infusion pump (Model 1023, Harvard Instrument Co.). The solutions perfused were iso-osmotic and contained 25 ng/ml of crystalline PGA with either mannitol or glucose at a concentration of 55.6 mM; the concentration of sodium and chloride was adjusted accordingly. The pH of both solutions was adjusted to 7.2.

The ten TS and eight control subjects were perfused at random, on two consecutive days, with the mannitol or the glucose-containing solutions. An equilibration period of 60 min was allowed

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in all instances, followed by three 20-min collections from the intermediate and distal apertures of the tube at a rate of 1 ml/min. Staggering of collections was not performed. Polyethylene glycol (PEG 4000) was used as a nonabsorbable marker at a concentration of 1%; its concentration was determined by a modification of the method of Hydén.⁵ PGA was measured microbiologically using *Streptococcus fecalis*.⁶ Results were expressed as per cent PGA absorbed by a 30-cm segment of jejunum.

The statistical significance of the data was evaluated with the Student's *t* test.

RESULTS

Figure 1 shows the results obtained when control and TS subjects were perfused with a mannitol-saline solution. Absorption of PGA was $47.3 \pm 5.8\%$ (mean \pm SEM) and $28.1 \pm 5.4\%$ for the control and TS subjects, respectively. There was considerable overlapping between the per cent of PGA absorbed by both groups.

Figure 2 depicts the results obtained when the same subjects were perfused with a glucose-saline solution. PGA absorption was $58.8 \pm 4.3\%$ (mean \pm SEM) and $9.8 \pm 3.1\%$ for the control and TS subjects, respectively. A clear-cut separation was observed between both groups with differences in absorption significant to the $p < 0.001$ level.

The mean geometrical luminal concentrations for both PGA and glucose were similar in the TS and control subjects.

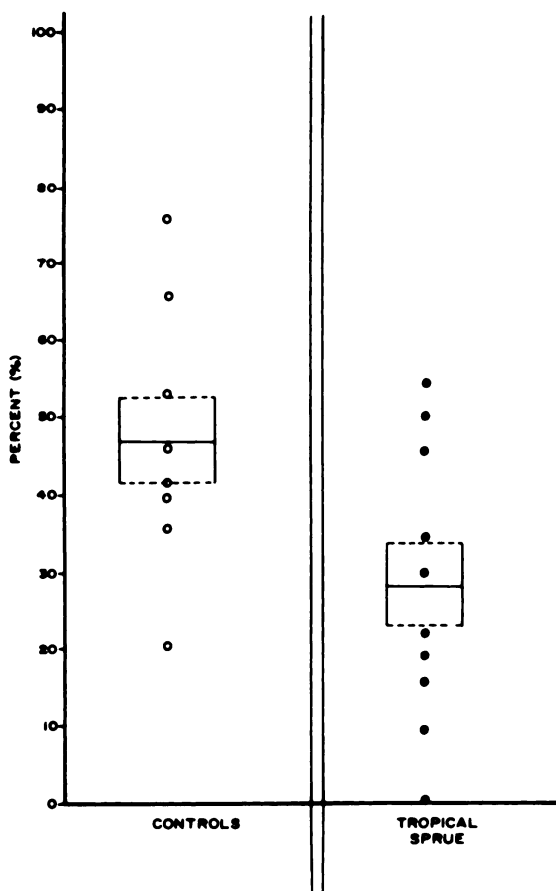


Fig. 1. Transport of pteroylglutamic acid from a mannitol (55.6 mM) saline solution by control (open circles) and tropical sprue (closed circles) subjects. Bar and dotted lines represent mean \pm SEM.

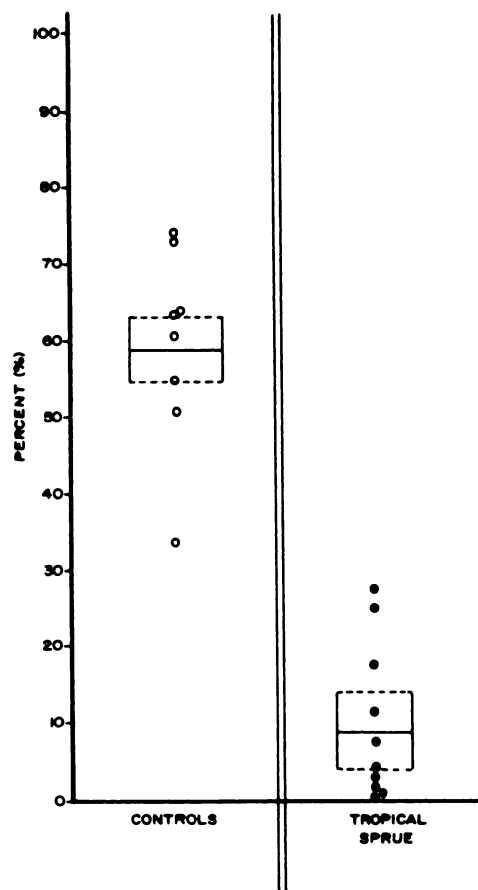


Fig. 2. Transport of pteroylglutamic acid from a glucose (55.6 mM) saline solution by control (open circles) and tropical sprue (closed circles) subjects. Bar and dotted lines represent mean \pm SEM.

DISCUSSION

The results obtained utilizing conventional methods for the assessment of PGA absorption in folate-deficient subjects with TS have been highly variable.¹ Pharmacologic as well as physiologic doses of the vitamin have been used in an attempt to demonstrate PGA malabsorption in this syndrome. Jeejeebhoy and co-workers⁷ and Klipstein¹ demonstrated a subnormal rise of serum folate levels (assayed with *S. fecalis*) following the oral administration of 15 $\mu\text{g}/\text{kg}$ of the vitamin in 38%–75% of the subjects studied. The absorption of more physiologic doses of PGA, ranging from 25 to 200 μg , has been tested either by assaying fecal excretion of tritium-labeled PGA⁸ or by incremental rises of serum concentrations using *L. casei*.⁹ The latter studies were less successful in demonstrating PGA malabsorption than the ones performed with the pharmacologic doses.

Marker perfusion techniques have been successfully applied by several investigators to assess PGA absorption in humans.^{3,10,11} Its use in subjects with untreated TS has heretofore been limited to the studies performed by Gerson who observed normal absorption of PGA in two subjects with this syndrome perfused with a glucose-free solution; impaired absorption became readily apparent, however, when glucose or galactose were added to the perfusate.¹²

Our results confirm his observations and demonstrate, in a larger number of subjects, that malabsorption of physiologic doses of PGA is demonstrable in all subjects with TS when the appropriate techniques are utilized.

REFERENCES

1. Klipstein FA: Folate in tropical sprue. *Br J Haematol* 23:119, 1972
2. Cooper H, Levitan R, Fordtran JS, Ingelfinger FJ: A method for studying absorption of water and solute from the human small intestine. *Gastroenterology* 50:1, 1966
3. Gerson CD, Cohen N, Hepner GW, Brown N, Herbert V, Janowitz HD: Folic acid absorption in man: Enhancing effect of glucose. *Gastroenterology* 61:224, 1971
4. Corcino JJ, Maldonado M, Klipstein FA: Intestinal perfusion studies in tropical sprue: 1. Transport of water, electrolytes, and d-xylose. *Gastroenterology* 65:192, 1973
5. Hydén S: Turbidometric method for determination of higher polyethylene glycols in biological materials. *Ann R Agric Coll Sweden* 22:139, 1955
6. Chanarin I, Anderson BB, Mollin DL: The absorption of folic acid. *Br J Haematol* 4:156, 1958
7. Jeejeebhoy KN, Desai HG, Noronha JM, Antia FP, Parekh VD: Idiopathic tropical diarrhea with or without steatorrhea (tropical malabsorption syndrome). *Gastroenterology* 51:333, 1966
8. Klipstein FA: Absorption of physiologic doses of folic acid in subjects with tropical sprue responding to tetracycline therapy. *Blood* 34:191, 1969
9. Hoffbrand AV, Necheles TF, Maldonado N, Horta E, Santini R: Malabsorption of folate polyglutamates in tropical sprue. *Br Med J* 2:543, 1969
10. Hepner GW, Booth CC, Cowan J, Hoffbrand AV, Mollin DL: Absorption of crystalline folic acid in man. *Lancet* 2:302, 1968
11. Halsted CE, Robles EA, Mezey E: Decreased jejunal uptake of labeled folic acid (^3H -PGA) in alcoholic patients; roles of alcohol and nutrition. *N Engl J Med* 285:701, 1971
12. Gerson CD: Glucose and intestinal absorption in man. *Am J Clin Nutr* 24:1393, 1971



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