Effect of Intraluminal pH on the Absorption of Pteroylmonoglutamic Acid

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Summary

The administration of pteroylmonoglutamic acid and either sodium bicarbonate or phenytoin sodium in normal subjects and epileptics results in lower serum levels of folic acid than when administered alone. Bicarbonate administration was shown to produce a more alkaline pH in the jejunum, while an alkaline pH was found in some epileptics on phenytoin therapy. The physicochemical mechanisms whereby folic acid absorption is reduced in an alkaline medium are considered, and the implications on folate absorption in other disease states are discussed. The mechanism by which phenytoin sodium alters the intrajejunal milieu is obscure.

Introduction

The association of megaloblastic anaemia with anticonvulsant therapy was first reported by Mannheimer *et al.* (1952). Since then low serum folate levels with macrocytosis have often been reported in patients on anticonvulsant therapy, notably primidone and phenylhydantoinates (Hawkins and Meynell, 1958; Klipstein, 1964). The mechanism by which folate metabolism is disturbed under these circumstances is uncertain. It has been suggested that competitive inhibition occurs at some stage in the folate metabolic pathway (Girdwood and Lenman, 1956; Newman and Sumner, 1957; Herbert and Zalusky, 1962). Dietary deficiency seems unlikely, since some of the patients have been well nourished (Hawkins and Meynell, 1958). Normal absorption has been found in most series with 5 mg folic acid, but with much smaller doses impaired absorption has been claimed (Dahlke and Mertens-Roesler, 1962).

Pteroylmonoglutamic acid is a weak acid, so that alteration of pH could have a pronounced effect on the availability of any absorbable substance within certain ranges. The studies described here investigate the effect of phenytoin sodium and sodium bicarbonate on jejunal pH and on the absorption of synthetic pteroylmonoglutamic acid *in vivo*.

Methods

Folic Acid Absorption Studies.—Folic acid absorption curves were obtained by estimating the serum folate activity in the fasting state and at half, one, and two hours after an oral 5-mg dose of folic acid, *Lactobacillus casei* being used as test organism (Herbert, 1966). Two male and three female

patients diagnosed as having idiopathic epilepsy and receiving phenytoin sodium for more than three years were studied. All were well nourished and taking an adequate diet, and at the time of investigation had normal blood counts and normal serum levels of vitamin B₁₂, folic acid, and iron. Fifteen normal subjects were investigated to establish the normal limits of the test of folic acid absorption, the results being compared with those obtained from the epileptic patients. Absorption tests were also carried out on the five epileptic patients and six volunteers (drawn from the control group of normal subjects) on two further occasions when either a single dose of 100 mg phenytoin sodium or 10 g sodium bicarbonate was given simultaneously with 5 mg folic acid orally. In one volunteer sodium bicarbonate induced colic and diarrhoea; the results obtained were not included in the mean absorption curve for this group. None of the epileptics took their regular anticonvulsant on the day of investigation. Absorption curves were also obtained from a normal subject after the simultaneous administration of 5 mg folic acid and 10 g sodium bicarbonate, or Garoin (phenytoin 100 mg + phenobarbitone 50 mg), and after administration of Garoin three times a day for 10 days.

Human Intraluminal pH Studies.—The Cambridge combined glass/calomel electrode was used with a Pye pH meter and Duplex chart recording system to provide permanent records of the traces of intraluminal conditions of the duodenum and upper jejunum (Benn and Cooke, 1970). The intestinal intraluminal pH of the five patients on long-term treatment with phenytoin sodium, including three who had had megaloblastic anaemia, was determined at various sites. In addition, the intestinal intraluminal pH of two volunteers was studied before and during the administration of 10 g sodium bicarbonate orally. In all cases the intestinal pH studies were conducted after an overnight fast, and none of the epileptics received anticonvulsants on the day of investigation.

Results

Folic Acid Absorption Studies.—The results obtained in the folic acid absorption studies are shown in Fig. 1. The range obtained in 15 normal control subjects is depicted, on which is superimposed the mean level for that range. Impairment of folate absorption occurred in normal controls when 5 mg folic acid was administered simultaneously with 10 g sodium bicarbonate. In the epileptic group impaired absorption of folic acid below the range of the control group was observed, and this effect was unchanged by the administration of 5 mg folic acid with 10 g sodium bicarbonate. In the normal subject pronounced impairment of folate absorption was observed at the end of the 10-day course of anticonvulsant therapy. A similar effect was observed with the simultaneous administration of either 10 g sodium bicarbonate or 100 mg Garoin with the oral dose of folic acid (Fig. 2).

Intraluminal pH Studies.—The intestinal intraluminal pH studies in five epileptics on long-term anticonvulsants is compared with the range normally encountered in control subjects in Fig. 3. The three patients with the most alkaline intraluminal pH had had folic-acid-deficient megaloblastic anaemia previously. The jejunal intraluminal pH traces

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FIG. 1—Mean and range of levels of serum folic acid (*L.casei*) after administration of 5 mg folic acid orally to 15 normal subjects. The dotted line (closed circles) represents the mean serum folic of five normal subjects after 5 mg folic acid + 10 g sodium bicarbonate. The solid line (open squares) represents the control findings in five epileptics on long-term anticonvulsant therapy after 5 mg folic acid. The dotted line (open squares) represent the serum levels when folic acid was administered with 10 g sodium bicarbonate to the five epileptics.

FIG. 2—Serum folic levels in a normal subject after 5 mg folic acid (closed circles), 5 mg folic acid and 10 g bicarbonate (open circles), 5 mg folic + Garoin 100 mg (open squares), and 5 mg folic acid after 300 mg Garoin daily for 10 days (crosses).

obtained in two normal volunteers after the administration of doses of 10 g sodium bicarbonate are shown in Fig. 4. In both of them sodium bicarbonate produced protracted alkalinization of the jejunal contents, and return to normal intraluminal pH was delayed.

Discussion

The site of optimal absorption has been established as the proximal jejunum both in rats (Burgen and Goldberg, 1962; Herbert and Shapiro, 1962; Yoshino, 1968) and in man (Chanarin *et al.*, 1958; Cox *et al.*, 1958; Chanarin and Bennett, 1962). Different workers have produced evidence in favour of active transport (Burgen and Goldberg, 1962; Herbert, 1967; Hepner *et al.*, 1968) and passive diffusion (Turner and Hughes, 1962; Spencer and Bow, 1964; Yoshino, 1968). It has been suggested that both mechanisms exist at different levels of the small bowel in the rat (Hepner, 1969). The absorption of folic acid is a complex phenomenon affected by

FIG. 3—Shaded area covers the range of pH encountered in normal subjects. The points represent the intraluminal pH in five epileptic patients on long-term anticonvulsant therapy. Each point was taken at a time when tracing was constant for at least seven minutes. The three with the most alkaline pH had had a folic-acid-deficient megaloblastic anaemia.

FIG. 4—Intra-jejunal pH tracings of two normal subjects. The break in tracings represents the point at which radiological verification of the position of the electrode was obtained, following which 10 g sodium bicarbonate was administered.

dissociation at varying pH, water transport, ionic flux, glucose transport, and solvent drag (Smith *et al.*, 1970a, 1970b), but since previous experimental work failed to control these factors many of the conclusions are questionable.

Pteroylmonoglutamic acid is weakly acidic and exists in solution as the neutral compound, monanion, dianion, and trianion, the relative amounts of which vary with the pH of the solution. It is extremely important, therefore, to consider which forms are concerned in any transport mechanism, and what effect the ambient pH may have on dissociation (Matty and Blair, 1968). Studies on the transport of weakly acidic drugs across the rat intestinal wall suggest that only the neutral acid is transported and that this form is transported through the lipid barrier (Pohland *et al.*, 1951; Schanker *et al.*, 1958; Hogben *et al.*, 1959). Furthermore, these studies showed that zones of greater acidity than that of intestinal chyme exist at the mucosal interface of the rat intestine. Such zones, or "microclimates," have a virtual pH of 5.3 in the rat when the bathing solution is at pH 7.4 (Schanker *et al.*, 1958), but the mechanism whereby these are procured are as yet obscure. Matty and Blair (1968) suggested that folic acid should be transported as the neutral compound across the lipid barrier. At pH 5.0 only about 1% folic acid in aqueous solution is present in the neutral form, and with increasing alkalinity, even less. Therefore, any absorptive processes will be limited by conditions which exist within the microclimate which in its turn will be influenced by mucosal or intraluminal conditions.

Various mechanisms have been proposed to explain the impairment of metabolism of pteroylmonoglutamic acid by phenytoin. It was proposed that intestinal conjugase activity

was impaired by the anticonvulsant (Hoffbrand and Necheles, 1968; Rosenberg *et al.*, 1968), but though conjugase activity was definitely impaired by phenytoin sodium *in vitro*, the study was not performed at the normal intraluminal pH of the jejunum. Furthermore, impairment of conjugase activity should have no effect on the absorption of pteroylmonoglutamic acid. More recently, phenytoin has been shown to have no effect on conjugase activity with a pure C^{14} -labelled triglutamate (Baugh and Krumdieck, 1969). It has been claimed that the conjugase system is of minor significance since all diets when assayed in the presence of ascorbate contain adequate amounts of monoglutamate to satisfy daily body requirements (Hurdle *et al.*, 1968). It is probable, therefore, that some other mechanism exists whereby phenytoin inhibits the absorption of monoglutamate.

Though it has been suggested that intraluminal pH changes may be of importance (Butterworth, 1968), the role of intraluminal and juxtamucosal pH in folic acid absorption has received little attention. Studies described here emphasize the effect that such changes may have on the absorption of pteroylmonoglutamic acid for the intraluminal pH of a group of epileptics treated with oral phenytoin sodium were more alkaline. Under these circumstances less pteroylmonoglutamic acid would be in a form suitable for absorption.

Proprietary preparations of phenytoin (Epanutin) in solution have a pH in the region of 12.0; sodium bicarbonate solution has a pH of 8.0. In our experience the effect of phenytoin sodium on pteroylmonoglutamate absorption was mimicked by the oral administration of sodium bicarbonate, and similar alterations in intraluminal pH were produced in two normal subjects. In clinical situations such alterations in the jejunum may be a significant factor in folate malabsorption. For example, in adult coeliac disease, where folate malabsorption often occurs, some untreated patients have an intraluminal jejunal pH between pH 7.5 and 8.5 (Benn and Cooke, 1970) so that dietary monoglutamate absorption is impaired sufficiently to precipitate folate deficiency.

In conclusion, evidence presented in our study suggests that changes in the bulk phase of intraluminal pH may have a profound effect on the absorption of pteroylmonoglutamic acid in epileptics and in normal subjects.

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References

- Baugh, C. M., and Krumdieck, C. L. (1969). *Lancet*, 2, 519.
 Benn, A., and Cooke, W. T. (1970). *Scandinavian Journal of Gastroenterology*. In press.
 Burgen, A. S. V., and Goldberg, N. J. (1962). *British Journal of Pharmacology and Chemotherapy*, 19, 313.
 Butterworth, C. E., jun. (1968). *British Journal of Haematology*, 14, 339.
 Chanarin, I., Anderson, B. B., and Mollin, D. L. (1958). *British Journal of Haematology*, 4, 156.
 Chanarin, I., and Bennett, M. C. (1962). *British Medical Journal*, 1, 985.
 Cox, E. V., Meynell, M. J., Cooke, W. T., and Gaddie, R. (1958). *Gastroenterology*, 35, 390.
 Dahlke, M. B., and Mertens-Roesler, E. (1967). *Blood*, 30, 341.
 Girdwood, R. H., and Lenman, J. A. R. (1956). *British Medical Journal*, 1, 146.
 Hawkins, C. F., and Meynell, M. J. (1958). *Quarterly Journal of Medicine*, 27, 45.
 Hepner, G. W. (1969). *British Journal of Haematology*, 16, 241.
 Hepner, G. W., Booth, C. C., Cowan, J., Hoffbrand, A. V., and Mollin, D. L. (1968). *Lancet*, 2, 302.
 Herbert, V. (1966). *Journal of Clinical Pathology*, 19, 12.
 Herbert, V. (1967). *American Journal of Clinical Nutrition*, 20, 562.
 Herbert, V., and Shapiro, S. S. (1962). *Federation Proceedings*, 21, 260.
 Herbert, V., and Zalusky, R. (1962). *Journal of Clinical Investigation*, 41, 1263.
 Hoffbrand, A. V., and Necheles, T. F. (1968). *Lancet*, 2, 528.
 Hogben, C. A. M., Tocco, D. J., Brodie, B. B., and Schanker, L. S. (1959). *Journal of Pharmacology and Experimental Therapeutics*, 125, 275.
 Hurdle, A. D. F., Barton, D., and Searles, I. H. (1968). *American Journal of Clinical Nutrition*, 21, 1702.
 Klipstein, F. A. (1964). *Blood*, 23, 68.
 Mannheimer, E., Pakesch, F., Reimer, E. E., and Vetter, H. (1952). *Medizinische Klinik*, 47, 1397.
 Matty, A. J., and Blair, J. A. (1968). *Lancet*, 2, 978.
 Newman, M. J. D., and Sumner, W. D. (1957). *Blood*, 12, 183.
 Pohland, A., Flynn, E. H., Jones, R. G., and Shive, W. (1951). *Journal of the American Chemical Society*, 73, 3247.
 Rosenberg, I. H., Godwin, H. A., Streiff, R. R., and Castle, W. B. (1968). *Lancet*, 2, 530.
 Schanker, L. S., Tocco, D. J., Brodie, B. B., and Hogben, C. A. M. (1958). *Journal of Pharmacology and Experimental Therapeutics*, 81, 123.
 Smith, M. E., Matty, A. J., and Blair, J. A. (1970a). *Gut*, 11, 368.
 Smith, M. E., Matty, A. J., and Blair, J. A. (1970b). *Biochimica et Biophysica Acta*, 219, 37.
 Spencer, R. P., and Bow, T. M. (1964). *Journal of Nuclear Medicine*, 5, 251.
 Turner, J. B., and Hughes, D. E. (1962). *Quarterly Journal of Experimental Physiology*, 47, 107.
 Yoshino, T. (1968). *Journal of Vitaminology*, 14, 21.