Formylation of Folate as Step in Physiological Folate Absorption

JANET PERRY, I. CHANARIN

British Medical Journal, 1973, 2, 588-589

Summary

Oral administration of folate analogues to rats is followed by a rise in plasma folate detectable only by micro-biological assay with Lactobacillus casei, suggesting methylation of folate during absorption. When using the everted rat gut technique with folate (PteGlu), dihydrofolate (H₂PteGlu), or tetrahydrofolate (H₄PteGlu) on the mucosal surface formyl folate (10-CHO-PteGlu, 10-CHO-H₄PteGlu, 5-CHO-H₄PteGlu) and methylfolate (5-CH₃-H₄PteGlu) are recovered from the serosal fluid. This indicates that absorption of PteGlu is followed by formylation and that the formyl group is further reduced to methyl, 5-CH₃-H₄PteGlu passing on to portal blood.

Introduction

Oral doses of reduced forms of folate monoglutamates such as dihydropteroylglutamic acid (H₂PteGlu), and tetrahydro-pteroylglutamic acid (H₄PteGlu) in man are followed by a rise in plasma methylenetetrahydrofolate (5-CH₃-H₄PteGlu) (Baker et al., 1965; Cohen, 1965; Chanarin and Perry, 1969; Perry and Chanarin, 1970; Pratt and Cooper, 1971; Whitehead et al., 1972; Nixon and Bertino, 1972). In man, reduction and methylation of folate occurs in the small gut (Chanarin and Perry, 1969; Perry and Chanarin, 1970). Olinger et al. (1972), using isolated rat gut, reported that 3H-PteGlu was converted to methylenetetrahydrofolate and that this conversion was interfered with by methotrexate.

In this paper we report evidence that folate is formylated as a first step and that methylation presumably arises from reduction of the formyl group.

Materials and Methods

Pteroylglutamic acid labelled with tritium on the 3rd and 5th positions of the para-aminobenzyol ring (Amersham) was used. H₂PteGlu and H₄PteGlu were prepared from 3H-folate by reduction with ascorbate and dithionite (Blakey, 1960; Silverman and Noronha, 1961).

5-Formyl-tetrahydrofolate (Lederle) was generally labelled with tritium by an exchange with tritiated water in the presence of a platinum catalyst (Amersham) and purified by chromatography on A25 DEAE-Sephadex followed by G-15 Sephadex (Nixon and Bertino, 1971). Tritium-labelled 10-formyl-H₂PteGlu and 5-CH₃-H₄PteGlu were prepared from the 5-formyl compound (Rabinowitz, 1963; Chanarin and Perry, 1967).

Folate analogues were identified by chromatography on A25 DEAE-Sephadex using a 0.9 by 27 cm column and elution by a gradient system consisting of 1.0 mol/l. phosphate buffer in the reservoir and 0.1 mol/l. phosphate buffer in the mixing chamber. Both buffers were pH 6.0 with 0.1 mol/l. 2-mercaptoethanol. Five-millilitre fractions were collected, radioactivity was counted, and microbiological activity determined using Lactobacillus casei, Streptococcus faecalis, and Pedicoccus cerevisiae. Marker compounds as prepared above were used for further identification of folate analogues. Radioactivity was counted on a Wallac liquid scintillation counter.

Male Sprague-Dawley rats weighing 250 g were used to prepare everted sacs (Wilson and Wiseman, 1954). Three 15-cm segments, starting from the pyloric end of the gut, were prepared from each animal. One millilitre of Krebs Ringer bicarbonate solution pH 6.1 was introduced into each sac and the sacs were incubated in 25 ml Krebs Ringer bicarbonate containing glucose 28 mmol/l. The folate compounds under test were added to the mucosal fluid at a concentration of 10 nmol, and the sacs exposed to O₂-CO₂ (95/5%) for three minutes and incubated for one hour at 37°C. At the end of this period ascorbate (pH 6.0) was added to both serosal and mucosal fluids to give a 1% concentration. Folate analogues in these fluids were separated by chromatography and identified.

Results

When 5 µg H₂PteGlu were given orally to three rats there was...
an increase in serum *L. casei* activity from 15 ng/ml to 40 ng/ml after one hour. There was no detectable increase in the activity on *S. faecalis* assay. This suggests that H₄PteGlu has been converted to 5-CH₂H₂PteGlu by the intact animal during intestinal absorption.

The result of an experiment using everted sacs of rat gut wherein PteGlu was added to the mucosal fluid is shown in fig. 1. The serosal fluid contained three folate analogues—

![Diagram](image_url)

FIG. 1—5 μg PteGlu were placed on the mucosal side of an everted rat small gut preparation. After one hour mucosal and serosal fluids were collected and folate analogues identified (see text). Only PteGlu was present on the mucosal surface but after passage through the gut 10-CHO-PteGlu and 5-CHO-H₄PteGlu were found.

namely, PteGlu, 5-CH₂H₂PteGlu, and 10-CHO-PteGlu. The latter was active with *L. casei* and *S. faecalis* but not with *P. cerevisiae*. Folates peaks corresponded to peaks of tritium activity indicating derivation from the labelled mucosal folate.

A study with H₄PteGlu added to the mucosal fluid is shown in fig. 2. 5-CHO-H₂PteGlu and 10-CHO-folates were present in the serosal fluid. In addition to 10-CHO-H₂PteGlu some 5-CHO-H₂PteGlu was present. Similar results were obtained when H₂PteGlu was added to the mucosal fluid.

**Discussion**

Our results differ from those of Olinger et al. (1972) in that in addition to methyltetrahydrofolate we found various formyl analogues of folate. Tetrahydrofolate, in fact, does not accept a methyl group in toto but only single carbon units at the formylaldehyde or formate state of reduction (Blakley, 1969). The accumulation of formyl derivatives as well as methyltetrahydrofolate in the serosal fluid of the everted sac as opposed to the intact animal is probably due to a negative feedback mechanism following a pile up of the methyl-form in the gut intestinal cells. Under normal circumstances the formyl group is further reduced to methyl. Butterworth (1973) recently reported the appearance of formylfolate in absorption studies in dogs.

With PteGlu as substrate 10-CHO-PteGlu appeared in the serosal fluid indicating that formylation can occur in the absence of reduction. Recently polyglutamate formation has been found in the liver of methotrexate-treated guinea pigs in the absence of reduction of the pteridine ring (Corrocher and Hoffbrand, 1972).

We are uncertain as to the significance of 5-formyl-tetrahydrofolate. This could arise as an artefact by conversion from the 10-formyl. Though oral 5-formyltetrahydrofolate gives rise to plasma 5-methyl-tetrahydrofolate (Chanarin and Perry, 1969; Perry and Chanarin, 1970; Pratt and Cooper, 1971; Nixon and Bertino, 1972), the latter reported that the 5-formyl group was removed and replaced by a formyl from another source. Should it be a physiological intermediary it would then be processed as described by Nixon and Bertino (1972).

We wish to thank Lederle Laboratories for the supply of folic acid.

**References**


