The effect of phenytoin on the absorption of synthetic folic acid polyglutamate

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SUMMARY The absorption of synthetic pteroyltriglutamate has been measured in nine normal students with and without the anticonvulsant drug phenytoin. It has been shown that phenytoin has no effect on the absorption of this folate polyglutamate. The reasons are discussed for the disparity between this result and those reported in the literature when folate polyglutamates derived from yeast were used.

Patients on anticonvulsant therapy may develop megaloblastic anaemia due to folic acid deficiency (Badenoch, 1954; Hawkins and Meynell, 1958). It has been suggested that folate deficiency is due to interference with the intestinal absorption of folate by the anticonvulsant drugs (Meynell, 1966; Dahlke and Mertens-Roesler, 1967). Eighty per cent of the folates in the diet are in the form of folate polyglutamates (Butterworth, Santini, and Frommeyer, 1963) which are deconjugated by intestinal enzymes during absorption (Rosenberg and Streiff, 1967). Hoffbrand and Necheles (1968) and Rosenberg, Godwin, Streiff, and Castle (1968) demonstrated that phenytoin impaired the absorption of folate polyglutamate derived from yeast but not of free folic acid itself. These authors also showed that the anticonvulsant drug could inhibit the intestinal folate conjugase *in vitro*. These findings suggested that the mechanism of folate malabsorption was due to inactivation of folate deconjugating enzymes in the intestinal mucosa. Baugh and Krumdieck (1969), using synthetic folate polyglutamates, were unable to demonstrate any evidence of intestinal folate deconjugase inhibition by phenytoin when studied *in vitro*. They did not, however, examine the effect of phenytoin on the absorption of synthetic folate polyglutamate.

In this communication we report the results of the effects of phenytoin on the absorption of folate triglutamate.

Subjects and Methods

Nine normal students volunteered for this study. Their ages ranged from 22 to 24 years and they had

no history of any haematological or gastroenterological disorders.

An oral loading dose of 5 mg was fed to each subject one week before the first absorption test. A second absorption test was performed one week after the first test. Each absorption test was performed after an overnight fast.

A fasting blood sample was obtained at 9.00 am, the student then drank 300 ml of water containing 320 μ g of pteroyltriglutamate. At one of the two absorption tests each subject was also fed a suspension of 200 mg of phenytoin in 25 ml of water three minutes before drinking the folate solution. Whether the phenytoin was taken at the first or second absorption test was decided randomly. Blood samples were then obtained by venipuncture at one, two, and three hours after the oral dose. The 'peak rise' in the serum folate level was determined by subtracting the fasting value from the highest serum folate level obtained after the oral pteroyltriglutamate.

The triglutamate form of folic acid (pteroyltriglutamate) was prepared by the method of Krumdieck and Baugh (1969). Thin-layer and paper chromatography showed it to be in excess of 95% pure. There was no detectable contamination with pteroyl-di or mono-glutamate. In addition microbiological assay with *Streptococcus faecalis* (ATCC 8043) showed the preparation to be free of pteroylmonoglutamate. The oral dose of pteroyltriglutamate was measured by an extinction coefficient (320 μ g pteroyltriglutamate is equivalent to 200 μ g of free folic acid).

The serum folates were assayed by the method of Davis, Nicol, and Kelly (1970) using the chloramphenicol-resistant strain of *Lactobacillus casei* (ATCC 7469).

Results

The values for the 'peak rise' of the serum folate obtained following oral pteroyltriglutamate with and without phenytoin are shown in the Table.

Case No.	Peak Rise in Serum Folate (mg/ml) after 320 µg Pteroyltriglutamate		Difference
	Without Phenytoin	With 200 mg Phenytoin	
1	4.1	2.5	+1.6
2	12.1	7.5	+4.6
3	4.1	5.0	-0.9
4	9.5	7.5	+0.2
5	6.4	2.0	+2.4
6	3.5	3.0	+0.5
7	8.0	15.0	-7.0
8	10.0	8.5	+1.5
9	4.0	4.0	0
Mean difference 0.32 (± 1.08 SE)			

Table Peak rise in serum folate levels after oral dose of pteroyltriglutamate with and without phenytoin in nine normal students

The mean of the differences between the peak rise obtained in the absence and in the presence of phenytoin was 0.32 (± 1.08 SE) which demonstrates that the paired results are similar to a high degree of significance.

Discussion

It has been demonstrated in this study that phenytoin has no adverse effect on the absorption of a synthetic polyglutamate *in vivo*. This finding substantiates the work of Baugh and Krumdieck (1969) who showed *in vitro* that phenytoin did not inhibit the intestinal folate deconjugase enzyme(s). These enzymes are necessary for the breakdown of the folate polyglutamates, a process which occurs before or during folate absorption.

Hoffbrand and Necheles (1968) and Rosenberg *et al* (1968) showed that phenytoin inhibited the absorption of partially purified folate polyglutamate derived from yeast. In our study the dose of phenytoin used was twice that used by the above authors but no effect could be demonstrated on a synthetic 95% pure folate triglutamate.

In both of the former studies the subjects on whom the folate tolerance tests were performed were not preloaded with folic acid to saturate their tissue stores. Clarke (1953) and Chanarin, Anderson, and Mollin (1968) have shown that it is essential to give a loading dose of folic acid in order to saturate the

tissues before the tolerance test if uniform serum levels are to be obtained.

This discrepancy in techniques may explain the differences in the results of this study as compared with those of Hoffbrand and Necheles (1968) and Rosenberg *et al* (1968). These authors also demonstrated that the deconjugase activity of small intestinal biopsies can be suppressed *in vitro*; however, even at concentrations of phenytoin in excess of those likely to occur *in vivo*, deconjugase activities of at least 25% of the original levels were still recovered. The present study would suggest that the deconjugase activity, even if it is partially suppressed *in vivo* by phenytoin, is not impaired to the extent of altering the rate of folate polyglutamate absorption at a physiological dose level.

Another possibility, albeit unlikely, which could explain the discrepancy in the reported results, is that yeast polyglutamate contains an inhibitor of folate absorption (Swendseid, Bird, Brown, and Bethell, 1947) and that this is potentiated by phenytoin.

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