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SHORT COMMUNICATIONS

Effect of diphenylhydantoin and ethanol feeding on the synthesis of rat liver folates from exogenous pteroylglutamate [^{3}H]

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TWO AGENTS known to interfere with folate metabolism were assessed with respect to their influence on the synthesis of tritiated pteroyl poly-γ-L-glutamates from parenteral [3′5′9 (*n*)^{3}H]pteroylglutamate (K salt for L,*N*-[4-{[(2-amino-4-hydroxy-6-pteridinyl) methyl]amino}benzoyl]glutamic acid) in rat liver. The mechanism of action of the anticonvulsant phenytoin (diphenylhydantoin, "Dilantin", "Epanutin") on folate metabolism is uncertain. While there is some evidence that it inhibits folate "conjugase" (γ-glutamyl carboxypeptidase) enzyme(s) from human jejunal mucosa *in vitro*,[1,2] experiments in this laboratory[3] have shown this agent to have no significant effect on the intestinal absorption of the synthetic pteroyltriglutamate by man *in vivo*. More recent evidence[4] clearly indicates an antagonistic relation *in vitro* between anti-convulsant drugs which reduce brain respiration and 5-formyltetrahydropteroylglutamate and noradrenaline which stimulate cerebral oxygen consumption.

The occurrence of folate deficiency megaloblastic anaemia in severe alcoholism[5] is apparently due to mal-absorption of folate,[6] and impaired utilization[7,8] in addition to the usual dietary deficiency.

Previous studies with rats[9–11] have established that exogenous [3′5′9 ^{3}H]pteroylglutamate is incorporated mainly into liver pteroylpenta and hexa glutamates specifically 10CHO- and $5CH_3H_4$-$PteGlu_{5,6}$ [(−), 10 formyl 5,6,7,8 tetrahydropteroylglutamyl-γ-glutamyl-γ-glutamyl-γ-glutamyl-γ-glutamate; (−), 10 formyl 5,6,7,8 tetrahydropteroylglutamyl-γ-glutamyl-γ-glutamyl-γ-glutamyl-γ-glutamyl-γ-glutamate; (+), 5 methyl 5,6,7,8 tetrahydropteroylglutamyl-γ-glutamyl-γ-glutamyl-γ-glutamyl-γ-glutamate; (+), 5 methyl 5,6,7,8 tetrahydropteroylglutamyl-γ-glutamyl-γglutamyl-γ-glutamyl-γ-glutamyl-γ-glutamate]. That the endogenous distribution is similar has been established by microbiological assay.

Adult Wistar rats were fasted overnight and injected intra-peritoneally with 25 μCi [3′5′9 ^{3}H] PteGlu (62 mCi/mg) (Radiochemical Centre, Amersham, Bucks, U.K.). Animals received intramuscular injections of 1 or 2 mg Epanutin Parenteral (Parke–Davis, Hounslow, U.K.) 3 hr previous

TABLE 1. INCORPORATION OF PARENTERAL [3′5′9 ^{3}H] PteGlu K INTO LIVERS OF EPANUTIN AND ETHANOL TREATED RATS

	wt (g)	μCi (g liver)	% Dose (g liver)	5 + 10CHO H_4PteGlu (%)	$5CH_3$ H_4PteGlu (%)
Normal $\bar{X}$(8) (SD)	7·11		4·61 (± 0·84)	22·9	77·1
Phenytoin	8·45	0·76	3·05	30	70
	8·88	1·13	4·50	18	82
	7·32	0·72	7·20	30	70
	7·46	1·03	10·30	24	76
$\bar{X}$(4) (SD)	8·03		6·25 (± 3·33)*	25·5	74·5
Ethanol	9·20	0·97	3·86	29	71
	10·13	0·10	2·10	17	83
	8·41	0·23	2·30	—	—
	8·68	0·31	3·10	—	—
X(4) (SD)	9·11		2·84 (± 0·42)†		

* $P < 0·2$.
† $P < 0·01$.

to tritium injection. Animals exhibiting a marked preference (5–18:1, v/v) for 8% ethanol over drinking water during a 14-day observation period were also included in the study. The animals were sacrificed 24 hr after injection and the livers removed, divided and weighed. One portion of each liver was autolysed in 2·5% potassium ascorbate pH 4·5 to yield pteroylmonoglutamates while the other portion was extracted by boiling and autoclaving at pH 9·0 followed by $KMnO_4$ oxidation to yield *p*-aminobenzoylpoly-γ-L-glutamates. Column separations of these mixtures respectively by QAE-Sephadex A25 (quaternary anion exchanger) (Pharmacia, Uppsala, Sweden) and DEAE-cellulose (Whatman DE52) anion exchange chromatography were carried out as described previously.[11]

In Table 1 are presented data on incorporation of [3′5′9 ^{3}H]PteGlu into liver pteroylmonoglutamates and the relative quantities of the fractioned PteGlu derivatives. Since the liver is a major site of folate metabolism and storage it is not surprising that such a large percentage of the administered dose appeared in the liver.

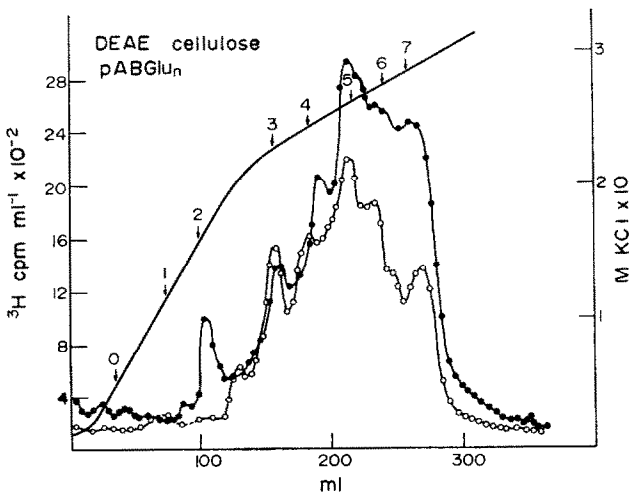

FIG. 1. DEAE cellulose chromatography: *p*-aminobenzoylpoly-γ-L-glutamate derivatives of liver folates synthesized from [3′5′9 ^{3}H]PteGlu in two rats (●,○) dosed with 1mg Epanutin. Numerals refer to elution positions of synthetic standards.

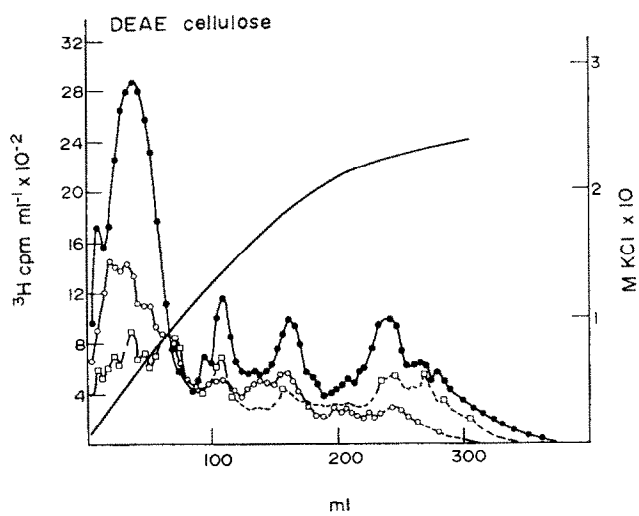

FIG. 2. DEA cellulose chromatography of tritiated poly-γ-glutamyl derivatives from livers of three animals on ethanol dietary (○,●, □). As in Fig. 1.

Rats given Phenytoin appeared to give a greater than normal 24 hr incorporation of the tritiated dose ($P < 0.2$) while those on ethanol incorporated much less tritium ($P < 0.01$). In the phenytoin treated there was no clear difference from the normal in the type folates synthesized the predominant form being $5CH_3H_4PteGlu_5$ though substantial amounts of tetra, hexa and hepta were also observed (Fig. 1). Two additional animals receiving 2 mg phenytoin showed the same pattern.

In the ethanol treated animals, the monoglutamate substituents were as normal but polyglutamate synthesis appeared to be impaired (Fig. 2). There may be a relationship between the lower level of tritium incorporation in these animals and their reduced capacity for producing polyglutamate folates. In similar studies with methotrexate (L,*N*-[4-{[2,4 diamino-6-pteridinyl) methyl, methyl]-amino}benzoyl]glutamic acid) treated animals tritium incorporation into liver folates was very effectively reduced and the small percentage of label remaining in the liver at 24 hr was normally distributed among polyglutamates with no remaining exogenous PteGlu.[12] These latter results suggested that biosynthesis of polyglutamate derivatives of folate may be necessary to retain exogenous monoglutamate forms within the cells. The ethanol treated animals employed in this study were not at the stage where structural changes in the hepatic tissue were evident to light microscopy, thus ethanol appears to directly affect the biosynthesis of liver pteroylpolyglutamates. Accordingly, this finding suggests a further mechanism for the well known association of folate deficiency and chronic alcoholism,[5] namely that alcohol in preventing the formation of pteroylpolyglutamates inhibits the storage of folate in the body. This substantiates previous work[13] which demonstrated that folate replete alcoholics when placed on a folate deficient diet with ethanol became folate deficient and developed megaloblastic haemopoiesis more rapidly than when they took a similar diet without ethanol.

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Effect of acute and chronic administration of lithium on steady-state levels of mouse brain choline and acetylcholine*

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THE THERAPEUTIC efficacy of lithium in the treatment of the manic phase of manic-depressive psychosis is well established.[1,2] In addition, lithium prophylaxis may be beneficial in preventing recurrent

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