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SHORT COMMUNICATION

Further observations on the site of action of amethopterin

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THE reduction of both FA* and DHFA to tetrahydrofolic acid is inhibited *in vitro* by the folic acid antagonists.[1] These reductive steps can be measured indirectly in the intact mouse by determining the concentration of liver CF after the administration of a test dose of FA or DHFA. It has already been shown that a small dose of amethopterin (0·05 mg/kg) completely prevented the conversion of exogenous FA to liver CF.[2] This report deals with observations on the effects of amethopterin on the metabolism of DHFA.

When DHFA (25 mg/kg) was given to (C57 × DBA)F_1 male mice there was a rise in liver CF of approximately two to three times pretreatment concentrations. Unlike FA, however, the administration of amethopterin 24 hr earlier had no effect on the amount of CF produced from exogenous DHFA. In Fig. 1, the effect of amethopterin upon liver CF derived from FA and DHFA is shown;

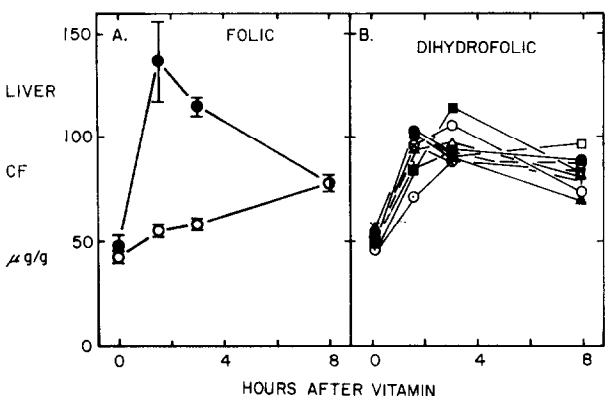

FIG. 1. A. Liver CF derived from 25 mg of FA per kg given 24 hr after saline (●———●) and 0·1 mg of amethopterin per kg (○———○).

B. Liver CF derived from 25 mg of DHFA per kg given 24 hr after saline (●———●), and amethopterin, 0·1mg/kg (○———○), 0·5 mg/kg (▲———▲), 1 mg/kg (△———△), 5 mg/kg (■———■), 10 mg/kg (□———□), 50 mg/kg (▽———▽), 100 mg/kg (▽———▽), and 500 mg/kg (○———○).

Each point represents the mean for 2 mice. In A, the individual values are indicated by the bars at the ends of the vertical lines through each point. The variance in B was of the same magnitude, but the lines were not included because of the number of points. All compounds were given subcutaneously. Liver CF was determined microbiologically after autolysis.[2]

0·1 mg of amethopterin per kg prevented the conversion of exogenous FA to liver CF, but doses of amethopterin ranging from 0·1 to 500 mg/kg did not inhibit the production of CF from exogenous DHFA.

* The following abbreviations are used: FA, folic acid; DHFA, dihydrofolic acid; CF, citrovorum factor.

Toxicity studies in the mouse confirmed these observations. Previous work showed that the administration of FA 1 hr before amethopterin increased the LD_{50}, whereas a small dose of amethopterin (0·1 mg/kg) given 24 hr earlier abolished this protective action.[2] In the present studies, administration of DHFA 1 hr before the LD_{50} injections not only increased the LD_{50} but also nullified the added toxicity produced by a large dose of amethopterin (100 mg/kg) given 24 hr earlier (Table 1, Experiment 1). Experiment 2 further demonstrates that, although both FA and DHFA afford some protection against amethopterin when given 1 hr earlier, only DHFA retains its protective properties when given 1 hr after amethopterin.

TABLE 1. EFFECT OF FA AND DHFA ON TOXICITY OF AMETHOPTERIN IN THE MOUSE*

Experiment 1			Experiment 2		
Treatment	Time (hr)	Amethopterin LD_{50} (mg/kg)	Treatment	Time (hr)	Amethopterin LD_{50} (mg/kg)
None	—	200	None	—	250
DHFA†	−1	450	FA†	−1	320
Amethopterin‡	−24	<30	DHFA†	−1	630
Amethopterin‡ + DHFA†	−24 / −1	180	FA† + DHFA†	−1 / −1	180 / 360

* LD_{50} determinations were done as described by Cornfield and Mantel,[9] using 6 groups of 6 mice for each determination.

† 25 mg/kg.

‡ 100 mg/kg.

These results indicate that the conversion of DHFA to liver CF in the mouse, as well as the ability of the animal to convert exogenous DHFA into some substance that protects against the toxicity of amethopterin, was not impaired by the administration of amethopterin. In marked contrast to these observations, both the conversion of FA to liver CF and the protection against the subsequent administration of amethopterin afforded by FA were prevented by a small dose of amethopterin.

Observations on the reduction of FA and the inhibition of this process by FA antagonists in various animals suggest that significant species differences exist. The studies by Zakrzewski and Nichol of folic and dihydrofolic reductases in a preparation from chick liver led them to conclude that a single enzyme performs both reductions and that both processes are equally inhibited by amethopterin.[3] Noronha and Sreenivasan, working with the rat, were able to separate the two reductive steps;[4] they found that folic reductase activity was localized in the supernatant fraction of liver homogenates, whereas dihydrofolic reductase activity was associated with the mitochondrion. Aminopterin inhibited the latter reaction, but its effect upon the former was not reported. More recently, Mead *et al.* have found that DHFA reversed the antileukemic effect and toxicity of amethopterin in mice bearing leukemia L1210 in a manner comparable to that of CF.[5]

The present observations indicate that in the mouse the conversion of exogenous DHFA to liver CF and related compounds is unaffected by the antagonist amethopterin. The fact that the conversion of FA is inhibited under these conditions suggests that two different enzymes are involved. The possibility that a single enzyme mediates both reductions cannot be excluded, but such a mechanism would imply that the inhibition by amethopterin could be reversed *in vivo* by DHFA but not by FA. In view of the tight binding of amethopterin to folic reductase,[6] the failure of folinic acid to prevent amethopterin fixation in liver,[7] and the prolonged period of inhibition of the reduction of FA produced by amethopterin,[8] this action by DHFA appears unlikely.

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