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18 September 1961

On the Site of Action of Amethopterin

Abstract. In the liver of the intact mouse, the conversion of exogenous folic acid to compounds with citrovorum-factor activity is inhibited completely by an amount of amethopterin similar to that bound to the enzyme folic acid reductase in vitro. Because this amount of amethopterin is several thousand times smaller than the LD_{50}, the toxic effects produced by the larger doses must be mediated via some additional mechanism.

Both folic and folinic (5-formyl-5,6,7,8-tetrahydrofolic) acids can protect mice from aminopterin toxicity (*1*). While folinic acid is effective when given simultaneously or even after the drug, folic acid must be given about 1 hour before aminopterin in order to provide any protection. During the period of 1 hour after the administration of folic acid, the folic acid is converted to compounds with citrovorum-factor activity, which can then serve to protect against aminopterin (*2*). A priming dose (nontoxic) of aminopterin abolishes the protection afforded by folic acid by preventing its reduction to more active materials. Folinic acid, because it is already reduced, is unaffected by prior administration of aminopterin and can substitute for the biologically active derivatives of folic acid. The measurement of liver citrovorum factor after the administration of folic acid provides an in vivo assay of the enzymes responsible for this conversion.

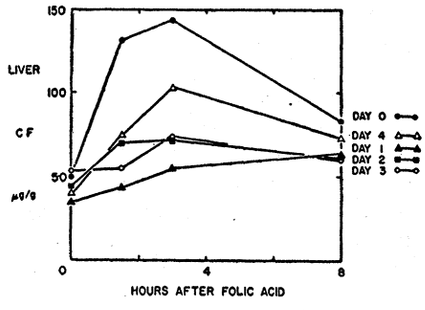

Fig. 1. The effect of amethopterin (0.05 mg/kg) on liver citrovorum factor derived from folic acid (25 mg/kg). Folic acid was given without amethopterin (day 0) and after amethopterin (days 1, 2, 3, and 4). Each point represents the mean for two mice. Amethopterin and folic acid were given subcutaneously.

The citrovorum-factor content of the livers of (C57 × DBA)F_1 male mice was determined by incubation of acetone powders with ascorbate and histidine and subsequent microbiological assay with *Pediococcus cerevisiae* (ATCC No. 8081), as described elsewhere (*2*). The influence of amethopterin (0.05 mg/kg) on the liver citrovorum factor after administration of folic acid (25 mg/kg) on the days indicated is presented in Fig. 1. In the animals that received no amethopterin (day 0), liver citrovorum factor increased from 50 to 140 μg/g in the first 3 hours after folic acid was given. On subsequent days, after administration of amethopterin, this response was abolished and had not been completely re-established by day 4, the last day of observation. Thus, in this experiment, the conversion of folic acid to citrovorum factor was inhibited completely by a very small dose of amethopterin.

The degree of inhibition of the conversion of folic acid can also be determined by observing the protective effect of previously administered folic acid on the toxicity of amethopterin. The data summarized in Table 1 show that administration of folic acid (25 mg/kg) 1 hour before administration of amethopterin increased the LD_{50} from 200 to 350 mg/kg. The administration of amethopterin (0.1 mg/kg) 24 hours before the LD_{50} injections abolished this protective effect.

Inhibition of the conversion of folic acid to liver citrovorum factor was produced by administration of 0.05 mg of amethopterin per kilogram of mouse, or 1 μg for a 20-g mouse. If all of the drug were localized in the liver, the concentration would be 2 mμmole/g of liver. Since the amount of folic acid reductase in 1 g of mouse liver can bind 0.8 mμmole of amethopterin in vitro (*3*), these results suggest that at this low dose of amethopterin most of the drug was bound to this enzyme. The disappearance of the protective action of previously administered folic acid after such a small dose of amethopterin further demonstrates the effectiveness of amethopterin in inhibiting the action of this enzyme.

Table 1. Protective effect of previously administered folic acid on the toxicity of amethopterin.

Prior treatment	Time before amethopterin administration (hr)	Amethopterin LD_{50} (mg/kg)
None		200
Folic acid (25 mg/kg)	1	350
Amethopterin (0.1 mg/kg) and folic acid (25 mg/kg)	24	180
	1	

If doses of amethopterin several thousand times smaller than the LD_{50} completely inhibit the conversion of folic acid to citrovorum factor in the intact mouse, larger doses cannot increase the degree of inhibition and therefore must produce toxicity via some additional mechanism. It is not possible to account for all the effects of the folic acid antagonists solely on the basis of inhibition of this conversion process. Because all these effects can be reversed by administration of folinic acid, the additional sites of action may involve the further metabolism of tetrahydrofolic acid and its derivatives (*4, 5*).

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References and Notes

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An Overview of Sleep as an Experimental Variable (1940–1959)

Abstract. Less than one half of 1 percent of the psychological literature relates to sleep. Although there has been a relative decline in such research, the central nervous system and pathological aspects have recently received increased attention. The United States is producing less than 17 percent of the research on sleep.

In a recent review of the research literature on sleep, some statistics of interest regarding this research area were assembled. The review covered the primarily psychological research since 1941, since Kleitman's book, pub-