THE INHIBITION OF DEHYDROGENASES BY FOLIC ACID AND SEVERAL OF ITS ANALOGS*

W. H. Vogel, R. Snyder, and M. P. Schulman

Department of Pharmacology
University of Illinois College of Medicine
Chicago 12, Illinois

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The inhibition of alcohol dehydrogenase and several other metallodehydrogenases by ortho-phenanthroline (1, 10-phenanthroline) has been studied extensively. The action of this and other chelating agents has been described in a recent review of metallodehydrogenases (1). Ortho-phenanthroline may inhibit these enzymes by competing with DPN (or DPNH) for a zinc or other metal moiety of the molecule, or its action may not be exerted primarily at a metal site.

It was recently reported from this laboratory that folic acid and aminopterin inhibited alcohol dehydrogenases derived from yeast and horse liver (2). In view of the known complexing of folic acid with zinc (3), it was plausible to assume that folic acid and aminopterin inhibited the alcohol dehydrogenases by a metal chelation. This suggested the possibility of inhibiting other dehydrogenases with folic acid and related compounds. The results of these studies are the basis of this report. Folic acid (pteroylglutamic acid), aminopterin (4-amino-pteroylglutamic acid) and methotrexate (amethopterin, 4-amino-10-methylpteroylglutamic acid) inhibited glutamic dehydrogenase, lactic dehydrogenase, malic dehydrogenase and glucose-6-phosphate dehydrogenase. Under identical experimental conditions, the inhibition produced by these compounds occurred at much lower concentrations than that found with ortho-phenanthroline. Leucovorin (5-formyl-5,6,7,8-tetrahydrofolic acid) was not inhibitory to the metallodehydrogenases.

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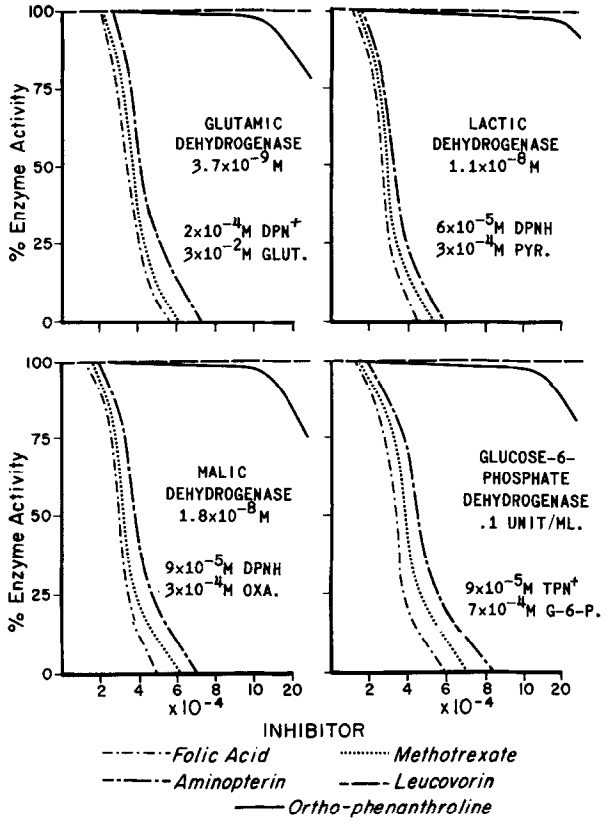
The references to the methods of assay are given with each enzyme: glutamic dehydrogenase (4), lactic dehydrogenase (5), malic dehydrogenase (6), glucose-6-phosphate dehydrogenase (7). The enzymes were crystalline preparations obtained commercially. In all cases, enzymic activity was assessed by observing the oxidation or reduction of the pyridine nucleotides at 340 mμ. The above enzymes require DPN except glucose-6-phosphate dehydrogenase which is TPN-dependent. Folic acid, aminopterin and ortho-phenanthroline were obtained commercially; methotrexate and the calcium salt of leucovorin were kindly supplied by the Lederle Laboratories. All compounds were dissolved in the appropriate assay buffer except calcium leucovorin. It was dissolved in water, phosphate buffer was then added and the precipitated calcium phosphate was removed by centrifugation.

Figure 1 represents the results calculated as per cent enzyme activity versus inhibitor concentration. Under the experimental conditions described above, folic acid, aminopterin and methotrexate inhibited the four enzymes at concentrations as low as 2×10^{-4} M. and the inhibition was complete at 8×10^{-4} M. These concentrations of ortho-phenanthroline were not inhibitory. To initiate inhibition, it was necessary to increase the concentration of ortho-phenanthroline to about 1×10^{-3} M. Fifty per cent inhibition of the dehydrogenases by folic acid, methotrexate and aminopterin was achieved with inhibitor concentrations of 3 to 4×10^{-4} M. Leucovorin was not at all inhibitory in the concentrations tested. Liver alcohol dehydrogenase was also inhibited in an identical fashion by folic acid, aminopterin and methotrexate while leucovorin was again without effect. It appears, therefore, that folic acid and several of its analogs are relatively non-specific inhibitors of these dehydrogenases. Additional experimentation will decide whether or not these compounds can inhibit other enzymes.

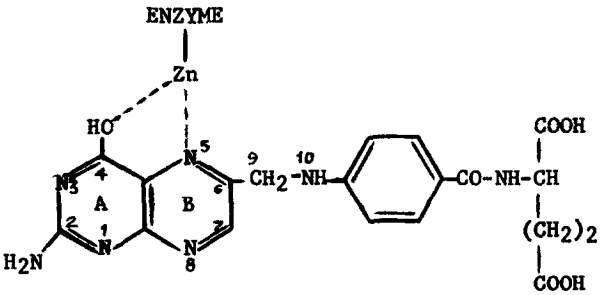
The binding of DPN to alcohol dehydrogenase is thought to involve the adenine moiety of the coenzyme (8,9). Since the pteridine ring of folic acid resembles adenine structurally, it may also bind the zinc of

Fig. 1 Inhibition of Dehydrogenases by Folic Acid and Analogs



the enzyme. Other possible sites of binding in the folic acid molecule have been rejected on the following grounds. Since leucovorin was not inhibitory, the carboxyl groups of the glutamate portion could not be likely binding sites. This was supported by other studies in which high concentrations of free glutamate (10^{-3} M) also did not inhibit alcohol dehydrogenase. The N_{10} site appears not to be involved in binding since methotrexate was inhibitory and bears a methyl substituent on N_{10}. An unoccupied N_5 position is needed for binding because a one-carbon unit at this site (e.g. as in leucovorin) resulted in full enzyme activity. The non-planar nature of reduced ring B in leucovorin as well as the formyl group at N_5 may be responsible for the absence of binding. Another reduced folate substituted at N_5, i.e. 5-methyl-5,6,7,8-tetrahydrofolic

acid (kindly provided by Dr. J. M. Buchanan of M.I.T.) also did not inhibit liver alcohol dehydrogenase. Thus, substitution at N_5 and reduction of ring B did not favor binding. The amino group must participate in binding as effectively as an hydroxyl function because aminopterin and methotrexate inhibited as well as folic acid. This is not unexpected since both are strong electron donors and would react similarly. On the assumption that zinc may be important at the active site of certain dehydrogenases, it may be chelated to the pteridine moiety of folic acid as follows:



It is equally possible to conclude that folic acid, aminopterin, and methotrexate interact with the dehydrogenases to alter the conformation of the active site.

It would be of considerable clinical interest if some fraction of aminopterin and methotrexate toxicity may be related to chelation and inactivation of (metallo)dehydrogenases. Folic acid *per se* is not toxic in relatively large doses in the intact organism (10) perhaps because it is readily reduced to a tetrahydrofolic derivative and adds a one-carbon unit at N_5. Our data demonstrate that chelation or binding correlates with a free N_5 position, an oxidized B ring, and a strong electrophilic group at C_4. To our knowledge, reduction and N_5 substitution of aminopterin and methotrexate have not been detected in tissues. Consequently, these analogs would not lose their chelating properties and ability to inhibit dehydrogenases in the intact organism.

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