2, 4-DIAMINO-5, 6, 7, 8-TETRAHYDROQUINAZOLINE, A NEW PTERIDINE ANTAGONIST

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Summary: The growth of the flagellate, Crithidia fasciculata, is inhibited by 2, 4-diamino-5, 6, 7, 8-tetrahydroquinazoline. This inhibition is reversed by biopterin. Folic acid does not reverse the inhibition but increases the effectiveness of biopterin in reversing the inhibition at low concentrations of the inhibitor.

Crithidia fasciculata and its close relatives are the only organisms presently known to have a nutritional requirement for unconjugated pteridines (Cowperthwaite, et al., 1953; Guttman and Wallace, 1964). For this reason these organisms are particularly valuable in testing compounds which are potential pteridine analogs, since in this way one can be certain that an inhibitor is acting in this system.

It was knowledge of the fact that inhibition by a variety of 4-substituted-2, 6-diaminopyridines could be reversed by biopterin (Markees, et al., 1960; 1968) that made possible the understanding of the relationship of biopterin to lipid metabolism (Kidder and Dewey, 1963; Dewey and Kidder, 1966; Dewey, et al., 1967). It seemed possible that further knowledge might be gained using another type of pteridine analog. The compound used, 2, 4-diamino-5, 6, 7, 8-tetrahydroquinazoline (DTQ), was synthesized by Dr. Noel Nugent in the Oak Ridge National Laboratories and

sent to us as a possible pteridine antagonist. Tests carried out with C. fasciculata have shown that the inhibitory effects of DTQ are reversed by biopterin and that there is some involvement of folic acid in the reversal.

Crithidia fasciculata was depleted of pteridines as previously described (Dewey and Kidder, 1966) and the media and growth conditions used were the same, with various additions as noted below.

In a medium containing folic acid at 1 μg/ml as the sole source of pteridines, C. fasciculata cleaves folic acid to yield 2-amino-4-hydroxy-6-hydroxymethylpteridine which is then converted to biopterin (Kidder, et al., 1967). In such a medium DTQ causes half maximal growth inhibition at 0.1 μg/ml; indicating interference with the conversion of folate to biopterin. The addition of leucovorin (calcium salt) at 2 μg/ml to this medium causes little reversal (half maximal inhibition at 0.2 μg/ml.).

Either thymidine or thymine can replace folic acid for the growth of Crithidia if biopterin is present (Kidder and Dutta, 1958). In a medium containing thymidine at 40 μg/ml and biopterin at optimal concentrations the organisms grow to only about half the level obtained in the presence of folic acid, but can be serially transplanted for periods of many months.

In the thymidine medium and varying concentrations of DTQ, as the concentration of biopterin is increased, a family of curves is obtained (Fig. 1) with rather peculiar characteristics. Growth at a concentration of DTQ of 2 μg/ml is inhibited to approximately the same extent at all concentrations of biopterin. There is a progressive release of inhibition at higher concentrations of inhibitor and of biopterin.

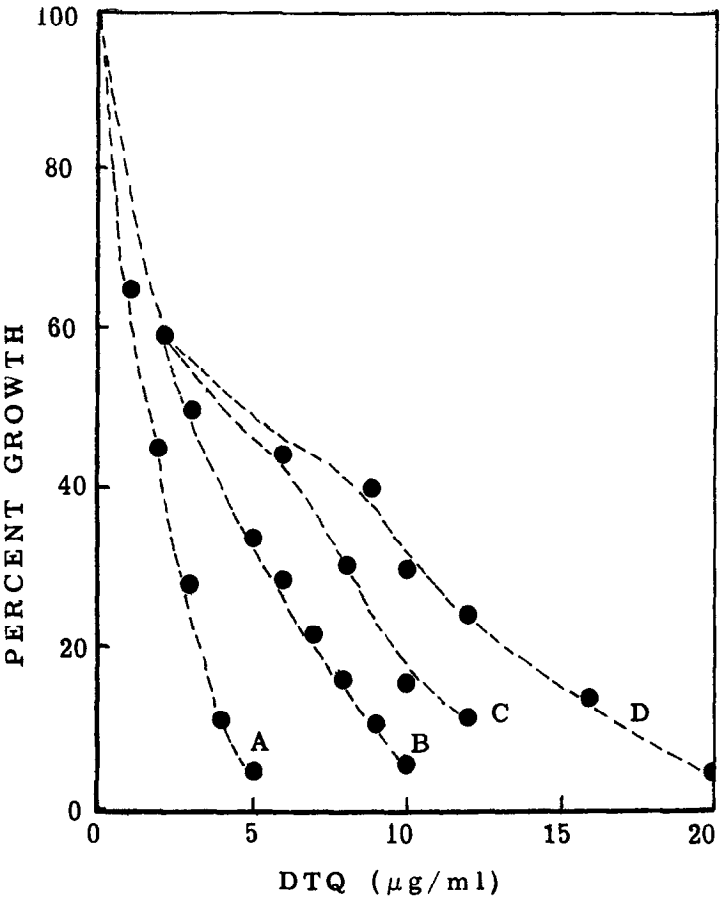

Fig. 1. Response of Crithidia fasciculata to 2, 4-diamino-5, 6, 7, 8-tetrahydroquinazoline (DTQ) in a medium containing no folic acid with thymidine at 40 μg/ml and biopterin as follows (μg/ml): A=0.001; B=0.002; C=0.004; D=0.008.

When the biopterin concentration is held constant and increasing amounts of folic acid are added to the medium, the greatest effect of folic acid is at inhibitor concentrations of 2-6 μg/ml (Fig. 2). It would appear that the presence of folic acid in relatively high concentrations (0.1 μg/ml) is necessary for biopterin to be effective as is shown in Fig. 3. Using the optimal concentration of folic acid and increasing amounts of biopterin, release is now more nearly proportional to the

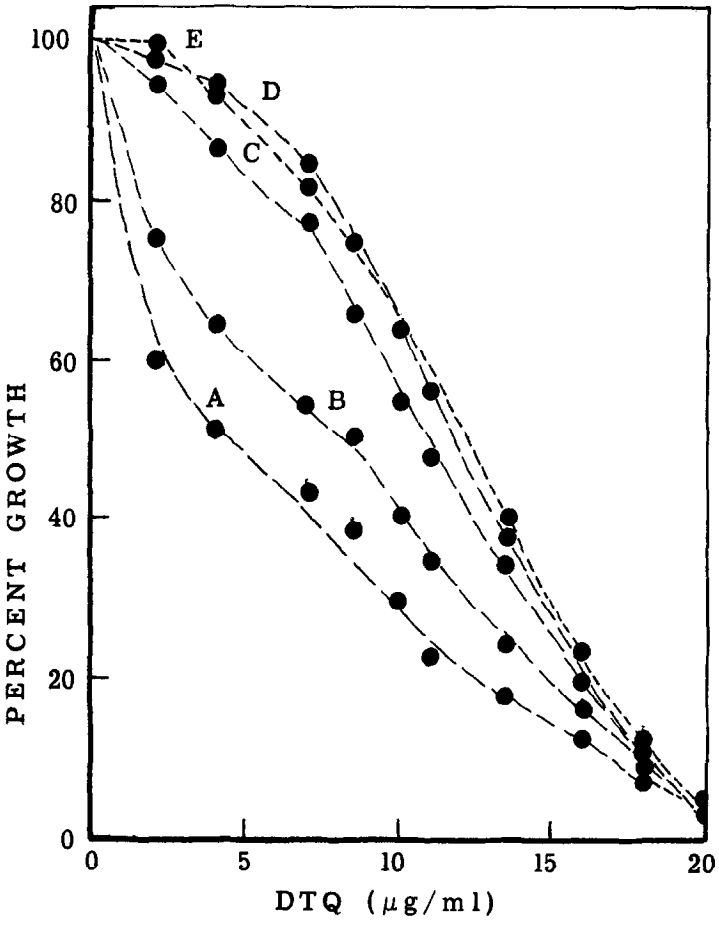

Fig. 2. Response of Crithidia fasciculata to 2, 4-diamino-5, 6, 7, 8-tetrahydroquinazoline (DTQ) in a medium containing 0.008 μg/ml of biopterin; 40 μg/ml thymidine and folic acid (μg/ml) as follows: A=0; B=0.001; C=0.01; D=0.1; E=1.0.

biopterin concentration. However, as the concentration of biopterin is doubled, the amount of DTQ required for half-maximal inhibition is increased by a factor of only 1.5-1.6 rather than 2. When the concentration of inhibitor required for half-maximal growth response is plotted against the logarithm of the biopterin concentration a straight line relationship is obtained (Fig. 4). This effect might have been predicted

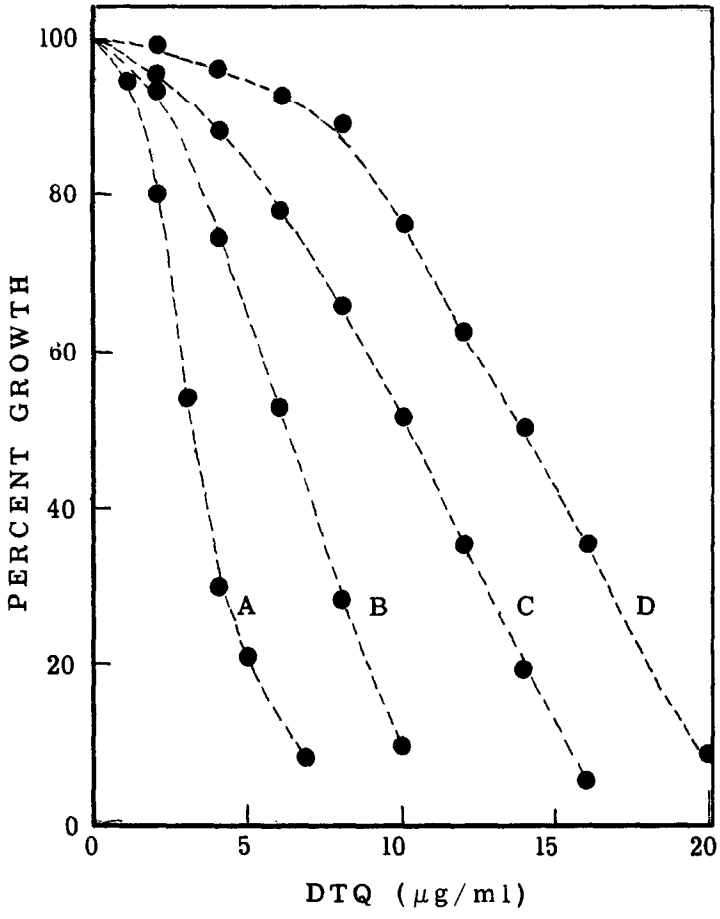

Fig. 3. Response of Crithidia fasciculata to 2, 4-diamino-5, 6, 7, 8-tetrahydroquinazoline (DTQ) 40 μg/ml thymidine, 0.1 μg/ml folic acid and biopterin as follows (μg/ml): A=0.001; B=0.002; C=0.004; D=0.008.

inasmuch as the dose-response of Crithidia to biopterin is also logarithmic (Fig. 5).

While it is apparent that DTQ is inhibiting the growth of Crithidia by affecting the metabolism of biopterin, it is also clear that folic acid is in some way involved. This cannot be as a precursor of biopterin or other unconjugated pteridine because folic acid is very effective

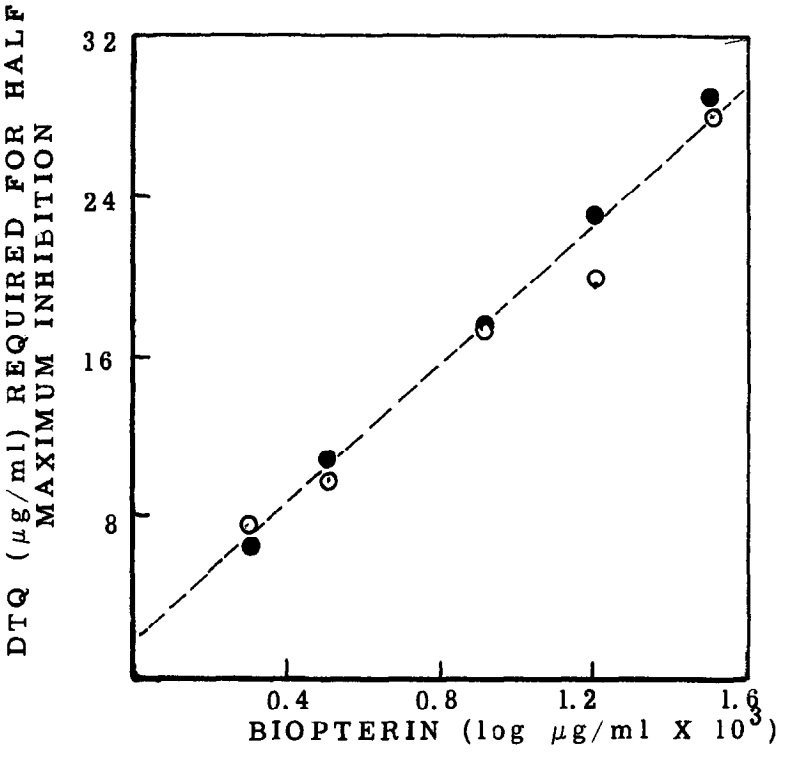

Fig. 4. Effect of biopterin, in the presence of folic acid, 0.1 μg/ml (○) and 1.0 μg/ml (●), on the inhibition of Crithidia fasciculata by 2, 4-diamino-5, 6, 7, 8-tetrahydroquinazoline (DTQ).

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in reversal at levels (0.01-0.10 μg/ml), which are insufficient to permit growth on folic acid as a biopterin precursor (Kidder, et al., 1967). Furthermore, a level (1.0 μg/ml) of folic acid capable of supplying the unconjugated pteridine requirement is no more effective than 0.1 μg/ml.

Since all of the compounds known to require folic acid derived cofactors in their biological formation (methionine, purines, thymidine and serine) are already present in the medium, there remains some unknown function(s) of folic acid, which is not essential for the growth of the organism, but which is necessary for growth to a high population

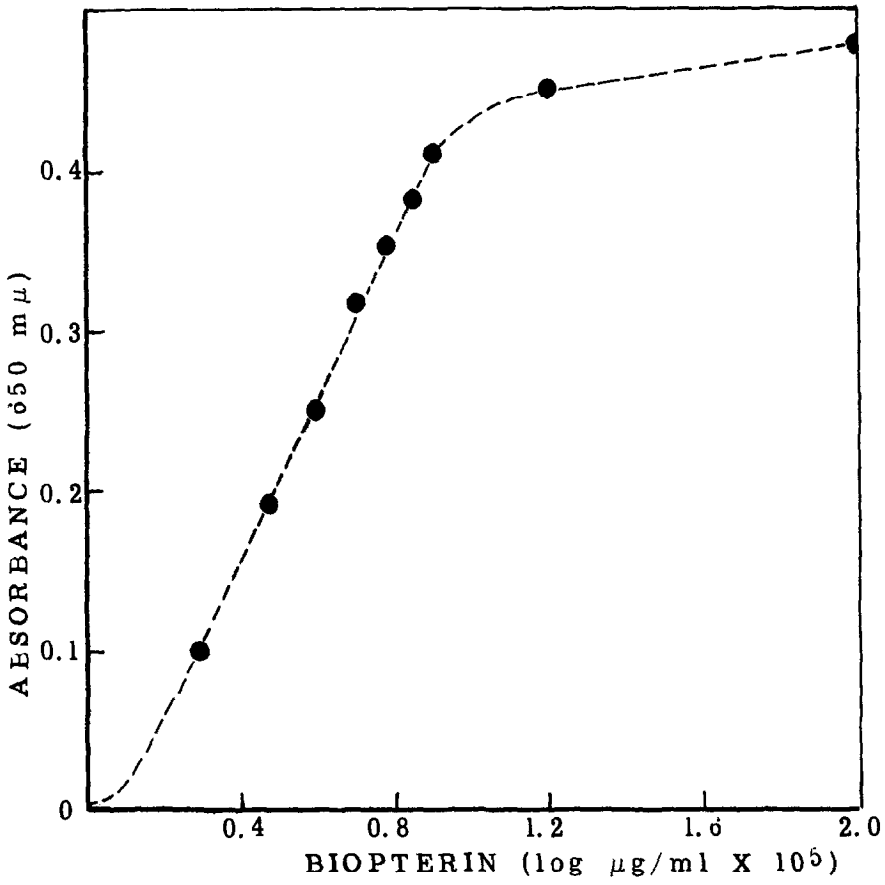

Fig. 5. Dose response of Crithidia fasciculata to 6-biopterin. Medium contained folic acid (0.001 μg/ml)

density and for the utilization of biopterin in reversal of inhibition by DTQ.

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