RELATIONSHIP BETWEEN PYRIMIDINE AND LIPID BIOSYNTHESIS AND UNCONJUGATED PTERIDINE*

G. W. Kidder and Virginia C. Dewey

Biological Laboratory, Amherst College
Amherst, Mass.

Received June 25, 1963

The trypanosomid flagellate, Crithidia fasciculata, exhibits a nutritional requirement for an unconjugated pteridine, which can be most efficiently satisfied by either 2-amino-4-hydroxy-6-dihydroxypropylpteridine (biopterin of Patterson, et al., 1955) or 2-amino-4-hydroxy-6-trihydroxypropylpteridine (neopterin of Rembold and Buschmann, 1963). The active cofactor is probably similar to that described by Kaufman (1962). Only when folic acid is included in the medium at extremely high levels (0.1 - 1 γ/ml) can unconjugated pteridine be omitted. This was interpreted as indicating a precursoral relationship between the conjugated and the unconjugated form (Kidder and Dutta, 1958). The need for folic acid can be by-passed by the addition of thymine (Nathan, et al., 1956; Kidder and Dutta, 1958; Dewey et al., 1959) in which case total cell yields are reduced to about one half. Other nucleic acid pyrimidines can be synthesized by the organism either in the presence or absence of folic acid; if unconjugated pteridine is available.

Recently unconjugated pteridines have been implicated as the hydroxylation cofactor for tyrosine formation from phenylalanine (Kaufman, 1962) and as a cofactor for the degradative oxidation of glyceryl ethers

* Supported by grants (AM 01005) and CA 02924) from the National Institutes of Health, U. S. Public Health Service and a grant (T-130D) from the American Cancer Society.

(Tietz, et al., 1963). Both of these reactions require molecular oxygen.

That other functions for unconjugated pteridines exist is evidenced by the fact that while tyrosine cannot be synthesized by Crithidia it still requires unconjugated pteridine (Cowperthwaite, et al., 1953; Kidder and Dutta, 1958).

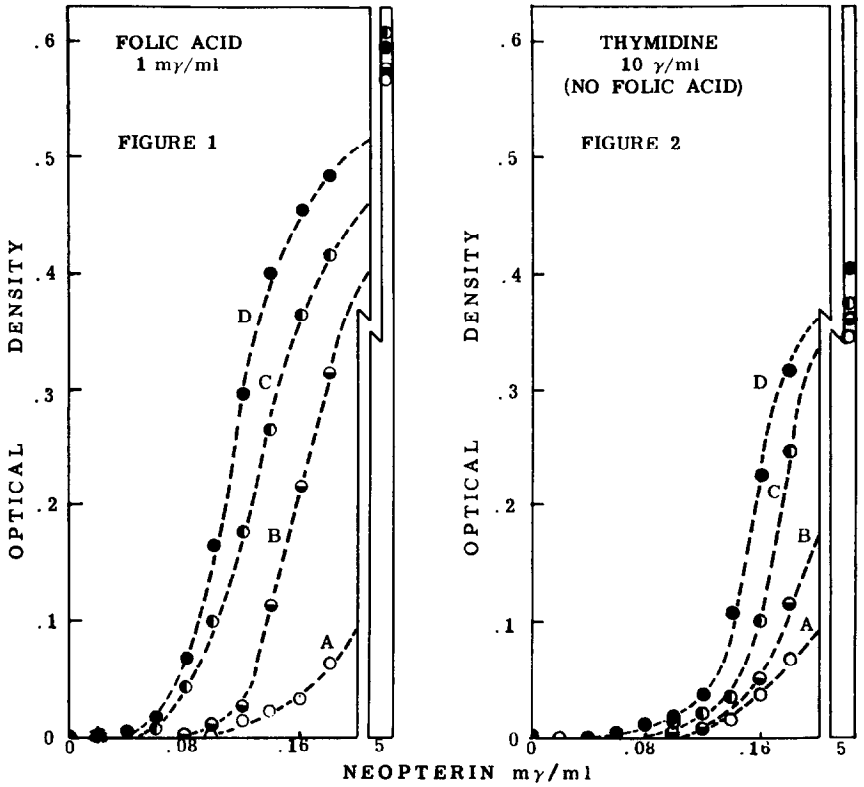
We now wish to report that evidence has been obtained implicating the cofactor formed from either biopterin or neopterin in the biosynthesis of nucleic acid pyrimidines, unsaturated fatty acids and sterols in Crithidia fasciculata.

In medium I of Kidder and Dutta (1958), from which all pyrimidines, purines (except adenine) and pteridines have been omitted, growth does not occur (4 day incubation) when pteridine-depleted organisms are used for the inoculum. Growth response occurs in relation to the concentration of unconjugated pteridine when either folic acid (1 mγ/ml) or thymidine (10 γ/ml) is added, although yields are reduced in the latter case (Figs. 1 and 2, curves A). The addition of uracil, cytosine, their nucleosides or nucleotides produces marked sparing of the unconjugated pteridine (Figs. 1 and 2, curves B). Orotic acid, dihydoorotic acid and dihydrouracil were inactive, although the first two may encounter permeability difficulties in the alkaline medium, used of necessity for hemin solubility.

The addition of lipid material (animal lecithin in Figs. 1 and 2, curves C) exhibits even greater sparing. This effect can be produced by unsaturated fatty acids (a mixture of oleic and linoleic acids) and by a saponified lipid fraction of Crithidia cells.

When both pyrimidine and lipids are present together the sparing effect is additive (Figs. 1 and 2, curves D).

In preliminary experiments we have found that an unsaponified



A = No addition; B = Cytidine (40 γ/ml); C = Animal lecithin (200 γ/ml; D = Cytidine + Animal lecithin

fraction of Crithidia lipids (containing sterols) also shows neopterin sparing ability, either alone or when added with animal lecithin and cytidine.

We interpret these results to mean that Crithidia can synthesize pyrimidines, desaturate fatty acids and synthesize sterols only in the presence of pteridine cofactor. It is possible that the unknown cofactor for the desaturation of fatty acids by the Mycobacterium system reported by Fulco and Bloch (1962), an oxygen requiring reaction, is an unconjugated pteridine, inasmuch as these compounds are extremly active, are somewhat unstable and would be expected to be present in materials of natural origin. Similarily the pteridine cofactors may play a role in the oxygen requiring reactions (Bloch, 1962) in the bio-

synthesis of steroids. It may be, at least in our system, that the oxidation of dihydroorotic acid is oxygen dependent and requires a pteridine cofactor leading to the biosynthesis of nucleic acid pyrimidines. The nature of the enzymes concerned in this reaction in obligate aerobic organisms is unknown and conceivably could involve hydroxylation followed by dehydration, for it is known that biosynthetic pathways in aerobic and anaerobic organisms are different in some cases (Bloch, 1962). Alternatively, it cannot be ruled out that the pteridine cofactor may act as a hydrogen acceptor. The inactivity of orotic acid could only be explained on the grounds of impermiability, and the true situation must await evidence from cell-free experiments.

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