REPLACEMENT OF THE BIOPTERIN REQUIREMENT OF CRITHIDIA

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The requirement of the trypanosomid flagellate, Crithidia fasciculata, for an unconjugated pteridine (biopterin) has been demonstrated (Patterson, Broquist, Albrecht, von Saltza and Stokstad, 1955; Patterson, Milstrey and Stokstad, 1956; Kidder and Dutta, 1958). Analogs of biopterin were prepared by the method of Petering and Schmitt (1949) by coupling a variety of hexoses with a number of suitably susbstituted pyrimidines (Table 1.). Organisms depleted of pteridines were used to inoculate media containing the reaction products. In no case was the crude product isolated from the reaction mixtures or any fraction obtained

Table 1.

Activity of analogs of biopterin for Crithidia fasciculata.

Pyrimidine	Sugar	Product	Activity*
2, 4, 5-tri-NH_2-6-OH-pyrimidine	l-arabinose	2-NH_2-4-OH-6-(l-erythro)-tri-OH-propylpteridine	.0002
"	d-galactose	2-NH_2-4-OH-6-(d-lyxo)-tetra-OH-butylpteridine	.0006
"	l-mannose	2-NH_2-4-OH-6-(l-arabo)-tetra-OH-butylpteridine	.0005
"	d-glucose	2-NH_2-4-OH-6-(d-arabo)-tetra-OH-butylpteridine	.002
4, 5, 6-tri-NH_2-pyrimidine	d-galactose	4-NH_2-6-(d-lyxo) tetra-OH-butylpteridine	10-100
2, 4, 5, 6-tetra-NH_2-pyrimidine	"	2, 4-NH_2-6-(d-lyxo)-tetra-OH-butylpteridine	0.1
2, 4, 5-tri-NH_2-6-CH_3-pyrimidine	"	2-NH2-4-CH_3-6-(d-lyxo)-tetra-OH-butylpteridine	1.0

*amount (γ/ml) required for half-maximal growth.

during purification procedures inhibitory. All showed replacement activity, usually of a low degree (except the product of the reaction of 2, 4, 5-triamino-6-hydroxypyrimidine with d-galactose).

In the hope that it might be possible to find a pyrimidine which would produce inhibition reversible by biopterin, a number of compounds was tested, including 2, 4, 5-triamino-6-hydroxypyrimidine, the precursor of biopterin in chemical synthesis (Table 2). It was found that this pyrimidine will replace biopterin for the growth of Crithidia, although rather inefficiently. Upon the addition of either l-arabinose or l-rhamnose to media containing the pyrimidine an effect is noted (Fig. 1) which may be sparing of the coenzymatic activity of biopterin or an increased efficiency of utilization of the pyrimidine. Other sugars, either pentoses or hexoses, had no effect or were inhibitory. The thio analog of this pyrimidine also replaced, but with very low activity.

Table 2.

Effect of pyrimidines on growth of Crithidia fasciculata.

Pyrimidine	Concentration	Activity*
6-methyl-	100 γ/ml.	-
4, 5-diamino-6-oxy-2-thio-	1-10 γ/ml.	Replacement
4, 5-diamino-2, 6-dioxy-	100 γ/ml.	-
4, 5, 6-triamino-2-oxy-	"	-
4, 5, 6-triamino-	"	-
2, 4, 5-triamino-6-hydroxy-	0.1 γ/ml.	Replacement
2, 4, 5-triamino-6-methyl-	1-10 γ/ml.	Inhibition
Uracil	100 γ/ml.	-
5-Nitrouracil	"	-
4-methyluracil	"	-

*- = no activity. Replacement or inhibition occurs at the concentrations indicated.

Of the other pyrimidines tested only one, 2, 4, 5-triamino-6-methylpyrimidine, produced inhibition. This effect was reversed by

biopterin (Fig. 2). When this pyrimidine was condensed with galactose, however, a compound with growth promoting properties was produced (Table 1).

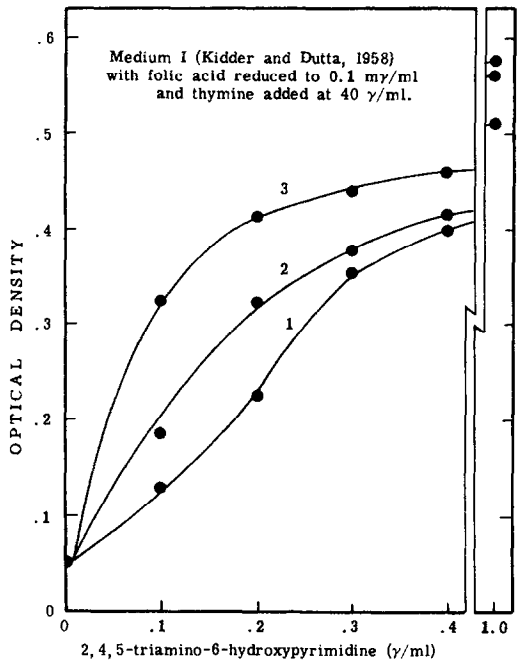

Fig. 1

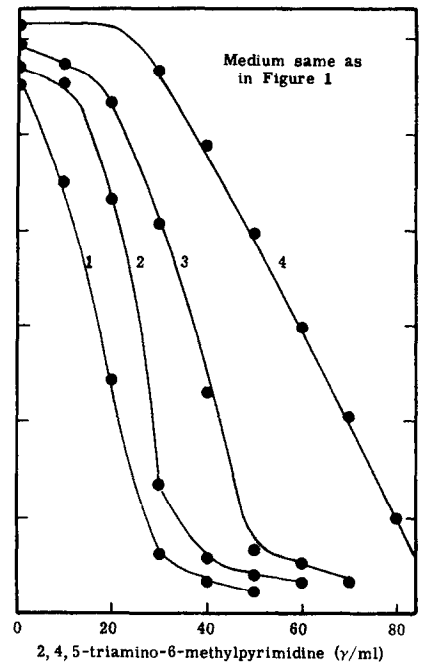

Fig. 2

FIGURE 1. (1 = control; 2 = l-rhamnose added at 1 mg/ml; 3 = l-arabinose at 1 mg/ml)
FIGURE 2. (1 = biopterin 0.5 mγ/ml; 2 = 1 mγ/ml; 3 = 2 mγ/ml; 4 = 5 mγ/ml)

It appears that Crithidia is deficient only in the capacity to synthesize the appropriate pyrimidine rather than the ability to synthesize a pteridine. The flagellate, however, does not require the addition of such pyrimidines as uracil or cytosine to the medium and requires thymine only in the absence of folic acid. Cultures have been maintained for a number of months in media containing thymine and a minimal amount of biopterin instead of folic acid. Growth is about half the maximal growth obtained in the presence of folic acid. Increasing the

amount of biopterin in the medium or the incubation period does not improve growth.

References

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Received July 1, 1959