FOLIC ACID SPARING BY TRIAMINO PYRIMIDINES*

G. W. Kidder and Virginia C. Dewey

Biological Laboratory, Amherst College
Amherst, Mass.

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Unlike Lactobacillus casei (Stokstad, 1941), Streptococcus faecalis (stokes, 1944) and Crithidia fasciculata (Kidder and Dutta, 1958) Tetrahymena pyriformis does not grow in a medium lacking folic acid, even when the medium is supplemented with purines, thymine and a number of other compounds known to be synthesized by enzymes requiring some form of folic acid as a cofactor.

The requirement of Crithidia for an unconjugated pteridine (biopterin), which can be formed biosynthetically from certain triamino-pyrimidines (Dewey, Kidder and Butler, 1959) or from folic acid (Kidder and Dutta, 1958), led us to investigate the possibility that Tetrahymena may also utilize in its metabolism an unconjugated pteridine which it can synthesize readily from folic acid. A number of pyrimidines was tested, therefore, for their ability to spare the folic acid requirement of this organism.

Tetrahymena pyriformis W was grown in medium A (Dewey, Parks and Kidder, 1950) containing Tween 80 at 10 mg/ml, from which folic acid was omitted. Dose responses to folic acid were studied in

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this medium with and without the additions of various supplements, as indicated. Organisms used for inoculation had been partially depleted of folic acid reserves by continued growth in low folic acid (0.5 mγ/ml) medium. The pyrimidines which were tested for folic acid sparing activity were 2, 5, 6-triamino-4-hydroxypyrimidine, (TAHP), 2, 6-diamino-4-hydroxy-5-formyl-aminopyrimidine (FAP) and 2, 6-diamino-4-hydroxy-5-acetylaminopyrimidine (AAP). They were sterilized by filtration and added to the medium aseptically.

All three pyrimidines proved to be active in sparing the folic acid requirement of Tetrahymena, although FAP was most active in this respect. The superiority of FAP over TAHP may well be due to its greater stability. In Table 1. is shown the response of Tetrahymena to folic acid with and without the addition of FAP. This formylamino pyrimidine also shows sparing activity in the presence of thymine (or thymidine)

Table 1.

Response of Tetrahymena to folic acid in media with and without supplementation

ADDITIONS TO BASAL MEDIUM	FOLIC ACID mγ/ml						
	0	0.02	0.06	0.10	0.14	0.18	1.0
	optical density						
NONE	.02*	.03	.03	.04	.05	.08	.65
PYRIMIDINE**	.04*	.04	.05	.05	.07	.12	.68
S***	.16*	.25	.37	.47	.52	.59	.65
PYRIMIDINE + S	.28*	.37	.51	.62	.65	.67	.65

* Will not grow in second transplant

** 2, 6-diamino-4-hydroxy-5-formylaminopyrimidine (1 γ/ml)

*** Supplement = glycine (100 γ/ml), thymidine (10 γ/ml), tyrosine ethylester (50 γ/ml), hydroxyproline (50 γ/ml), cysteine (10 γ/ml), creatine (40 γ/ml), PABA (.001 γ/ml), cyanocobalamin (.001 γ/ml), stigmasterol (5 γ/ml), biopterin (.001 γ/ml)

and glycine as well as in the presence of a mixture of compounds (S in Table 1) known to, or suspected of sparing folic acid. Concentrations of FAP as low as 0.1 γ/ml show sparing activity which increases up to a concentration of 1 γ/ml, above which no further increase in sparing is obtained. This small amount of pyrimidine which produces maximal sparing effect, as well as the fact that either TAHP or the acetylamino derivative (AAP) are also active, seems to rule out the possibility that the formyl compound is acting as a one carbon donor (in the synthesis of formyltetrahydrofolic acid, for example).

Crithidia can utilize TAHP to replace biopterin (Dewey, et al., 1959) but the formylamino compound is inactive. This inactivity probably indicates the organisms' inability to deacylate FAP. Tetrahymena, on the other hand, appears to possess deacylases capable of releasing TAHP from either FAP or AAP.

At the present we interpret these results to mean that Tetrahymena utilizes TAHP for the synthesis of a pteridine (not biopterin, which has no activity for Tetrahymena) also derived from folic acid when it is the only available source. Although the folic acid sparing effects of these pyrimidines are not large they are consistant. The order in which sparing compounds are added is important in determining the magnitude of the effect produced. It should be noted, as further evidence for the synthesis of unconjugated pteridines by Tetrahymena, that culture media in which the organisms have grown and which contained small amounts of folic acid as the only source of pteridines, yield non-folic acid fractions with high biopterin-replacing activity for Crithidia.

The above results suggest an explanation for earlier observations (Dewey and Kidder, 1953) in which it was found that folic acid analogs,

in which the glutamic acid residue was replaced by other amino acids, were capable of sparing, but not replacing, the folic acid requirement of Tetrahymena. Such compounds could act as a source of unconjugated pteridines equally as well as could folic acid.

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