A FOLIC ACID LINKED SYSTEM IN BACTERIAL CELL WALL SYNTHESIS?

Sir:

Much progress has been made in identifying the constituents of bacterial cell walls and in elucidating the biochemical reactions involved in cell wall synthesis[6]. However, a folic acid linked system has not been reported to be involved in cell wall synthesis. This communication presents evidence that a folic acid mediated reaction may indeed be involved, directly or indirectly.

Addition of trimethoprim[7] to cells of *Proteus vulgaris* growing as described previously[2], results in morphological changes dependent on trimethoprim concentration (Table 1). Trimethoprim concentrations above 50 μg/ml do not increase the number of cells that become spheroplasts. The morphological changes caused by different concentrations of trimethoprim are unlike those induced by a classical inhibitor of bacterial cell wall synthesis such as penicillin. Low concentrations of penicillin cause sensitive gram-negative cells to elongate and enlarge. At somewhat higher concentrations, these cells form hernias. With increasing concentrations, the hernias enlarge, and with higher concentrations spheroplasts are formed. Low concentrations of trimethoprim cause cells to elongate and enlarge. However, hernias never occur prior to spheroplast formation in response to increasing trimethoprim concentrations. The cells enlarge into spheroplasts.

Table 1. Morphological changes induced in *Proteus vulgaris* by trimethoprim

μg/ml	Frequency of morphological changes
0.1	Slight elongation and increase in girth of all cells
0.25	Infrequent ovoid cell: no spheroplasts
0.5	<1 % large ovoid cells: no spheroplasts
1.0	10 % large ovoid cells: no spheroplasts
2	10 % spheroplasts
4~10	40~50 % spheroplasts
50→	60~80 % spheroplasts

All cells not noted as spheroplasts or ovoid were markedly elongated and wider in girth.

Trimethoprim is a known inhibitor of dihydrofolic acid reductase[7]. Other known inhibitors of this enzyme, *i. e.*, 2,4-diamino-5-butoxy-pyrimidine[3], amethopterin[5], and aminopterin[5] also induce spheroplast formation in this strain of *P. vulgaris* but are only one eighth, one tenth and one twenty-fifth as active on a weight basis as trimethoprim. 2, 4-Diamino-N, N-dimethylquinazoline sulfonamide (British Patent Specification 1,143,290, Feb. 19, 1969), and 4,6-diamino-1, 2-dihydro-2, 2-dimethyl-1-(1-naphthylmethoxy)-1,3,5-triazine, both of which belong to groups known to be inhibitors of the enzyme[5], are also spheroplast inducers. These two compounds are approximately one tenth as active as trimethoprim. 2-Hydrazino-3-mercaptoquinoxaline (German Patent 1,104,962, Dec. 28, 1959) also is only one tenth as active as trimethoprim.

Trimethoprim potentiates the antibacterial activity of sulfonamides *in vitro* and *in vivo*[1] which is not surprising since these compounds inhibit sequential steps leading to folic acid synthesis. Sulfadiazine and sulfaquinoxaline also potentiate the spheroplasting activity of trimethoprim. These two sulfa drugs have no observable morphological or inhibitory effect on *P. vulgaris* cells when used alone at concentrations from 0.01 μg/ml up to 400 μg/ml, the highest level tested in this complex medium. However, sulfadiazine at 1 μg/ml or sulfaquinoxaline at 5 μg/ml potentiates the spheroplasting action of trimethoprim at 1 μg/ml.

Folinic acid reverses the spheroplasting activity of trimethoprim. However, 1 mg/ml of the compound is required to reverse the effect of 1 μg/ml of trimethoprim. This ratio may be due to cellular impermeability to folinic acid.

Trimethoprim concentrations up to 400 μg/ml did not induce cells of a strain of *Escherichia coli* or a strain of *Vibrio percolans* to form spheroplasts. However, cells of these two bacteria did double in length and increase in girth. Thus the *P. vulgaris* used in this study is exquisitely sensitive to trimethoprim.

NICKERSON and WEBB[4] reported structural changes induced in bacteria growing in presence of aminopterin. The compound

caused *E. coli* and *Aerobacter aerogenes* to form long filamentous cells without cross walls. *Serratia marcescens* responded to aminopterin by forming swollen coccoidal cells as well as distorted filaments. These changes are indicative of an effect on cell wall formation. It appears that there is a folic acid linked system that affects bacterial cell wall synthesis.

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