Synthesis and Activity of Phthalylglutamic Acid Analogs as Potential Folic Acid Antagonists in Bacteria

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Four phthalylglutamic acid analogs were found to have no activity as inhibitors of bacterial growth in chemically defined media. More specifically, the compounds were not active as folic acid antagonists in bacteria. Cell permeability studies on these substances have not been conducted yet; consequently, this phenomenon is not ruled out as the mechanism whereby these compounds are inactive.

FAIGLE *et al.* (1) found that the principal metabolic products of *N*-(2,6-dioxo-3-piperidyl)phthalimide (thalidomide) in the dog were hydrolytic products and consisted chiefly of glutamic acid derivatives. *N*-Phthalyl-D,L-glutamic acid and *N*-(*o*-carboxybenzoyl)-D,L-glutamic acid were shown to be two of the main hydrolytic products. Interestingly, thalidomide is the only sedative thus far described whose metabolites are glutamic acid derivatives. Kempner (2) reported that thalidomide antagonized the growth and sexual development of cockerels and suggested that thalidomide or certain of its metabolites may be folic acid antagonists. A clear illustration of the structural similarities between folic acid and some thalidomide metabolites also was shown by Faigle *et al.* (1). These workers suggested that the neurotoxic and embryotoxic effects of thalidomide or products of its metabolism may be due to an antagonism of glutamic acid or glutamine. This view was also held by Roath *et al.* (3), who showed that both isomers of thalidomide and certain degradation products of the drug inhibited development of human leucocytes. In addition, Frank *et al.* (4) found that thalidomide inhibited the growth of certain protozoa, and that this inhibition was reversed by nicotinic acid, adenine dinucleotide, and menadione.

On the basis that folic acid antagonists are known to be teratogenic (5) and that in many instances congenital malformations in animals are associated with vitamin deficiencies (primarily folic acid and riboflavin) and the suggestion of others that thalidomide metabolites may function as folic acid antagonists, it was of interest to test this hypothesis in a bacterial system. For this purpose, four phthalylglutamic acid analogs were synthesized and tested for possible antagonism of folic acid utilization and synthesis in a microorganism which requires preformed folic acid and in one which synthesizes all of its own vitamins and amino acids.

The test compounds, *N*-phthalyl-D,L-glutamic acid anhydride, *N*-phthalyl-D,L-glutamic acid, and *N*-(*o*-carboxybenzoyl)-D,L-glutamic acid, were synthesized according to the methods of King and Kidd (6). The pure isomer, *N*-(*o*-carboxybenzoyl)-L-glutamic acid, was prepared according to the method of Nefkens *et al.* (7) for *N*-phthalyl-L-glutamic acid, followed by partial alkaline hydrolysis. This isomer had a corrected melting point of 159.5 to 160.5°, and $[\alpha]_D^{26} = -29°$ ($c = 1$, methanol). Identity of all compounds was confirmed by melting point determinations, elemental analyses for carbon, hydrogen, and nitrogen, infrared and NMR spectra, and optical rotations.

All compounds were tested for their ability to inhibit the growth of *Escherichia coli* B in the synthetic medium of Davis and Mingioli (8). In addition, they were tested for their ability to inhibit utilization of folic acid in Difco's folic acid assay medium, a modified medium of Capps *et al.* (9), using *Streptococcus faecalis* (ATCC 8043), a folic acid dependent bacterium. Neutralized solutions of test compounds, and of folic acid where applicable, were sterilized by Seitz filtration and portions added to previously autoclaved culture tubes containing graded levels of sterile distilled water and basal medium. Only freshly prepared solutions of test compounds were used. Final assay volumes were 6 ml. per culture tube (18 × 150 mm.). In the preparation of the inocula, precautions were taken to minimize the carry-over of preformed folic acid by appropriate washing of the cultures and by the use of small inocula. Incubation periods of 18–20 hours at 35° were used with both cultures. Growth was measured turbidimetrically as absorbance using a Coleman junior spectrophotometer at a wavelength of 620 mμ.

All of the glutamic acid analogs studied, at final concentrations of 1.0, 10, and 100 mcg./ml., fail to inhibit the growth of *S. faecalis* when cultured in the presence of 0.001 mcg./ml. of folic acid (a level required for about half maximal growth). At the concentrations tested, the ratios of inhibitor to metabolite screened were 1000:1, 10,000:1, and 100,000:1 (weight basis). In contrast, control tubes containing 4-amino-N^{10}-methylpteroylglutamic acid, a known folic acid antagonist, showed 64 and 95% inhibition of growth at inhibitor to metabolite ratios of only 1:1 and 8:1.

Growth of *E. coli* (a bacterium capable of synthesizing all of its own vitamins and amino acids) was not inhibited by concentrations up to 100 mcg./ml. of the glutamic acid analogs tested.

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