
COMMUNICATIONS TO THE EDITOR

RELATIONSHIP OF THE FOLINIC ACID GROUP AND THE LEUCONOSTOC CITROVORUM FACTORS

Sir:

The reported nutritional requirement of *Leuconostoc citrovorum* 8081 for thymidine¹ or a factor(s) in refined liver extracts² suggested the possibility that the organism required an anti-pernicious anemia principle. In the isolation of thymidine,³ this factor and erythrotin,⁴ a member of the vitamin B₁₂ group and probably identical with vitamin B₁₂, were separated quantitatively. While neither of these fractions possess the activity of the original liver extract for *Leuconostoc citrovorum* in a previously described medium⁵ supplemented with pyridoxine, the two fractions on recombination possessed the full activity. The thymidine containing fraction could be replaced by 0.03–0.1 γ per cc. of crystalline thymidine while the erythrotin-containing fraction could not be replaced by crystalline erythrotin. However, concentrates of the folinic acid⁶ were found to be highly effective in replacing the erythrotin fraction, and the relative potencies of these fractions determined by *Lactobacillus casei* test and this modified test paralleled closely.

A concentrate of folinic acid 200,000 times as active as an enzymatic digest of liver in the *Lactobacillus casei* test, elicited a half-maximal response in the *Leuconostoc citrovorum* test at 0.0001–0.0002 γ per cc. In the absence of thymidine, 0.001 γ per cc. of this factor was required for the same response. Thymidine at a concentration of 10–20 γ per cc. can also replace the folinic acid group; consequently, the synergistic action of the folinic acid group and thymidine in stimulating the growth of the organism resulted in the high activity of purified liver extracts which we have previously found to contain large amounts of thymidine.³

Other factors associated with the folinic acid appear to be effective for this organism, but folic acid is essentially inactive under our testing conditions. Very mild acid hydrolysis destroys folinic acid but forms a compound with biological activities corresponding to folic acid.

This synergistic action and interchangeability of thymidine and the folinic acid group indicate the functioning of this group in the biosynthesis of thymidine as well as further involvement of thymidine concerning the biosynthesis of

the active coenzyme form of folinic acid for this organism.

THE BIOCHEMICAL INSTITUTE AND THE THOMAS J. BARDOS
DEPARTMENT OF CHEMISTRY, THE THOMAS J. BOND
UNIVERSITY OF TEXAS, AND THE JEAN HUMPHREYS
CLAYTON FOUNDATION FOR RESEARCH WILLIAM SHIVE
AUSTIN, TEXAS

RECEIVED SEPTEMBER 23, 1949

THE FOLINIC ACID GROUP, A SERIES OF NEW VITAMINS RELATED TO FOLIC ACID

Sir:

By application of *inhibition analysis* to a study of factors functionally related to *p*-aminobenzoic and folic acids, testing procedures for a wide variety of new factors occurring in refined liver extracts have been developed.^{1,2} One of these methods developed about two years ago, involved the prevention of the toxicity of methylfolic acid for *Lactobacillus casei* in a previously described medium³ supplemented with thymine, purines, folic acid (0.001 γ per cc.) and methylfolic acid (1 γ per cc.). Under these conditions, only the folic acid group was known to prevent competitively the toxicity of methylfolic acid. However, liver extracts, both crude and refined, which prevent the toxicity in a competitive manner, are approximately 15 times as active as can be accounted for on the basis of their folic acid content determined by assay with *Lactobacillus casei* in the absence of the inhibitor. A similar technique has been employed with *Streptococcus faecalis* R in demonstrating an unusual activity for formylfolic acid.⁴

With the aid of this assay based on this differential in activity, one of the active principles has been concentrated more than 200,000 fold from enzymatic digests of hog liver. A half-maximal response of *Lactobacillus casei* is obtained in the presence of 0.002 γ per cc. of the concentrate which is somewhat more active than folic acid under these testing conditions. Depending upon the time of incubation and response at which the comparison is made, the concentrate is from 10 to 100 times as active as folic acid in preventing the toxicity of methylfolic acid (1 γ per cc.) for *Streptococcus faecalis* R. On the basis of structure and functional relationship to folic acid, this active principle has been termed folinic acid. On the basis of estimated purity of the concentrate, folinic acid does not appear to be less active than folic acid in promoting the growth of either organism in the absence of the inhibitor.

(1) Snell, *et al.*, *J. Biol. Chem.*, **175**, 473 (1948).
(2) Sauberlich and Baumann, *ibid.*, **176**, 165 (1948).
(3) Shive, *et al.*, *THIS JOURNAL*, **70**, 2299 (1948).
(4) Shive, *Ann. New York Acad. Science*, in press, presented before the New York Acad. Science, Feb., 1949.
(5) Snell, *et al.*, *J. Biol. Chem.*, **143**, 519 (1942).
(6) Bond, *et al.*, *THIS JOURNAL*, **71**, 3852 (1949).

(1) Shive, *et al.*, *THIS JOURNAL*, **70**, 2299 (1948).
(2) Shive, *Ann. New York Acad. Science*, in press, presented before the New York Acad. of Science, Feb., 1949.
(3) Rogers and Shive, *J. Biol. Chem.*, **172**, 100, 751 (1947).
(4) Gordon, *et al.*, *THIS JOURNAL*, **70**, 878 (1948).