A Growth Factor for *L. citrovorum* Synthesized by Hemopoietic Tissue Reversing Aminopterin and Chloromycetin Inhibition.* (19732)

MARIAN E. SWENDSEID, PATSY D. WRIGHT, AND FRANK H. BETHELL.
(Introduced by C. C. Sturgis.)

From the Thomas Henry Simpson Memorial Institute for Medical Research, Ann Arbor, Mich.

When suspensions of leukocytes or myeloid elements of bone marrow obtained from human sources are incubated with folic acid under conditions similar to those described for rat liver tissue(1,2), a substance is formed which stimulates the growth of *L. citrovorum* without the addition of folinic acid† but which cannot be assayed accurately(3) using this vitamin as a standard curve. When turbidity readings are plotted against increasing concentrations of leukocyte extracts, a growth response curve is obtained which is distinctly different from folinic acid (Fig. 1). Treatment with hog kidney conjugase(4) does not alter the type of response curve, an indication that the factor is not a glutamyl conjugate of folinic acid(5).

Since this factor is produced by an enzymatic process in hemopoietic tissue in the presence of folic acid, it has been tentatively designated as *hemofolin* for reference purposes. Although it would appear that hemofolin is a hitherto undescribed derivative of folic acid, the possibility remains that it is a product of some reaction in which folic acid is an obligatory participant.

Hemofolin has also been characterized as to its role in reversing the inhibition of *L. citrovorum* produced by 4-aminopteroylglutamic acid (Aminopterin) and by chloramphenicol (Chloromycetin). The results in Table I show that both hemofolin and folinic acid reverse Aminopterin inhibition. In the case of some levels of chloromycetin inhibition, however, whereas folinic acid is inactive as a reversing agent, hemofolin will promote growth of the bacteria in the presence of the antibiotic.

These data assume importance from several standpoints. In the first place, the differing behavior of hemofolin and folinic acid in chloromycetin inhibition - reversal studies serves to emphasize the distinctiveness of hemofolin and to offer a further means of distinguishing it from folinic acid. The fact that hemofolin can overcome the growth inhibition of chloromycetin constitutes evidence relating the action of this antibiotic to folic acid metabolism in bacteria. Some preliminary results have indicated that such a relation exists in animal tissue as well(6).

These inhibition studies also suggest that the metabolically active form of the folic acid vitamins in *L. citrovorum* may be more closely identified with hemofolin than with folinic acid. The reversal of aminopterin by both folinic acid and hemofolin and of chloromycetin by hemofolin alone is interpreted to mean that folinic acid is a metabolic antecedent of hemofolin.

Attempts at concentration of hemofolin have been hampered by the extremely small amounts of the substance available. It has not been demonstrated to occur in leukocytes prior to incubation with folic acid, in liver tissue before and after folic acid incubation, or in kidney or intestine although its presence in some of these substances may be masked by the preponderance of other vitamin forms, There is some evidence that it occurs in certain types of yeast extract. It is hoped that the development of specific inhibition analysis technics will enable a more critical evaluation to be made as to whether hemofolin normally exists in natural materials.

For the most part, sources of the hemofolin-producing enzyme have been the peripheral leukocytes of persons with chronic leukemia. Assigning a value of 10 units to the amount of hemofolin necessary for optimal growth in *L. citrovorum,* from 250 to 700 units are formed per ml of leukocytes under the assay conditions employed. These include addition of folic acid and ascorbic acid with incubation

* Supported in part by a grant from Parke, Davis and Co.

† We are indebted to Dr. William Shive of the University of Texas for this material.

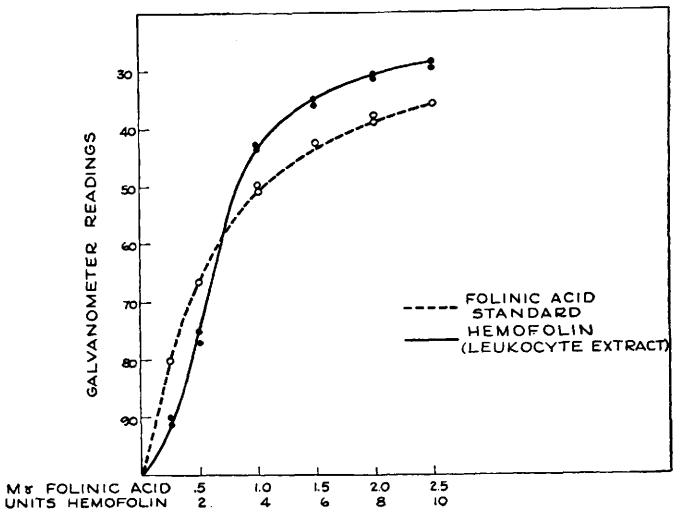

FIG. 1. 24 hr growth response of *Leuconostoc citrovorum* to a substance contained in leukocytes incubated with folic acid (hemofolin).

at 38°C in phosphate buffer pH 7.0. The enzyme for producing hemofolin has also been found in splenic tissue from various animals.

From the evidence presented as to its mode of formation, it might be presumed that hemofolin is closely related to or identical with an active coenzyme form of folic acid which is of special significance in hemopoietic cells. In this connection, it might be suggested that the ability to produce hemofolin is lacking in pernicious anemia. However, experiments have shown that bone marrow cells from untreated pernicious anemia patients can synthesize hemofolin where folic acid is the substrate.

TABLE I. Reversal of Aminopterin and Chloromycetin Inhibition of *L. citrovorum* by Hemofolin.

Inhibitor	Concentration, γ/tube	Amt for ½ max growth: Folinic acid, mγ/tube	Amt for ½ max growth: Hemofolin concentrate, units/tube
None	—	1.25	5
Aminopterin	25	3.40	10.9
	50	5.80	25
Chloromycetin	40	>250	14.4
	90	>250	51.8

Summary. An apparently new growth factor for *L. citrovorum* has been described. It is differentiated from folinic acid on the basis of growth-response curves, and reversal of chloromycetin inhibition. This factor is produced by homopoietic tissue incubated with folic acid and has been called hemofolin.

1. Nichol, C. A., and Welch, A. D., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 52.

2. Broquist, H. P., Stokstad, E. L. R., and Jukes, T. H., *J. Lab. and Clin. Med.*, 1951, v38, 95.

3. Sauberlich, H. E., and Baumann, C. A., *J. Biol. Chem.*, 1948, v176, 165.

4. Bird, O. D., Robbins, M., Vandenbelt, J. M., and Pfiffner, J. J., *J. Biol. Chem.*, 1946, v163, 649.

5. Swendseid, M. E., Ackermann, W. W., and Bethell, F. H., *J. Biol. Chem.*, 1951, v190, 791.

6. Swendseid, M. E., Swanson, A. L., and Bethell, F. H., *Fed. Proc.*, 1952, v2, 296.

Received July 14, 1952. P.S.E.B.M., 1952, v80.