PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 67–69 (1974)

Accumulation of Formimino-L-Glutamic Acid in the Free-Living Nematode *Caenorhabditis briggsae* as Related to Folic Acid Deficiency[1] (37749)

N. C. Lu, W. F. Hieb, and E. L. R. Stokstad

Department of Nutritional Sciences, University of California, Berkeley, California 94720

Formimino-L-glutamic acid (FIGLU), which is an intermediate of histidine degradation, is excreted in large amounts in the urine of folic acid deficient rats and humans (1–3). Urinary excretion of FIGLU can be raised by increasing the level of dietary histidine in rats (4). Luhby and Cooperman (5) have used the "histidine load" test for diagnosis of folic acid deficiency in humans. However, very little is known about histidine degradation in folic acid deficiency in lower animals. The present study was conducted with the axenically grown free-living nematode, *Caenorhabditis briggsae,* which requires folic acid for reproduction (6).

Materials and Methods. The chemically defined medium, *C. briggsae* maintenance medium (CbMM) (7), was obtained from Grand Island Biological Co., Grand Island, NY. Mass cultures were set up in 50 ml long neck flasks according to the method described by Tomlinson and Rothstein (8).

"Preculture." Preculture was carried out to grow folic acid deficient worms which were used as inoculum for the "histidine load" cultures. The 5 ml preculture contained CbMM, 50 μg/ml cytochrome *c* (Sigma Chemical Co., St. Louis, MO), 50 μg/ml β-sitosterol (Sigma Chemical Co.), and was supplemented with 10 mg/ml casamino acids (acid hydrolyzed casein) (Difco Laboratories, Detroit, MI). A concentration of 10 ng/ml folic acid (Sigma Chemical Co.) was used in the medium to ensure a reasonable growth of the worms. The preculture was stopped at Day 20. The worms from 10 ml were combined and were used as inoculum in the histidine load cultures.

"Histidine load" cultures. In addition to a basal concentration of 283 μg/ml L-histidine (1x) present in the original CbMM, another 566 μg/ml L-histidine (Calbiochem, Los Angeles, CA) was added in the "histidine load" media, resulting in a total concentration of 849 μg/ml L-histidine (3x). Four different combinations of the test media, two levels of histidine (1x and 3x), each with and without folic acid, were prepared in duplicate. The 5 ml basal medium contained CbMM, 50 μg/ml cytochrome *c*, 50 μg/ml β-sitosterol, 0 or 7.5 μg/ml folic acid, and was supplemented with 10 mg/ml casamino acids.

Tissue preparation and extraction. The worms were separated from the medium by light centrifugation for 5 min. The medium was immediately stored at −20°. The worms were washed twice with water and extracted by homogenizing in 1 *M* potassium phosphate buffer (pH 7.2); the supernatant was stored at −20°. FIGLU was then determined in both worm tissue extract and test medium by the enzymatic method described by Tabor, Colowick and Kaplan (9).

Results and Discussion. The final worm population and FIGLU in the basal control medium [+FA +His (1x)], the "histidine load" control medium [+FA +His (3x)], and the two folic acid deficient media [−FA +His (1x), −FA +His (3x)] were determined and the results are shown in Table I. The development of folic acid deficiency in worms on the two folic acid deficient media was clear. The final populations in these media were, respectively, 1/20 and 1/30 of that in the corresponding control medium. The population in the high histidine medium

[1] Supported in part by U.S. Public Health Service Grant No. AM-12625.

TABLE I. FIGLU Analysis in Folic Acid Deficient *C. briggsae* in the Presence of Increased Dietary Histidine.

Medium		Population on Day 41 (no. of worms/ml medium)		FIGLU (nmole/mg dry wt of worms)[b]			
				Medium		Worms	
FA (7500 ng/ml)	His[a] (283 μg/ml)	Flask no.: 1	2	1	2	1	2
+	1x	39,000	45,000	5	9	13	14
—	1x	2400	1900	Neg	Neg	349	474
+	3x	31,000	39,000	19	14	33	30
—	3x	1100	1400	215	Neg	1329	1223

[a] His (1x): L-histidine 283 μg/ml medium; His (3x): L-histidine 849 μg/ml medium.
[b] 40 ng/worm (dry wt) was used.

containing folic acid was only slightly lower than that in the normal control medium (low histidine plus folic acid). However, the average population in the folic acid deficient medium was further reduced about 40% when higher histidine concentration (3x) was used, indicating that increased amounts of histidine may increase the folic acid requirement of the nematode.

The FIGLU concentration in both medium and worms was determined and was expressed as moles per dry weight of a mixed population of worms. The dry weight of an average size worm (40 ng/worm) was determined by oven drying a mixed size population of worms at 108° overnight. Table I shows that folic acid deficient worms in the presence of a basal amount of histidine contained about 30 times as much FIGLU as those grown in the medium with adequate folic acid. The addition of histidine increased the FIGLU concentration in the worms two or three times in either case. However, the FIGLU concentration from media in which the folic acid deficient worms grew was, for the most part, undetectable, suggesting that the excretion of FIGLU by the worms is variable if it does occur at all. Therefore the major portion of the accumulated FIGLU remains within the worm tissue in spite of its considerable production following either folic acid deficiency or increased dietary histidine. It is thus apparent that folic acid is required for histidine degradation in this organism as evidenced by changes in FIGLU concentration.

It is interesting that Stifel, Rosensweig and Herman (10) reported that FIGLU inhibited the activities of some folate dependent enzymes (serine hydroxymethyltransferase, methylenetetrahydrofolate dehydrogenase, and formyltetrahydrofolate synthetase) as well as some glycolytic enzymes (hexokinase, pyruvate kinase, and FDP aldolase) *in vitro*. The accumulation of FIGLU in *C. briggsae* could be inhibitory to certain important enzymatic reactions and could thereby interfere with reproduction in the nematode.

Since folic acid is required by the nematode in histidine breakdown, the same as that found in humans and rats, the axenically grown free-living nematode could serve as a model organism for further studies in folic acid metabolism.

Summary. Formimino-L-glutamic acid (FIGLU) accumulates in the tissues of the axenically grown free-living nematode *Caenorhabditis briggsae* under conditions of folic acid deficiency and to an even greater extent when an increased amount of histidine is added to the medium. The results suggest that folic acid is required for the breakdown of histidine in this organism.

1. Silverman, M., Gardines, R. C., and Bakerman, H., J. Biol. Chem. **194**, 815 (1952).
2. Spivey-Fox, M. R., and Ludwig, W. J., Proc. Soc. Exp. Biol. Med. **108**, 703 (1961).
3. Broquist, H. P., J. Amer. Chem. Soc. **78**, 6205 (1956).
4. Rabinowitz, J. C., and Tabor, H., J. Biol. Chem. **233**, 252 (1958).

5. Luhby, A. L., and Cooperman, J. C., Proc. Soc. Exp. Biol. Med. **101**, 350 (1959).

6. Hansen, E. L., and Dougherty, E. C., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **16**, 304 (1957).

7. Dougherty, E. C., Hansen, E. L., Nicholas, W. L., Mollet, J. A., and Yarwood, E. A., Ann. N.Y. Acad. Sci. **77**, 176 (1959).

8. Tomlinson, G., and Rothstein, M., Biochim. Biophys. Acta **63**, 465 (1962).

9. Tabor, H., Colowick, S. P., and Kaplan, N. O., *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. 5, p. 784. Academic Press, New York (1962).

10. Stifel, F. B., Rosensweig, N. S., and Herman, R. H., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **31**, 712 (1972).

Received July 9, 1973. P.S.E.B.M., 1974, Vol. **145**.