Effects of Folic Acid on Aminopterin Toxicity in the Immature Mouse. (17726)

B. H. ERSHOFF, J. P. HOFFSTADT, AND H. B. MCWILLIAMS.
From the Emory W. Thurston Laboratories, Los Angeles, Calif.

It is now well established that by suitable structural alterations synthetic vitamin analogues can be prepared which are inhibitory or "toxic" to the organism. In most cases the inhibition can be alleviated by adequate amounts of the corresponding vitamin, thus suggesting that the inhibition is of the competitive variety. With 4-amino-pteroylglutamic acid (aminopterin), however, an absolute deficiency of folic acid can be produced(1) which fails to respond even to massive doses of this vitamin. Franklin *et al.*(2) found that mice died within one week when fed purified rations containing 1 mg of 4-amino-pteroylglutamic acid per kg of diet. Folic acid failed to counteract this effect even when administered in doses as high as 100 mg per kg of diet, *i.e.* at a metabolite to inhibitor ratio of 100:1. Similar results have been observed in other species. Goldsmith *et al.*(3) demonstrated that the injection of estradiol into newly metamorphosed female frogs is followed within 2 to 3 weeks by marked enlargement and coiling of the oviducts. This effect may be largely counteracted by the administration of 4-amino-pteroylglutamic acid. As in the case of mice, however, doses of folic acid 100 times as large as the inhibitor failed to modify the aminopterin effect. Similar findings have been reported for the chick and rat. Karnofsky *et al.*(4) found that 4-amino-pteroylglutamic acid profoundly stunted the growth of chick embryos and produced a number of developmental abnormalities. Doses of folic acid 4000 times as large as the inhibitor failed to counteract these effects. In the rat toxic doses of 4-amino-pteroylglutamic acid incited within 6 days the occurrence of anorexia, diarrhea, atony of the entire gastrointestinal tract, adrenal hyperplasia, and atrophy of the thymus, spleen and bone marrow, with resulting leukopenia. Folic acid did not prevent these effects even when administered at a metabolite to inhibitor ratio of 600:1, although it did prolong the period during which animals remained symptom free(5). On the other hand Oleson *et al.*(6) reported that folic acid in doses approximately 25 times greater than the inhibitor counteracted the growth-retarding effect of 4-amino-pteroylglutamic acid in the chick and rat; and Hertz and Tullner(7) observed that folic acid in doses 300 to 400 times greater than the inhibitor counteracted the effects of 4-amino-pteroylglutamic acid in inhibiting the estrogen induced growth in the female genital tract of the stilbestrol-treated chick and the estradiol-treated ovariectomized rat. In the present communication data are presented indicating that folic acid in sufficiently high dosage will also counteract the deleterious effects of 4-amino-pteroylglutamic acid in the immature male mouse.

Procedure. The basal ration employed in the present experiment consisted of dextrin* 58, casein† 25, cottonseed oil (Wesson) 10,

1. Philips, F. S., and Thiersch, J. B., *J. Pharm. and Exp. Ther.*, 1949, v95, 303.

2. Franklin, A. L., Stokstad, E. L. R., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 398.

3. Goldsmith, E. D., Schreiber, S. S., and Nigrelli, R. F., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 299.

4. Karnofsky, D. A., Patterson, P. A., and Ridgway, L. P., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 447.

5. Higgins, G. M., *Blood*, 1949, v4, 1142.

6. Oleson, J. J., Hutchings, B. L., and Subba-Row, Y., *J. Biol. Chem.*, 1948, v175, 359.

7. Hertz, R., and Tullner, W. W., *Endocrinology*, 1949, v44, 278.

8. Sure, B., *J. Nutrition*, 1941, v22, 499.

* White Dextrin Merck, N.F.V., Merck and Co., Inc., Rahway, N. J.

† Vitamin Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Sure's Salt Mixture No. 1(8)

§ Ruffex, Fisher Scientific Co., St. Louis, Mo.

TABLE I.
Effects of Folic Acid, B Vitamins, and Liver on the Growth and Length of Survival of Immature Mice Fed Toxic Doses of Aminopterin for 20 days.*

Dietary supplement	Avg initial body wt, g	% surviving*	Survival of decedents,† days	Gain in body wt in 20 days,† g
None	13.8	100	—	5.1 ± 1.1 (12)
"	14.7	0	8.4 ± .6	—
B complex vit. mixture (without vit. B_{12})	15.6	0	6.8 ± .3	—
Vit. B_{12}	12.2	0	7.3 ± .5	—
Whole liver	14.6	16.7	8.4 ± .7	0.5 ± 0.1 (2)
Liver conc. 1-20	14.9	0	8.2 ± .7	—
" residue	15.0	0	8.7 ± .5	—
Folic acid (90 mg/kg of diet)	14.1	25	12.9 ± .3	0.1 ± 0.9 (3)
" " (490 " " ")	14.6	100	—	8.2 ± 1.0 (12)

The values in parentheses indicate the No. of surviving animals on which the averages are based.

* Data are based on the top 12 of the 15 mice constituting each group. In all of the dietary regimens, except the first in which no aminopterin was fed, the dosage of aminopterin was 1 mg per kg of diet.

† Including standard error of the mean calculated as follows $\sqrt{\frac{\epsilon d^2}{n}} / \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

salt mixture‡ 5, and cellulose§ 2%. To each kg of the above diet were added the following synthetic vitamins: thiamine hydrochloride 20 mg, riboflavin 20 mg, pyridoxine hydrochloride 20 mg, nicotinic acid 60 mg, calcium pantothenate 60 mg, biotin 4 mg, folic acid 10 mg, p-aminobenzoic acid 400 mg, inositol 800 mg, 2-methyl-naphthoquinone 10 mg, and choline chloride 2 g. Each mouse also received once weekly a vitamin A-D concentrate, containing 25 U.S.P. units of vitamin A and 2.5 U.S.P. units of vitamin D, and 1.5 mg of alpha-tocopherol acetate. For the tests 135 male mice of the CC-1 strain¶ were selected at the age of 24 to 27 days and an average weight of 14.6 g. They were placed in metal cages with raised screen bottoms to prevent access to feces and were fed the following diets *ad libitum*: (a) basal ration alone (b) basal ration plus aminopterin. In addition to the basal ration plus aminopterin, diet (c) contained per kg twice as much thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, nicotinic acid, calcium pantothenate, biotin, folic acid, p-aminobenzoic acid, inositol and 2-methyl-naphthoquinone as was present in the basal ration; diet (d) contained 30 γ of vitamin B_{12}** per kg; diet (e) 10% of whole liver power;†† diet (f) 2.5% of liver concentrate 1:20;‡‡ diet (g) 10% of liver residue;§§ diet (h) 90 mg of folic acid per kg; and diet (i) 490 mg of folic acid per kg. Aminopterin (4-aminopteroylglutamic acid)‖‖ was incorporated in the above diets at a level of 1 mg per kg of diet. The supplements replaced an equal

‡ Napco Fish Oil Concentrate, assaying 800,000 U.S.P. units of vitamin A and 80,000 U.S.P. units of vitamin D per g.

¶ Animals were obtained from a local breeder. The CC-1 strain was derived from crossbreeding the Webster and ABC strain.

** Cobione (Crystalline Vitamin B_{12} Merck), Merck and Co., Rahway, N.J.

†† Whole Dried Liver Powder, Armour and Co., Chicago, Ill.

‡‡Liver Concentrate Powder 1-20, Wilson Laboratories, Chicago, Ill.

§§ Extracted Liver Residue, Wilson Laboratories, Chicago, Ill.

‖‖ We are indebted to Dr. E. L. R. Stokstad of Lederle Laboratories, Pearl River, N.Y., for providing the aminopterin employed in the present experiment.

amount of dextrin. Feeding of the 15 mice in each group was continued for 20 days or until death, whichever occurred sooner.

Results. The findings are summarized in Table I. To minimize variations in averages due to early deaths, infection and atypical responses on the part of individual mice, the data were computed on the basis of the top 12 animals in each group. In agreement with earlier findings(2) the mice lost weight and failed to survive when fed a purified ration containing 1 mg of aminopterin per kg of diet. The supplements of whole liver powder, water-soluble liver extract and water-insoluble liver residue were without significant effect. Doubling the B vitamin content of the experimental diet and adding vitamin B_{12} also proved ineffective. Only folic acid of all of the substances tested exerted a protective effect. A diet containing 100 mg of folic acid per kg of diet prolonged slightly the average survival time of the mice. Folic acid at a level of 500 mg per kg of diet, however, completely counteracted the deleterious effects of aminopterin on growth and survival under conditions of the present experiment.

Summary. Immature male mice lost weight and failed to survive when fed a purified ration containing 1 mg of aminopterin per kg of diet. These effects were completely counteracted by folic acid during an experimental period of 20 days when administered at a level of 500 mg per kg of diet.

Received February 17, 1950, P.S.E.B.M., 1950, v73.

Antimicrobial Action of Terramycin *in Vitro* and *in Vivo*. (17727)

GLADYS L. HOBBY, NANCY DOUGHERTY, TULITA F. LENERT, ELIZABETH HUDDERS AND MARY KISELUK.

From the Research Laboratories of Chas. Pfizer & Co., Inc., Brooklyn, New York.

In a recent communication(1), the isolation of a new antibiotic, terramycin, has been reported. Terramycin is a highly active antimicrobial agent which is effective both *in vitro* and *in vivo* against certain of the pathogenic bacteria and certain of the rickettsiae (1,2). The studies reported herein were carried out in an attempt to evaluate more extensively the antimicrobial action of terramycin *in vitro* and its chemotherapeutic action in acute experimental infections in animals.

In vitro activity. 1. Materials and methods. Partially purified and crystalline terramycin,* terramycin hydrochloride, terramycin sulfate, and 2 forms of the sodium salt of terramycin (pH 8.5 and 9.8)† have been used to date in determining the sensitivity of various microorganisms to terramycin. Aureomycin

1. Finlay, A. C., Hobby, G. L., P'an, S. Y., Regna, P. P., Routien, J. B., Seeley, D. B., Shull, G. M., Sobin, B. A., Solomons, I. A., Vinson, J. W., and Kane, J. H., *Science*, 1950, v111, 85.

2. Rose, H. M., 1950, personal communication.

*All of the terramycin used throughout this study was prepared by the Departments of Biochemical and Chemical Research and Development, Chas. Pfizer & Co., Inc. We are indebted especially to Dr. B. A. Sobin and Dr. P. P. Regna for the preparation of large quantities of the antibiotic for this study during the early stages of its development, and to Mr. J. H. Kane and Dr. R. A. Patelski for their advice and assistance throughout the study.

† The pH of the sodium salt of terramycin in dilute aqueous solution is approximately 9.8. It was observed early in the study of terramycin that dilute solutions of its sodium salt may be adjusted to pH 8.5 without precipitation of the antibiotic. It is recognized that such solutions probably consist of mixtures of terramycin and its sodium salt. Although this form cannot be prepared readily as a solid product, its pH and its satisfactory solubility have made it a more desirable preparation for certain preliminary laboratory studies than terramycin hydrochloride (pH 1.5), terramycin sulfate (pH 1.5), or the true sodium salt of terramycin (pH 9.8).