have been efficacious by itself, since the studies of Castle and his associates have shown adequately that intrinsic factor alone produces no significant hematopoietic effect in human patients with pernicious anemia in relapse. Whether the pig had developed a deficiency of intrinsic factor, of course, cannot be stated, although a gastric analysis after stimulation with histamine, about 3 weeks prior to the initiation of therapy, indicated a high total acidity with absence of free hydrochloric acid. Studies designed to answer some of these questions are now in progress.

Summary. Interference with the metabolism of pteroylglutamic acid in the pig, through the use of a crude chemical antagonist, interrupts growth and significantly inhibits the formation of erythrocytes and of granulocytes. This interference is removed, despite continued feeding of the antagonist, by administration of a crude source of extrinsic factor (essentially free of PGA), together with normal human gastric juice. This finding affords an experimental animal with which to study the mechanism of action of antianemic substances and their functional relation to folic acid; also, a suitable bioassay tool is offered for guiding the isolation of antianemic factors of unknown chemical composition.

The administration of a purified diet similar to that successfully used by Cartwright *et al.*[10] failed to produce a failure in growth and in hematopoiesis in swine. It is suggested that this failure may possibly be attributable to the presence of biotin in the diet employed in this study.

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Acceleration of Pteroylglutamic Acid Deficiency in Mice and Chicks by a Chemical Antagonist.

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In another communication[1] the effect on rats of a synthetic preparation "antagonistic" to pteroylglutamic acid was described. The present article describes the results obtained with mice and with chicks which received the same preparation. An acceleration of pteroylglutamic acid deficiency was observed, and the effect was prevented by adding pteroylglutamic acid to the diet at levels sufficient to overcome the action of the antagonistic preparation.

Experimental. Rockland female mice, 5 to 6 weeks old, were kept in wire-floored cages. Five animals were used per group. The following basal diet was fed (diet 1) glucose (Cerelose), 72 g; washed casein (Labco), 20 g; salt mixture,[2] 4 g; corn oil (Mazola) *plus* vitamins A, D and E, 3 g; succinylsulfathiazole, 1 g; choline chloride, 0.1 g; inositol, 0.1 g; niacinamide, 5 mg; calcium pantothenate, 5 mg; thiamine HCl, 1 mg; riboflavin, 1 mg; pyridoxine HCl, 1 mg; *p*-aminobenzoic acid, 1 mg; 1-acetoxy-2-methyl-4-naphthyl sodium phosphate, 0.5 mg; biotin, 0.02 mg. Three grams of the corn oil preparation contained vitamin A (acetate), 1500 U.S.P. units; vitamin D (Delsterol), 200 A.O.A.C. chick units; mixed tocopherols, 34 mg.

The antagonist was prepared by condensing 2,4,5-triamino-6-hydroxypyrimidine and *p*-aminobenzoyl-*l*(+)-glutamic acid with 2,3-dibromobutyraldehyde in the reaction described elsewhere.[3] The preparation was carried out by Dr. M. E. Hultquist and Dr. J. M. Smith, Jr.* The reaction product was used without purification. A similar product, using *p*-aminobenzoyl-*d*(—)-glutamic acid has been stated to have "displacing" activity for pteroylglutamic acid in the growth

1 Franklin, A. L., Stokstad, E. L. R., Belt, M., and Jukes, T. H., *J. Biol. Chem.*, 1947, in press.

2 Hawk, P. B., and Oser, B. L., *Science*, 1931, **74**, 369.

TABLE I.
Growth of Mice on Diet 1 as Affected by Various Supplements.

Group No.	Supplement per kilo of diet 1	Body wt (g) 0	1	2	3	4	5	6 wk
1	None	15	18	21	21	23	24	24
2	10 g crude antagonist	16	17	19	19	17	16	16
3	10 g crude antagonist + 0.1 g pteroylglutamic acid	16	20	22	22	26	25	26

TABLE II.
Hematological Observations with Mice Receiving Diets Described in Table I.

Group No.	Hemoglobin, g per 100 cc at 4 wk	at 6 wk	White cells ($\times 10^3$), per mm^3 at 4 wk	at 6 wk	Differential count at 4 wk, %* N	L	M	E
1	16.9	17.6	9.3	12.1	24	73	2	0 to 1
2	15.9	13.0	3.3	3.3	21	77	1	0
3	17.4	18.5	10.1	12.6	23	76	0 to 1	0 to 1

* N = Neutrophils; L = Lymphocytes; M = Monocytes; E = Eosinophils.

of *S. faecalis* R.[4]

The supplements fed are described in Table I. The mice on the basal diet (Group 1) did not develop any signs of deficiency when the experiment was terminated at the end of 6 weeks. At this time the 2 surviving animals in Group 2, which received the antagonist, were in a moribund condition. The animals in this group were emaciated, but the chromodacryorrhea and very ruffled fur which were observed in rats on a similar diet[1] were not found. When the 2 surviving mice in Group 2 were killed and autopsied, the mouths were normal in contrast to the results with rats. The livers were yellow, but appeared normal in size and texture. The uteri were atrophic. Other data are summarized in Tables I and II, which show that complete protection was given by pteroylglutamic acid. The data in Table II indicate that the formation of white cells of both the myeloid and lymphoid series was depressed equally by the antagonist. In this respect the mice differed from rats, in which species the reduction of the granulocyte count is more conspicuous than the reduction of the lymphocyte count in pteroylglutamic acid deficiency.[1,5]

In experiments with chicks the basal diet had the following composition: Glucose (Cerelose), 58.5 g; purified casein (Labco), 20 g; gelatin, 8 g; calcium gluconate, 5 g; cystine, 0.4 g; choline chloride, 0.2 g; inositol, 0.1 g; bone ash, 2 g; NaCl, 0.6 g; KH_2PO_4, 0.45 g; K_2HPO_4, 0.6 g; $MgSO_4$, 0.25 g; $MnSO_4 \cdot 4H_2O$, 0.05 g; ferric citrate, 0.05 g; $CuSO_4 \cdot 5H_2O$, 2 mg; $Al_2(SO_4)_3 \cdot 18H_2O$, 1.6 mg; zinc acetate, 1.4 mg; KI, 0.6 mg; cobalt chloride, 0.4 mg; nickel chloride, 0.2 mg; calcium pantothenate, 5 mg; niacinamide, 5 mg; riboflavin, 1 mg; pyridoxine HCl, 1 mg; thiamine HCl, 1 mg; *p*-aminobenzoic acid, 1 mg; 1-acetoxy-2 methyl-4-naphthyl sodium phosphate, 0.5 mg; biotin, .02 mg; to which were added vitamin A (acetate), 1500 U.S.P. units; vitamin D, 200 A.O.A.C. units; mixed tocopherols, 34 mg dissolved in corn oil (Mazola) to a total of 3 g. Day-old New Hampshire chicks were placed on the diets. The usual signs of pteroylglutamic acid deficiency were

[3] Angier, R. B., Boothe, J. H., Hutchings, B. L., Mowat, J. H., Semb, J., Stokstad, E. L. R., SubbaRow, Y., Waller, C. W., Cosulich, D. B., Fahrenbach, M. J., Hultquist, M. E., Kuh, E., Northey, E. H., Seeger, D. R., Sickels, J. P., and Smith, J. M., Jr., *Science*, 1946, **103**, 667.

[*] Calco Chemical Division, American Cyanamid Company, Bound Brook, N.J.

[4] Martin, G. J., Tolman, L., and Moss, J., *Arch. Biochem.*, 1947, **12**, 318.

[5] Spicer, S. S., Daft, F. S., Sebrell, W. H., and Ashburn, L. L., *U. S. Pub. Health Rep.*, 1942, **57**, 1559.

TABLE III.
Effect of Various Additions to a Basal Purified Diet, Deficient in Pteroylglutamic Acid (PGA), on Growth and Hemoglobin Formation in Chicks.

Experiment No.	Group	Supplement per kilo of diet	Weights and number of survivors (in parentheses) at various ages: 1 day g	14 days g	28 days g	Hemoglobin, 28 days, g%
1	1	None	46 (11)	91	116 (11)	6.3
1	2	0.1 mg PGA	44 (10)	102	177 (10)	8.2
1	3	0.1 " " + 1 g antagonist	45 (6)	85 (6)	95 (2)	4.7
1	4	10 " " + 1 " "	45 (6)	145	353 (6)	9.6
1	5	1 " "	44 (10)	127	310 (10)	10.0
2	6	None	38 (10)	75 (9)	80 (2)	
2	7	10 g antagonist	36 (5)	57 (4)	—*	
2	8	0.1 mg PGA	40 (10)	81 (8)	167 (5)	
2	9	0.3 " "	39 (10)	100	253 (10)	
3	10	None	43 (10)	95(10)	166 (7)	7.7
3	11	1 mg PGA	45 (10)	148 (9)	321 (9)	9.1
3	12	1 " " + 10 g antagonist	43 (10)	96 (8)	106 (3)	8.4

* All dead by three weeks.

TABLE IV.
Observations on Red and White Cell Counts in Chicks in Experiment 3 as Affected by PGA Deficiency.

Experiment No.	Group	Supplement per kilo of diet	Erythrocyte count cells per mm³ (× 10⁶): 14 days	28 days	White cell count cells per mm³ (× 10³): 14 days	28 days
3	10	None	1.0	1.6	18.6	12.6
3	11	1 mg PGA	2.0	2.4	24.9	19.9
3	12	1 " " + 10 g antagonist	1.7	1.8	11.7	6.0

noted on the basal diet. These included slow growth, poor feathering and low hemoglobin content of the blood. When pteroylglutamic acid was added to the diet at an adequate level the growth was rapid and the birds were normal in appearance. Addition of the antagonist together with a suboptimal level of pteroylglutamic acid resulted in growth slower than that obtained with the basal diet, but with a higher level of pteroylglutamic acid the depressing effect of the antagonist on growth was completely reversed. When the antagonist was added alone, all the birds were dead by 3 weeks. Hemoglobin determinations in one experiment indicated the production of anemia by the antagonist and its reversal by pteroylglutamic acid. These results are summarized in Table III.

Summary. Mice on a purified diet with added succinylsulfathiazole developed no signs of pteroylglutamic acid deficiency within 6 weeks. When a crude synthetic preparation of a pteroylglutamic acid antagonist was added to the diet a syndrome appeared which was characterized by slow growth, anemia and leucopenia. Pteroylglutamic acid prevented the appearance of the syndrome. The development of pteroylglutamic acid deficiency in chicks on a purified diet was aggravated by adding the antagonist to the diet, and the effects of the antagonist were reversed by pteroylglutamic acid.