Replacement of Pteroylglutamic Acid by Folinic Acid for the Chick.* (18509)

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The synthesis of a compound related to, but not identical with, pteroylglutamic acid (PGA) has been reported by Shive *et al.*(1). The compound(2) is synthesized by hydrogenation of formylfolic acid. It contains 4 more hydrogen atoms and appears to be identical with the naturally-occurring folinic acid, as described by Bond *et al.*(3). Both the natural folinic acid(4) and the synthetic folinic acid(1) have been shown to satisfy the nutritional requirements of *Leuconostoc citrovorum* 8081 for a factor described by Sauberlich and Baumann(5). An excellent review of the subject has recently been published(6). Broquist *et al.*(7) reported that concentrates of the *L. citrovorum* factor replaced PGA for the chick. The synthesis and isolation of a crystalline material which is active in place of the *L. citrovorum* factor for this organism was recently announced by Brockman *et al.* (8). These authors also stated that this material was active for the chick in place of PGA, but gave no data.

In the light of this evidence, it was of interest to determine whether the synthetic folinic acid is physiologically active for the chick in place of PGA.

Experimental. Day-old White Leghorn male chicks from the Poultry Division flock were used. They were sexed at time of hatching and raised in electrically-heated, metal brooders with wire screen floors. Feed was supplied *ad libitum* and the water changed daily. Six chicks were used per group and the experiments were terminated at the end of 4 weeks. The basal ration (SIF), low in PGA, consisted of 56.5% cerelose, 20% casein, 8% gelatin, 5% cellulose, 4% soybean oil, 6% salts 2M†, 0.3% DL-methionine, 0.2% choline chloride, and the following vitamins‡ (mg/kg): thiamine hydrochloride, 4; riboflavin, 8; calcium pantothenate, 20; pyridoxine hydrochloride, 6; nicotinic acid, 100; biotin, 0.2; α-tocopherol acetate, 5; and 2-methyl 1,4-naphthoquinone, 1. To each kg of diet were added 10,000 I. U. of vit. A and 1000 A.O.A.C. units of vit. D_3. The synthetic folinic acid used in these trials was prepared and supplied by Dr. William Shive of the University of Texas.

Results. The results are shown in Table I. In the first trial the addition of 0.5 mg of folinic acid/kg of ration gave a growth response equal to that of an equal amount of PGA. The feed efficiency figures were also similar. That 0.5 mg of folinic acid/kg or 0.5 mg of PGA was probably not adequate in this ration is shown by the difference in weights of these groups and the group receiving 2 mg of PGA/kg. In the first trial the basal ration contained no added vit. B_{12},

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† Salts 2M(g/600 g): $CaCO_3$-150, K_2HPO_4-90, Na_2HPO_4-73, $Ca_3(PO_4)_2$-140, NaCl-88, $MgSO_4 \cdot 7H_2O$-50, ferric ammonium citrate (17-18% iron)-4, $MnSO_4 \cdot 4H_2O$-4.2, KI-0.4, $ZnCl_2$-0.2, and $CuSO_4 \cdot 5H_2O$-0.2.

‡ We are indebted to Merck and Co., Rahway, N. J., for crystalline vitamins, Lederle Laboratories, Pearl River, N. Y., for the pteroylglutamic acid, and to U. S. Industrial Chemicals, Inc., N. Y., for DL-methionine.

TABLE I. Studies with Synthetic Folinic Acid.

Trial	Group	Supplement/kg of basal ration SIF	Avg wt, g, 4 wk	Feed efficiency*
1	1	0	120	2.63
	2	.5 mg folinic acid	226	2.01
	3	.5 mg PGA	231	1.96
	4	30 mcg cryst. vit. B_{12}	127	2.51
	5	1 mg cryst. vit. B_{12}	142	2.33
	6	2 mg PGA	269	2.13
			26 days	
2†	1	0	106	2.66
	2	.1 mg folinic acid	126	2.57
	3	.1 mg PGA	105	3.08
	4	.4 mg folinic acid	209	2.09
	5	.4 mg PGA	167	2.21
	6	2 mg PGA	211	2.13

* Feed efficiency—Total feed consumed / wt gained

† Trial 2—30 mcg cryst. vit. B_{12}/kg added to basal ration.

which has been reported to spare PGA(9).

In trial 2, two different levels of folinic acid were compared to similar levels of PGA. The growth responses and feed efficiency figures show that the folinic acid was fully as active as PGA; whether it may be even more active will require more work to fully determine.

It is clearly evident that folinic acid should be added to the list of known compounds of the folic acid group which are active for the chick.

Summary. Studies on the biological activity of synthetic folinic acid show that it may replace pteroylglutamic acid in the diet of the chick.

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Effect of Administration of ACTH and Cortisone upon Blood Glutathione Levels (18510)

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Conn and his associates(1,2) reported that the administration of ACTH resulted in diminished glucose tolerance and glycosuria which were associated with a decrease of blood glutathione (GSH) and an increased excretion of urinary uric acid. Conn, Lewis, and Johnston(3) showed that the administration of GSH to a normal individual receiving ACTH produced a dramatic but transitory reduction in the hyperglycemia and glycosuria. This observation, as Conn *et al.*(1) have indicated, is of interest inasmuch as the diabetogenic activity of alloxan, a possible purine metabolite, can be decreased by GSH as well as other substances containing the thiol group. Kinsell *et al.*(4) found an increased urinary excretion of sulfur-containing compounds following the administration of ACTH. Lazarow and Berman(5) noted that the injection of cortisone into normal rats led to a decrease in blood GSH which appeared to correlate with the degree of glycosuria. Lazarow (6) found that GSH potentiated the action of cortisone and, in contrast to its action during ACTH therapy reported by Conn *et al.* (3), produced an increased glycosuria when injected into cortisone diabetic rats.

The observations of Conn *et al.*(1,2) upon

* Lt. j.g. (M.C.) U.S.N.

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