Excretion of Formiminoglutamic Acid as an Index of Vitamin B_{12}, Folic Acid, and Methionine Deficiencies.*‡ (27041)

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Excretion of large amounts of formiminoglutamic acid (FGA), a metabolite of histidine, has been observed in Vit. B_{12} deficient rats(1) and chicks(2) fed diets borderline with respect to methionine requirement. In each species, supplemental methionine caused a reduction of FGA excretion to negligible levels, similar to those of the Vit. B_{12} supplemented animals. After a histidine load, high levels of FGA have been excreted by human beings deficient in folic acid(3-5), whereas patients with pernicious anemia failed to excrete significant amounts of FGA under the same conditions(3,4). However, Zalusky and Herbert(5) reported high excretion rates of FGA in 2 of 6 Vit. B_{12} deficient patients. In the present studies, methionine lowered excretion of FGA more effectively in chicks deficient in Vit. B_{12} than in chicks deficient in folic acid. Chicks that were moderately to severely deficient in methionine did not excrete significant amounts of FGA when folic acid and Vit. B_{12} were present in the diet.

Methods. Day old female New Hampshire chicks were distributed into groups of 6 to 8 each and placed in electrically heated batteries with screen wire floors. They received food and water *ad libitum*. Diet C62(6), which was fed in some experiments, was a purified diet that contained 30% of soybean protein,† 0.3% of L-cystine, and 24% of fat. When folic acid was omitted from Diet C62, the chicks grew almost as well as the controls. The soybean protein was found to be grossly contaminated with folic acid-active material. The following procedure was satisfactory for removing most of this activity. A 5% suspension of the protein in distilled water was held at pH 8 or slightly above for 5 minutes by addition of 6N NaOH. The protein was allowed to settle and the aqueous layer was siphoned off. The remaining liquid was removed by a cloth filter press; the protein was washed several times with distilled water and finally with ethanol. The protein was forced through a 14 mesh sieve, spread in thin layers on enameled trays, and dried in a forced air oven at 50°C for 2 hours. About 85% of the initial protein was recovered after the extraction process. Total folic acid activity, determined with *Lactobacillus casei*, was 375 mμg/g for one batch of starting protein and activity after extraction was 25 mμg/g. This specially extracted protein was satisfactory for producing severe folic acid deficiency in chicks and in the complete diet it was equal to the original protein in supporting normal growth. Omission of Vit. B_{12} from Diet C62 resulted in a severe deficiency which was the same with either the original or the extracted protein.

Two diets were used for producing a deficiency of methionine. One of these, Diet C69M, was exactly like Diet C62 except that L-cystine was omitted and 100 g of the soybean protein and 10 g of glucose in each kg of diet were replaced with 110 g of amino acid mixture 13,§ which simulated the composition of the soybean protein except methionine and cystine were omitted. Diet C68M contained (g/kg of diet): vitamin-free casein 100, gelatin 80, amino acid mix 5 (minus methionine) 115, L-arginine•HCl 8, corn oil 40, hydrogenated vegetable oil (Crisco) 200, Fox-Briggs Salts N 60 (7), choline chloride 2, crude glucose (Cerelose) 395, and vitamins at the same levels as

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† Cl Assay Protein purchased from Archer-Daniels-Midland Co., Cincinnati, Ohio.

§ Amino acid mix 13 (minus cystine and methionine) contained in g: DL-alanine 3.6, L-arginine•HCl 8.3, DL-aspartic acid 6.2, L-glutamic acid 19.3, glycine 4.1, L-histidine•HCl 2.6, DL-isoleucine 13.0, L-leucine 7.5, L-lysine•HCl 6.8, DL-phenylalanine 5.0, L-proline 2.5, DL-serine 6.9, DL-threonine, 7.8, L-tryptophane, 2.0, L-tyrosine 3.4, DL-valine 11.0.

TABLE I. Effects of Methionine Supplementation and Variation of Histidine Intake Upon Excretion of Formiminoglutamic Acid by Folic Acid Deficient, Vitamin B_{12} Deficient, and Control Chicks.*

Day	L-Histidine·HCl %	DL-Methionine %	Folic acid deficient Body wt g	Folic acid deficient FGA†	Vit B_{12} deficient Body wt g	Vit B_{12} deficient FGA†	Control Body wt g	Control FGA†
1	1	0	92	79	116	91	205	15
2	1	0	94	96	120	119	220	16
3	1	1	104	41	132	13	240	4
4	1	1	114	41	147	7	260	1
5	2	1	116	69	160	20	280	2
6	2	1	121	81	174	44	299	4
7	4	1	124	122	188	50	318	6
8	4	1	126	232	197	91	328	30

* Average of values from 2 experiments, 12 chicks/treatment, 22 days of age on day 1. Chicks were fed Diet C62, complete or deficient as indicated, prepared with specially extracted soybean protein.
† μmoles FGA/mole of histidine consumed/24 hr.

those in Diet C62. Amino acid mix 5(8) was formulated to simulate the amino acid composition of casein.

When the chicks were 3 to 4 weeks old, they were placed in individual cages. The procedures for collection of excreta and determination of FGA have been described(6). During each collection period, L-histidine·HCl was incorporated in the diet at a level of 1%, except in experiments presented in Table I, where higher levels were also fed.

Results. Data on FGA excretion of chicks deficient in folic acid and Vit. B_{12} are presented in Table I. With 1% L-histidine·HCl added to the diet (days 1 and 2), excretion of FGA was in the same elevated range for both deficient groups. The controls excreted much less. Addition of 1% DL-methionine to the diet caused a more pronounced drop in FGA excretion of Vit. B_{12} deficient chicks than that in the folic acid deficient group. The excretion of the control animals was also decreased. As the histidine supplement was increased to 2 and 4% of the diet, FGA excretion rose in both deficient groups and the amounts of FGA excreted were much higher for the folic acid deficient than for the Vit. B_{12} deficient chicks. Only on day 8 did the higher intake of histidine cause an increase in excretion of FGA by the control chicks. The daily food intake of the folic acid deficient chicks ranged from 7 to 12 g/100 g body weight, that for Vit. B_{12} deficient chicks from 9 to 12 g, and for the controls 7 to 11 g. Initial mean body weights of the 2 deficient groups were not too different; however, growth rate of the Vit. B_{12} deficient chicks was much greater than that of the folic acid deficient chicks, once the methionine supplement was added to the diet.

The effect of methionine deficiency upon FGA excretion is presented in Table II. Chicks fed either Diet C68M or C69M were severely

TABLE II. Excretion of Formiminoglutamic Acid by Methionine Deficient, Methionine-Vitamin B_{12} Deficient, and Control Chicks.*

Diet	DL-Methionine supplement %	Age, 3 wk No. chicks	Age, 3 wk Body wt g	Age, 3 wk FGA†	Age, 4 wk No. chicks	Age, 4 wk Body wt g	Age, 4 wk FGA†
C68M‡	0	—	—	—	16	107	1.2
"	.2	—	—	—	7	270	.9
"	.6	—	—	—	15	364	.2
C69M‡	0	15	84	14	8	93	16
"	.15	8	154	1.9	—	—	—
"	.7	14	214	.5	8	305	.2
C69M, No B_{12}	0	8	75	48	6	93	60

* When number of chicks exceeds 8, values are the average of 2 experiments. With Diet C69M, the data for 3 and 4 weeks are from chicks in different experiments.
† μmoles FGA/mmole histidine consumed/24 hr.
‡ By calculation Diet C68M contained (g/kg diet) cystine 1 and methionine 4; Diet C69M contained cystine 1.2 and methionine 2.

deficient in methionine as indicated by the very poor growth and feathering. Their long primary wing feathers curved upward near the ends and the upper wing covert feathers curled outward from the wing. With each diet the smaller methionine supplements resulted in intermediate rates of growth. Excretion of FGA was very low by all chicks fed Diet C68M irrespective of methionine supplementation. Chicks fed Diet C69M excreted small amounts of FGA; but with each level of methionine supplementation, FGA excretion was very low. When Vit. B_{12} was omitted from the methionine-low Diet C69M, growth was similar to that obtained with Diet C69M; however, the FGA excretion was markedly elevated by this omission of Vit. B_{12}.

When choline was omitted from Diet C62, the chicks grew slowly and developed perosis. Mean daily excretion of FGA was 6 and 8 μmoles FGA/mmole histidine/day for choline deficient chicks in 2 experiments.

Discussion. To a limited extent these data support the studies with human beings in which high FGA excretion was associated with folic acid deficiency but not with Vit. B_{12} deficiency(3,4). For example, when the chicks received 1% L-histidine•HCl and 1% DL-methionine supplements (days 3 and 4, Table I), FGA excretion of the Vit. B_{12} deficient chicks was almost as low as that of the controls, whereas, the folic acid deficient chicks excreted large amounts of FGA. On the other hand, subsequent elevation of dietary histidine in presence of the methionine supplement caused FGA excretion of the Vit. B_{12} deficient chicks to rise higher than that of the controls. Thus it would appear that the relative differences between histidine and methionine intakes may provide an explanation for the low FGA excretion reported for human beings (3,4) and the high values reported for chicks (2) and rats(1) deficient in Vit. B_{12}. It is difficult to compare the histidine loads received by the young growing chicks and the human beings (chiefly adults); however, the following calculations provide a very rough basis for comparison. In relation to body weight, total histidine intake of the chicks fed 1% L-histidine•HCl (approximately 1.5 g/kg body weight/day) was about 7 times that for a human being receiving a daily dose of 15 g L-histidine•HCl, as recommended by Luhby *et al.* (3), assuming a 65 kg man with a daily protein consumption of 65 g. In relation to protein intake under the same conditions, only 5.2% of the chicks' total protein plus free amino acid intake was histidine while the proportion of histidine would be around 18% for man. Presumably the folic acid and Vit. B_{12} deficient humans received adequate methionine. It would be of interest to study the effect of methionine-low diets upon FGA excretion in the Vit. B_{12} deficient patients. It is significant that increasing the L-histidine•HCl supplement of the Vit. B_{12} deficient chick to 2 and 4% of the diet caused appreciable excretion of FGA even in the presence of 1% methionine whereas the controls were able to metabolize these higher levels of histidine almost completely.

Unquestionably the severity of the folic acid and Vit. B_{12} deficiency states influences the excretion of FGA and the response to supplemental methionine. Silverman and Pitney(1) found that a 1% supplement of methionine caused greater reduction in FGA excretion by folic acid deficient rats than was observed in the present study with chicks; however, their deficient rats were growing at the same rate as the controls and presumably were less severely deficient than these chicks. Although the folic acid deficient and the Vit. B_{12} deficient chicks grew at similarly slow rates they cannot necessarily be regarded as being equally deficient. When the Vit. B_{12} deficient chicks received supplemental methionine, they were able to utilize the diet more effectively for growth. Their total daily food intake increased, but remained fairly constant in relation to body weight. The greater utilization of histidine for tissue protein synthesis probably did not affect the rate of FGA excretion since the requirement of histidine for the growing chick is 1.5 g/kg diet. With the 1% supplement of L-histidine•HCl, total histidine content of the diet was 15.9 g/kg and with the 4% supplement, 40.2 g/kg. Confirmation that growth rate did not affect FGA excretion has ben provided by a preliminary experiment in which food intake of the Vit. B_{12} deficient and control groups was limited to about $\frac{3}{4}$ of the expected intake beginning the day the methionine supplement was incorporated in the diet. The FGA excretion pattern of these chicks was similar to that of their

corresponding groups in Table I (unpublished data).

The function of tetrahydrofolic acid in the metabolism of the formimino group of FGA has been delineated by Tabor and Wyngarden (9). No such role has been described for Vit. B_{12}. From the data in this paper it seems possible that Vit. B_{12} affects utilization of FGA in some way in addition to that involving the metabolism of methionine. The 1% level of methionine did not cause FGA excretion of the Vit. B_{12} deficient chicks to stay at the same low level as that of Vit. B_{12} supplemented chicks when histidine was fed at the highest levels. Only 0.3% supplemental methionine is required to support normal growth of B_{12} deficient chicks with this diet(10). Secondly, the chicks that were severely deficient in methionine did not excrete large amounts of FGA until Vit. B_{12} was omitted from the diet. Perhaps either methionine or Vit. B_{12} or both modify the cofactor role of folic acid in the metabolism of the formimino group by the chick in a manner like that demonstrated in the rat by Noronha and Silverman(11).

Summary. Day old chicks were fed a diet borderline in methionine for 3 to 4 weeks. They excreted large amounts of formiminoglutamic acid (FGA), a metabolite of histidine, when either folic acid or Vit. B_{12} was omitted from the diet. FGA was excreted in very small amounts by control chicks fed the complete diet. Incorporation of supplemental methionine at 1% of the diet caused a marked reduction in FGA excretion of Vit. B_{12} deficient chicks and a moderate reduction in the folic acid deficient chicks. Further elevation of dietary histidine and continuation of the supplemental methionine resulted in increased FGA excretion which was relatively greater in the folic acid deficient than in the Vit. B_{12} deficient group. Only on the last day of the experiment was the excretion elevated for the control group. Chicks fed diets severely deficient in methionine did not excrete large amounts of FGA until Vit. B_{12} was omitted.

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Inhibition of Aminonucleoside Nephrosis in Rats. II. Effect of Nucleic Acid Precursors and L-triiodothyronine.* (27042)

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The mechanism by which the aminonucleoside (P-A) of puromycin induces the classical nephrotic syndrome in rats is still unknown. Since adenine, a primary precursor of RNA in rat, has been shown partially to block the nephrotoxicity of P-A(1,2), it seemed worthwhile to evaluate the protective action of other RNA and DNA intermediates.

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Methods. Female rats, descendants of the