Fellman(13) have demonstrated *in vitro* inhibition of dopa decarboxylase by phenylpyruvic, phenyllactic, and phenylacetic acids. Since these derivatives of phenylalanine are known to be present in excessive quantities in phenylketonuria and this change is reversed by a diet low in phenylalanine content (13), the present data would appear to provide evidence for the *in vivo* inhibition of dopa decarboxylase.

Pare *et al.*(14) have indicated that the decrease of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in phenylketonuria is due to an inhibition of 5-hydroxytryptophan decarboxylase by phenylalanine metabolites. These findings have recently been confirmed in normal rats made phenylketonuric by feeding excessive amounts of both phenylalanine and tyrosine(15). Studies carried out *in vitro* by Yulwiler(16) and Hess *et al.* (17) have indicated that the pyridoxal-phosphate dependent decarboxylases may represent closely related or identical enzyme systems. The similarity between the changes caused by the derivatives of phenylalanine upon 5-hydroxytryptophan and dopa decarboxylase would seem to provide further *in vivo* evidence for this hypothesis.

Summary. Data have been presented showing a decrease of norepinephrine and epinephrine in the plasma and of dopamine, norepinephrine, and epinephrine in the urine of phenylketonuric children. These changes were reversed when the patients were treated with a low-phenylalanine diet. Since plasma tyrosine levels remained unchanged, this decrease appears to be caused by inhibition of dopa decarboxylase by the derivatives of phenylalanine. This would provide further *in vivo* evidence that the pyridoxal-phosphate dependent decarboxylases represent related or identical enzyme systems.

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Effects of Vitamin B_{12} and Methionine on Excretion of Formiminoglutamic Acid by the Chick.* (26735)

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Young rats fed a diet deficient in Vit. B_{12} were found by Silverman and Pitney(1) to excrete large amounts of formiminoglutamic acid (FGA), a metabolite of histidine that normally is excreted in negligible amounts in urine. They found that supplements of either Vit. B_{12} or methionine caused a drop in FGA

* Part of these data were presented at Fifth International Congress on Nutrition, Sept. 1-7, 1960, Washington, D. C.

TABLE I. Effect of Vit. B_{12} Deficiency upon Excretion of Formiminoglutamic Acid.*

Vit. B_{12}, mg/kg diet	Body wt ± S.E., g	Histidine intake ± S.E., mmole/day	μmoles FGA ± S.E.	
			Per day	Per mmole histidine
0	114 ± 3.3	1.1 ± .07	113 ± 13.9	108 ± 14.5
.1	215 ± 8.8	2.0 ± .11	20 ± 5.0	11 ± 2.7

* Avg of values from 3 exp., total of 21 chicks for each treatment, 22 days of age.

excretion to low or zero levels. This Vit. B_{12}-methionine relationship in the rat was closely analogous to that observed by Fox *et al.*(2,3) in young chicks; *i.e.*, growth was poor in the absence of Vit. B_{12} but good upon supplementation of the diet with either the vitamin or methionine. The diets used in both the rat and the chick studies contained high levels of fat and were borderline in the sulfur-containing amino acids. FGA excretion pattern of the Vit. B_{12} deficient chick was investigated because it seemed that this parameter of deficiency could be useful in elucidating some of the interrelationships between Vit. B_{12}, methionine, and fat. It was found that the Vit. B_{12} deficient chicks excrete large amounts of FGA and that this excretion rate is markedly reduced by methionine.

Methods. Day-old female New Hampshire chicks were placed in electrically heated batteries with screen wire floors. They were fed diet C62(4), a purified soybean protein diet that contained 24% of fat. Vit. B_{12} was omitted from the diet except as indicated in Table I. After 3-5 wks, the chicks were placed in individual cages and the excreta were collected and assayed for FGA as previously described(4,5). The number of chicks per experimental group varied between 5 and 8. During the collection periods, L-histidine HCl was added to the diet at a level of 1% to accentuate the differences in FGA excretion. Supplements of methionine and related compounds were incorporated into the diet for 24 hr intrevals as indicated in Tables II and III. In the experiments reported in Table IV, chicks were raised on a Vit. B_{12} deficient diet exactly like C62 except that fat content was lowered to 4%; glucose was added to compensate for the weight of fat (200 g/kg of diet) that was omitted. FGA excretion was determined while the chicks received the low level of dietary fat and also during the 2 days following an increase in dietary fat to 24%. In some experiments food intake was restricted on certain days. When this was done, the chicks received one-third of the day's ration at the beginning of the day and the remaining two-thirds 8 hr later.

In addition to colorimetric assays for FGA many extracts of excreta were also subjected to paper chromatography and paper electrophoresis. Components of the extract were separated in duplicate on Whatman 3MM paper by ascending chromatography for 16 hr. The developing solvent was the butanol phase which separated after mixing n-butanol 40%, glacial acetic acid 10%, and water 50% by volume. The strips were dried and one strip for each sample was exposed to the atmosphere over ammonium hydroxide for 1 hr to hydrolyze the formimino group from

TABLE II. Effect of Methionine Supplementation for One Day upon Excretion of FGA by Vit. B_{12} Deficient Chicks.*

Day	DL-methionine, % of diet	Body wt, g	Histidine intake, mmole/day	μmoles FGA	
				Per day	Per mmole histidine
1	0	203	2.0	103	52
2	0	212	1.9	111	58
3	1	231	2.6	14	5
4	0	232	1.8	270	150
5	0	239	1.9	121	64

* Mean values for 15 chicks from 2 experiments. Chicks were 31 days of age on day 1.

TABLE III. Effect of Compounds Related to Methionine upon Excretion of FGA by Vit. B_{12} Deficient Chicks.* (μmoles FGA/day/mmole histidine intake.)

Day	Supplement†	DL-methionine‡	DL-homocysteine thiolactone HCl	DL-homocystine	L-cystine	Choline chloride	Betaine
1	0	67	113	39	35	68	46
2	0	72	111	34	51	45	59
3	+	35	111	39	50	48	72
4	0	211	113	68	66	74	60
5	0	120	103	46	43	44	48
No. of chicks		5	7	13	5	13	12

* When more than 7 chicks received a given supplement, the results are the avg of 2 experiments; ages ranged between 21 and 31 days.

† The methionine supplement (Table II) was incorporated at a level of 1% of the diet; all supplements in this table were equimolar or equivalent in methyls (choline and betaine) to methionine.

‡ Food intake of each chick was limited on day of methionine supplementation to amount of diet consumed the previous day.

the FGA. Excess ammonia was dissipated from the strips by forced air at room temperature for ½ hr. All strips were then dipped in a solution of 0.2% ninhydrin in acetone. FGA does not stain with ninhydrin whereas the glutamic acid formed during hydrolysis stains blue. Other pairs of strips were sprayed with the same alkaline ferricyanide-nitroprusside reagent and sodium borate solution as used for the colorimetric assay(5). These reagents form a pink color with compounds having a formimino group. Electrophoresis was carried out with the pyridine-acetate buffer of Knowles(6) in Spinco Model R migration chambers at 400 volts for two and one-half hr. A sample stripper was used to apply 0.01 ml of sample across each strip of 3 cm wide Whatman 3MM paper. The same volume of a standard solution containing 1 μmole FGA/ml was used as a control. Following electrophoresis, the strips were dried and cut in half longitudinally. One of each pair was exposed to ammonia vapor and then both strips were stained in the same manner as described above.

Results. The Vit. B_{12} deficient chicks grew slowly and excreted more than 5 times as much FGA as chicks in the control group receiving Vit. B_{12} (Table I). This difference was highly significant ($P<0.01$) when total mean daily excretions were compared as well as when FGA values were related to dietary histidine intake. Injection of Vit. B_{12} decient chicks with 1 γ Vit. B_{12}/day has been reported to cause FGA excretion to decline to minimal levels after 2 days(4). The same effect has been observed when the diet of the Vit. B_{12} deficient chicks was supplemented with 0.1 mg Vit. B_{12}/kg of diet (unpublished data).

When Vit. B_{12} deficient chicks were given a DL-methionine supplement (incorporated in the diet at a level of 1%) for 24 hr, there was an immediate and marked drop in FGA excretion (Table II). On the day following methionine supplementation, FGA excretion rose to a level which was more than double the daily excretion during the pre-supplementation period. Then the amount of FGA dropped to the original level on the fifth day of the experiment. This pattern of FGA excretion is also shown in the photograph of the paper strips (Fig. 1) on which the com-

TABLE IV. Effect of Dietary Fat Level upon Excretion of FGA by Vit. B_{12} Deficient Chicks.*

Day	Dietary fat, %	Body wt, g	Histidine intake, mmole/day	μmole FGA Per day	μmole FGA Per mmole histidine
		Exp. 1 (ad libitum)			
1	4	180	2.6	65	25
2	4	190	2.5	87	35
3	24	195	1.7	103	61
4	24	200	1.9	108	57
		Exp. 2 (restricted food intake†)			
1	4	171	1.4	157	112
2	4	173	1.4	144	103
3	24	178	1.4	167	119
4	24	183	1.4	158	113

* 5 chicks in Exp. 1, 8 in Exp. 2; all 21 days old on day 1 of each experiment. All chicks received the low fat diet prior to 21 days of age.

† Amount of diet offered each chick each day was 75% of the avg amount consumed by the group on the day prior to day 1.

ponents in extracts of excreta were separated electrophoretically. These were from one chick during successive days of the above experiment. No formimino-containing compound other than FGA has been detected during this work by either the chromatographic or electrophoretic procedures described above.

The chicks ate more of the diet on the day when the methionine supplement was given (Table II). This increase may have influenced the excretion of FGA on the day following supplementation. Food intake on the day of methionine supplementation was therefore limited to the same amount of diet as that eaten on the day prior to the methionine supplement (Table III). In this experiment methionine did not lower FGA to as low a level as that seen in Table II. A possible explanation may lie in the fact that the chicks ate all of their diet before the end of the 24 hr period and part of the "post-methionine" high rate of FGA excretion may have been included in this day's excretion. However, the rise in FGA excretion on the day following supplementation was similar to that in Table II.

Several compounds biochemically related to methionine were tested for their effect upon FGA excretion (Table III). Each was incorporated into the diet for one day at a level equimolar to the 1% supplement of methionine. The first 2 days of each of these experiments serve as the basis of comparison for assessing the effect of each of the supplements. None of these compounds (DL-homocysteine thiolactone HCl, DL-homocystine, L-cystine, choline chloride, or betaine) reduced FGA excretion. In fact FGA excretion was slightly higher on the day that betaine was given. When either homocystine or choline chloride was given, there was a moderate increase in mean FGA excretion on the day following the supplement; however, these effects were small when compared with those of methionine.

Chicks raised to 3 weeks of age on the Vit. B_{12}-free diet that contained only 4% of fat excreted large amounts of FGA (Table IV). When the fat content of this diet was increased to 24% there was a moderate increase in FGA excretion. There was a decrease in the weight of food ingested when the fat content of the diet was increased, probably due chiefly to the higher caloric density of the high fat diet. A second experiment was conducted in which food intake was restricted during the initial 2-day FGA measurement period. It was thus possible to keep food intake constant during the time that both the low and the high fat diets were fed. In this experiment there was no change in FGA excretion when the dietary fat intake was increased.

Discussion. The dramatic effect of a methionine supplement in decreasing the high rate of FGA excretion by Vit. B_{12} deficient chicks confirms the findings that Silverman and Pitney obtained with the rat(1). The mechanism of this effect has been discussed (1.7); however, it is still largely a matter for conjecture. In these experiments only the intact methionine molecule was effective. The very rapid effect of the methionine supplement as compared to that of Vit. B_{12} suggests that methionine may be directly involved in the metabolism of the formimino group arising from dietary histidine. The marked increase in FGA excretion following removal of the methionine supplement apparently was not related to the increased food intake that occurred when additional methionine was incorporated in the diet (Table III). A relatively small proportion of the dietary histidine was excreted as FGA by the deficient chicks. It is therefore possible that the Vit. B_{12} deficient chicks had become adapted to use efficiently the small amount of available methionine for metabolism of the formimino group and that this efficiency was temporarily lost when the 24 hr high methionine supplementation period ended. This may account for the observed excretion pattern (Table II, Fig. 1).

FGA excretion of individual Vit. B_{12} deficient chicks has been found to be fairly constant from day to day; however, somewhat larger differences in control values have been found between different experiments (days 1 and 2, Table III). These experiments were carried out over a period of several months and variability in the chicks may have been a factor. The 2 experiments reported in Table IV were conducted almost

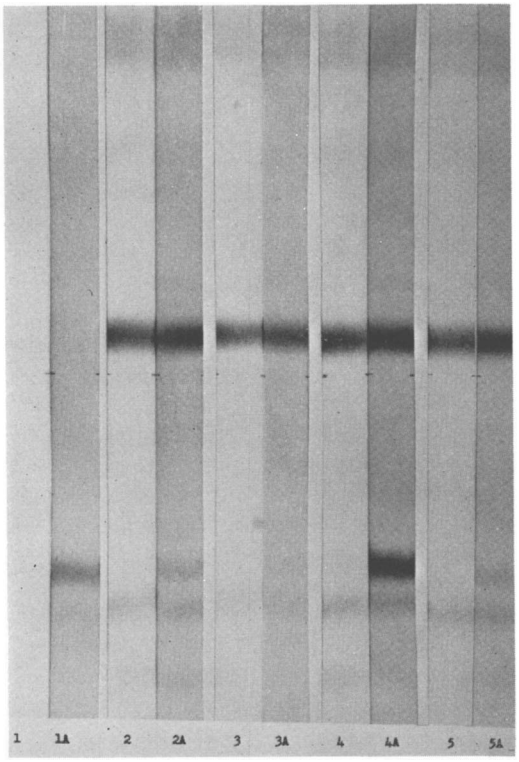

FIG. 1. Separation of amino acids by electrophoresis. The strip of each pair marked "A" was exposed to ammonia for 1 hr prior to staining with ninhydrin. The origin was marked with pencil on each side of each pair at the center of each strip. During electrophoresis the position of the lower ends of the strips as seen in the photograph were at the anode side. The patterns resulted from application of the following solutions: (1) standard FGA, 1 μmole/ml, (2) extract from vit. B_{12} deficient chick, 0.8 μmole FGA/ml (by colorimetric analysis), (3) same chick, day of 1% methionine supplement, 0.08 μmole FGA/ml, (4) same chick, first day after methionine supplement removed, 4.85 μmoles FGA/ml, (5) same chick, second day after methionine supplement removed, 0.9 μmole FGA/ml. Each day's excreta were made to a total volume of 50 ml.

successively. These data offer the suggestion that restriction of food intake may increase excretion of FGA. This possibility is being investigated further.

It is difficult to explain the differences in FGA excretion pattern between the 2 Vit. B_{12} deficient animal species and human subjects with pernicious anemia. Luhby *et al.* (8) and Spray and Witts(9) found that pernicious anemia patients excreted insignificant amounts of FGA after daily histidine loading tests of 15 and 2 g, respectively. In view of the results with animals, it is suggested that the methionine intake of the patients may have been sufficient to mask the effect of their Vit. B_{12} deficiencies upon FGA excretion. It is significant that a high level of dietary fat was not necessary to cause Vit. B_{12} deficient chicks to excrete large amounts of FGA. This is contrary to the earlier observations on growth of the chick fed this Vit. B_{12} deficient diet(3). The level of dietary fat is probably not involved in the differences between man and the chick.

Summary. Young chicks fed a diet deficient in Vit. B_{12} for 3-5 weeks excreted large amounts of formiminoglutamic acid (FGA) when compared to control chicks that received Vit. B_{12} in the diet. The high excretion rate of FGA by the Vit. B_{12} deficient chicks was reduced to very low levels by incorporation of 1% DL-methionine into the diet for 24 hr. This effect was not observed with supplements of DL-homocysteine thiolactone HCl, DL-homocystine, L-cystine, choline chloride, or betaine. Although a high level of dietary fat had previously been found necessary to cause poor growth in the absence of Vit. B_{12}, FGA excretion was quite high with either 4 or 24% of fat in the diet.

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