The Role of Histidine in the Anemia of Folate Deficiency

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The amino acid histidine is metabolized to glutamic acid in mammalian tissue. Formiminoglutamic acid (FIGLU) is an intermediary in this reaction, and tetrahydrofolic acid is the coenzyme that converts it to glutamic acid. A test for folate deficiency concerns the measurement of urinary FIGLU excretion after a histidine load. It was observed that folate-deficient individuals receiving the histidine for the FIGLU test made hematological response that alleviated the anemia associated with this deficiency. This was unusual in that a biochemical test to determine the deficiency results in a beneficial effect for one aspect of the deficiency. The studies reported in this paper give a metabolic explanation for this phenomenon. Urine was collected for 24 hr from 25 folate-deficient subjects, 10 vitamin B_{12}-deficient subjects, and 15 normal controls. Urinary excretion of histidine was a mean of 203 mg with a range of 130–360 mg for the folate-deficient subjects; 51.5 mg with a range of 30–76.6 mg for normal subjects; and 60.0 mg with a range of 32.3–93.0 mg for the vitamin B_{12}-deficient subjects. All the folate-deficient subjects subsequently made a hematological response to the histidine administered for the FIGLU test. No hematological response was observed in the vitamin B_{12}-deficient individuals. When folic acid was given to folate-deficient subjects who received no histidine, urinary histidine levels returned to normal levels rapidly and this was followed by a hematological response. Others have shown that volunteers fed a histidine-free diet developed anemia. In normal subjects, histidine is excreted much more in the urine than other essential amino acids are. Hemoglobin protein contains 10% histidine. Under normal conditions, dietary histidine can supply sufficient histidine to prevent anemia. When the dietary intake is diminished or the urinary excretion is greatly increased, anemia results. It is concluded that folate deficiency causes histidine depletion through increased urinary excretion of this amino acid. Feeding histidine replenishes tissue levels of histidine, resulting in hemoglobin regeneration. Folic acid administration results in return of histidine to normal urinary levels. Thus, a combination of folic acid histidine would be beneficial for folate deficient individuals. Exp Biol Med 227:998–1000, 2002

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The amino acid histidine is metabolically converted to glutamic acid in mammalian tissue in the following sequence: histidine → urocanic acid → formiminoglutamic acid (FIGLU) → glutamic acid.

The conversion of FIGLU to glutamic acid requires tetrahydrofolic acid as a coenzyme (1). A method for determining folate deficiency is based on this reaction. A metabolic load of histidine, dissolved in a suitable liquid, is administered orally to a subject suspected of having this deficiency, urine is collected for 24 hr, and the FIGLU excretion is determined by an enzymatic method (2, 3).

It was reported that subjects with macrocytic, megaloblastic anemia who were shown to have folate deficiency by the excretion of elevated FIGLU after the histidine load in this test made a rapid hematological response without the administration of folic acid (4).

This was a unique occurrence in that the test for a vitamin deficiency resulted in the correction of a sign of this deficiency, namely the anemia. Those with vitamin B_{12} deficiency who did not excrete elevated FIGLU failed to show such a response.

A study was undertaken to find a metabolic explanation for this effect of histidine, and the results are reported in this paper.

Materials and Methods

Subjects. Twenty-five individuals with folate deficiency, 10 with vitamin B_{12} deficiency, and 10 normal subjects as controls were studied.

Folate deficiency was determined by FIGLU excretion after a histidine load, serum, and whole blood levels of folic acid and response to physiological doses of folic acid.

Vitamin B_{12} deficiency was determined by serum vitamin B_{12} levels, urinary excretion of methylmalonic acid, the Shilling test for intrinsic factor, and hematological response to appropriately administered physiological levels of vitamin B_{12}. Details of these methods are given in Reference 1.

FIGLU Assays. Each subject received a metabolic load of 15 g L-histidine hydrochloride monohydrate divided into three doses of 5 g dissolved in apple juice, 4 hr apart. Urine was collected for 24 hr after the first dose in a bottle containing 10 ml of HCL and 10 ml of toluene. FIGLU was measured by the enzymatic methods previously described (2, 3). Excretion of FIGLU greater than 35 mg in 24 hr

indicated folate deficiency. This level of excretion increases with the severity of the deficiency.

Histidine Assays. A basal 24-hr urine excretion was collected before the histidine load. After neutralization, the samples were diluted with distilled water as necessary for the assay. The microbiological assay for histidine used (5) was modified so that the final volume in the test tubes was 2 ml consisting of 1 ml of medium and 1 ml of diluted urine.

All the tests in this study were standard laboratory diagnostic tests. All the subjects were ambulatory and none took vitamin supplements.

Results

The basal urinary excretion of histidine and FIGLU and for FIGLU after a histidine load are shown in Table I. In all cases, the basal urinary excretion of histidine exceeded that of FIGLU. The histidine excretion for the normal controls was a mean of 51.5 mg/24 hr, and that for the vitamin B_{12}-deficient subjects was 60.0 mg/24 hr, which were not significantly different. The basal urinary excretion of the folate-deficient subjects was a mean of 203 mg/24 hr, which was significantly higher that of normal controls, 51.5 mg/24 hr, $P < 0.01$ and that of vitamin B_{12}-deficient subjects at 60.0 mg/24 hr, $P < 0.01$.

Each subject with elevated basal histidine excretion and FIGLU excretion after a histidine load of over 35 mg/24 hr made a hematological response 4 to 5 days after the histidine dose (Table II). As in the original study (4), the degree of response depended on the degree of anemia. The reticulocyte response ranged from 4% to 13.5%, and the hemoglobin with an initial range of 7.5–10.0 g/100 ml increased to 10.6–13.8 g/100 ml. Seven days after the histidine dose, the folate-deficient patients were given 1 mg of folic acid i.m. for three consecutive days, and their histidine and FIGLU levels returned to levels similar to that of the normal controls.

These data show that histidine administered to folate-deficient subjects was responsible for alleviating their anemia even though they still exhibited biochemical evidence of folate deficiency. In two additional subjects with macrocytic, megaloblastic anemia who showed no response to three consecutive doses of vitamin B_{12}, their urinary histidine excretion of 210 and 302 mg/24 hr was not diminished. They were then given 1 mg of folic acid i.m. for three consecutive days. After 2 days of 1 mg of folic acid administration, the urinary histidine excretion was 55 and 60 mg/24 hr, respectively, and after 5 days, they made a satisfactory hematological response.

The subjects with vitamin B_{12} deficiency did not excrete higher than normal levels of histidine and they made no hematological response to histidine administration.

Table II. Hematological Response to Histidine Administration in Folic Acid-Deficient Patients

	Before Histidine		After Histidine	
	Reticulocytes %	Hemoglobin G/100 ml	Reticulocytes %	Hemoglobin G/100 ml
Range	0.2–1.4	7.5–10.0	4.0–13.5	10.6–13.8
Mean	0.5	8.1	8.9	12.1

Note. Values 5 days after dose.

Discussion

Histidine used as a metabolic load in the FIGLU test not only detects the folate deficiency, but it also alleviates the low hemoglobin level associated with this deficiency. This is an unusual situation in which a diagnostic test also cures the anemia.

The 24-hr urinary histidine excretion collected before the histidine load was three to four times that found in normal controls. There is a relationship between histidine and anemia. Histidine constitute 10% of the amino acid composition of hemoglobin (6). Normal adults were fed a histidine-free diet and after 20 days, they developed a decrease in hemoglobin levels (7). In a study with folate-deficient rats, there was a decrease in histidase (histidine-ammonia lyase) in their livers (8). If this also occurs in humans, less histidine would be metabolized to glutamic acid, thus increasing the urinary histidine excretion.

The urinary excretion of histidine in normal urines exceeds that of other essential amino acids (9, 10). The level of this excretion was similar to those found in the present

Table I. Urinary Excretion of Histidine and FIGLU in Subjects with Folate or Vitamin B_{12} Deficiency and in Normal Controls

Number	Diagnosis	Basal urinary excretion mg/24 hr histidine (mean ± SE[b]; range)	Basal urinary excretion mg/24 hr FIGLU (mean ± SE[b]; range)	Urinary excretion after histidine load[a] mg/24 hr FIGLU (mean ± SE[b]; range)
25	Folate deficiency	203 ± 21.3 (130–360)	16.9 ± 8.5 (0–33.6)	113.1 ± 16.8 (43.9–421)
10	Vitamin B_{12} deficiency (Addisonian pernicious anemia)	60.0 ± 16.5 (32.3–93.0)	5.8 ± 2.3 (0–7.4)	16.1 ± 8.2 (19–27.6)
15	Normal controls	51.5 ± 14.8 (30–76.6)	4.1 ± 1.5 (2.3–7.5)	16.8 ± 3.8 (6.1–29.7)

[a] Folate-deficient range >35 mg/24 hr.
[b] SEM.

study. This high urinary excretion may be explained by the results of a study in which healthy volunteers were infused with an amino acid mixture. The glomerular filtration of histidine was greater than that of the other amino acids. However, the renal reabsorption that was almost complete for the other amino acids was less than complete for histidine (11). Under normal conditions, the diet compensates for the loss of histidine, but cannot meet the increased demands in folate deficiency where the excretion is three to four times that in normal individuals. It is apparent that folate deficiency results in a histidine deficiency and that histidine alleviates the anemia.

Folates are essential for purine and pyrimadine synthesis. Under normal conditions, histidine provides one-carbon units for this synthesis during the metabolism of FIGLU to glutamic acid (1). In folate deficiency, this pathway is blocked. Therefore, it is essential to provide folic acid to correct the enzymatic functions of the folate enzymes. As a result of this study, we recommend a regimen that we have adopted: This is to give folate-deficient individuals 1 mg of folic acid and 5 g of histidine for 3 to 5 days to correct the deficiency. After this, a good diet can provide sufficient folate and histidine to prevent a deficiency.

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