A STUDY OF THE METABOLISM OF 4-AMINO-5-IMIDAZOLECARBOXAMIDE (AIC) IN FOLIC ACID DEFICIENCY IN RATS

SHARAD D. DEODHAR, M.D., PH.D., AND GERALD PITTMAN, B.S.* Division of Pathology

I is well known that folic acid deficiency is an important and fairly common cause of macrocytic anemia. At the present time, clinical states of folic acid deficiency can be studied by two laboratory methods. One method involves direct determination of the concentration of folic acid in the serum, by bacteriologic and other technics that are cumbersome and not fully reliable. The other method involves determination of the urinary excretion of formiminoglutamic acid (figlu), a metabolite of the amino acid, histidine, which requires folic acid for its catabolism. This latter method lacks the desired specificity for detecting folic acid deficiency, and it has been reported¹ that the test is often positive also in B_{12} deficiency and in chronic disease of the liver.

The well-known role of folic acid in converting the compound, 4-amino-5-imidazolecarboxamide (AIC), into the corresponding purine derivative (Fig. 1) suggested the possibility that this naturally occurring purine precursor might provide yet another valuable tool in the study of folic acid deficiency. Various investigators^{2, 3} have shown that AIC is converted into the purine moiety of nucleic acids in experimental animals and also in man. In the absence of folic acid, this conversion does not occur, and it was shown many years ago that in bacterial cultures treated with sulfonamides (folic acid antagonists) AIC accumulated in significant amounts.⁴ Little information is available on the fate of AIC in well-defined folic acid deficiency in experimental animals or in man. Accordingly, our investigation was undertaken to study the fate of AIC in rats made folic acid deficient by treatment with 4-amino-N₁₀-methyl pterylglutamic acid,[†] a well known folic acid antagonist.

MATERIALS AND METHODS

The materials comprised the hydrochloride derivative of AIC⁺ obtained commercially, and Methotrexate. The experimental animals were 37 Sprague-Dawley male rats that each weighed in the range of 150 to 200 gm. The methods involved the quantitative determination of the urinary AIC as a diazotizable amine, by the Bratton-Marshall test;⁵ and hematologic

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^{*} Senior medical student, University of Missouri Medical School, Columbia, Missouri.

[†] Methotrexate, Lederle Laboratories.

[‡] Calbiochem, Inc., Los Angeles, California.



Fig. 1. Conversion of 4-amino-5-imidazolecarboxamide (AIC) into purine derivative.

determinations of hemoglobin, and leukocyte and erythrocyte counts by the usual tests.

The rats were divided into two groups, a normal, control group of 12 rats, and a group of 25 rats in which folic acid deficiency was experimentally produced. This latter group was treated with Methotrexate injected intraperitoneally three times a week in graduated dosages per kilogram of body weight. For the first two weeks a dose of 1 mg. per kilogram of body weight was given; this was increased to 2 mg. for the next two weeks, and then to 3 mg. for the last five to six weeks. Body weight, blood hemoglobin, leukocyte and erythrocyte determinations were made biweekly on all rats in both groups. Specimens of blood were obtained from the tail. During the early period of treatment with Methotrexate, nine rats died, presumably from acute drug toxicity, leaving 16 rats available for study in this folic acid deficient group.

When the hemoglobin, and leukocyte and erythrocyte determinations indicated folic acid deficiency, a dose of 30 mg. per kilogram of body weight of AIC, in a saline solution, was injected intraperitoneally in both groups of rats. During the next 24 hours after AIC administration, all food was withheld, and the rats were given saline only. Each rat was kept in a metabolic cage. Urine was collected at from four- to six-hour intervals and the amount of AIC in the urine was determined. Urine was collected during 24 consecutive hours, although AIC excretion occurred mostly in the first two to six hours, and usually no AIC could be detected in the 16- to 24-hour collection. At the end of the experiment the animals were killed and postmortem examinations were performed.

RESULTS

After two weeks of treatment with Methotrexate, the body weight, blood hemoglobin content and blood cell counts began to decrease significantly, and by 9 to 10 weeks the average values for these blood constituents were considerably lower than were those for the control animals (*Table 1*).

At autopsy the specimens of bone marrow of the rats in the treated

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Group of rats	Body weight, gm.	Blood determinations			AIC
		Hemoglobin, gm./100 ml.	Leukocytes, no./cu.mm.	Erythrocytes, no./cu.mm.	percentage of admin- istered dose, %
Methotrexate treated (folic acid deficient), average value of 16 rats	286 (250-342)†	4.2 (3.2-5.8)†	2400 (1800-3200)†	1.1×10^{6} (0.8-1.5 × 10 ⁶)†	55* (42–68)†
Normal control, average value of 12 rats	396 (360-520)†	14.0 (11.5-16.2)†	12,500 (8,200-15,200)†	5.2×10^{6} (4.2-6.0 × 10 ⁶)†	18.6 (15-24)†

Table 1.—AIC urinary excretion in normal and in folic acid deficient rats

* This figure is significantly different from the "control figure" (P value <0.01).

[†] The bracketed values indicate the range for each value in the respective group of rats.

group showed a definite megaloblastic change compatible with folic acid deficiency. The urinary AIC excretion was considerably greater in the Methotrexate treated group (55 percent) than in the control group (18 percent). This difference in the AIC excretion could not be explained on the basis of altered renal or hepatic handling of AIC in the treated group. Twenty-four-hour volumes of urine and blood urea concentrations were in the normal ranges in these animals. Although specific liver function tests were not performed, gross and microscopic morphologic studies showed no significant changes in the liver or in the kidneys of the treated group of rats.

COMMENT

The results of this study are consistent with the view that the utilization of AIC for purine synthesis is folic acid-dependent; in folic acid deficiency, the conversion is inhibited and a much greater portion of administered AIC is excreted in the urine. A study of the urinary excretion of administered AIC may thus provide a useful approach in the clinical evaluation of folic acid deficiency. To date, Herbert and associates⁶ have reported the only clinical study concerning the use of AIC. The results of their study on a small number of patients were inconclusive. However, in view of the sound, theoretic basis of this approach, a thorough and extensive clinical investigation is warranted. Unfortunately, the commercially available synthetic AIC has not yet been released for clinical use, and clinical studies are not yet possible. This compound, though, should be kept in mind for future studies of patients for folic acid deficiency.

SUMMARY

A known metabolite in purine biosynthesis, 4-amino-5-imidazolecarboxamide (AIC), was administered to 12 normal rats and to 16 rats made folic acid deficient by treatment with Methotrexate, and the AIC urinary ex-

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cretion was studied. The folic acid deficient group excreted a considerably higher proportion (approximately threefold) of administered AIC as compared with the control group. These results are consistent with the known role of folic acid in the conversion of AIC into the purine moiety of nucleic acids; thus, in folic acid deficiency, this conversion is inhibited, and unchanged AIC is primarily excreted by the kidneys. It is proposed that AIC may provide a valuable agent for the clinical evaluation of conditions related to folic acid deficiency.

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