The Nutritional Status of Folic Acid in Persons with Leukemia and Its Possible Relation to Effects of Aminopterin Therapy

By Marian E. Swendsen, Ph.D., Ann Louise Swanson, B.S., Muriel C. Meyers, M.D., and Frank H. Bethell, M.D.

Since 1948 there have been many clinical reports on the therapeutic trial of metabolic antagonists of folic acid in leukemia.1-4 These studies show that in a certain number of patients a partial, temporary remission follows the administration of a folic acid antagonist. These remissions occur for the most part in cases of acute leukemia, particularly those in the younger age group, whereas the results of treatment in chronic leukemia are in general unsatisfactory. Moreover, in patients with chronic leukemia, the administration of an antagonist is usually accompanied by early severe toxic manifestations such as erythema, stomatitis, hemorrhage and anorexia. Therefore, it appears that a factor of general systemic nature which conditions the individual response to folic acid antagonists must vary in different types of leukemia. It seemed possible that the metabolic availability of folic acid itself might be such a factor. Accordingly, an attempt has been made to determine the nutritional status with respect to folic acid in a series of cases of chronic and acute leukemia.

Methods and Clinical Material

The criterion for folic acid nutritional adequacy which has been used is based on a widely employed method for vitamin nutrition studies: measurement of the urinary excretion of the vitamin in question following a test dose, a lowered vitamin excretion being indicative of some degree of nutritional deficiency. There is direct evidence that such a method is valid for assessing folic acid nutritional adequacy as it has been found that untreated pernicious anemia patients, who subsequently respond hematologically to folic acid therapy and who presumably are deficient in this vitamin, excrete a much smaller proportion of the test dose than do normal subjects.5-8

Accordingly, the excretion of folic acid or folic acid-like compounds was measured for a twenty-four hour period following the administration of a 5 mg. test dose to patients with various types of leukemia. Folic acid determinations were made by a microbiologic assay procedure using S. faecalis as the test organism.8

Thirty-one cases of lymphocytic or granulocytic leukemia including 18 with chronic and 13 with acute forms of the disease were used as subjects for the study. The patients were all adults and were selected on the basis of their suitability for metabolic studies and the absence of evidence of renal impairment or gross dietary inadequacies.

Experimental Results

Previous studies from several laboratories have shown that the folic acid excretion of normal subjects under ordinary dietary conditions is less than 10 μg.
per twenty-four hours. Since supplementation with from 3 to 5 mg. of folic acid results in a urinary excretion of about 30 per cent of the test dose, any difference in folic acid excretion as a result of variations in dietary intake is insignificant in the interpretation of these data. Therefore, the urinary excretion of folic acid after giving 5 mg. of the vitamin to a series of patients with leukemia is recorded as a direct percentage of the administered dose. Figure 1 compares the data obtained in normal subjects; patients with acute leukemia, granulocytic or lymphocytic; and patients with chronic leukemia, granulocytic or lymphocytic.

The range of variation of folic acid excretion is greater for the patients with both acute and chronic leukemia than for the 10 normal individuals. However,

THE RANGE OF VARIATION OF FOLIC ACID EXCRETION IS GREATER FOR THE PATIENTS WITH BOTH ACUTE AND CHRONIC LEUKEMIA THAN FOR THE 10 NORMAL INDIVIDUALS. HOWEVER,

the average excretion of folic acid for both types of leukemia is much less than for normals (1.5 mg. per 24 hours) and, furthermore, the vitamin excretion is significantly less for chronic (0.80 mg. per 24 hours) as compared to acute leukemia (1.14 mg. per 24 hours). A statistical analysis of these data indicates that there is less than one possibility in one hundred that any of these differences in average excretion is due to chance.

In vitamin nutrition studies, where vitamin test losses are given and urinary excretion measured, a percentage excretion value which is lower than any obtained in normal individuals, is set up as being indicative of some degree of nutritional inadequacy. In general, the correlation is made that the lower the percentage excretion value obtained for the subject under investigation, the more severe is the deficiency state. For the series of cases reported here, none of the 10 normal subjects excreted less than 25 per cent of the test dose of folic acid. When the leukemia cases are considered in the same manner, it is found that 19

FIG. 1.—Percentage of 5 mg. dose of folic acid excreted in 24 hours by normal subjects and by patients with acute and chronic leukemia.

<table>
<thead>
<tr>
<th>%</th>
<th>NORMAL SUBJECTS</th>
<th>ACUTE LEUKEMIA</th>
<th>CHRONIC LEUKEMIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AV. = 1.50 MG. | AV. = 1.14 MG. | AV. = 0.80 MG.|
PER. 24 HRS.  | PER. 24 HRS.  | PER. 24 HRS.  |

o — GRANULOCYTIC
△ — LYMPHOCYTIC
of the 31 excrete less than 20 per cent of the test dose and 7 of the 31 excrete less than 15 per cent. The data for the various types of leukemia are recorded in Table 1. It is to be noted that all of the cases excreting less than 15 per cent of the test dose are cases of chronic leukemia.

Therefore, if the percentage excretion of a test dose of folic acid is to be taken as a measure of the vitamin nutritional adequacy, then an actual depletion of folic acid occurs in leukemia and this depletion is more frequently associated with the chronic than with the acute form of the disease.

### Table 1.—Correlation of Various Types of Leukemia with Percentage Excretion of Folic Acid Test Dose

<table>
<thead>
<tr>
<th>Type of leukemia</th>
<th>No. of cases</th>
<th>No. of cases excreting less than 20% of test dose</th>
<th>No. of cases excreting less than 15% of test dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic granulocytic</td>
<td>11</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Chronic lymphocytic</td>
<td>7</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Acute granulocytic</td>
<td>10</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Acute lymphocytic</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>19</td>
<td>7</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Based on folic acid excretion studies, evidence has been obtained that depletion of body stores of folic acid occurs in many cases of leukemia. As a possible explanation, it is suggested that the patient with leukemia has an increased requirement for the vitamin due to his hypermetabolic state, or perhaps more precisely, to the production of large numbers of immature leukocytes with relatively high folic acid concentrations. Since patients with chronic leukemia more commonly show evidence of greater folic acid depletion than those with acute leukemia, it appears that duration of the disease may be a factor in exhausting tissue reserves of the vitamin.

The evidence of folic acid depletion occurring more frequently and with a greater degree of severity in cases of chronic leukemia correlates the nutritional inadequacy of this vitamin with the severe toxic reactions to Aminopterin (4-aminopteroylglutamic acid) occurring in these patients. It would appear that Aminopterin itself might be retained in the body to a greater degree in chronic leukemia and become distributed throughout the body tissues. Data indicating that this may be true are presented in a subsequent paper dealing with Aminopterin excretion.

Evidence has been presented by Farber that in some cases of acute leukemia in children folic acid may be a controlling factor in the rate of proliferation of leukemic cells. Although folic acid depletion may have a retarding influence on cell growth it should be emphasized that no attempt is made to interpret the data presented in this paper in terms of qualitative or quantitative cytological changes. Moreover, it should not be inferred that folic acid depletion is associated specifically with leukemia to the exclusion of other forms of disseminated malignant disease. However, folic acid nutrition in leukemia acquires significance in the light of therapeutic considerations pertaining to the use of folic acid antagonists.
The correlation of folic acid depletion with chronic leukemia, which cannot be satisfactorily treated with folic acid antagonists, suggests that it may be possible by measuring folic acid excretion following a test dose of the vitamin to predict the subsequent response of a given patient to Aminopterin administration.

**SUMMARY**

Folic acid excretion following test doses of the vitamin has been measured in 31 cases of leukemia. Although the percentage excretion values varied widely, the average values were significantly lower in cases of both chronic and acute leukemia than in a series of normal subjects. However, this evidence of depletion of body stores of folic acid occurred more frequently and with a greater degree of severity in cases of chronic leukemia, particularly, those with lymphocytic involvement. This suggests that the degree of folic acid nutritional depletion is related to the varying clinical results obtained with antagonist therapy in chronic and acute leukemia.

**REFERENCES**

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