Folic Acid Activity in Leukemia and Cancer

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SUMMARY

The serum folic acid activity (SFAA) was measured using Lactobacillus casei as the test organism. The SFAA was lower for patients with malignant tumors than for the control group. The lowest values were for malignant lymphoma and leukemia. No relation was found between the SFAA and either the spread of the tumors, the therapy administered, the age of the patient or the degree of wasting. Although the SFAA was significantly lowered in leukemia, the folic acid activity (FAA) was higher in white cells of such patients than in those of healthy controls.

The FAA was also determined in chicks during development of virus-induced leukemia.

Low SFAA has been found in cases of malnutrition and intestinal malabsorption (2, 12, 16) and in experimentally induced folic acid deficiency (11). Low values have also been found in megaloblastic anemia of pregnancy (12, 16) and in conditions accompanied by increased blood-cell formation (5, 19).

Following the report by Hoogstraten et al. (15) of a low folic acid activity in serum (SFAA) in some cases of leukemia we undertook a systematic study of the SFAA level, determined microbiologically, in normal subjects and those with leukemia, malignant lymphoma and other tumors. Part of the material of this study has been described in a preliminary report (22).

Hellman et al. (9) have recently reported low SFAA in 10 and borderline levels in another 10 out of 35 cases of solid tumors.

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METHODS

SFAA.—Ten milliliters of blood was obtained from each human subject. Serum samples were diluted with 5 X 10^{-2} M sodium phosphate buffer (pH 6.1), in which 50 mg/100 ml of ascorbic acid had been freshly dissolved, and the SFAA was determined microbiologically (1, 12) using Lactobacillus casei.

The serum-buffer solution was autoclaved for 2 min. at 118°C. Coagulated proteins were centrifuged down and the clear supernatant was diluted 1:2.5 with distilled water. Of the diluted serum 0.5 ml, 1.0 ml, and 2.0 ml were each added in duplicate to 2.0 ml of the double-strength, basal medium and distilled water was added to a final volume of 4.0 ml. Tubes were sterilized by autoclaving at 118°C. for 2 min. and, after cooling, inoculated along with the standard folic acid assay tubes.

L. casei (ATCC 7469) was maintained in a yeast extract-protease-peptone medium (1) and stored at 4°C. After not more than 1 week 1 drop of the stored liquid culture was transferred to 10 ml of the fresh maintenance medium, incubated 18 hr. at 37°C. and stored at 4°C. On the day prior to the assay one drop of an 18-hr. culture was transferred to 4.0 ml of single-strength basal medium (13), supplemented with 400 μg of folic acid (FA) and incubated at 37°C. for 6 hr. This culture was centrifuged and washed twice with 5.0 ml of freshly prepared 0.9 % saline. It was then resuspended in 5.0 ml of saline; 1.0 ml of this suspension was diluted 1:25 with saline. The assay tubes were inoculated with one drop of the diluted suspension.

A standard FA curve was prepared for each assay: 2.0 ml double strength basal medium was dispensed into test tubes. From 0.2 ml to 2.0 ml of the standard FA
solution was added in triplicate to provide 40, 80, 160, 240, 320, and 400 \( \mu g \) FA. The final volume was made up to 4.0 ml with distilled water. Blanks with no added FA were included. The tubes were plugged with cotton and autoclaved for 2 min at 118°C, cooled, inoculated, and incubated at 37°C in a water bath for 20 hr. The growth was measured in a Beckman B spectrophotometer at 600 nm. The uninoculated blank control was set at 100% transmittance and the relative transmittance of other tubes determined. Transmittance was plotted against the logarithm of the FA concentration. FA activities of serum samples were calculated using a standard curve.

Chick blood was drawn from a wing vein and 0.1 ml of the plasma was similarly assayed. The FAA difference for chicks was calculated in relation to the FAA value before inoculation, and for the uninoculated chicks in relation to the FAA from the first day of the experiment. A positive difference indicated an increase and a negative difference a decrease of FAA.

**Separation of cells.**—About 9 ml of blood was added to 1 ml of 6% dextran (m.wt. 250,000, AB Pharmacia) and 0.2 ml of 5% ethylenediamine tetraacetate (EDTA) in a heparinized tube. Agglutinated red cells were permitted to sediment for 45 min and the supernatant (S1) siphoned off and centrifuged at low speed for 2–3 min. The second supernatant (S2) contained mainly white cells and platelets. This supernatant (S2) was recentrifuged for 1 min at high speed (about 5000 rpm). The sediment (S3) was studied with a white cell count in the conventional manner, a differential count to determine the contamination with erythrocytes, and a bioassay. From the bottom of the first sediment (S1) a sample was obtained consisting of erythrocytes only; the absence of leukocyte contamination was checked in smears stained by the May-Grünewald Giemsa method. In this sample the erythrocyte FFA was measured; the value obtained in this manner was used for correction of the erythrocyte contamination in the white cell sample. No correction for contamination by platelets was made.

**RESULTS**

*Normal human controls.*—The SFAA values in 46 normal fasting controls ranged from 2.5 to 7.5, with a mean of 4.6 \( \mu g/\text{ml} \). This is lower than some previous values (10,12) but comparable with others (3,4, 8).

### TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Patients</th>
<th>Mean SFAA level (( \mu g/\text{ml} ))</th>
<th>S. E. of the mean</th>
<th>Significance of difference with respect to controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>46</td>
<td>4.60</td>
<td>0.20</td>
<td>—</td>
</tr>
<tr>
<td>Leukemia</td>
<td>40</td>
<td>2.66</td>
<td>0.27</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Malignant</td>
<td>14</td>
<td>2.27</td>
<td>0.14</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>lymphoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other malignant tumors</td>
<td>64</td>
<td>3.42</td>
<td>0.21</td>
<td>( P &lt; 0.001 )</td>
</tr>
</tbody>
</table>

* S.E., standard error.

### TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>Mean SFAA level (( \mu g/\text{ml} ))</th>
<th>S. E. of the mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiotherapy</td>
<td>30</td>
<td>3.3</td>
<td>0.34</td>
</tr>
<tr>
<td>No radiotherapy</td>
<td>34</td>
<td>3.5</td>
<td>0.25</td>
</tr>
<tr>
<td>ESR: 0–50 mm/hr.</td>
<td>35</td>
<td>3.6</td>
<td>0.29</td>
</tr>
<tr>
<td>51–130 mm/hr.</td>
<td>27</td>
<td>3.4</td>
<td>0.30</td>
</tr>
<tr>
<td>General condition:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>good</td>
<td>43</td>
<td>3.5</td>
<td>0.24</td>
</tr>
<tr>
<td>poor</td>
<td>19</td>
<td>3.5</td>
<td>0.42</td>
</tr>
<tr>
<td>Hemoglobin: ≤12.2 gm/100 ml</td>
<td>34</td>
<td>3.0*</td>
<td>0.26</td>
</tr>
<tr>
<td>&gt;12.2 gm/100 ml</td>
<td>28</td>
<td>4.0*</td>
<td>0.31</td>
</tr>
<tr>
<td>Age: 60 years</td>
<td>17</td>
<td>3.2</td>
<td>0.39</td>
</tr>
<tr>
<td>61–70 years</td>
<td>28</td>
<td>3.5</td>
<td>0.33</td>
</tr>
<tr>
<td>&gt;70 years</td>
<td>19</td>
<td>3.3</td>
<td>0.40</td>
</tr>
</tbody>
</table>

* The abbreviations used are: ESR, erythrocyte sedimentation rate; S.E., standard error.

* The difference between those two groups is almost significant (\( P < 0.05 \)). There was no significant difference between the other groups.

**Cases of leukemia.**—In this group the values ranged from 0.8 to 7.0 \( \mu g \). The mean SFAA for these patients, (2.66 \( \mu g/\text{ml} \)) was significantly lower than that found in the controls (\( P < 0.001 \); Table 1).

**Cases of malignant lymphoma.**—All SFAA values in this group fell below the normal mean of 4.6 \( \mu g/\text{ml} \), the values ranging from 1.3 to 3.5 \( \mu g/\text{ml} \). The mean for the group, 2.27 \( \mu g/\text{ml} \), was significantly lower than that for the normal controls (\( P < 0.001 \); Table 1).

**Cases of other malignant tumors.**—In this group of 64 patients the values ranged from 1.1 to 8.5 \( \mu g/\text{ml} \). The mean SFAA of 3.4 \( \mu g/\text{ml} \) was also significantly lower than that for the normal controls (\( P < 0.001 \); Table 1).

Of these 64 cases 30 had radiotherapy less than 1 month before the SFAA determination and the mean SFAA for this group was 3.3 \( \mu g/\text{ml} \); the mean for the 34 not irradiated was 3.5 \( \mu g/\text{ml} \) (Table 2).

The series was divided into a group of 36 cases in which, according to clinical and x-ray examination, the tumor was restricted to the primary site and possibly the area of the regional lymph nodes, and a group of 28 with a more extensive spread. The means of the two groups were identical (3.4 \( \mu g/\text{ml} \)).

Grouping with respect to the "degree of emaciation" gave 44 patients in a good, and 20 in a poor, general condition. This grouping was performed by one of the authors at the time of sampling and before the results of the study were known. The means for the two groups were almost identical (Table 2).

There was no difference when the grouping was made with respect to the origin of the tumor, but the series is too small to permit of any definite conclusions on the association between this factor and the SFAA level.

It is important to note that the patients with malig-

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The difference in SFAA between the age groups was evident for white blood cells from cases of acute leukemia. However, in the cases of malignant tumors no correlation between folic acid activity and nutritional state of the patient has been observed by Hellman et al. (9) in a series of 35 cases of carcinoma. In the present series, however, there was no relationship between the degree of wasting and the SFAA (Table 2). An acceleration of iron metabolism has been observed in cases of small malignant tumors (14, 20). The present findings indicate decreased production, or accelerated metabolism, of another nutrient—folic acid. Although the serum FAA was significantly lower, the value for WBC from cases of acute leukemia was significantly higher than for such cells from normal controls. The increase in FAA in white cells was based on only a few observations, and may be due to differences in cell size. The finding is, however, consistent with previous results relating increased *Pediococcus cerevisiae* activity and decreased uptake of C14 from formate into ribonucleotides of leukemic cells (6, 23).

**DISCUSSION**

Whether the low SFAA level in cases of malignant tumors is a consequence of the disease and has no effect on the development of the pathologic picture, or whether it is a factor in the wasting observed in these cases, was not established by this investigation. A possible explanation is a decrease in the intake of folic acid. Some correlation between folic acid activity and nutritional state of the patient has been observed by Hellman et al. (9) in a series of 35 cases of carcinoma. In the present series, however, there was no relationship between the degree of wasting and the SFAA (Table 2). An acceleration of iron metabolism has been observed in cases of small malignant tumors (14, 20). The present findings indicate decreased production, or accelerated metabolism, of another nutrient—folic acid. Although the serum FAA was significantly lower, the value for WBC from cases of acute leukemia was significantly higher than for such cells from normal controls. The increase in FAA in white cells was based on only a few observations, and may be due to differences in cell size. The finding is, however, consistent with previous results relating increased *Pediococcus cerevisiae* activity and decreased uptake of C14 from formate into ribonucleotides of leukemic cells (6, 23).

**REFERENCES**


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