A STUDY OF THE RELATION BETWEEN FOLIC ACID AND TISSUE ASCORBIC ACID* 

BY MORTON A. SCHWARTZ AND J. N. WILLIAMS, JR. 

(From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, Wisconsin) 

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Many instances of a close relationship between folic acid and ascorbic acid have been reported in the literature. Johnson and Dana (1) have shown that ascorbic acid relieves many of the abnormalities noted in a folic acid deficiency syndrome. Woodruff and Darby (2) have reported that folic acid aids in correcting the abnormal tyrosine metabolism of the scorbutic guinea pig. Nichol and Welch (3, 4) observed that ascorbic acid stimulates the conversion of folic acid by rat liver slices to an essential growth factor for Leuconostoc citrovorum. Recently, Williams (5, 6) has reported that the lowered choline oxidase activity of the livers of rats fed aminopterin is stimulated in vitro by the addition of ascorbic acid. It has also been shown by Williams (7) that the ascorbic acid content of the rat liver is decreased markedly by dietary aminopterin. It was suggested that folic acid or the L. citrovorum factor (LCF) is possibly involved in the maintenance of ascorbic acid in the rat. 

In the present studies we have investigated this problem further by studying the effect of an aminopterin-induced folic acid deficiency upon the level of ascorbic acid in rat liver and the possible reversal of this effect by supplementing the animals with various levels of folic acid and synthetic LCF. If such a reversal could be effected, the direct involvement of folic acid (or LCF) in the maintenance of tissue ascorbic acid could be demonstrated, although the lack of a reversal by folic acid or LCF would not rule out this possibility, since the utilization of both substances might be inhibited by dietary aminopterin (4, 8). 

EXPERIMENTAL 

Male, 225 to 250 gm. rats of the Holtzman strain were used as experimental animals. The basal ration consisted of 18 per cent casein (Labco), 4 per cent Salts IV (9), 2 per cent vitamin mix (10), 5 per cent corn oil, and 71 per cent sucrose. A number of rats were placed on this diet, while 

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others were given the same ration to which aminopterin \(^1\) was added. The aminopterin was included in the vitamin mix, so that 100 gm. of vitamin mix contained 20 mg. of aminopterin. Within about 10 days the rats on the aminopterin ration showed the typical deficiency syndrome. Porphyrin appeared around the eyes and nose, diarrhea was evident, and the animals became inactive. When diarrhea or porphyrin secretion appeared, the rats were separated into five groups. The rats receiving the basal ration without aminopterin were divided into two groups at the same time. Thus there were seven groups in all, five deficient and two normal.

The two normal groups were treated as follows: Group I received the basal ration and served as a normal control; Group II was supplemented with 200 \(\gamma\) of folic acid per rat per day for 3 days (after about 10 days on the basal ration) in order to observe whether higher levels of the vitamin over that included in the basal ration had any effect on the liver ascorbic acid level. The five deficient groups were treated as follows: Group III was sacrificed without supplementation 3 days after the first deficiency symptoms appeared and served as a negative control; Group IV was supplemented with 60 \(\gamma\) of folic acid per rat per day; Group V was supplemented with 60 \(\gamma\) of LCF per rat per day; Group VI was supplemented with 500 \(\gamma\) of folic acid per rat per day; and Group VII was supplemented with 500 \(\gamma\) of LCF per rat per day. In every case the supplementations were given for 3 days after the first deficiency symptoms appeared and were administered by intraperitoneal injections of a water solution of folic acid or LCF. At the end of the supplementation period the rats were sacrificed by decapitation, and the ascorbic acid content of the livers was determined by the method of Roe and Oesterling (11) as modified by Bolomey and Kemmerer (12) and Bolin and Book (13).

**RESULTS AND DISCUSSION**

The results of the ascorbic acid analyses are presented in Table I. The method of analysis differentiates between ascorbic acid and dehydroascorbic acid, but, since the dehydroascorbic acid was always present in negligible amounts, only the values for ascorbic acid are reported in Table I. Because of the high mortality of all the groups fed aminopterin, both supplemented and unsupplemented, the experiment was set up several times in order to obtain enough surviving rats for significant averages. Therefore, the results in the figures are actually the results for several complete feeding experiments. From Table I it can be observed that die-

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Vitamin aminopterin lowers the rat liver ascorbic acid level very markedly (compare Groups I and III). Also it can be seen that, when the normal rats are supplemented with excess folic acid, no change in the ascorbic acid content of the livers occurred. The value for ascorbic acid of the animals which received the supplement of 60 γ of folic acid (Group IV) is only slightly higher than the value obtained with the negative controls (Group III). The same observation holds true for those animals which were supplemented with 500 γ of folic acid (Group VI) and 500 γ of LCF (Group VII). The ascorbic acid content of those animals which received 60 γ of LCF was slightly below the value for the ascorbic acid content of the livers of the negative controls (compare Groups V and III). Although the stand-

TABLE I
Effect of Aminopterin and Supplements of Folic Acid and LCF on Rat Liver Ascorbic Acid

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of animals</th>
<th>Ration</th>
<th>Supplement per rat per day*</th>
<th>Ascorbic acid in liver γ per gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>9</td>
<td>Basal</td>
<td></td>
<td>275 ± 11†</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>“”</td>
<td>200 γ folic acid</td>
<td>268 ± 8</td>
</tr>
<tr>
<td>III</td>
<td>9</td>
<td>“” + aminopterin</td>
<td>60 γ folic acid</td>
<td>165 ± 4</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>“” + “”</td>
<td>60 “ LCF</td>
<td>184 ± 12</td>
</tr>
<tr>
<td>V</td>
<td>9</td>
<td>“” + “”</td>
<td>500 “ folic acid</td>
<td>172 ± 5</td>
</tr>
<tr>
<td>VI</td>
<td>8</td>
<td>“” + “”</td>
<td>500 “ LCF</td>
<td>184 ± 9</td>
</tr>
<tr>
<td>VII</td>
<td>8</td>
<td>“” + “”</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The supplements were given after the first deficiency symptoms appeared.
† Standard error of the mean.

ard errors of the ascorbic acid determinations in all groups are relatively small, it is believed that the differences in Groups III to VII are not significant. In any event, Table I clearly shows that the different levels of folic acid and LCF did not increase the liver ascorbic acid to any value approaching normal.

Parallel with the failure of the supplements to reverse the action of aminopterin on rat liver ascorbic acid content was the failure of the supplements to enable the rats to recover from the folic acid deficiency. Many of the animals of the groups treated with aminopterin supplementation died during the 3 day supplementation period. These animals still displayed symptoms of folic acid deficiency, as did those animals which survived the supplementation period, and were sacrificed.

In agreement with other workers (4, 14, 15), high levels of folic acid are not able to reverse the toxic action of aminopterin. The rats in the present experiments were getting a maximum of 40 γ of aminopterin per day.
This implies that they received about 400 g of aminopterin before LCF supplementation began (assuming an average of 10 gm. of ration was consumed per rat per day for 10 days). Therefore, upon administering 500 g of LCF a day, the maximum initial LCF to aminopterin ratio was greater than 1:1. Nichol and Welch (4) reported a restoration of growth within a few days when LCF and aminopterin were given simultaneously in a ratio of roughly 1:1 at a 25 g level to rats kept for about 6 weeks on a folic acid-free ration. From a comparison of these results and those presented here, it appears that in an aminopterin-induced folic acid deficiency there is an increased resistance to the reversal of the deficiency by LCF. LCF, fed from the beginning with the aminopterin in a ratio of 1:1, may prevent aminopterin toxicity, but it cannot overcome the effects of aminopterin once the aminopterin-induced deficiency has reached an advanced stage, as shown in these experiments.

SUMMARY

1. Dietary aminopterin markedly reduces the ascorbic acid content of rat liver.
2. Leuconostoc citrovorum factor and folic acid at levels of 60 g per rat per day and 500 g per rat per day have little effect on the low ascorbic acid levels of rat liver induced by feeding aminopterin once the deficiency symptoms have developed.
3. These levels of folic acid and LCF are also unable to overcome the folic acid deficiency syndrome induced by aminopterin as measured by appearance and mortality, once the symptoms of the deficiency have appeared.

BIBLIOGRAPHY

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