A STUDY ON THE RELATIONSHIP BETWEEN L-ASCORBIC ACID AND PURINE METABOLISM IN VIVO

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A recent finding from this laboratory indicated that the incubation in vitro of purified xanthine oxidase with trace amounts of L-ascorbic acid resulted in a marked inhibition of the enzymatic activity (1, 2). Since the physiological mode of action of L-ascorbic acid is still obscure, it seemed desirable to determine whether ascorbic acid would, in the intact animal, exert a similar effect on xanthine oxidase. Should ascorbic acid inhibit xanthine oxidase in vivo, animals receiving high ascorbic acid supplements should produce uric acid and allantoin at a diminished rate. Therefore, this problem was studied by supplementing guinea pigs with varying dosages of ascorbic acid and measuring the liver xanthine oxidase activity, the rates of urinary uric acid and allantoin excretion, and the blood uric acid and allantoin levels in each animal.

EXPERIMENTAL

Three groups of adult 450 gm. male guinea pigs were maintained ad libitum on a Purina rabbit chow basal ration; this ration is sufficiently scorbutigenic to induce symptoms of scurvy in 2 weeks. Each group was housed for 18 days in a group metabolism cage placed over a paraffinized funnel and received daily the indicated oral supplement of L-ascorbic acid. The ascorbic acid supplements were dissolved in a 75 per cent sucrose solution to increase acceptability by the guinea pigs and were fed to each animal daily by means of a pipette. Daily urine collections were made under toluene, washings of the funnel being combined with the urine. The urines were stored in the frozen state until analyzed. At the end of the 18 day period the animals were stunned by a blow on the head and exsanguinated and their sera were analyzed for uric acid and allantoin. The levels of urinary and serum uric acid were estimated according to a slightly modified uricase procedure of Wolfson et al. (3). Urine allantoin was measured by the procedure of Young and Conway (4), and serum allantoin was estimated from a tungstic acid filtrate of serum by a slight modification of their colorimetric procedure (5).

As 0.5 mg. of L-ascorbic acid is the approximate daily requirement for guinea pigs of this size (6), in order to ascertain whether or not high ascor-
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bic acid levels result in a shift in purine metabolism a 10- and 100-fold increase of the required level was orally administered daily. The results are depicted in Table I. As there was no significant change in the urinary uric acid and allantoin levels throughout the experimental period, these values are expressed as the mean of the eighteen daily excretions by each group. It is apparent that the excretion of uric acid approximated 1.0 mg. per 100 gm. of guinea pig per day, that the allantoin excretion approximated 3.7 mg. per 100 gm. of guinea pig per day, and that these values were not significantly influenced by varying the ascorbic acid intake between 0.5 mg. and 50.0 mg. daily. The serum concentrations of uric acid and allantoin for the experimental groups likewise do not change significantly with increasing ascorbic acid intake.

**Table I**

*Effect of l-Ascorbic Acid upon Serum Concentration and Urinary Excretion of Uric Acid and Allantoin*

Seven guinea pigs were used in each experimental group.

<table>
<thead>
<tr>
<th>Ascorbic acid supplement (mg. per day)</th>
<th>Urinary uric acid (mg. per 24 hrs. per 100 gm. animal)</th>
<th>Urinary allantoin (mg. per 24 hrs. per 100 gm. animal)</th>
<th>Serum uric acid (mg. per cent)</th>
<th>Serum allantoin (mg. per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.920 ± 0.091*</td>
<td>3.62 ± 0.46*</td>
<td>0.54 ± 0.23*</td>
<td>1.43 ± 0.24*</td>
</tr>
<tr>
<td>5.0</td>
<td>1.016 ± 0.094</td>
<td>3.80 ± 0.65</td>
<td>0.59 ± 0.19</td>
<td>1.59 ± 0.40</td>
</tr>
<tr>
<td>50.0</td>
<td>1.021 ± 0.094</td>
<td>3.69 ± 0.49</td>
<td>0.62 ± 0.22</td>
<td>1.20 ± 0.08</td>
</tr>
</tbody>
</table>

* Standard deviation of the mean.

To determine in a more direct manner whether high dosages of ascorbic acid will inhibit the xanthine oxidase activity in the intact animal, and to preclude the possibility of a limited intestinal absorption of the ascorbic acid, the following experiment was undertaken: 300 gm. male guinea pigs were maintained *ad libitum* on a Purina rabbit chow ration. They were divided into four groups, each receiving daily the indicated supplement of ascorbic acid by subcutaneous injection. The ascorbic acid was prepared daily in a 0.1 M potassium phosphate buffer at pH 7.2. After 10 to 14 days on this regimen the animals were sacrificed and the xanthine oxidase activity of their livers measured manometrically at 37° according to the method of Axelrod and Elvehjem (7).

The results of the liver enzyme activities of animals receiving increasing levels of ascorbic acid are found in Table II. Liver xanthine oxidase activity is expressed as the mean value for all the guinea pigs in a group with their standard deviation. The mean values indicate that the liver xanthine oxidase activity was diminished following high dosages of ascorbic acid; however, there are large variations in xanthine oxidase activity among the animals within each group which result in large standard deviations.
A non-parametric median statistical analysis of the data, however, shows a probability of <0.05, thus indicating that there is a significant difference in the liver xanthine oxidase activities of the various groups.

**Table II**

<table>
<thead>
<tr>
<th>Ascorbic acid, mg. per 100 gm. body weight per day</th>
<th>No. of animals</th>
<th>Liver xanthine oxidase, µl. O₂ per 20 min. per 284 mg. liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7</td>
<td>30.0 ± 4.8*</td>
</tr>
<tr>
<td>0.99</td>
<td>6</td>
<td>28.6 ± 4.8</td>
</tr>
<tr>
<td>9.9</td>
<td>6</td>
<td>25.9 ± 5.7</td>
</tr>
<tr>
<td>99.0</td>
<td>5</td>
<td>22.4 ± 6.2</td>
</tr>
</tbody>
</table>

* Standard deviation of the mean.

**DISCUSSION**

These data indicate that under conditions in vivo liver xanthine oxidase activity can be significantly inhibited by the administration of high levels of ascorbic acid. However, this relationship does not seem to be of physiological significance, as the ascorbate dosage required for significant inhibition of the enzyme is several hundred fold the daily requirement of the animal for the vitamin. The minor enzymatic inhibition realized is not reflected in the blood levels or rates of excretion of uric acid or allantoin.

These findings in the animal experiment are in contrast to previous findings in vitro, wherein incubation of purified xanthine oxidase with ascorbic acid resulted in a marked diminution of the rate of uric acid formation (2). It may be that under conditions in vivo the ascorbic acid-xanthine oxidase complex does not form as readily as under conditions in vitro. This might be due to rapid metabolism of ascorbic acid by the animal or to a protection of the xanthine oxidase in the animal by some unknown mechanism.

**SUMMARY**

A study has been made on the relationship between l-ascorbic acid and purine catabolism in vivo. High levels of ascorbic acid administered to guinea pigs inhibit their liver xanthine oxidase activities to a minor degree. Blood levels and rates of excretion of uric acid and allantoin, however, are independent of the ascorbic acid intake.

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**BIBLIOGRAPHY**

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