ASCORBIC ACID CATABOLISM IN GUINEA PIGS*

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(Received for publication, February 11, 1957)

There have been numerous reports suggesting that the metabolism of ascorbic acid in humans and in animals is affected by stress. However, detailed information which would permit an analysis of the underlying mechanism is not available. From the variety of effects cited, it would appear that no single mechanism can explain all of the findings. It has been suggested that increased ascorbic acid requirements exist in stress either because of increased utilization (2) or because of increased catabolism (1). These two factors are not synonymous, since utilization implies the retention of the intact ascorbic acid molecule or dehydroascorbic acid, and catabolism infers the destruction of the molecule beyond the dehydro-ascorbic acid stage. A choice between these mechanisms is, moreover, difficult or impossible to make under ordinary circumstances because the products of complete catabolism of ascorbic acid cannot be specifically determined, except with tracer techniques (4, 5).

The availability of ascorbic acid-1-C\(^{14}\) made it possible to resolve some of these difficulties and investigate certain aspects of this important problem in more detail. The present communication deals specifically with the fate of ascorbic acid after equilibration with body ascorbic acid, in contradistinction to the metabolism of newly administered ascorbic acid in the intact guinea pig.

In one series of experiments, the rate of ascorbic acid loss by the diphtheria-intoxicated guinea pig was investigated. The effects of the toxin in guinea pigs are consistent and reproducible. Among the consequences produced by its administration is a decrease in ascorbic acid in several tissues (6), characteristically pronounced in the adrenal glands. Scorbutive guinea pigs also show lowered resistance to the toxin (7). Preliminary work had shown that urinary excretion of ascorbic acid radioactivity did not differ significantly between toxin-treated and control guinea pigs, although there was some reason to believe that respiratory

* This study was supported in part by funds provided under contract No. AF 18(600)-638, with the School of Aviation Medicine, USAF, Randolph Air Force Base, Texas.

1 Among recent reviews on this topic are those by Meiklejohn and Passmore (1), Pirani (2), and Lloyd and Sinclair (3).
radioactivity might be depressed in the former group (8). Such animals offered the opportunity to test ascorbic acid metabolism in stress in more detail.

The metabolism of ascorbic acid in scorbutic guinea pigs was studied for similar reasons. It might be anticipated that the scorbutic guinea pig in some way "adopts" itself to restricted intake of the vitamin by more efficient conservation of its body or dietary ascorbic acid. Experiments were undertaken to test the possibility that the biological half life of ascorbic acid in such animals differs from that of normal control guinea pigs.

EXPERIMENTAL

_Materials_—Radioactive ascorbic acid was synthesized from high specific activity NaC\(^{14}\)N essentially as previously described (9). The carboxyl-labeled product had a specific activity of \(7.04 \times 10^6\) c.p.m. per mg. (absolute), and was routinely administered in doses of 5 mg. per kilo of body weight, dissolved in 1.0 ml. of distilled water, by intraperitoneal injection.

Diphtheria toxin was generously provided by Dr. D. G. Brigham of Parke, Davis and Company, Detroit, Michigan. The toxin was administered in doses of 1 minimal lethal dose, subcutaneously, in a total of 0.15 ml. of solution with saline, 12 hours before injection of ascorbic acid-1-C\(^{14}\) and the start of the metabolic experiment.

_Animals_—Male guinea pigs were maintained on Purina rabbit chow, a scorbutigenic diet, and water _ad libitum_. In addition, they received a daily oral dose of 30 mg. of ascorbic acid per kilo of body weight until 24 hours before the start of the experiment, or until the start of the scorbutigenic regimen. For this regimen, guinea pigs were maintained on the same diet, without the oral supplements, for a period of 21 days. This diet rapidly and invariably has been found to produce scurvy, when fed alone. The administration of ascorbic acid both prevents and cures the hypovitaminosis.

_Methods_—Body ascorbic acid was determined on a sample of tissue obtained in the following manner. The animal was decapitated, exsanguinated, and skinned, and the intestinal contents were removed. The tissues, with skin excepted, were then thoroughly ground; the sample analyzed represented an aliquot portion of this material, which was further ground with sand and then treated by the method of Roe et al. (10), as

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\(^2\) The minimal lethal dose is that dose of diphtheria toxin which, when injected subcutaneously, will kill a guinea pig of 250 gm. in weight in 4 or 5 days. The observed survival period was 4 to 6 days in these experiments, owing to greater variability in the weights. Only one animal was lost prematurely.
modified by Lowry et al. (11). Preparation of the tissues was carried out at 2°.

The metabolism apparatus was a slightly modified version of that used by Jackel et al. (12). Collection of respiratory carbon dioxide samples, conversion of aliquots to barium carbonate, the conversion of urine and ascorbic acid aliquots to barium carbonate, and the counting methods were based on previously reported methods, and are cited by the same authors (12). All determinations were made in duplicate, and counted to a probable error of ±5 per cent in the most unfavorable cases. The use of ascorbic acid-1-C¹⁴ of high specific activity in fairly large quantities resulted in high sample radioactivity. A favorable sample count to background ratio made the attainment of greater statistical accuracy simple in most cases. A windowless flow counter, calibrated with samples prepared from the National Bureau of Standards Beta-Ray Standard,
was used. Corrections for background and self-absorption, volume and time corrections, and corrections for small variations in administered radioactivity were made as necessary to permit comparison of the data. All samples had a constant area, and were mounted on copper, under the same conditions as those employed during the determination of the correction factors for the particular counting arrangement.

Procedure—In general, the experiments consisted of the administration of radioactive ascorbic acid to guinea pigs which had received their last ascorbic acid supplement 21 hours previously, or to scorbutic guinea pigs. Diphtheria toxin was administered, when called for, 12 hours before the start of the metabolism run. The animal was then placed in the chamber and the metabolism apparatus permitted the collection of respiratory CO\textsubscript{2} without interruption for any desired period, in suitable fractions, as well as the quantitative collection of urine.

After determination of the radioactivity excreted in any particular interval, the data were converted to “per cent of radioactivity remaining in the animal,” and plotted on semilogarithmic paper (Fig. 1). The data become linear approximately 30 hours after administration of the radioactive material. This is interpreted to indicate the completion of equilibration.

RESULTS AND DISCUSSION

The rate of excretion of radioactivity can be equated to the fate of the animal’s ascorbic acid only when certain limiting conditions are met. It is essential that the administered labeled vitamin should become completely mixed with the body ascorbic acid. At that time, the specific activity of the ascorbic acid will be equal in all tissues. Moreover, this specific activity will then remain constant, provided that no further ascorbic acid is fed, since guinea pigs are incapable of synthesizing the vitamin. It is also required that no appreciable part of the radioactive ascorbic acid should have been converted to other radioactive compounds which tend to accumulate in the body. This could give an erroneous impression as to the rate of ascorbic acid breakdown, since determination of that rate depends upon measurements of excreted radioactivity. A number of previously reported experiments (8) and numerous unpublished experiments performed subsequently have shown that this condition is adequately fulfilled. Without exception, it was possible to isolate substantially all the radioactivity as unchanged ascorbic acid from a variety of tissues, within the precision of the methods. This shows that no more than 5 per cent, and probably less, is present as radioactivity other than ascorbic acid radioactivity. Experiments \textit{in vitro} with liver homogenates showed that ascorbic acid may be degraded via dehydroascorbic and diketogulonic.
acids (13), although this may not be the major catabolic pathway (14). However, the rates of breakdown (15), as well as the relatively low tissue concentrations (14), suggest that interference from an accumulation of these compounds can scarcely be expected.

Under these conditions, it is evident that the excretion of radioactivity is, in fact, directly proportional to the decrease in body ascorbic acid. Furthermore, the rate constant for the excretion of radioactivity must be equal to the rate constant for the decrease of body ascorbic acid. This makes a comparison of the relative catabolism rates particularly simple.

The data presented in Fig. 1 show that the excretion of radioactivity may be expressed by

$$k = \frac{2.303}{\Delta t} \log_{10} \left( \frac{\text{ascorbic acid}_t}{\text{ascorbic acid}_i} \right)$$

It is now only necessary to know the total body ascorbic acid at any time after the start of the experiment to permit the evaluation of the rate data in terms of actual amounts of ascorbic acid rather than of ratios. Such data will be shown below.

Normal Guinea Pigs—Fig. 1 represents typical data from these metabolism experiments; corresponding rate constants, shown in Table I, are based on the periods starting about 30 hours after the administration of radioactive ascorbic acid, since the curves become linear at that time.

The calculated mean biological half life of ascorbic acid in normal guinea pigs, based on these constants, is 100 hours, ranging from 70 (Animal 3) to 144 hours (Animal 15). The average half life based on all the animals included in this report is 99 hours. These values apply only to "body ascorbic acid" and not to "administered ascorbic acid." Further details regarding this distinction will be part of a subsequent communication.

Total body ascorbic acid was measured in three normal control guinea pigs at the conclusion of the metabolism run. From these values and the rate constants for the corresponding animals, the ascorbic acid content at the start of the experiment (4 hours after the last oral dose of ascorbic acid) was calculated. Values for body ascorbic acid obtained in this way were 44.0, 44.2, and 37.3 mg. per kilo of body weight. In other words, the approximate body level attained by a 30 mg. per kilo daily oral ascorbic acid supplement is 40 mg. per kilo. Since no symptoms of hypovitaminosis C in male guinea pigs have ever been found under these conditions, it may be assumed that this is a "normal" value. However, tissue saturation is attained at a much higher intake (16).

Scorbutic Guinea Pigs — The rate of catabolism of a group of four scorbutic guinea pigs was determined, and then redetermined after realimentation. These animals served, therefore, as internal controls. Data from
this group are shown in Table I. While the number of animals used was limited, excellent agreement of the data is apparent. The rate of catabolism not only follows the same pattern as that in normal animals, but the rate constants also appear to be unaffected significantly. Because of this, it becomes possible to calculate the body ascorbic acid levels of guinea pigs at various times after supplementation has ceased.

### Table I

**Body Ascorbic Acid Depletion Rate Constants**

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Weight (gm.)</th>
<th>k x 10^3 hr.</th>
<th>Animal No.</th>
<th>Weight (gm.)</th>
<th>k x 10^3 hr.</th>
<th>Animal No.</th>
<th>Weight (gm.)</th>
<th>k x 10^3 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>1</td>
<td>366</td>
<td>6.58</td>
<td>1-B</td>
<td>369</td>
<td>11.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>366</td>
<td>9.94</td>
<td>4</td>
<td>285</td>
<td>5.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>370</td>
<td>7.87</td>
<td>5</td>
<td>285</td>
<td>4.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>376</td>
<td>6.81</td>
<td>6</td>
<td>398</td>
<td>10.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>320</td>
<td>7.63</td>
<td>7</td>
<td>305</td>
<td>8.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>412</td>
<td>4.83</td>
<td>10</td>
<td>379</td>
<td>5.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>370</td>
<td>6.25</td>
<td>11</td>
<td>356</td>
<td>7.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>313</td>
<td>6.39</td>
<td>12</td>
<td>482</td>
<td>8.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>402</td>
<td>6.12</td>
<td>13</td>
<td>512</td>
<td>7.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td><strong>6.9</strong></td>
<td></td>
<td><strong>5.6</strong></td>
<td> </td>
<td></td>
<td><strong>7.5</strong></td>
<td> </td>
</tr>
<tr>
<td><strong>S.d.</strong></td>
<td></td>
<td>±1.3</td>
<td> </td>
<td>±0.5</td>
<td> </td>
<td></td>
<td>±2.1</td>
<td> </td>
</tr>
</tbody>
</table>

These rate constants are based upon experiments with intact guinea pigs. Animals 15, 16, 17, and 18 served as internal controls.

When an unsupplemented scorbutigenic diet was fed, dentine deposition in guinea pig incisors was found to be impaired within 7 days (17). During this time, body ascorbic acid may be expected to decrease on the average, by nearly 69 per cent. For the present group of animals, this means an approximate decrease to less than 13 mg. per kilo. After 3 weeks, when severe gross symptoms of scurvy will have been found under the conditions of this experiment, the level will have fallen to a calculated average of 1.2 mg. per kilo.

These results suggest an explanation for the finding that the previous intake of ascorbic acid, provided that it was adequate to prevent scurvy, had little bearing on the survival time of guinea pigs on a scorbutigenic
diet (18). Because of the exponential nature of the curves, a guinea pig with an initial level of 40 mg. per kilo will, after 21 days, contain only approximately 0.6 mg. per kilo more than another pig with an initial level of 20 mg. per kilo.

*Diphtheria Toxin-Treated Guinea Pigs*—Two groups of animals were examined for the effect of diphtheria toxin stress on ascorbic acid catabolism. For the first group, a procedure similar to that used for the normal group was followed, with the exception that 1 minimal lethal dose of toxin was administered. Since rate measurements in effect started 30 hours after the injection of labeled ascorbic acid, as pointed out above, such determinations included the period of time when the toxin showed very drastic effects. Data from this group are included in Table I. The similarity of the rate constants to those of normal animals is apparent.

In a second set of experiments, Animals 16, 17, and 18, which had been previously examined twice, i.e. while scorbutic and after realimentation, were maintained on the usual supplement of ascorbic acid for 4 weeks. Thereafter, a third metabolism experiment was performed, in this case after the administration of toxin. No alteration of rate constants is discernible.

In a limited number of animals from Group 1 of the toxin-treated guinea pigs, body ascorbic acid was also determined. The values found, 43.7, 40.1, 39.8, and 35.0 mg. per kilo, do not differ significantly from those reported above for normal animals. This indicates that not only are the rate constants unaltered under these circumstances, but also the amount of ascorbic acid catabolized. In other words, no significant changes in body ascorbic acid had taken place between the time of administration of the toxin and injection of radioactive ascorbic acid, and no differences were observed subsequently. It appears that stress does not necessarily affect the rate of ascorbic acid catabolism.

These experiments demonstrate that the biological half life of body ascorbic acid is unchanged in scorbuty, as well as during toxin stress, under the conditions employed. Because of this, the procedures used cannot show the existence of a deficiency. Hence, augmented “need” for ascorbic acid in the toxin-treated guinea pigs cannot be ruled out, despite unaltered tissue stores and catabolism rate constants. For the scorbutic guinea pig, this is self-evident.

**SUMMARY**

1. The effect of toxin stress and scorbuty on the rate of catabolism of ascorbic acid has been investigated.

2. The rate of catabolism followed first-order kinetics in normal, scorbutic, and diphtheria-intoxicated guinea pigs. The rate constants for the
catabolism of ascorbic acid in intact guinea pigs were unaffected by the level of body ascorbic acid or toxin stress.

3. The evidence indicated that the biological half life of ascorbic acid in the guinea pig is neither decreased in stress nor increased in scurvy. Regardless of the magnitude of the body ascorbic acid level, a definite fraction per day was lost by the animal, this predictable quantity being independent of the test conditions employed.

4. Depletion of body ascorbic acid at the time of impaired dentine formation was calculated to average about 69 per cent. At the time of the appearance of gross symptoms of scurvy, the body level will not be much more than 1 mg. per kilo of body weight. When fed a 30 mg. daily oral ascorbic acid supplement per kilo of body weight, a level of approximately 40 mg. per kilo is established in male guinea pigs.

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