PHYSIOLOGICAL ROLE OF ASCORBIC ACID

I. ASCORBIC ACID METABOLISM UNDER DECOMPRESSION STRESS

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(Received May 30, 1959)

Ascorbic acid is considered to be a vitamin which has a special intimate relation with reactions of living bodies generally observed during stress. In order to study the physiological rôle of this vitamin, one of the most efficient methods is to investigate its significance in the dynamics of metabolism or its effects when administered during stress. On the other hand, it is well known that the administration of ascorbic acid prior to exposing the experimental subject to a low pressure environment enhances its tolerance. (1, 9, 20, 27). It is also reported that the demand for ascorbic acid is changed when the test subject is exposed to a similar form of stress (14, 15). However, in these experiments, what was described as decompression load contained also the condition of hypoxia which inevitably accompanies decompression. Therefore, the difference between the significance of ascorbic acid in an anaerobic metabolic process without change in pressure and the significance of the vitamin in decompression itself has not been clarified. Furthermore, although the relationship between the load and ascorbic acid can be recognized in these studies, the rôle of ascorbic acid or the mechanism of its action has not hitherto been systematically studied.

From this point of view, the hypoxia factor was especially eliminated in the present experiment, and the influence of decompression alone on ascorbic acid metabolism was studied. Further, in a separate group an experiment was made in order to ascertain the effect of the inhalation of pure oxygen which is preparatorily used to prevent the formation of nitrogen bubbles in the body. The latter is regarded as a main cause of decompression symptoms. From the changes observed in the content of ascorbic acid in the urine and the body, the authors were able to confirm the importance of the physiological rôle played by this vitamin when the body is subjected to a state of decompression or pure oxygen inhalation.

EXPERIMENTAL

As experimental animals, thirty male rats of the Wister breed weighing 105—292 g were used after they had been fed on the same usual diet for at least
more than seven days in the laboratory. They were divided into three equal
groups, A, B and C. Group A was the control, Group B was subjected to a
200 mm Hg non-hypoxic decompression for 20 minutes, and Group C was given
a preliminary pure oxygen inhalation for 2 hours after which it was subjected
to the same decompression as that of Group B. Decompression was carried
out in a small-sized low pressure mechanism made in the laboratory. It is
capable of decompressing to 15 mm Hg within about two minutes. The volume
of the decompression chamber was about 6.5 l.

After the temperature in the decompression chamber had reached 20°, a
few experimental animals were placed in the chamber and in order to elimi-
nate the hypoxia factor accompanying decompression, the air within the cham-
ber was replaced by approximately 150 l of pure oxygen, after which the
pressure within the chamber was rapidly reduced to 200 mm Hg within 46
seconds. This condition was maintained for 20 minutes, after which the ori-
ginal pressure was revived instantaneously. To absorb CO₂ in the low pres-
sure chamber, soda-lime was laid on the bottom of this vessel.

In Group C, prior to bringing the animals in the decompression chamber,
they were placed in a non-permeable polyethylene bag (10 l in volume) for 2
hours in order to drive out the nitrogen from the body tissues, based on the
experiments of Behnke (1) on men. As soon as the original pressure was
recovered, the animals were anaesthetized with 5 mg of pentobarbital sodium
per 100 g body weight by hypodermic injection. This anaesthetic is said to
have no influence on both the pituitary-adrenocortical system (2) and the as-
corbic acid (21) content in the adrenals.

Fig. 1 shows the course of the experiments to ascertain the changes in
the ascorbic acid content of the adrenals, liver and blood. Fig. 6 shows the
course of the experiments to ascertain the changes of ascorbic acid in the
urine.

The quantity of ascorbic acid was measured by the dinitrophenyl hydra-
zine method as described by Fujita and Teruuchi (9) which is considered
highly efficient for fractional determination of dehydroascorbic acid and ascor-
bic acid and is also said to have a high specificity for ascorbic acid. The
colorimetry was made with Hitachi's photoelectric colorimeter at 520 m, 0.15
mm in slit width, using a 10 × 10 mm absorption cell.

The standard calibration curve for the concentration range of 0.1—3.0 mg
per 100 ml was found to be a straight line, crossing the original point. The
factor for calculating the concentration (mg/100 ml) from E was found to be
3.75. The dilutions of the tissue extracts used were 20, 20 and 64, for liver,
adrenals and blood, respectively. For the urine the absolute value excreted
per hour was calculated.

Since it was found that the contents of the vitamin in adrenals showed
no essential difference between the right and left one (23), one was used to mea-
sure the content of total ascorbic acid, i.e., the sum of ascorbic acid (AA),
dehydroascorbic acid (DHA) and diketogulonic acid (DGA), and the other for
DHA plus DGA. Samples of the liver were always taken from the left lobe,
one gram of which was used for measuring the total ascorbic acid content.
and another one gram for measuring the content of DHA plus DGA.

Two ml of blood were taken from the heart by puncturing through the diaphragm from the abdominal side. One ml was used for the measurement of total ascorbic acid content and the remainder for measuring the content of DHA plus DGA. For determining the content in the urine the animals were divided into three equal groups and each group put into a plastic container from the bottom of which urine was collected every 2 hours. The contents of the vitamin were determined and the average of the three animals was calculated. The experimental animals were also put in this urine-collecting vessel during both oxygen inhalation and decompression experiments. The amount of ascorbic acid was obtained by subtracting the value of DHA plus DGA from the total value.

Similar experiments were made on ascorbic acid metabolism when pure oxygen was inhaled for 2 hours under normal pressure.
RESULTS

Table I shows the changes of the contents of each form of ascorbic acid in the adrenals, liver and blood in Groups A, B and C as expressed as the mean values of each group. The AA contents in each group in the adrenals are shown in Fig. 2.

The content in Group B decreased to 81.9% of that of Group A and that in Group C to 58.3%. Statistic calculation showed that the level of significance between Groups A and B was $0.02 > \alpha > 0.01$, that between A and C and that between B and C were $0.01 > \alpha$, showing all significant differences. The ratio of AA to DHA+DGA is 98.7 : 1.3 in A, 99.2 : 0.8 in B, and 98.9 : 1.1 in C, no significant changes being observed.

In the liver (Fig. 3) the decrease of the vitamin was observed in both Groups B and C as compared to A, the values of the vitamin in Groups B and C being 72.2 and 62%, respectively. The level of significance between A and B was $0.02 > \alpha > 0.01$, that between A and C $0.01 > \alpha$, both showing significant differences, whereas that between B and C was $0.2 > \alpha > 0.1$, showing scarcely any significant differences. The ratios of AA to DHA+DGA in the Groups A, B and C were found to be 95.4 : 4.6, 98.3 : 1.7 and 97.8 : 2.2, respectively, indicating very little significance of these differences.

The tendency of the change in the contents of ascorbic acid in blood was found to differ from that in adrenals and liver (Fig. 4). Group B showed a value of 133.8% of that of Group A. On the contrary, Group
### Table I

*Change in Contents of Each Form of Ascorbic Acid in the Adrenals, Liver and Blood of Rats during Decompression*

The figures indicate the mean values of each group.

The figures in parentheses indicate the relative values, taking the values of A as 100.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Adrenals</th>
<th>Liver</th>
<th>Blood</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>DHA</td>
<td>DGA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mg</td>
<td>mg</td>
<td>mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 ml</td>
<td>100 ml</td>
<td>100 ml</td>
</tr>
<tr>
<td>A</td>
<td>4</td>
<td>340.2</td>
<td>0.14</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>278.6</td>
<td>0.10</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(82)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>198.2</td>
<td>0.06</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(58)</td>
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<td></td>
</tr>
</tbody>
</table>

A, control. B, 200 mm Hg pure oxygen load. C, 200 mm Hg pure oxygen load after inhalation of pure oxygen.

Body weight of the subject, 172-292 g. Weight of each adrenal, 11.5-24.0 mg. Weight of liver, 5.5-9.0 g.

### Table II

*Changes in Urinary Ascorbic Acid in Each Group of Rats during Decompression Experiment*

The figures indicate the mean values of each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>I urine</th>
<th>II urine</th>
<th>III urine</th>
<th>IV urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>DHA</td>
<td>DGA</td>
<td>AA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mg</td>
<td>mg</td>
<td>mg</td>
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<td>100</td>
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<td>100</td>
</tr>
<tr>
<td>A</td>
<td>6</td>
<td>0.013</td>
<td>0.012</td>
<td>0.001</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(92)</td>
<td>(8)</td>
<td></td>
<td>(115)</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>0.008</td>
<td>0.006</td>
<td>0.002</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100)</td>
<td>(75)</td>
<td>(25)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>0.008</td>
<td>0.006</td>
<td>0.003</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100)</td>
<td>(75)</td>
<td>(25)</td>
<td></td>
</tr>
</tbody>
</table>

*a The quantity of total ascorbic acid in the first urine of each group was expressed as 100.

*b The quantity of AA of Group A in the third urine was expressed as 100.
C showed a value of 31% of that of Group A. The level of significance between Groups A and B was 0.01 > ρ > 0.001, and that between A and C, as well as that between B and C 0.001 > ρ, showing all significant differences. Further, the ratio of AA to DHA+DGA was 94.2:5.8 in A, 89.5:10.5 in B, and 85.9:14.1 in C, but the distribution of each individual value was too widespread to have any significance.

Table II shows the mean values of each group of the change in the amount of the ascorbic acid excreted in the urine in every two hours. Fig. 6 indicates the successive change in the amount of ascorbic acid in the urine of each group.

Group A has a tendency of show-
ing a mountain-type curve whose peak is represented by the third urine. It is of interest that in Group C, a similar tendency appears, and in Group B a marked increase is seen in the third and fourth urine. In the adrenals, liver and blood, the change in the contents of ascorbic acid at the time when decompression load ended, was examined. It is therefore of convenience that similarly in urine special attention is paid to only the third urine which is closely related to the decompression load. The change in the concentration of ascorbic acid in the third urine is shown in Fig. 7. Taking the AA concentration of Group A as the standard, Group B showed a value of 292.6%, whereas Group C showed almost the same value as that of Group A. Another remarkable characteristic in the urinary ascorbic acid excretion is the relative increase of DHA + DGA. The ratios of AA to DHA + DGA in Groups A, B and C were 45.5 : 54.5, 42.9 : 57.1 and 32.5 : 67.5, respectively.

The above findings may be summarized as follows. Under a condition of non-hypoxic decompression, ascorbic acid levels in the adrenals and liver fall, whereas those in the blood and urine rise remarkably. However, when a similar decompression is loaded after a preliminary inhalation of pure oxygen, the ascorbic acid levels in the blood fall, as in the case with those in the liver and adrenals, but the urinary excretion of the vitamin fails to rise. Objective findings observed (Table III) in the experimental animals at the end of decompression may give valuable clues to the physiological explanation of the differences in the ascorbic acid metabolism seen among the three groups.

A characteristic finding in this Table is that a relatively strong reactions were observed in Group B, whereas hardly any abnormalities were found in Group C. In the group which was allowed to inhale pure oxygen under normal pressure the ascorbic acid levels in the adrenals, liver, blood and urine showed no significant difference from those of the control group.

### DISCUSSION

It is a well-known (6, 28) fact that the external factor of hypoxia or decompression acts as a stressor, causing the living body to bring about non-specific general adaptation syndrome. It is quite natural that what plays a leading rôle in such a condition of stress is the reaction of pituitary-adrenal system, and that with the reactions occurring secondarily, many kinds of adaptation reactions not directly related to the pituitary-adrenal system take place.

In the experiments under low pressure on humans (15) or guinea pigs (14) performed by Krasno and Ivy, the change of the ascorbic acid levels observed
in blood, urine and tissues may be regarded as the result of such a direct or indirect adaptation reaction, but it is quite natural that such changes in metabolism of ascorbic acid depend upon the condition of the stressor, and the kind of experimental animals used, and also on individual differences.

It is noteworthy that in the pituitary gland, adrenals and other endocrine organs the concentration of ascorbic acid is generally very high as compared to other tissues (4, 21). It is also generally considered (12, 17, 18, 22, 24, 25) by the two following facts that ascorbic acid performs some rôle in corticosteroidogenesis. (a) Ascorbic acid plays some rôle to prevent the oxidation of adrenaline secreted from the adrenal medulla at quite an early stage of stress, i.e., during the emergency reaction to give it stability (3, 11, 27, 30). (b) In the alarm reaction prior to the secretion of corticosterone by the adrenal cortex a decrease in the ascorbic acid content is observed.

The finding that ascorbic acid in the adrenals decreases more in Groups B and C than in Group A, obviously shows that such adrenal activity has been enhanced in the former groups. But the finding that the decrease is more marked in Group C than in Group B does not mean that the stressor of Group C has brought about greater adrenal activity than that of Group B.

In Group B, during decompression, the adrenals having high fat content, 30—54% dry weight (5), are liable to produce air bubbles resulting in the obstruction of capsular arterioles and large venous medullary channels, thus causing circulatory disturbances or the stressor of this group causes a disturbance in the utilization of ascorbic acid in the adrenal tissue, thus delaying the speed of utilization of the vitamin. In the adrenals DHA+DGA was found only in very minute quantities or none at all. This may be due to the fact, as Slusher (26) and Salomon (21) have pointed out, that ascorbic acid in a high state of oxidation is released into the adrenal veins causing only a slight amount to be retained in the adrenals.

The influence of decompression is expected to bring about a change both in permeability of the cell membranes and of the capillary walls, and in the osmotic pressure in the tissues, disturbance in oxidation and reduction processes or degeneration of collagen, and the liver (17, 26) which is regarded as the main supply of ascorbic acid, is expected to respond to the necessities given above and to the demand of other endocrine organs. The finding that the decrease in Group B is a little less than that of Group C, may be explained easily if it is taken into account that it has something to do with the change in blood, but the stressor in Group B causes a disturbance in the utilization of ascorbic acid in the periphery, causing a stagnation of the vitamin in the blood stream which in turn slows down the rate of mobilization of the vitamin from the liver. Of course, it may also be connected with the disturbance of utilization of the vitamin in the liver tissue itself. The finding that there is scarcely any oxidized form of ascorbic acid in the liver is assumed to suggest the rôle of the liver to be a supplying organ of the vitamin.

Ascorbic acid in the blood is regarded (3) as a “threshold” substance like glucose. Its threshold value is 1.0—1.8 mg/100 ml (8, 10, 13, 16, 29) in the case of normal adults. As to the threshold value of rats, there is no report avail-
able, but the finding that the ascorbic acid level in the blood of Group B showed 133.8% of that of the control group and the urinary ascorbic acid 292.6% suggests that the ascorbic acid level exceeded by far the threshold value to the excessive excretion.

On the contrary, in Group C ascorbic acid in the blood showed a value of 31% of the control group, the urinary excretion of the vitamin being almost the same value as the control group. This seems to suggest the following fact. Oxygen that had been saturated excessively as a result of the substitution of oxygen for nitrogen in body fluid and tissues by inhaling pure oxygen beforehand, promoted the oxidation and utilization of the vitamin during decompression. At the same time the formation of nitrogen bubble was prevented by such pretreatment. It is a very advantageous condition resulting from adaptation under low pressure (Table ‡V). These findings have revealed a new benefit of inhaling pure oxygen under normal pressure which has hitherto been considered to have only the effect of preventing nitrogen escape.

SUMMARY

1. A study was made on the physiological rôle of ascorbic acid when a living body is subjected to decompression with or without previous treatment of pure oxygen inhalation.

2. After a non-hypoxic decompression of 200 mm Hg was loaded on rats for 20 minutes, the animals showed severe symptoms and at the same time the ascorbic acid levels in adrenals, liver, blood and urine showed 81.9, 72.2, 133.8 and 292.6% respectively as compared with that in the control groups. This shows that this form of stress causes a disturbance in the utilization of ascorbic acid in the periphery and gives rise to a mobilization of the vitamin, which in turn causes a stagnation of the vitamin in the blood stream. The excess ascorbic acid is excreted in the urine resulting in the increase of urinary excretion of the vitamin.

3. When the animals had been allowed to inhale pure oxygen under normal pressure for two hours in order to replace nitrogen beforehand, and the similar decompression were loaded, they exhibited practically no abnormal symptoms. Ascorbic acid levels in the adrenals, liver, blood and urine showed 58.3, 62, 31 and 97.5% respectively as compared with that of the control groups, suggesting that the inhalation of pure oxygen prior to decompression not only prevents the formation of nitrogen bubbles and symptoms due to decompression, but also has a favorable influence on the oxidation of ascorbic acid in the peripheral tissues and enhances its utilization, thus increasing tolerance to low pressure.

ACKNOWLEDGEMENT

The authors wish to thank Prof. Ryoichi Sugimoto for his kind guidance, and for the revision of this paper.
REFERENCES