EFFECT OF ASCORBIC ACID ON INCREASE IN ESTERIFIED CHOLESTEROL IN ADRENAL GLANDS OF GUINEA PIGS FED ON CHOLESTEROL DIET

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It was demonstrated by Zaffaroni et al.1) and Werbin et al.2) that cholesterol is a precursor of adrenal cortical hormones. Since the most of cholesterol in the adrenal cortex is in the esterified form, it is easy to assume that the first step in the transformation reaction from cholesterol to cortical hormones may be the esterification process. On the other hand, Sayers et al.3) discussed the important role of ascorbic acid in the formation of cortical hormones. However, in what respect ascorbic acid is related has not been elucidated.

In the present study, experiments were performed in the guinea pig to see if cholesterol feeding increases the esterified fraction of adrenal cholesterol, and, if so, how ascorbic acid influences on this increase.

EXPERIMENTAL MATERIALS AND METHODS

Female guinea pigs weighing 200-300 g were used. Soyabean-curd refuse and vegetables were given as a normal diet and heated soyabean-curd refuse without vegetables as an ascorbic acid deficient diet. The positive urinary blood reaction was taken as an indicator of ascorbic acid deficiency. After the blood reaction became positive the animals were fed for the additional 1 or 2 days and used for experiments. Free cholesterol was precipitated as digitonide and removed, the amount of ester cholesterol remained was determined by using Liebermann-Burchard reaction,4) and the amount of free cholesterol was obtained by subtracting the amount of ester cholesterol from that of total cholesterol.

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RESULTS

1. Level of Adrenal Cholesterol in Normal Guinea Pigs.

The average level of total cholesterol in the adrenal glands of normal guinea pigs was 4.6% and that of ester cholesterol 4.0% as shown in Table 1. The data indicate that the most (86.8%) of cholesterol in the adrenal glands is in the esterified form.

Table 1

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>No. of Rate</th>
<th>Total Cholesterol</th>
<th>Ester Cholesterol</th>
<th>Free Cholesterol</th>
<th>Ester/Total Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>13</td>
<td>3.7 5.2 4.6</td>
<td>3.1 4.5 4.0</td>
<td>0.6 0.7 0.6</td>
<td>86.8</td>
</tr>
<tr>
<td>Asorb. Acid defic. Diet</td>
<td>13</td>
<td>3.1 4.3 3.7</td>
<td>2.7 3.7 3.1</td>
<td>0.4 0.6 0.6</td>
<td>86.1</td>
</tr>
<tr>
<td>Cholest. Diet</td>
<td>10</td>
<td>5.2 6.5 5.7</td>
<td>4.6 5.5 5.0</td>
<td>0.6 1.0 0.7</td>
<td>87.0</td>
</tr>
<tr>
<td>Asorb. Acid defic. and Cholest. Diet</td>
<td>10</td>
<td>4.5 5.3 4.8</td>
<td>2.9 3.3 3.1</td>
<td>1.6 2.0 1.7</td>
<td>64.5</td>
</tr>
<tr>
<td>Asorb. Acid defic. and Cholest. Diet, Asorb. Acid Inject.</td>
<td>10</td>
<td>4.7 6.3 5.4</td>
<td>4.2 5.4 4.7</td>
<td>0.5 0.9 0.7</td>
<td>87.5</td>
</tr>
</tbody>
</table>

2. Level of Adrenal Cholesterol in Ascorbic Acid Deficient Guinea Pigs.

Since all of the guinea pigs which had received the ascorbic acid deficient diet described above gave the positive urinary blood reaction on the 11th-12th day, they were kept on the deficient diet for 13 days and used for the experiment. The results are shown in Table 1.

The level of total cholesterol in the adrenal glands of the ascorbic acid deficient guinea pigs was 3.7%, which is less than the normal value. Ester cholesterol also decreased to 3.1%, which is much less than the normal value.

3. Level of Adrenal Cholesterol in Guinea Pigs Fed on Cholesterol Diet.

The normal diet supplemented with cholesterol in daily dose of 0.2 g was given for 13 days and the amount of adrenal cholesterol was determined. The results are shown in Table 1.

Total cholesterol was 5.7%, which indicates marked increase as compared with the normal and the ascorbic acid deficient groups. Ester cholesterol increased to 5.0%, the figure being much larger than those in the normal and the ascorbic acid deficient groups.
4. Level of Adrenal Cholesterol in Guinea Pigs Fed on Ascorbic Acid Deficient and Cholesterol Supplemented Diet.

In this experiment the effect of the ascorbic acid deficient diet plus cholesterol in the dose of 0.2 g/day was examined. The results are shown in Table 1.

The average total cholesterol was 4.8%. The figure is larger than that for the ascorbic acid deficient group (3.7%) but is smaller than that for the normal diet plus cholesterol group (5.7%). The average content of ester cholesterol was 3.1%, which is the same as in the ascorbic acid deficient group.

5. Level of Adrenal Cholesterol in Guinea Pigs Received Ascorbic Acid Deficient, Cholesterol Supplemented Diet and Injection of Ascorbic Acid.

It was shown that ester cholesterol in the adrenal glands decreased with the ascorbic acid deficient diet. The supplementation of cholesterol to the above diet did not elevate the level of ester cholesterol as shown in the result 4, while cholesterol supplementation to the normal diet caused remarkable increase in ester cholesterol (the result 3). Since this difference in the effect of cholesterol supplementation was considered to be due to the existence of ascorbic acid in the diet, an experiment was conducted in guinea pigs fed on the ascorbic acid deficient diet with added cholesterol and given the injection of ascorbic acid. Cholesterol was added to the diet at a level of 0.2 g/day and ascorbic acid was injected subcutaneously in a dose of 25 mg/day. The animals were kept for 13 days. The level of adrenal cholesterol is shown in Table 1.

The content of total cholesterol was 5.4%, and that of ester cholesterol 4.7%. It will be noted that the ester fraction is remarkably increased as compared with that in the ascorbic acid deficient and cholesterol supplemented group (3.1%).

SUMMARY AND DISCUSSION

The effect of ascorbic acid on the increase in ester fraction of adrenal cholesterol in the cholesterol fed guinea pigs was examined. The results are summarized as follows:

1) The average content of total cholesterol in the adrenal glands of the normal guinea pigs was 4.6%, of which 4.0% (that is, about 87% of total cholesterol) was in the esterified form.

2) In the ascorbic acid deficient guinea pigs, total cholesterol in the adrenal glands was 3.7% on the average, of which 3.1% was esterified. These figures are much less than the normal values.

3) In the guinea pigs fed on the normal diet supplemented with cholesterol (0.2 g/day), the average total cholesterol in the adrenal glands was 5.7%, of
which 5.0% (that is, 87% of total cholesterol) was esterified. The both fractions are remarkably increased.

4) In the guinea pigs fed on the ascorbic acid deficient diet supplemented with cholesterol (0.2 g/day) and developed ascorbic acid deficiency, total cholesterol in the adrenal glands was 4.8%, of which 3.1% was esterified. These figures are less than those in the guinea pigs fed on the normal diet plus cholesterol. Especially, the esterified fraction hardly showed any increase as compared with that in the normal animals.

5) Guinea pigs were fed on the ascorbic acid deficient diet supplemented with cholesterol and given the injection of ascorbic acid. Total cholesterol in the adrenal glands in these animals was 5.4% and the ester cholesterol 4.7%, the figures indicating a remarkable increase as compared with those without injection of ascorbic acid.

6) From the above results it is concluded that ascorbic acid supplementation augments the increase in total cholesterol in the adrenal glands of guinea pigs. Especially, the increase in ester cholesterol is augmented remarkably. These data are considered to offer supporting evidence for the assumption that the first step in the transformation reaction from cholesterol to adrenal cortical hormones is the esterification process as described in the beginning. It was also demonstrated in this study that the esterification of cholesterol is augmented by ascorbic acid. The fact that ascorbic acid enhances the function of adrenal cortex as generally recognized may be explained by the augmentation of the increase in cholesterol ester in the adrenal glands by ascorbic acid.

REFERENCES