EFFECT OF SCURVY ON CHOLESTEROL AND ASCORBIC ACID IN GUINEA PIG ADRENALS

BY SACHCHIDANANDA BANERJEE AND CHANDICHARAN DEB

(From the Department of Physiology, Presidency College, Calcutta, India)

(Received for publication, November 8, 1950)

It has been shown earlier (1) that the metabolism of carbohydrate is disturbed in scorbutic guinea pigs as evidenced by diminished glucose tolerance, deposition of liver glycogen, and insulin content of the pancreas. Although the adrenalin content of the adrenals of scorbutic guinea pigs is increased, it has been shown by removing the adrenal medulla that adrenalin is not responsible for the lower glucose tolerance observed in scurvy (2). It has also been reported that the altered carbohydrate metabolism observed in scorbutic guinea pigs is not due to either hypo- or hyperthyroidism (3). The adrenal cortex is intimately related to the carbohydrate metabolism. It was, therefore, of interest to find out whether the hormone of the adrenal cortex is affected in scorbutic guinea pigs. Because of the presence of a small quantity of the steroid hormone in the adrenal gland, it is difficult to estimate it. Cholesterol and ascorbic acid, however, appear to be related to the formation of the adrenal cortical hormone. Injection of the adrenocorticotropic hormone of the pituitary in rats and guinea pigs has been found to lower both the ascorbic acid and cholesterol content of the adrenal glands (4). In the present investigation total cholesterol and ascorbic acid concentrations of adrenal glands of scorbutic guinea pigs and paired, fed, normal guinea pigs have been estimated with the purpose of finding out the function of the adrenal cortex in scorbutic guinea pigs.

EXPERIMENTAL

Female guinea pigs, varying between 150 and 210 gm. in weight, were fed a scorbutic diet (5) with a daily supplement of 5 mg. of ascorbic acid per animal for 1 week. The animals which grew were selected, divided into two groups, housed in individual cages, and paired evenly so that the weights of the animals in each pair, one from each of the groups, were about the same. The animals of one of the groups were fed ad libitum the scorbutic diet only. The food consumption by each of the animals of this group was measured and the equivalent amount of food was given to the corresponding animal of the second group, which received in addition a daily oral supplement of 5 mg. of ascorbic acid. All the animals received in addition 2 drops of a concentrate of vitamins A, D, and K twice
a week. At the end of 20 days the animals were fasted overnight and killed on the 21st day by decapitation. The adrenals were removed. Cholesterol was estimated in the left adrenal gland, and the right gland was used for the estimation of ascorbic acid.

_Estimation of Adrenal Cholesterol_—The left adrenal gland was disintegrated, spread as a thin paste over a washed filter paper 7 cm. in diameter, and dried in an electric oven at 80° for 45 minutes. The filter paper was then rolled and placed inside a glass thimble with perforated walls. The thimble was suspended from the lower end of a condenser, which was fitted with a ground glass joint into a 125 cc. conical flask containing 8 cc. of acetone. Cholesterol in the adrenals was extracted with acetone on a boiling water bath for 2 hours. The volume of the extract was made up to 10 cc. with acetone. To 0.5 cc. of the acetone extract in a test-tube 2.5 cc. of a mixture of alcohol and acetone (1:1) and 4 drops of a 30 per cent solution of potassium hydroxide were added. The mixture was placed in a water bath at 40° for half an hour, neutralized with 10 per cent acetic acid with phenolphthalein indicator, poured into an evaporating basin, and evaporated to dryness in an electric oven kept at 80°. The residue was extracted three times with 2 cc. portions of hot chloroform. The volume of the extract was made up to 6 cc. with chloroform in a 10 cc. glass-stoppered graduated cylinder and 0.2 cc. of pure sulfuric acid, followed by 2 cc. of acetic anhydride, was added to it. The mixture was shaken for 15 seconds, placed in a water bath at 20° for 5 minutes, and then at room temperature (30°) for another 5 minutes for the development of color. The color was estimated in a Lumetron photoelectric colorimeter with a 620 mμ filter.

_Estimation of Adrenal Ascorbic Acid_—The weighed right adrenal gland was macerated with 3 cc. of 10 per cent trichloroacetic acid and sea sand in a glass mortar and transferred to a graduated centrifuge tube; the volume was made up to 6 cc. by washing the mortar with distilled water and the mixture was centrifuged. Ascorbic acid in the extract was estimated by titration against a standardized solution of 2,6-dichlorophenol indo-phenol.

**DISCUSSION**

From Table I it will be seen that both cholesterol and ascorbic acid concentrations of adrenals are significantly diminished in the scorbutic guinea pigs. The low value of adrenal cholesterol of guinea pigs, as reported by Sayers _et al._ (4), may possibly be due to imperfect extraction of cholesterol from the gland. Cholesterol is completely extracted in the method described by us. The decrease in the cholesterol and ascorbic acid concentrations of adrenals in scorbutic guinea pigs, along with the low glycogen
content of the liver and values of normal fasting blood sugar (1), indicates indirectly that the secretion of the adrenal cortical hormone is diminished in scurvy. McKee, Cobbey, and Geiman (6) observed that the injection of cortical hormone in scurbutic guinea pigs did not increase the deposition of glycogen in the livers of these animals. This possibly indicates that the disturbed carbohydrate metabolism, as observed in scurbutic guinea pigs, is due to the combined effect of diminished secretion of both cortical hormone and insulin.

**TABLE I**

<table>
<thead>
<tr>
<th>Weight at death</th>
<th>Ascorbic acid per gm.</th>
<th>Cholesterol per gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>right adrenal</td>
<td>left adrenal</td>
</tr>
<tr>
<td>Pair No.</td>
<td>Normal</td>
<td>Scorbutic</td>
</tr>
<tr>
<td>1</td>
<td>212</td>
<td>184</td>
</tr>
<tr>
<td>2</td>
<td>232</td>
<td>185</td>
</tr>
<tr>
<td>3</td>
<td>260</td>
<td>231</td>
</tr>
<tr>
<td>4</td>
<td>223</td>
<td>185</td>
</tr>
<tr>
<td>5</td>
<td>223</td>
<td>195</td>
</tr>
<tr>
<td>6</td>
<td>204</td>
<td>195</td>
</tr>
<tr>
<td>7</td>
<td>266</td>
<td>232</td>
</tr>
<tr>
<td>8</td>
<td>228</td>
<td>180</td>
</tr>
<tr>
<td>9</td>
<td>254</td>
<td>202</td>
</tr>
</tbody>
</table>

**SUMMARY**

1. The effect of vitamin C deficiency on the cholesterol and ascorbic acid concentrations of adrenal glands has been studied in guinea pigs by the paired feeding technique.

2. Both the cholesterol and the ascorbic acid concentrations of the adrenals are diminished in scurbutic guinea pigs.

3. A method of extraction of cholesterol from adrenals has been described.

4. The altered carbohydrate metabolism observed in scurbutic guinea pigs has been suggested to be due to the combined deficiency of adrenal cortical hormone and insulin.
BIBLIOGRAPHY

EFFECT OF SCURVY ON
CHOLESTEROL AND ASCORBIC ACID
IN GUINEA PIG ADRENALS
Sachchidananda Banerjee and Chandicharan Deb


Access the most updated version of this article at http://www.jbc.org/content/190/1/177.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/190/1/177.citation.full.html#ref-list-1