RELATION OF SCURVY TO THE ADRENALIN CONTENT OF THE ADRENAL GLANDS OF GUINEA PIGS

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It has been reported from this laboratory (1) that a deficiency of vitamin C in guinea pigs leads to changes in carbohydrate metabolism, as evidenced by glycosuria, a diabetic type of the glucose tolerance curve, and depletion of the glycogen content of the liver. We have also found that there is a fall in the insulin content of the pancreas (2), an increase in the size and also the number of islets, and degranulations of the β-cells of the pancreas (3) of scorbutic guinea pigs.

It is well known that adrenalin and insulin produce opposite glycemic effects (Cori and coworkers (4, 5)). It was therefore of particular interest to examine the effect of scurvy on the adrenalin content of the adrenal glands of guinea pigs.

McCarrison (6) reported that the adrenalin content of the adrenals of scorbutic guinea pigs is decreased. Ohata (7) also noted a definite reduction in the adrenalin content of the adrenal glands of scorbutic guinea pigs and presented evidence that this was not purely an effect of inanition. Deutsch and Schlapp (8) likewise reported a decrease in the adrenalin content of the adrenal glands of guinea pigs in scurvy. Mouriquand and Leulier (9), however, found no lowering of the adrenalin content of the adrenals of guinea pigs in avitaminosis C. Guha (10) also observed no change in the adrenalin content of the adrenals of scorbutic guinea pigs.

In view of the conflicting data cited above it was decided to reinvestigate the adrenalin content of the adrenal glands of scorbutic guinea pigs. A preliminary report of this work has been published elsewhere (11).

EXPERIMENTAL

Two groups of young guinea pigs of weights varying between 250 gm. and 400 gm. were selected. One of the groups was fed ad libitum a scorbutic diet consisting of crushed barley 64 parts, crushed gram¹ 20 parts, casein 12 parts, calcium carbonate 3 parts, and common salt 1 part for 22 to 25

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¹ Cicer arietinum (Leguminosae) is a pulse commonly cultivated as human food in India.
days until the animals developed scurvy. The second group of guinea pigs received the above diet for 15 days with the daily oral supplement of 5 mg. of ascorbic acid per animal. The animals of both the groups were fed 0.2 cc. of a concentrate of vitamins A and D (adexoline, Glaxo) twice a week during the experimental period.

**Table I**

*Determination of Adrenalin in Normal and Scorbutic Guinea Pigs*

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Average weight per animal (gm.)</th>
<th>Weight of pooled adrenals (mg.)</th>
<th>Weight of adrenal per 100 gm. body weight (mg.)</th>
<th>Ascorbic acid per 1 gm. of adrenal (mg.)</th>
<th>Adrenalin per 1 gm. of adrenal (γ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scorbutic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>310</td>
<td>1140</td>
<td>91</td>
<td>0.032</td>
<td>859</td>
</tr>
<tr>
<td>4</td>
<td>374</td>
<td>1120</td>
<td>93</td>
<td>0.038</td>
<td>675</td>
</tr>
<tr>
<td>6</td>
<td>292</td>
<td>1700</td>
<td>96</td>
<td>0.021</td>
<td>707</td>
</tr>
<tr>
<td>7</td>
<td>340</td>
<td>2840</td>
<td>119</td>
<td>0.048</td>
<td>630</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>100</td>
<td>0.035</td>
<td>719.2</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>311</td>
<td>720</td>
<td>57</td>
<td>0.915</td>
<td>502</td>
</tr>
<tr>
<td>4</td>
<td>310</td>
<td>860</td>
<td>69</td>
<td>0.732</td>
<td>230</td>
</tr>
<tr>
<td>4</td>
<td>237</td>
<td>560</td>
<td>59</td>
<td>0.828</td>
<td>459</td>
</tr>
<tr>
<td>4</td>
<td>323</td>
<td>760</td>
<td>59</td>
<td>1.184</td>
<td>263</td>
</tr>
<tr>
<td>4</td>
<td>397</td>
<td>980</td>
<td>62</td>
<td>1.351</td>
<td>315</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>61.2</td>
<td>1.002</td>
<td>353.8</td>
</tr>
</tbody>
</table>

**Table II**

*Statistical Analysis*

All values of *t* are highly significant.

<table>
<thead>
<tr>
<th></th>
<th>Weight of adrenal per 100 gm. body weight (mg.)</th>
<th>Ascorbic acid per 1 gm. of adrenal (mg.)</th>
<th>Adrenalin per 1 gm. of adrenal (γ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference of means</td>
<td>38.8</td>
<td>0.967</td>
<td>365.4</td>
</tr>
<tr>
<td>Standard error of difference</td>
<td>6.187</td>
<td>0.13078</td>
<td>74.66</td>
</tr>
<tr>
<td><em>t</em></td>
<td>6.27</td>
<td>7.39</td>
<td>4.89</td>
</tr>
</tbody>
</table>

The animals were fasted overnight. They were killed by a blow on the head and the neck veins were cut. The adrenals were carefully freed from the connective tissue and transferred to a weighed bottle containing normal saline and a few drops of 10 per cent trichloroacetic acid. The adrenal glands of several animals were pooled and extracted with trichloroacetic acid by the method of Barker and Marrian (12).
Of the different chemical methods for the estimation of adrenalin, Folin and Trimble’s reagent (13) as used by Barker et al. (14) was found to be most satisfactory in our hands. It was shown by Guha (10) and Birch et al. (15) that Folin’s tungstic acid reagent gives a blue color with ascorbic acid as well as with adrenalin. Rees (16) observed that the blue color obtained with Folin’s reagent was proportional to the amount of ascorbic acid present. When the ascorbic acid in the adrenal gland extracts was first determined by titration with 2,6-dichlorophenol indophenol and the blue value corresponding to the amount of ascorbic acid present was subtracted from the total blue value of the sample, the true adrenalin content of the sample was obtained. This result was found by Rees to correspond to that determined biologically. Von Euler (17) and Schild (18) also obtained no significant difference in the adrenalin content of the adrenals of guinea pigs when it was determined by the biological and colorimetric methods. Rees’ modified method for the colorimetric estimation of adrenalin was therefore used in the present investigation on the trichloroacetic acid extract of adrenal glands. The results are shown in Table I and the statistical analysis is given in Table II.

**DISCUSSION**

It will be noted from the data of Table I that the adrenal glands of the scorbutic guinea pigs were found to be much higher in adrenalin than were those of normal control animals. This is in contrast to the findings of previous investigators cited earlier. It is therefore of interest to compare the method used in this study with those used by others.

McCarrison (6) used Folin’s reagent for the estimation of adrenalin and, since it is known (10, 15, 16) that Folin’s reagent also gives a blue color with ascorbic acid, a relatively higher adrenalin value for the adrenals of normal guinea pigs might have resulted from the fact that the normal glands contain larger amounts of ascorbic acid. In scorpy the ascorbic acid content is greatly diminished (Table I), so that the diminution of the adrenalin value might have been apparent rather than real. Ohata (7), who also noted a reduction of the adrenalin content in scorpy, estimated adrenalin with iodic acid which likewise reacts with ascorbic acid. On the other hand Mouriquand and Leulier (9) who used mercuric chloride, which gives a red coloration with adrenalin and no coloration with ascorbic acid, obtained no decrease in the adrenalin content of the adrenal glands in scorpy. Guha (10) who determined adrenalin biologically also found no difference in the adrenalin content under the two conditions. These workers, however, used guinea pigs which were in the extreme stages of scorpy. On the other hand, our animals were killed when they were just falling in weight and were in the early stages of scorpy. Deutsch and
Schlapp (8) determined adrenalin content biologically and found a reduction in the adrenalin content of the adrenals in scurvy. These results which differ from that of Guha (10) are difficult to reconcile with our own. We do not know whether the different diets used by us had anything to do with it. The size of the adrenal glands has been found to be considerably increased in scurvy. This was also observed by Deutsch and Schlapp (8), McCarrison (6), Michaud (19), and Baldwin et al. (20). We have not used the paired feeding technique in our experiments and it might be argued that the results are due to inanition. However, in the experiments of Baldwin et al. (20) the paired feeding technique was employed and, although the adrenalin content of the adrenals was not determined, they observed the same increase in the weight of the gland as we have observed. It seems unlikely, therefore, that the increase in the adrenalin content in scurvy is the result of inanition. The increase in the adrenalin content of the adrenals in scurvy is of particular interest in relation to our earlier finding (2) that the insulin content of the pancreas is decreased. The action of vitamin C on the secretion of insulin appears to be specific in some degree, as the insulin content of the pancreas is not altered in vitamin B1 deficiency, which also affects carbohydrate metabolism (21).

The lowered glucose tolerance and the lower glycogen content of the liver of the scorbutic guinea pigs as compared to normal animals may be partly due to an increased action of adrenalin in the absence of the opposing action of insulin. The lower glycogen content of the liver in scurvy might also be due in part to the decrease in adrenal cortical hormone in scorbutic guinea pigs observed by Giroud et al. (22, 23), since adrenal cortical hormone has been shown by Grattan and Jensen (24) to promote the deposition of glycogen in the liver.

SUMMARY

The effect of scurvy on the adrenalin content of the adrenal glands of guinea pigs was studied. A significant increase in the adrenalin content of the adrenals of scorbutic guinea pigs was noted, in contrast to the earlier findings of a diminution of the insulin content of the pancreas in scurvy.

Standard adrenalin was obtained by courtesy of Dr. B. Mukerji, director of the Biological Standardisation Laboratory, Government of India. Hoffmann-La Roche, Inc., made a free gift of 2,6-dichlorophenol indophenol.

BIBLIOGRAPHY

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