EFFECT OF SCURVY ON GLUTATHIONE AND
DEHYDROASCORBIC ACID IN GUINEA
PIG TISSUES

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(Received for publication, August 27, 1951)

Hypofunction of the islands of Langerhans in scorbutic guinea pigs, as
evidenced by diminished insulin content (1) and degranulation of the
β-cells (2), results in lowered glucose tolerance and decreased deposition
of glycogen in the liver (3). Insulin contains a large amount of cystine
(4) and it is probable that under the influence of enzymes cystine is taken
up by the β-cells to form the insulin molecule. In the body cystine may
be derived either from methionine or cysteine, and glutathione, the nat-
urally occurring tripeptide, contains cysteine. If the glutathione content
of the body is diminished, it may interfere with the normal synthesis of
insulin. Certain enzymes of the body depend on —SH groups for their
activity, and, if the —SH groups are inactivated, the enzymes become
inactive (5). The activity, however, may be restored in most cases by
the addition of glutathione (6). Glutathione thus is supposed to protect
the —SH groups of enzymes. In scurvy, if the glutathione content of
tissues is diminished, the β-cell sulfhydryl enzymes, necessary for the
synthesis of insulin, may not be well protected from the toxic action of
unknown substances leading to the hypofunction and the diminished
secretion of insulin.

Intravenous injection of dehydroascorbic acid in rats leads to chronic
hyperglycemia and glycosuria (7). Cysteine and glutathione, if injected
intravenously just before the injection of dehydroascorbic acid, can pre-
vent this diabetogenic action (8). In order to find out whether the im-
paired carbohydrate metabolism in scurvy is related to either increased
dehydroascorbic acid or diminished glutathione content of the tissues,
these substances were estimated in both scorbutic and pair-fed normal
guinea pigs.

EXPERIMENTAL

The selection of the guinea pigs, the feeding of the scorbutic diet either
with or without the supplement of ascorbic acid by the paired feeding tech-
nique, and the method of killing the animals on the 21st day of the experi-
ment were the same as those described previously (9).
EFFECT OF SCURVY ON GLUTATHIONE

Extraction of Tissues—Tissues, after removal from the body, were wrapped in filter paper to remove the adherent blood and then dropped into weighed beakers containing 2 per cent sulfosalicylic acid. The beakers were weighed again and kept in a refrigerator. The tissues were ground with sea sand in a chilled glass mortar in sulfosalicylic acid solution and extracted with sulfosalicylic acid to a definite volume, and the

\[
\begin{array}{l}
\text{Average, normal} \quad 58 \pm 8.0 \\
\quad \text{Scurbutic} \quad 204 \pm 12.0 \\
\quad \text{Intestine} \quad 237 \pm 11.5 \\
\quad \text{Kidney} \quad 216 \pm 7.1 \\
\quad \text{Liver} \quad 206 \pm 15.0 \\
\quad \text{Kidney} \quad 172 \pm 4.8 \\
\quad \text{Liver} \quad 351 \pm 12.1 \\
\quad \text{Normal} \quad 31 \pm 6.6 \\
\quad \text{Scurbutic} \quad 142 \pm 2.0 \\
\quad \text{Intestine} \quad 132 \pm 12.2 \\
\quad \text{Kidney} \quad 174 \pm 10.7 \\
\quad \text{Liver} \quad 165 \pm 8.6 \\
\quad \text{Liver} \quad 352 \pm 7.6 \\
\quad \text{Normal} \quad 123 \pm 11.3 \\
\end{array}
\]

\[
\begin{array}{l}
\text{Difference of means} \quad 27 \pm 10.37 \\
\text{Standard error of difference} \quad 62 \pm 5.37 \\
\text{t} \quad 114 \pm 6.8 \\
\text{t} \quad 41 \pm 3.2 \\
\text{t} \quad 32 \pm 1.8 \\
\text{t} \quad 7 \pm 0.78 \\
\text{t} \quad 1 \pm 0.06 \\
\end{array}
\]

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\text{t} \quad 32 \pm 1.8 \\
\text{t} \quad 7 \pm 0.78 \\
\text{t} \quad 1 \pm 0.06 \\
\end{array}
\]
mixture was centrifuged. The centrifugate was used for the estimation of ascorbic acid, dehydroascorbic acid, and glutathione.

**Table II**

*Ascorbic Acid Values of Tissues of Guinea Pigs*

All values are expressed as mg. per 100 gm. of tissue.

<table>
<thead>
<tr>
<th>Pair No.</th>
<th>Adrenals</th>
<th>Pancreas</th>
<th>Spleen</th>
<th>Intestine</th>
<th>Kidney</th>
<th>Liver</th>
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<td>15.60</td>
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<tr>
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<td>Normal</td>
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<td>26.50</td>
<td>11.40</td>
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<tr>
<td></td>
<td>Scorbutic</td>
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<td>3.37</td>
<td>2.54</td>
<td>3.02</td>
<td>7.09</td>
</tr>
</tbody>
</table>

Average, normal 67.67 12.90 30.81 13.01 14.90 16.98

Difference of means 57.56 9.97 27.16 9.36 10.03 9.67

Estimation of Ascorbic Acid and Dehydroascorbic Acid—Ascorbic acid in the extract was estimated by titration against a standardized solution of 2,6-dichlorophenol indophenol. For the estimation of dehydroascorbic acid, hydrogen sulfide was passed for 5 minutes into an aliquot of the solution in order to reduce dehydroascorbic acid to ascorbic acid. The
excess of hydrogen sulfide in the solution was removed by passing a current of nitrogen and total ascorbic acid was estimated by titration with indophenol dye. Dehydroascorbic acid was calculated by deducting the value of ascorbic acid from the total ascorbic acid.

**Estimation of Glutathione**—Glutathione was titrated in 5 cc. aliquots of the extract with a standard solution of potassium iodate (0.001 N in 2 per cent sulfosalicylic acid) at 0–5° according to a slightly modified method of Leaf and Neuberger (10). The amount of potassium iodate solution which was equivalent to the amount of ascorbic acid present in the same aliquot of extract, as determined by titration against the indophenol dye, was deducted from the total amount of standard iodate required to titrate the solution. From the volume of the iodate standardized against a pure solution of glutathione, the values of glutathione were calculated. Blood glutathione was estimated according to the method of Woodward and Fry (11). The results are given in Tables I to III.

**Results**

The glutathione content of blood, adrenals, pancreas, and spleen was significantly diminished in scorbutic guinea pigs but the diminution was maximal in the pancreas. No significant change in the glutathione of liver, kidney, and small intestine could be observed in scorbutic guinea pigs. The ascorbic acid content of the tissues diminished significantly in

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**Table III**

*Dehydroascorbic Acid Values of Tissues of Scorbutic Guinea Pigs*

All values are expressed as mg. per 100 gm. of tissue.

<table>
<thead>
<tr>
<th>Pair No.</th>
<th>Adrenals</th>
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<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.56</td>
<td>3.25</td>
<td>0.12</td>
<td>1.27</td>
<td>1.26</td>
<td>3.00</td>
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<td>3.76</td>
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<td>1.15</td>
<td>0.67</td>
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<td>0.44</td>
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<td>0.13</td>
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<tr>
<td>6</td>
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<td>0.49</td>
<td>0.13</td>
<td>0.90</td>
<td>0.27</td>
</tr>
<tr>
<td>7</td>
<td>0.19</td>
<td>0.18</td>
<td>0.16</td>
<td>1.09</td>
<td>1.51</td>
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<td>8</td>
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</tr>
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</table>

Average... 2.90 1.26 1.04 1.01 1.23 1.92
±0.39 ±0.28 ±0.34 ±0.07 ±0.53 ±0.56

* Dehydroascorbic acid was absent in the tissues of pair-fed normal guinea pigs.
scorbutic guinea pigs. Dehydroascorbic acid, which was absent from the tissues of normal guinea pigs, was present in a considerable amount in the tissues of scorbutic guinea pigs.

DISCUSSION

Substances which are diabetogenic, and which also cause diminution in the insulin content of the pancreas, also lower the glutathione content of the tissues. Thus, after injections of alloxan (12, 13) and anterior pituitary extracts (14, 15), the glutathione content of blood is diminished. In human diabetes also, glutathione content of blood and pancreas is low (16). The diminished glutathione content of blood and tissues of scorbutic guinea pigs might, therefore, result in diminished insulin synthesis in the β-cells, as glutathione might supply the cystine of the insulin molecule.

It has been observed that glutathione combines with alloxan (17) and dehydroascorbic acid (8) and thereby prevents the diabetogenic action of these substances. Dehydroascorbic acid present in the tissues of scorbutic guinea pigs might combine with the glutathione of the tissues and thereby cause a fall in the available glutathione concentration in the β-cells of the pancreas. The protective rôle of glutathione on the sulfhydryl enzymes in the β-cells is thus further jeopardized, which might result in the death of the β-cells and a diminished secretion of insulin.

SUMMARY

The glutathione content of blood, adrenals, pancreas, spleen, liver, kidney, and small intestine was determined in scorbutic and pair-fed normal guinea pigs. Glutathione was significantly diminished in blood, adrenals, pancreas, and spleen, while in other tissues studied the value was not changed significantly. The lowering of the glutathione value in the pancreas of scorbutic guinea pigs was maximal.

The dehydroascorbic acid content of adrenals, pancreas, spleen, liver, kidney, and small intestine was determined in both scorbutic and pair-fed normal guinea pigs. Dehydroascorbic acid was absent in the tissues of normal guinea pigs, while it was present in considerable amounts in the tissues of scorbutic guinea pigs.

It has been suggested that the diminished insulin secretion in scorbutic guinea pigs may be due to the combined effect of diminished glutathione content and increased dehydroascorbic acid content of tissues in general and of pancreas in particular.

We are indebted to Hoffmann-La Roche, Inc., for the supply of 2,6-dichlorophenol indophenol.
BIBLIOGRAPHY

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