THE EFFECT OF DESICCATED THYROID, \(\alpha\)-DINITROPHENOL, AND CORTICAL HORMONE EXTRACT ON THE VITAMIN C CONTENT OF SOME ORGANS OF THE GUINEA PIG FED GRADED DOSES OF ASCORBIC ACID*

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Certain organs and glands of animals (whether susceptible to scurvy or not) have been shown by biological tests and reduction with the indophenol indicator to contain ascorbic acid (vitamin C) in higher concentration than most of the other tissues. Thus, the anterior lobe of the pituitary, corpus luteum, thymus, suprarenals, and, to a lesser extent the liver, are rich in the vitamin. The ascorbic acid content (1, 2) as well as the oxygen consumption (3) of organs decreased on the onset of scurvy. A close relationship has long been evident in vitamin C and the respiratory as well as the metabolic rate of the different organs. Localized concentration of vitamin C in the suprarenals has been ascribed for the maintenance of the normal functional activities of that organ (4).

It has been definitely established that the metabolic rate of organs is increased by administration of the thyroid hormone and by \(\alpha\)-dinitrophenol. The specific effect of the thyroid hormone on the calcium metabolism (5), as well as an increase in weight of the thyroid gland (6) in scorbutic guinea pigs, has been described.

The purpose of this investigation was to clear up the disputed relationship between the cortical hormone and vitamin C as well as to observe the effect of desiccated thyroid and \(\alpha\)-dinitrophenol on guinea pigs fed different amounts of vitamin C.

* The greater part of the material upon which this paper is based was presented before the meeting of the American Chemical Society at New York, April, 1935.
EXPERIMENTAL

Ascorbic acid, prepared from Hungarian red pepper in the fall of 1932, was purified by the methyl alcohol-dioxane method (7) in the fall of 1934. No attempt had been made at the time of preparation to purify this batch of crystals. The original product had been sealed in a glass tube in vacuo. After purification, the well formed white crystals were kept in vacuo in a desiccator over sulfuric acid. About twice a week a solution of the vitamin in ordinary distilled water was placed in vials, which were saturated with carbon dioxide gas, stoppered, and kept in an ice box till ready for use. The solution was fed daily to guinea pigs per os by means of a graduated dropper.

2,4-Dinitrophenol (c. r.) dissolved in distilled water was likewise given to the animals per os.

Cortical hormone extract\(^1\) was injected subcutaneously daily. The amount injected in the normal guinea pig should have been adequate (based on the assay) to prevent suprarenal deficiency in adrenalectomized animals.

Young guinea pigs about 300 gm. in weight were employed throughout the experiment. The technique as to the preliminary care of the animals, diet, weighing, scoring, etc., described in the Sherman and Smith monograph, was followed (8). 1 cc. of cod liver oil was given to each animal every week. The thyroid diet (0.5 per cent) contained 3 gm. of desiccated thyroid gland (Parke, Davis and Company) per 600 gm. of the basal diet.

At definite intervals, animals were etherized and the degree of scurvy estimated. The organs were immediately removed, dried between sheets of filter paper, weighed, minced with sand, and extracted with a sufficient amount of 4 per cent trichloroacetic acid. The filtered solution, after the residue was pressed, was then titrated rapidly with 2,6-dibromophenol indophenol blue to the first permanent trace of pink color. The indicator solution had previously been standardized against a known weight of pure ascorbic acid so that the amount of vitamin C in the tissues could easily be calculated (allowance being made for the blank in every case). This direct titration method, extensively used at the present time, agrees reasonably well with animal assay work.

\(^1\) I am greatly indebted to Dr. E. C. Kendall, Department of Biochemistry, Mayo Clinic, Rochester, Minnesota, for a generous supply of the extract and for the assay.
<table>
<thead>
<tr>
<th>Daily dose</th>
<th>Gland eaten daily</th>
<th>Average survival</th>
<th>Average gain in weight</th>
<th>Vitamin C content of organs</th>
<th>Weight of adrenals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. days gm.</td>
<td></td>
<td></td>
<td>Adrenals</td>
<td>Liver</td>
</tr>
<tr>
<td>Basal diet</td>
<td></td>
<td></td>
<td></td>
<td>mg. per organ</td>
<td>mg. per organ</td>
</tr>
<tr>
<td>Diet only</td>
<td>30</td>
<td>-133 18</td>
<td>0.006 0.160 0.016</td>
<td>0.02 0.012 0.007</td>
<td>353, 565</td>
</tr>
<tr>
<td>0.2 cc. cortin</td>
<td>31†</td>
<td>-84 20</td>
<td>0.015 0.183 0.007</td>
<td>0.04 0.009 0.006</td>
<td>385, 465</td>
</tr>
<tr>
<td>0.2 &quot; &quot; + 0.25 mg. ascorbic acid</td>
<td>32†</td>
<td>113 20</td>
<td>0.015 0.122 0.008</td>
<td>0.04 0.007 0.006</td>
<td>265, 320, 410</td>
</tr>
<tr>
<td>0.25 mg. ascorbic acid</td>
<td>52†</td>
<td>11 6</td>
<td>0.02 0.136 0.012</td>
<td>0.05 0.006 0.01</td>
<td>390, 448</td>
</tr>
<tr>
<td>5 &quot; &quot; &quot; + α-dinitrophenol</td>
<td>53†</td>
<td>252 0</td>
<td>0.084 0.315 0.08</td>
<td>0.20 0.011 0.06</td>
<td>365, 400, 480</td>
</tr>
<tr>
<td>Diet only</td>
<td>31</td>
<td>-133 20</td>
<td>0.01 0.10</td>
<td>0.04 0.006</td>
<td>425, 460, 503</td>
</tr>
<tr>
<td>0.5 mg ascorbic acid</td>
<td>28†</td>
<td>106 0</td>
<td>0.04 0.176 0.04</td>
<td>0.23 0.008 0.007</td>
<td>181</td>
</tr>
<tr>
<td>2 &quot; &quot; &quot;</td>
<td>21†</td>
<td>17 0</td>
<td>0.056 0.30</td>
<td>0.28 0.025</td>
<td>173, 210</td>
</tr>
<tr>
<td>5 &quot; &quot; &quot;</td>
<td>29†</td>
<td>168 0</td>
<td>0.095 0.76 0.127</td>
<td>0.47 0.035 0.15</td>
<td>222, 280</td>
</tr>
<tr>
<td>10 &quot; &quot; &quot;</td>
<td>29†</td>
<td>205 0</td>
<td>0.161 0.97 0.11</td>
<td>0.66 0.045 0.12</td>
<td>242, 245</td>
</tr>
<tr>
<td>20 &quot; &quot; &quot;</td>
<td>24†</td>
<td>94 0</td>
<td>0.112 1.28 0.13</td>
<td>0.61 0.08 0.14</td>
<td>183</td>
</tr>
<tr>
<td>Thyroid diet (0.5%)</td>
<td>80</td>
<td>-130 18</td>
<td>0.019 0.118</td>
<td>0.07 0.012</td>
<td>207, 312, 345</td>
</tr>
<tr>
<td>5 mg. ascorbic acid</td>
<td>120</td>
<td>-94 2(?)</td>
<td>0.085 0.352 0.04</td>
<td>0.17 0.03 0.11</td>
<td>382, 471, 618</td>
</tr>
<tr>
<td>10 &quot; &quot; &quot;</td>
<td>130</td>
<td>-85 2(?)</td>
<td>0.242 0.592 0.055</td>
<td>0.41 0.045 0.11</td>
<td>465, 673</td>
</tr>
<tr>
<td>20 &quot; &quot; &quot;</td>
<td>90</td>
<td>24† -86 1(?)</td>
<td>0.176 0.88 0.08</td>
<td>0.44 0.07 0.13</td>
<td>406</td>
</tr>
</tbody>
</table>

* Highest possible score 24.
† Etherized.
The results of the experiments are recorded in Table I and Fig. 1.

**Vitamin C and Cortical Hormone**—As evident from the data in Table I and the weight curves in Fig. 1, cortical hormone per se has no influence on the survival period of guinea pigs on a scorbutic diet, thus confirming previous work (9, 10). Loss in weight and failure of appetite in scorbutic guinea pigs occur generally after the 15th day. The survival period (usually ranging from 20 to 48 days) and degree of scurvy depend on a variety of factors, such as infection, fasting, the animal itself, etc. At necropsy, gastrointestinal hemorrhages are not always observed, though the other symptoms of scurvy are present to a marked degree.

The minimum protective dose of pure ascorbic acid necessary to insure protection against scurvy in guinea pigs is considered to be at least 0.5 mg. daily per os. This amount may, however, be
inadequate if the tooth structure method of assay is employed. If less than this minimum protective dose is given, a certain degree of scurvy is to be expected. Thus, in a 55 day test period, a fair degree of scurvy with little change in weight has been recorded by giving 0.25 mg. of fairly pure ascorbic acid per os (11). Similar results were obtained with 0.3 mg. (2). A marked difference in survival and scurbutic symptoms was obtained by Grollman and Firor (9) when 0.25 mg. of pure ascorbic acid was given per os or by interperitoneal injection to guinea pigs. It is chiefly to this wide variation in response by the different modes of administration that the reported beneficial results of Lockwood and Hartman (12) have been explained as due to the presence of vitamin C in the injected cortical hormone extracts. It is hardly conceivable, however, that sufficient ascorbic acid can be present in such extracts to account for this explanation in view of the solvents employed. The cortical hormone extract used in the present series of tests was devoid of ascorbic acid since 1 cc. of the extract, on titration with 2,6-dibromophenol indophenol blue before and after treatment with H₂S for 1 hour (followed by removal of the gas), gave the same titration value as with ordinary distilled water.

Certain limitations should, however, be recognized in the evidence presented by Lockwood and Hartman to describe the marked influence of cortical hormone extract in delaying the onset of scurvy symptoms. The slight differences in scurvy score with little change in weight in the 28 day test rather than a more extended period can be attributed to the individual variations of the animals. A greater degree of protection against scurvy may have been expected as regards the amount of orange juice fed (0.7 cc. of orange juice contains about 0.42 mg. of ascorbic acid).

The data presented in this paper indicate clearly that guinea pigs receiving 0.25 mg. of ascorbic acid (half the minimum protective dose) compared in every respect with those receiving the cortical hormone extract in addition to the same amount of vitamin C. Delay in the onset of scurvy symptoms was not observed. The vitamin C contents of the organs together with the usual data support this view. The slower drop in weight of the cortin-injected animals during the early part of the test is also indicated in the charts of those working on this subject (9, 12).

It is likewise evident from these tests that vitamin C in adequate
amount is essential for the proper utilization of the cortical hormone, whether elaborated by the intact gland or supplied as the extract by injection. Vitamin C ordinarily disappears at a marked rate from the suprarenals of guinea pigs (7), though traces may still be present in organs of animals dying of scurvy (4). Marked histological changes in the cortical portion of the gland (13), as well as hypertrophy of the suprarenals in scurvy (14), have been described. Since the functions of the suprarenal are markedly disarranged in scurvy, it may be expected that a brief temporary beneficial effect may be obtained if cortin be injected with less than the required amount of vitamin C. Cortin has been shown in curative tests (10) to be of no influence in scorbutic guinea pigs. In the case of suprarenalectomized dogs (which synthesize their own vitamin C), lack of sufficient cortical hormone is responsible for weakness, and loss in weight and appetite, while gastrointestinal hemorrhages as well as contracted bladder are characteristic symptoms at necropsy. It is not improbable, therefore, that guinea pigs dying of scurvy may also have acute suprarenal deficiency to complicate the clinical picture.

Effect of Thyroid Diet and \(\alpha\)-Dinitrophenol—The beading of the costochondral rib junctions is a familiar observation at necropsy of scorbutic animals. Evidence of this beading as well as a slight fragility of the hind leg bones was noticed on the 5th day of the thyroid diet. These symptoms are not present in guinea pigs fed the usual diet in the same time interval. This beading of rib junctions was not observed in the thyroid-fed guinea pigs when 2 mg. or more of vitamin C were given daily for a period of 30 days. The leg bones, though slightly brittle even when vitamin C was fed, did not appear to be of the same mushy type commonly found in scorbutic animals, but were nevertheless designated in Table I with (?) since no other signs of scurvy were noted. The influence of the thyroid hormone in scorbutic animals, especially after the functions of the suprarenals have been disarranged, should possibly be kept in mind in the usual method of scoring.

The amount of dried thyroid gland consumed by the animals was unusually large and the effects rather drastic. Under these conditions, however, more pronounced results are obtainable. The general vitality and appearance of the guinea pigs were more marked as the amount of ascorbic acid was increased. Too large
a consumption of the thyroid diet may lead to death. Kendall et al. (15) have definitely shown that more cortical hormone is required for suprarenalectomized dogs if thyroxine is also administered.

The data in Table I present some striking results. The ascorbic acid per gm. of tissue depends on the amount of vitamin C fed. The increase was more pronounced in the suprarenals and liver, with that of the spleen fairly constant. Appreciably lower values were obtained on the thyroid diet, which indicates that as the metabolic rate of the organs is increased more vitamin C is required. The increase in size and weight of the suprarenals of animals on the thyroid diet together with the ascorbic acid content of the gland when compared to the controls is evident.

α-Dinitrophenol has no influence on the survival period in scurvy. The amount fed to the animals (4 mg. per kilo of body weight) was of the same magnitude as used by others working with this substance. The ascorbic acid content of the liver and spleen was markedly low on the 5 mg. vitamin C level.

**SUMMARY**

Ascorbic acid, kept in vacuo for 2 years, does not lose its antiscorbutic potency.

Ascorbic acid in adequate amount is necessary for the proper utilization of the cortical hormone. Cortical hormone extract does not prolong the survival period of guinea pigs on a scorbutic diet. The extract as ordinarily prepared after treatment with ethyl ether and other solvents contains no significant amounts of ascorbic acid.

Large amounts of ascorbic acid promote the general vitality and appearance of guinea pigs on a thyroid diet. The vitamin C content of organs depends on the amount of ascorbic acid fed and decreases as the metabolic rate of the tissues is increased.

α-Dinitrophenol has no influence on the survival period in scor-

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2 One guinea pig on the 20 mg. of ascorbic acid dose, after eating about 125 mg. of dried thyroid gland daily, was found dead on the 19th day. Marked gastrointestinal hemorrhages and contracted bladder were observed at necropsy. The ascorbic acid content of the organs was high. Death in such instances should possibly be ascribed to suprarenal deficiency.
butic guinea pigs. It apparently has no harmful effects when 1 mg. per kilo of body weight are employed.

**BIBLIOGRAPHY**

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