EFFECT OF ASCORBIC ACID DEFICIENCY ON COLLAGEN CONTENT OF GUINEA PIG TISSUES*

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In their treatise on human scurvy (1), Aschoff and Koch suggested that the primary abnormality of this disease was the inability of the organism to produce intercellular substances. Wolbach and his collaborators (2–4) came to the same conclusion as the result of experiments performed in guinea pigs. Although they stated (5) that the scorbutic state was characterized by the inability of the guinea pigs to form and maintain intercellular materials, the evidence dealt mostly with the relation of vitamin C to the formation of intercellular substances. Many investigators have directed their attention to this aspect of connective tissue physiology and the experimental evidence is conclusive that, in the absence of ascorbic acid, there is a marked deficiency in the formation of connective tissue and its components.

Little has been written as to the fate of the preformed connective tissue in the vitamin C-deficient state. Höjer (6) wrote that in the guinea pig "there is general atrophy in scurvy of the connective tissue, and especially of its collagen substance." Aschoff and Koch described an inability to maintain established supporting structures in the human. When scurvy supervened on a recently healed wound, the newly formed collagen of the scar reverted to "precollagen;" yet the fibrous tissue bordering the incision remained normal (7).

Controversy still exists concerning the manner in which connective tissue is maintained in the body. Two main theories have been proposed: One view states that there is a dynamic equilibrium in which formation and destruction of the connective tissue occur simultaneously and continuously. The other theory maintains that there is constant anabolism of connective tissue unaccompanied by any appreciable breakdown. It is generally agreed that, in the absence of ascorbic acid, the anabolic processes are inhibited. Whether catabolism proceeds at its normal pace or whether it is accelerated in the absence of vitamin C has not been established. Ham and Elliott (8) proposed that in scurvy there was failure to...
form new connective tissue, rather than acceleration of the destructive phase. In order to establish clearly that vitamin C is essential for the maintenance of preformed collagen, it must be demonstrated that in its absence catabolism of the collagen is accelerated.

A chemical method is available for the quantitative estimation of collagen in tissues (9). It has been used to measure the collagen content of liver (9–14), heart and muscle (9, 15–18), aorta (19), kidney (9, 12), and spleen (9) of different species. This study was undertaken to establish the fate of the preformed collagen in the tissues of guinea pigs placed on an ascorbic acid deficient diet.

**Materials and Methods**

Thirty male, weanling guinea pigs were used as experimental animals. They were divided equally into the following groups.

**Group 1**—These animals received a diet consisting of ground Derwood rabbit chow that had been exposed to the air for at least 24 hours, supplemented with 5 percent ground dried yeast. Weekly, 0.2 cc. of wheat germ oil and 0.8 cc. of cod liver oil were fed orally. Water was given *ad libitum*. At the start of the experiment these animals weighed between 160 and 200 gm., with a mean weight of 185 gm. They were maintained on this diet for 24 to 38 days, at the end of which time they were sacrificed.

**Group 2. Age Controls for Group 1**—These guinea pigs received the same diet as Group 1, and, in addition, vitamin C was administered in the form of fresh greens daily. They were of equal body weight to Group 1 at the start of the experiment and were kept on the diet for 24 to 38 days.

**Group 3. Weight Controls for Group 1**—These animals received the same diet as Group 2 and were selected so that their body weights at the termination of the experiment were the same as those of Group 1.

The animals were sacrificed with a blow to the back of the skull. Their bodies were weighed and dissected and the organs were removed in the following manner and order.

**Skeletal Muscle**—A sample of approximately 2 gm. of adductor muscle of the leg was freed of fat, fascia, and tendon. In the animals in which the muscle of one leg was not sufficient, an additional sample was taken from the opposite side.

**Heart**—The heart was removed from the body and the pericardium was stripped away. The great vessels were severed at their bases. The cardiac chambers were opened and the blood was removed.

**Lungs**—The lungs were divested of the mediastinal structures by severance at their hila. The pulmonary vessels were allowed to drain free of blood.

**Liver**—The diaphragm, portal structures, and gallbladder were separated
from the liver; the hepatic blood was drained and the remaining organ was weighed to the nearest 0.1 gm.

Kidneys—The capsules were stripped away from the surface of the kidneys and the renal vessels and ureters were severed at the uretero-pelvic junction. The kidneys were sectioned longitudinally and the blood was wiped away.

Spleen—The spleen was removed, free of its vessels, fat, and mesentery. Quantitative chemical determinations of the collagen content of the various tissues were made (9). In each instance the entire organ was used for the measurement, except for the skeletal muscle and liver of which 2 to 4 gm. of samples were used. The tissues were utilized within 1 hour after the animals were sacrificed. Rarely, the tissues were stored in sealed containers for no longer than 24 hours at 0° prior to use.

**Table I**

<table>
<thead>
<tr>
<th>Group No. (10 guinea pigs each)</th>
<th>Body weight</th>
<th>Lungs</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Weight</td>
<td>Collagen, wet weight</td>
</tr>
<tr>
<td></td>
<td>gm*</td>
<td>gm*</td>
<td>per cent*</td>
</tr>
<tr>
<td>1. Scorbutic</td>
<td>176</td>
<td>1.66</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>±13</td>
<td>±0.10</td>
<td>±0.05</td>
</tr>
<tr>
<td>2. Age controls</td>
<td>358</td>
<td>2.13</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>±14†</td>
<td>±0.07†</td>
<td>±0.09†</td>
</tr>
<tr>
<td>3. Weight controls</td>
<td>173</td>
<td>1.51</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>±11</td>
<td>±0.09</td>
<td>±0.13</td>
</tr>
</tbody>
</table>

* Includes standard error of the mean.
† Indicates significant difference (P ≤ 0.01) from the mean of the scorbutic group.

Results

**Progress of Vitamin C Deficiency**—The animals on the scorbutogenic diet (Group 1) gained weight during the first 2 weeks of the experiment and appeared healthy and active. In the 3rd and successive weeks, their weights became stabilized and then declined, so that at the termination of the experiment the mean weight of this group was 176 gm., with a range of 130 to 255 gm. The animals were listless and apathetic; gingival hemorrhages, subcutaneous ecchymoses, and swelling and reddening of the joints of the extremities were present. Examination of the tissues revealed hemorrhages in the skeletal muscles and periarticular soft tissues. The animals of Group 2 gained weight normally and appeared healthy. They
were sacrificed at the same time as the scorbutic animals and weighed be-
tween 300 and 440 gm., with a mean of 358 gm. They displayed none of
the stigmata of the scorbutic state. The animals of Group 3 were selected
from a normal population so that they were equivalent in weight to the

**TABLE II**

Mean Collagen Content of Kidneys and Spleen

<table>
<thead>
<tr>
<th>Group No. (10 guinea pigs each)</th>
<th>Body weight</th>
<th>Kidneys</th>
<th></th>
<th></th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm.*</td>
<td>gm.*</td>
<td>per cent*</td>
<td>mg.*</td>
<td>gm.*</td>
</tr>
<tr>
<td>1. Scorbutic</td>
<td>176</td>
<td>2.81</td>
<td>0.41</td>
<td>11.5</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>±13</td>
<td>±0.15</td>
<td>±0.03</td>
<td>±0.8</td>
<td>±0.05</td>
</tr>
<tr>
<td>2. Age controls</td>
<td>358</td>
<td>3.18</td>
<td>0.66</td>
<td>21.0</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>±14†</td>
<td>±0.15</td>
<td>±0.07†</td>
<td>±3.1†</td>
<td>±0.08</td>
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<tr>
<td>3. Weight controls</td>
<td>173</td>
<td>1.98</td>
<td>0.41</td>
<td>8.2</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>±11</td>
<td>±0.11†</td>
<td>±0.02</td>
<td>±0.9</td>
<td>±0.03</td>
</tr>
</tbody>
</table>

* Indicates significant difference (P ≤ 0.01) from the mean of the scorbutic group.

**TABLE III**

Mean Collagen Content of Heart and Skeletal Muscle

<table>
<thead>
<tr>
<th>Group No. (10 guinea pigs each)</th>
<th>Body weight</th>
<th>Heart</th>
<th></th>
<th></th>
<th>Skeletal muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm.*</td>
<td>gm.*</td>
<td>per cent*</td>
<td>mg.*</td>
<td>per cent*</td>
</tr>
<tr>
<td>1. Scorbutic</td>
<td>176</td>
<td>0.61</td>
<td>1.39</td>
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<td>1.87</td>
</tr>
<tr>
<td></td>
<td>±13</td>
<td>±0.04</td>
<td>±0.17</td>
<td>±0.40</td>
<td>±0.23</td>
</tr>
<tr>
<td>2. Age controls</td>
<td>358</td>
<td>1.00</td>
<td>1.05</td>
<td>10.50</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>±14†</td>
<td>±0.04†</td>
<td>±0.06</td>
<td>±0.90</td>
<td>±0.21†</td>
</tr>
<tr>
<td>3. Weight controls</td>
<td>173</td>
<td>0.55</td>
<td>0.82</td>
<td>4.51</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>±11</td>
<td>±0.03</td>
<td>±0.07†</td>
<td>±0.51†</td>
<td>±0.06†</td>
</tr>
</tbody>
</table>

* Indicates significant difference (P ≤ 0.01) from the mean of the scorbutic group.

**Chemical Data**—The data giving the weights of the various organs, their
collagen content, and the total amount of collagen in each organ are listed
in Tables I to III.

**Lungs**—The lungs of the ascorbic acid-deficient animals (Group 1) were
significantly smaller and contained less collagen than the lungs of the age controls (Group 2). However, no significant differences of these values from those of the weight controls (Group 3) were observed.

Liver—Significant diminution of the weight and total collagen content of the livers of the deficient animals from that of the age controls was recorded. These values were not different from those of the weight controls.

Kidneys—The kidneys of the scorbutic animals were of approximately the same weight as the comparable organs of their age controls, but were heavier than the kidneys of the weight controls. However, the mean percentage and total collagen content of the kidneys of Group 1 resembled the values of the weight controls and were significantly less than those of the age controls.

Spleen—Very small amounts of collagen (not greater than 3.5 mg.) were present in the spleens of the different groups. No significant differences between the groups could be distinguished.

Heart—The hearts of the scorbutic animals were similar in weight to those of the weight controls and were less than the age controls. The percentage and total collagen content of the hearts resembled those of age controls and were significantly greater than in the weight controls.

Skeletal Muscle—There was marked loss of muscle tissue in the scorbutic animals. The relative amount of collagen (per cent) was markedly increased in the experimental group over either of the control groups.

DISCUSSION

In the development of the ascorbic acid-deficient state the guinea pigs underwent marked changes of nutrition. The appetite of the animals diminished sharply and the weight loss that ensued was severe. During the course of these experiments, the animals of Group 1 at first gained and then lost weight, and at the termination of the experiment they were of the same weight approximately as at the start, 3 to 5 weeks previously. It has been shown that organ weights increase with age in the guinea pig (20) and that variations of the collagen content of these organs are to be expected at different age and weight levels. Therefore, in the interpretation of the results obtained from scorbutic animals, the effects of weight loss and vitamin deficiency alone had to be distinguished. The proper selection of control groups helped to eliminate this factor. By the selection of animals of equal age which were allowed to develop normally, a comparison could be made in which both weight loss and vitamin deficiency played a part. The second group of controls, normal animals of

1 Elster, S. K., and Lowry, E. L., to be published.
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the same weight, allowed a comparison in which vitamin deficiency alone played the major rôle. This technique has been utilized previously in the study of capillary permeability in scurvy (21).

In none of the tissues examined was less collagen found in the scorbutic animals than in both of the control groups. For the most part, the organs resembled in weight and collagen composition the corresponding organs of the weight controls and were less than the age controls. Some exceptions may be cited in that the kidneys grew normally, whereas the other organs remained small. No explanation for this phenomenon is offered. In the cardiac and skeletal muscular tissues, the relative amount of collagen was increased. Although it was not possible to weigh the entire muscle mass of the body, it was obvious that the bulk of the weight loss in the animals was due to the loss of muscle substance, a condition that was described previously (22). The relative increase of the connective tissue was due to accretion rather than to an increase of the collagen. The collagen content of the heart of the scorbutic animals resembled that of the age controls rather than of the weight controls. Since the heart specimens contained valves, chordae tendineae, and auricles, structures composed of large amounts of dense, compact collagen, one may think of these sites as containing relatively “stable” collagen. This is in contrast to the state of the connective tissue of the other organs in which the collagen is deposited in more delicate fibers and is, therefore, more susceptible to resorption.

From these studies, it can be seen that in no organ has there been greater loss of collagen than of other tissue constituents during the course of ascorbic acid deficiency. In muscular tissue, rather, the collagen was less sensitive to destruction than the muscle itself. That ascorbic acid is essential for the formation of collagen in vivo cannot be disputed. Once formed, the fiber seems to be independent of further vitamin C nutrition, except in so far as it is destroyed by the usual catabolic actions of the body and requires resynthesis. In the true sense, therefore, ascorbic acid may not be essential for the maintenance of preformed connective tissue.

The rôle of ascorbic acid in collagen formation has not been well elucidated. Meyer (23) has suggested that vitamin C may be incorporated into chondroitinsulfuric acid which may then be used in collagen synthesis. Penney and Balfour (24) have shown that hyaluronic acid, a component of connective tissue ground substance, was not formed in the scorbutic state in the guinea pig. Ascorbic acid has been demonstrated to decrease the viscosity of collagen in vitro in the presence of hydrogen peroxide (25). Studies (26, 27) have yielded conflicting results as to the rôle of vitamin C in fiber formation in vitro. The adrenal cortical hormones have become of increasing interest and importance in connective tissue physiology. Ragan and his collaborators (28) have recently demonstrated that corti-
sone inhibited the formation of granulation tissue, a condition also seen in the ascorbic acid-deficient state. The relation between these two facts is a fertile field for further investigation.

SUMMARY

Male, weanling, guinea pigs were made scorbutic and chemical measurements of the collagen content of the lungs, liver, kidneys, spleen, heart, and skeletal muscles were made. Comparable determinations were performed in normal age and weight controls.

There was a significant decrease of the collagen content of the lungs, liver, and kidneys as compared with age controls, but no significant difference from normal animals of the same weight. A relative (per cent) increase of the collagen content of the heart and skeletal muscle was noted. The total collagen content of the heart was not significantly different from that of the age controls, but was greater than in the weight controls. The collagen content of the spleen was unaffected.

In no instance was less collagen found in the tissues of scorbutic guinea pigs than in normal animals of the same weight.

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BIBLIOGRAPHY

2. Wolbach, S. B., and Howe, P. R., Arch. Path. and Lab. Med., 1, 1 (1926).
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