THE EFFECT OF ASCORBIC ACID DEFICIENCY ON THE COLLAGEN CONCENTRATION OF NEWLY INDUCED FIBROUS TISSUE*

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Recent chemical studies (1, 2) have shown that in neither acute nor chronic scurvy is there a diminution of the concentration or of the total amount of collagen in the formed tissues of guinea pigs. This result is in conflict with the broad generalization that ascorbic acid is necessary for the maintenance of collagen (3). It is, however, consistent with the results of most studies of experimental scurvy which have revealed the necessity for vitamin C during formation of collagen (4, 5) but which in general have presented no clear cut evidence concerning the effect of a deficiency of the vitamin on preformed collagen. Hunt (6), however, has presented evidence which suggested to him that newly induced collagen might require ascorbic acid for its maintenance. He observed that the collagen present in a recently healed wound reverted to precollagen when the animal became scorbutic. He also cited instances from the older literature in which scars of long healed wounds were reported to have broken down, presumably due to scurvy. The question thus arose whether induced new collagen, in contrast to the collagen of normal tissues, required ascorbic acid for its maintenance. This paper presents a study of collagen concentration in new fibrous tissue formations as represented by healed skin wounds, by new subcutaneous fibrous tissue, and by new perirenal fibrous tissue and the effect thereon of scurvy.

EXPERIMENTAL

Procedure and Results

The general procedure was to induce new tissue formation in 300 to 400 gm. guinea pigs by one of the several methods described below. The new fibrous tissue was permitted to develop normally for about 14 to 28 days, during which time the animals were maintained on a stock guinea pig ration supplemented with 50 mg. of ascorbic acid per os every other day. At the end of this period of tissue formation the guinea pigs were separated into three groups. The animals of Group 1 were killed and collagen deter-

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mined immediately; those of the other two groups were placed on Mac-
donald's scorbutigenic Diet 5 (7).¹ One of these latter groups (Group 2)
was allowed to develop scurvy until moribund, at which time the animals
were killed;² the other group (No. 3) was fed 50 mg. of ascorbic acid every
other day during this period and the animals were killed at about the same
time as the scorbutic animals.

The collagen concentration in the new fibrous tissue formations of the
animals of all three groups was determined by the method of Lowry et al.
(8), with modifications used in previous experiments (2). An aliquot of
the entire healed area was used for these analyses. A piece of the new
tissue and a costochondral junction were fixed for histological examina-
tion.³

Wounds—Since evidence for the breakdown of collagen of healed
wounds during scurvy has been presented by Hunt (6), similar material
was used in the present study. However, in order to have adequate ma-
terial for chemical analysis it was necessary to make the wounds more
extensive than in his experiments. A piece of skin about 25 mm. in di-
амeter, together with all underlying tissue down to the muscle layer, was
cut from one flank. Sulfadiazine was sprinkled into the wound and the
edge was marked with a tattoo. The wounds healed over in 3 to 4 weeks,
although occasionally a small scab-crusted area remained; at this time the
guinea pigs were separated into the three groups described above.

Healing, as indicated by epibhelization of the scar tissue and hair growth,
continued in the group on the scorbutigenic diet (Group 2), as well as
in the control group (Group 3). In no case was there ulceration of
the wounds. At the end of the experiment (47 to 63 days) only the presence
of the tattoo permitted demarcation between old and new tissue. As
may be seen in Table I, the collagen content of the wounds of the animals
in Group 2, which had developed acute scurvy, was in no case lower than
that of comparable wounds analyzed before the guinea pigs were placed
on a scorbutigenic régime (Group 1). Deposition of new collagen con-
tinued after the wounds healed over, as indicated by the collagen concen-
tration of Groups 2 and 3, which was higher than that of Group 1. The
collagen concentration in the scorbutic guinea pigs (Group 2) was 2 per
cent less than that of animals maintained with ascorbic acid for the same
period (Group 3), a small difference which reflects a decreased collagen
formation in the wounds as a result of scurvy (4). It appears from these

¹ Dr. E. W. Crampton of Macdonald College, Quebec, very kindly made available
an adequate supply of this diet.
² The diagnosis of acute scurvy was confirmed by gross and microscopic exami-
nation.
³ We are indebted to Mr. John Boldosser of the Department of Pathology and
Oncology for histologic preparations.
data that ascorbic acid was not needed to maintain the collagen which had formed during the period of normal healing.

Subcutaneous Fibrous Tissue—Many substances, when introduced into the organism, provoke a foreign body reaction, with subsequent formation of fibrous tissue. Of several materials tried, two proved suitable for the present study. These were dicetyl phosphate (9) and an Irish moss extractive, either of which, when injected subcutaneously as a 1 per cent suspension, induced formation of new tissue. After the new tissue had developed normally for 14 days, the guinea pigs were separated into groups as previously described. The results of collagen assays of this new tissue, as given in Table II, show that no decrease in collagen concentration occurred as a result of scurvy. However, in these experiments in which an irritant was the stimulus to collagen formation, as in the wound experiments, further formation of new collagen appeared somewhat inhibited in the scurbitic animals. During the experimental period the new tissue was slowly resorbed and so after 40 to 50 days less material was available for analysis than at 14 days. This occurred irrespective of whether ascorbic acid was administered or not.

Fibrous Tissue of Renal Capsule—Page (10) has described the use of cellophane to produce a perinephritis with resultant formation of a relatively thick, fibrous, renal capsule. This technique was applied to guinea pigs, but because of the high mortality when both kidneys were wrapped in cellophane most of the data to be reported were based on experiments in which only the left kidney was so treated. (Polythene sheeting was

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**Table I**

**Effect of Scurvy on Collagen Concentration of Healed Skin Wounds**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of guinea pigs</th>
<th>Days on adequate diet</th>
<th>Days on scorbutigenic diet</th>
<th>Collagen concentration, per cent of fresh tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>23 (21-23)</td>
<td></td>
<td>10.6 ± 0.52</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>26 (21-28)</td>
<td>34 (24-56)†</td>
<td>17.6 ± 0.78</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>60 (57-63)</td>
<td></td>
<td>19.6 ± 0.36</td>
</tr>
</tbody>
</table>

* These data are presented as the mean plus or minus its standard deviation.
† Skin wounds originally 25 mm. in diameter were permitted to heal for 21 to 28 days before placing the guinea pigs on a scorbutigenic diet.
used in place of cellophane in about half of the experiments with the same results.) The thickened capsule which formed could be readily separated from the kidney and weighed from 150 to 600 mg. If the capsule weighed more than 400 mg., it was individually assayed for collagen, but, if it weighed less than this, two capsules were pooled for analysis.

In addition to the thickening of the renal capsule a layer of fibrous tissue formed about the outside of the plastic wrapping. The collagen in this tissue, referred to as “capsule about plastic,” was also determined.

The results of collagen determinations on these tissues (Table III) are in striking contrast to those of the previous experiments, for here we ob-

### Effect of Scurvy on Collagen Concentration of New Subcutaneous Fibrous Tissue

<table>
<thead>
<tr>
<th>Material injected</th>
<th>Group No.</th>
<th>No. of guinea pigs</th>
<th>Days on adequate diet</th>
<th>Days on scorbutigenic diet</th>
<th>Collagen concentration, per cent of fresh tissue</th>
<th>Approximate amount of new tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irish moss extract</td>
<td>1</td>
<td>10</td>
<td>14</td>
<td>27 (24-30)†</td>
<td>1.57 ± 0.13</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>14</td>
<td></td>
<td>1.84 ± 0.22</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9</td>
<td>45 (38-47)</td>
<td></td>
<td>2.13 ± 0.13</td>
<td>2.6</td>
</tr>
<tr>
<td>Dicetyl phosphate</td>
<td>1</td>
<td>8</td>
<td>14</td>
<td>25 (22-33)†</td>
<td>1.71 ± 0.10</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12</td>
<td>14</td>
<td></td>
<td>2.01 ± 0.17</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12</td>
<td>45 (28-56)</td>
<td></td>
<td>2.50 ± 0.21</td>
<td>3.5</td>
</tr>
</tbody>
</table>

* These data are presented as the mean plus or minus its standard deviation.
† New tissue, formed in response to the injection of a 1 per cent suspension of Irish moss extract or of dicetyl phosphate, was permitted to develop for 14 days before placing the guinea pigs on a scorbutogenic diet.

### Effect of Scurvy on Collagen Concentration of New Fibrous Tissue Induced by Wrapping Kidney in Plastic

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Group No.</th>
<th>No. of analyses</th>
<th>Days on adequate diet</th>
<th>Days on scorbutigenic diet</th>
<th>Collagen concentration, per cent of fresh tissue</th>
<th>Approximate weight of capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perirenal capsule</td>
<td>1</td>
<td>8</td>
<td>17 (11-21)</td>
<td>26 (16-29)†</td>
<td>2.28 ± 0.21</td>
<td>280</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>14</td>
<td></td>
<td>1.03 ± 0.41†</td>
<td>360</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>45 (35-52)</td>
<td></td>
<td>2.11 ± 0.16</td>
<td>390</td>
</tr>
<tr>
<td>Capsule about plastic</td>
<td>1</td>
<td>6</td>
<td>16 (11-21)</td>
<td>25 (16-29)†</td>
<td>2.70 ± 0.22</td>
<td>280</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>14</td>
<td></td>
<td>1.61 ± 0.23†</td>
<td>360</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>45 (35-52)</td>
<td></td>
<td>2.65 ± 0.12</td>
<td>390</td>
</tr>
</tbody>
</table>

* These data are expressed as the mean plus or minus its standard deviation.
† Fibrous tissue capsules were allowed to develop for 14 days before placing the guinea pigs on a scorbutogenic diet.
‡ This mean is significantly different from the mean of Group 1 ($P < 0.05$).
serve that the collagen concentration of the new tissue in the scorbutic
guinea pigs was lower than in the normal animal. The small amount of
capsule available decreased the reliability of these analyses. However,
evaluation of histologic sections stained for collagen was confirmatory, for
the capsular tissue from scorbutic animals appeared, in general, to have
considerably less collagen than the normal. A possible interpretation of
this apparent difference in behavior of the collagen of various tissues in
ascorbic acid deficiency will be discussed below.

DISCUSSION

Is ascorbic acid required for the maintenance of formed collagen? Pre-
vious studies (1, 2) showed clearly that scurvy resulted in little or no
breakdown of organ collagen and the present data suggest a similar pat-
tern for the collagen of new fibrous tissues. The conclusion in the case
of the latter tissues must, however, be somewhat equivocal, for, although
the collagen concentration of neither healed wounds nor of new subcuta-
neous tissue decreased in acute scurvy, that of the fibrous tissue capsules
formed in response to wrapping the kidney in plastic was significantly
lowered. While these data might suggest that collagens from different
tissues are dissimilar with respect to the requirement of ascorbic acid for
maintenance, we are inclined to think that the differences are not inherent
in the tissues but might be due to differences in the experimental con-
ditions. When either dicetyl phosphate or Irish moss extract was injected
subcutaneously, the material, and thus the fibrogenic stimulus, gradually
disappeared and, as a result of resorption, the total amount of new tissue
decreased between the 14th and 45th day. However, when the kidneys
were wrapped, the plastic remained present throughout the experiment
as a continuing stimulus to new tissue formation. Any new tissue which
formed during scurvy would be expected to have less collagen than normal,
since there is abundant histologic evidence (4) and confirming chemical
evidence\(^7\) that ascorbic acid is required for the formation of new collagen.
The low collagen concentration of the kidney capsules in scorbutic animals
might be due to such an admixture of collagen-poor new tissue. If this
process occurred, then the difference between the collagen of the kidney
capsules and other newly induced collagen and organ collagen is only an
apparent one.

The view that the maintenance (in contrast to formation) of collagen
does not require ascorbic acid is contrary to the view expressed or implied
by Hunt (6) and some others (3, 11) that there is a reversion of collagen
in scurvy. Changes which have been described as collagen degeneration
or collagen atrophy are best seen in growing tissues and especially in tis-
sues subject to physical stress. Follis has shown that such changes in

\(^7\) Unpublished.
bones were prevented by immobilizing the limb in a plaster cast (12). The presence of "degenerate" collagen in scurvy would appear to be the result of an abortive new collagen formation in response to the stimulus of growth or trauma. Hunt's experiment, which he believes shows that the newly formed collagen of a recently healed abdominal wound reverted to precollagen, may have a similar explanation, for the present data (Table I) show that collagen formation continues even though the wound has completely healed over. However, his conclusion was based on the study of the effects of scurvy on only one wound which had healed for 20 days in an adequately maintained guinea pig, and normal variability might have played a rôle in his observation. The limitations of the chemical methods used in the present study do not permit us to exclude the possibilities that either a very small fraction of the collagen (possibly that most recently laid down) requires ascorbic acid for maintenance or that a subtle change in the macromolecule occurs in scurvy (for discussion see Robertson (2)). Nevertheless, it would appear from evidence available at the present that ascorbic acid is not needed for the maintenance of formed collagen, but only for the replacement of that fraction destroyed by wear and tear.

SUMMARY

Formation of collagenous tissue was induced in guinea pigs by producing wounds, by subcutaneous injection of dicetyl phosphate or Irish moss extract, and by wrapping a kidney with plastic.

The collagen concentration of the healed wounds or of the new subcutaneous tissue was not lowered when the animals were made scorbutic; however, under the same conditions, the collagen concentration of the new tissue about the kidney decreased.

These results were discussed and it was suggested that the major portion of induced new collagen, like organ collagen, does not require ascorbic acid for maintenance.

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