HYALURONIDASE INHIBITOR IN BLOOD SERUM OF SCORBUTIC GUINEA PIGS

BY J. A. SCHACK,* RICHARD W. WHITNEY, AND MONROE E. FREEMAN

(From the Department of Basic Science and the Department of Chemistry and Physics, Army Medical Department Research and Graduate School, Washington)

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The chemical nature of the non-specific hyaluronidase inhibitor normally present in blood serum has not been elucidated, but quinoid and hydroquinoid compounds have been found to be most effective inhibitors of the depolymerization of hyaluronic acid by hyaluronidase in vitro (1, 2). Other researches have suggested that intermediate metabolites of reduced or oxidized quinoid type may appear in blood and urine during intense salicylate therapy, alkaptonuria, and incomplete tyrosine metabolism associated with ascorbic acid deficiency (2–7). The latter condition in guinea pigs was also marked by increased serum methemoglobin and the excretion of benzoquinone acetic acid in the urine (3). Since the presence of quinoid substances in serum and urine has not been correlated with the concentration of hyaluronidase inhibitor, these measurements have been undertaken.

Methods

The non-specific hyaluronidase inhibitor was determined by the method of Dorfman, Ott, and Whitney (8) with the addition of magnesium ion to the diluted serum samples according to Freeman, Whitney, and Dorfman (9). Methemoglobin was determined by a modification of the method of Drabkin and Austin (10). Water-soluble quinones were demonstrated by Fishberg's test (3). The presence of homogentisic acid was deduced from the darkening of alkaline urine samples exposed to air. Occasional animals were sacrificed for pathological confirmation of clinical scurvy.1

Weanling male and female guinea pigs were placed on a diet of ground Purina rabbit chow which had been exposed to the air for 24 hours in thin layers, supplemented by 5 per cent dried yeast and a semiweekly addition of cod liver oil and wheat germ oil. On analysis, the ascorbic acid content was found to be negligible.

Blood specimens were taken by cardiac puncture from groups of

* Present address, Michael Reese Hospital, Chicago 16, Illinois.
1 The authors are indebted to Lieutenant David Mintz, Army Institute of Pathology, for the histopathological examinations.
approximately thirty guinea pigs (weight 200 gm.) before initiation of the test diet and before, during, and after the appearance of clinical scurvy. Urine specimens were collected in 0.1 N HCl under petrolatum for 12 hour periods before and after the collection of the blood samples.

EXPERIMENTAL

Blood and urine specimens were obtained from two series of female guinea pigs while the animals were on a liberal diet of greens. After about 20 days on the deficient diet, when mild signs of scurvy appeared, the animals were bled and urine specimens were obtained. About 10 days later, when clinical scurvy had proceeded to the stage of bloody diarrhea and occasional death, blood samples were taken from the survivors.

**TABLE 1**

Concentration of Hyaluronidase Inhibitor in Sera of Normal and Scorbutic Guinea Pigs

The results are expressed in units of hyaluronidase inhibitor as defined by Dorfman *et al.* (8).

<table>
<thead>
<tr>
<th>Series</th>
<th>Normal diet</th>
<th>Deficient diet</th>
<th>Normal diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
<td>7 days</td>
<td>21-24 days</td>
</tr>
<tr>
<td>A, B</td>
<td>55</td>
<td>89</td>
<td>88</td>
</tr>
<tr>
<td>C</td>
<td>65</td>
<td>97</td>
<td>87</td>
</tr>
<tr>
<td>D</td>
<td>50*</td>
<td>70†</td>
<td>88</td>
</tr>
</tbody>
</table>

* After 100 mg. of ascorbic acid intramuscularly.
† After 0.25 gm. of L-tyrosine by mouth.

The data from these two groups (Table I, Series A and B) show a significant increase in the mean concentration of the hyaluronidase inhibitor with the onset of clinical scurvy, but the progression of scurvy was not accompanied by further increases in inhibitor concentration.

A comparable group of male guinea pigs (Series C, Table I) gave similar results.

Since withdrawal of ascorbic acid in Series A, B, and C, Table I, appeared to produce a rise in the inhibitor titer, the converse was examined by intramuscular injection of 100 mg. of ascorbic acid in a group of guinea pigs on a full diet of greens (Series D, Table I). The blood samples drawn 6 hours later showed no significant depression of inhibitor titer. These guinea pigs were continued on the full diet for an additional week and then placed on the deficient diet.

In view of the observations of Painter and Zilva (6) regarding the rapidity with which the biochemical defect in faulty tyrosine metabolism mani-
fested itself, an attempt was made to force the increase of inhibitor by the administration of tyrosine before clinical scurvy became evident. After 7 days on the deficient diet with no clinical evidence of scurvy, each guinea pig of Series D was given 0.25 gm. of L-tyrosine by mouth. The blood samples drawn 6 hours later showed a small rise in the mean titer of the

**Table II**

*Daily Excretion of p-Quinone in Urine of Scorbatic Guinea Pig*

<table>
<thead>
<tr>
<th>Days on deficient diet</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
<th>26</th>
<th>27</th>
<th>28</th>
<th>29</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Quinone, mg.%</td>
<td>4.2</td>
<td>1.26</td>
<td>2.52</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.2</td>
<td>0</td>
</tr>
<tr>
<td>Total quinone excreted, mg.</td>
<td>84</td>
<td>31.5</td>
<td>48.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>67</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table III**

*Urinary Excretion of Quinone-Like Substances and Concentration of Hyaluronidase Inhibitor in Sera of Normal and Scorbatic Guinea Pigs (Mean Figures, Series D)*

<table>
<thead>
<tr>
<th>Normal diet</th>
<th>Deficient diet</th>
<th>Normal diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>7 days</td>
<td>29 days</td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyaluronidase inhibitor units†</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>Methemoglobin, mg.%</td>
<td>0</td>
<td>1.1</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homogentisic acid</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>KI-oxidizing substance, mg. per 24 hrs.‡</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Methemoglobin induced by 2 cc. urine, %§</td>
<td>0</td>
<td>6.4</td>
</tr>
</tbody>
</table>

* 100 mg. of ascorbic acid were given intramuscularly at the beginning of the experiment and 0.25 gm. of L-tyrosine by mouth on the 7th day.
† Hyaluronidase inhibitor units defined by Dorfman et al. (8).
‡ Reported as benzoquinone acetic acid, Fishberg (3).
§ Per cent methemoglobin induced in a known amount of hemoglobin solution by addition of 2 cc. of cleared urine.

hyaluronidase inhibitor. This group then progressed to clinical scurvy. Blood and urine samples were taken from the surviving animals on the 29th day of the deficient diet, when they were immediately returned to a full diet. By the 50th day, they appeared sleek and normal in every respect and had gained weight for 10 days. The mean inhibitor concentration, last column of Table I, clearly showed a return to normal in the surviving animals that had recovered on the full diet.
HYALURONIDASE INHIBITOR IN BLOOD SERUM

While the earlier work of other investigators seems to have clearly established the presence of quinone-like compounds in blood and urine of scorbutic animals, it seemed advisable to verify the existence of similar conditions in these experiments. The daily urinary excretion of p-quinone is illustrated in Table II. The wide variation in daily output is similar to that reported by Fishberg (3) for human subjects.

Table III shows the appearance of quinone-like substances in the urine, the methemoglobin level, and concentration of hyaluronidase inhibitor in the serum of the animals in Series D. These data, typical of all series, seem to indicate a significant increase of quinoid-like substances in the urine at the time of maximum inhibitor titers, although direct and immediate correlation cannot be concluded.

DISCUSSION

The data demonstrate that the non-specific hyaluronidase inhibitor of guinea pig blood increased significantly as marked clinical scurvy appeared and returned to normal with recovery of the animals. Furthermore, the inhibitor level appeared to rise before the appearance of clinical scurvy if the animals were fed 0.25 mg. of tyrosine. These findings would lend considerable support to the hypothesis that compounds of the quinoid type, which are known to be active hyaluronidase inhibitors in vitro, may be closely related to the non-specific hyaluronidase inhibitors normally found in animal serum.

The results substantiate the findings of Fishberg (3) and Painter and Zilva (6) that the scorbutic guinea pig excretes significant amounts of methemoglobin-inducing substances and KI-oxidizing substances in the urine. The failure to demonstrate homogentisic acid excretion in any significant amounts is in agreement with the findings of Painter and Zilva.

The variation in the daily excretion of quinone-like substances by the individual guinea pig has been noted. In addition, there was variation in the magnitude of the individual response of guinea pigs to the onset of scurvy in terms of all the effects measured. However, the type of response was similar in all animals. Although no direct cause and effect relationship has been demonstrated by the data, it seems unlikely that the increase in concentration of the hyaluronidase inhibitor at the time of excretion of quinone-like substances is entirely fortuitous.

SUMMARY

1. The non-specific hyaluronidase inhibitor of normal guinea pig serum increased significantly with the onset of marked clinical scurvy and returned to normal values when the surviving animals were returned to a healthy condition.
2. Methemoglobin appeared in the blood and quinone-like compounds were excreted in the urine with the onset of scurvy and during the period of elevated level of hyaluronidase inhibitor.

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HYALURONIDASE INHIBITOR IN BLOOD SERUM OF SCORBUTIC GUINEA PIGS
J. A. Schack, Richard W. Whitney and Monroe E. Freeman


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