Studies of Tissue Response to Injections of Enzymes

V. Development of Subcutaneous Nodular Masses, and Plasma and Tissue Hexosamine Changes Following Subcutaneous Injection of Streptokinase

By Samuel T. Schlamowitz, M.D., and Arthur C. DeGraff, M.D.

The presence of proteolytic enzymes in blood has been known for a long time. A variety of methods and agents have been employed to activate these enzymes. Among these, is streptokinase, a bacterial kinase obtained from the hemolytic streptococcus. The subcutaneous "nodules" and significant hexosamine alterations which occurred following the single subcutaneous inoculation of crude streptokinase, resembled those obtained with partially purified streptokinase and with trypsin (crude and crystalline). Although it is possible that the results obtained with streptokinase were due to the direct conversion of the proteolytic enzyme to its active form, and that changes noted with trypsin were due to the nullification of the inhibitor with the concomitant liberation of the free and active enzyme, it is also very possible that both trypsin and streptokinase give rise to the liberation of a tissue activator, which in turn catalyzes the transformation of the proteolytic enzyme to its active form.

In 1893 Dastre noted that serum possesses proteolytic activity. The observation of Delezenne and Pozerski that serum treated with chloroform exhibited proteolytic activity has been repeatedly confirmed. A variety of agents and methods have been employed to activate the serum proteolytic enzymes. Christensen and MacLeod, Christensen and Ratnoff have demonstrated the conversion of plasminogen to plasmin, by means of the bacterial enzyme, streptokinase. Astrup and Permin demonstrated the activation of the serum proteolytic enzymes by means of a tissue kinase, "cytofibrinokinase," present in tissue washings of cells from various organs. The presence of an activator of the proteolytic enzymes, within the stroma of human erythrocytes, has been reported by Permin. In addition to confirming this observation, Tagnon demonstrated the presence of this activator in a particular fraction of mammalian tissue extract, and found the properties of the activator "to be compatible with the hypothesis that the activator is a kinase acting on the substrate plasminogen to transform it to plasmin." Ungar and Mist have reported the activation of the serum proteolytic enzymes of guinea pigs by means of specific antigen, peptone and certain polysaccharides among which were hyaluronic acid and chondroitin sulfuric acid.

This study was undertaken to ascertain whether the results previously obtained with partially purified streptokinase could be confirmed. It relates the microscopic and macroscopic tissue changes and chemical changes resulting from the subcutaneous injection of crude streptokinase in rabbits. It also affords an opportunity to compare these results with those noted following the subcutaneous injection of trypsin.

Materials and Methods

Nine young male albino rabbits were employed in this study. The animals fulfilled the criteria set forth in the previous studies with regard to age, weight, and clinical status. The methods for

* The limited supply of streptokinase was sufficient for the study of only 9 animals.
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the measurements of the local lesions, enzymatic activity, plasma and tissue hexosamine, and statistical analysis previously described were again employed in this study.

The untreated animals used in the previous studies served as controls, and the results obtained in this study were compared with those found in the animals treated with partially purified streptokinase, and trypsin.

**TABLE 1.—The Relation between the Occurrence, Size of the Subcutaneous Mass and Time after A Single Subcutaneous Injection of Streptokinase.**

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Streptokinase.† Crude streptokinase made up in a phosphate buffered saline solution (pH 7.4) was prepared so that 1 ml. of the solution contained 10,000 units of streptokinase.

**PROCEDURE AND RESULTS**

A single injection of 1 ml. of crude streptokinase solution containing 10,000 units was introduced subcutaneously into the shaved area of the plantar surface of each hind leg of each of the 9 rabbits. This area of inoculation is analogous to the human heel region. Within three to five days post inoculation, a distinct, firm, nontender, nonerythematous mass appeared at each of the sites in each test animal (table 1). These subcutaneous masses ("nodules") progressively enlarged to attain their maximum in 9 to 14 days, and then slowly regressed. However, at the end of the 30 day experimental period, the "nodules" were still detectable. No conventional signs of inflammative

† Crude streptokinase was generously contributed by Drs. MacLeod and Christensen, Department of Microbiology, New York University-Bellevue Medical Center, New York, New York.
Table 2.—Comparison of Mean Hexosamine Content* of the Injection Sites, "Nodules," Skin,† Tendon,‡ and Plasma following a Single Subcutaneous Injection of Streptokinase with That Following a Single Subcutaneous Injection of Trypsin.§

<table>
<thead>
<tr>
<th>Inoculant</th>
<th>Number of Animals Examined</th>
<th>Mean</th>
<th>σ</th>
<th>t</th>
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<td>Injection Sites and &quot;Nodules&quot;</td>
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<td></td>
<td></td>
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<tr>
<td>Untreated</td>
<td>27</td>
<td>5.16</td>
<td>±0.66</td>
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<td>6.63</td>
<td>±0.39</td>
<td>7.24</td>
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<td>7.08</td>
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<td>±1.73</td>
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<td>Skin†</td>
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<tr>
<td>Untreated</td>
<td>27</td>
<td>4.14</td>
<td>±1.04</td>
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<tr>
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<td>4.33</td>
<td>±0.47</td>
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<tr>
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<td>Tendon‡</td>
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<tr>
<td>Untreated</td>
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<td>4.13</td>
<td>±0.76</td>
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<td>Crude Trypsin</td>
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<td>4.45</td>
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<td>0.94</td>
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<td>Plasma</td>
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<td>Untreated</td>
<td>30</td>
<td>51.4</td>
<td>±6.80</td>
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<td>77.5</td>
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* The plasma hexosamine is expressed as mg. hexosamine per 100 ml. plasma. All other values are expressed as mg. per Gm. dry tissue, and cover the entire study period.
† Skin covering the quadriceps femoralis—a site distant from the injection site.
‡ Tendon of Achilles—subjacent to the injection sites.
§ The values for the trypsin have been reported in Circulation 1: 819–843, 1950.
P.P.S.: Partially Purified Streptokinase
C.S.: Crude Streptokinase

Discussion

Macroscopically, the subcutaneous masses which developed at the sites of the subcutaneously inoculated crude streptokinase, closely resembled those which occurred following the single subcutaneous injection of partially purified streptokinase and following the single subcutaneous injection of crude or crystalline trypsin. The developmental course of these firm, nontender, non-necrotic, nodular masses, seems to occupy a position intermediate between that caused by the partially purified streptokinase and crystalline trypsin. This is evidenced by the fact that in the animals treated with the partially purified streptokinase, the "nodules" were macroscopically detectable within three to five days; in the trypsin treated group, four to eight days were necessary for the appearance of the "nodules". The subcutaneous nodular masses, which developed following the single subcutaneous inoculation of crude streptokinase attained their maximum size in 10 to 18 days, whereas...
those in the partially purified group required 12 to 16 days\(^{19}\) and those in the trypsin series, 12 to 18 days.\(^{21,\ 22}\)

The significant elevation of the hexosamine content of the injection sites and "nodules" which occurred soon (24 hours) after the subcutaneous introduction of the crude streptokinase, closely resembled that which occurred in the series treated with partially purified streptokinase (table 2, fig. 1).\(^{19}\) In addition, the results obtained with crude and partially purified streptokinase closely resembled those noted in animals which received crude and crystalline trypsin (figs. 1 and 2, table 2). It is obvious that the insignificant amount of hexosamine present in the inoculating dose of the crude streptokinase (table 3) preparation, could hardly account for the significant hexosamine values obtained.

The presence of proteolytic enzymes in blood have been known for a long time.\(^1\) Likewise, it has been known for some time that treating plasma with chloroform, results in the production of proteolytic activity.\(^2\-\ 6\) This is due to the abolition of the plasma inhibitor and activation of the enzymes takes place, presumably by autocatalysis.\(^6\)

Such activation occurs when the proteolytic enzyme plasmin, (which is normally present in plasma in an inactive form called plasminogen) is shaken with chloroform.\(^6\, \ 17\) However, this type of activation is a slow process.\(^3\) Streptokinase, in contrast, catalyzes the transformation of plasminogen to plasmin rapidly.\(^17\)

Thus, as with partially purified streptokinase the inoculating dose of crude streptokinase was considered sufficient not only to overcome the high antiprotease titer present in the rabbits,\(^6\, \ 24\) but to insure an excess.

Although it is tempting to hypothesize that the tissue (macroscopic and microscopic)\(^{20}\) and chemical changes noted in the studies with the streptokinase preparations were due to the plasmin resulting from the conversion of plasminogen, one must keep in mind that these preparations contain desoxyribonuclease,\(^5\, \ 6\) and its exact role must first be clarified.

It is conceivable that the hexosamine and histologic alterations obtained with trypsin were due to the nullification of the effects of the plasma inhibitor, with the resultant liberation of free and active proteolytic enzyme. The fact that the inhibitor of plasmin is probably identical with plasma antitrypsin\(^{17}\) and that similar hexosamine and histologic changes were obtained with both trypsin and streptokinase would seem to suggest that there may be some relationship between trypsin and plasmin.

Attractive as these possibilities may be, a third consideration which must not be forgotten is that these substances (trypsin and streptokinase), by their subcutaneous introduction, give rise to the liberation of a tissue activator, identical or similar to those identified by Astrup,\(^14\) Permin,\(^15\) Tagnon,\(^16\, \ 17\) and Unger,\(^18\) which in turn causes the transformation of the inactive plasma proteolytic enzyme (plasminogen) to plasmin.

It is very possible that all of the above factors are simultaneously or successively involved.

**Summary**

1. Subcutaneous "nodules" which macroscopically resembled those obtained with a single subcutaneous injection of partially purified streptokinase and/or trypsin, developed on the plantar aspect of each of the hind legs of each of the test rabbits in three to five days following the single subcutaneous inoculation of crude streptokinase. As with those animals in the series treated with trypsin and partially purified streptokinase, no erythema, tenderness, eschar, necrosis, slough, breakdown or fixation occurred.

2. The nodular masses increased to a maximum and then regressed. The nodular masses were still present, however, at the termination of the 30 day experimental period.
3. The significant increase in the hexosamine content of tissue at the injection sites and in the "nodules," which occurred within 24 hours of the single subcutaneous injection of crude streptokinase, persisted throughout the entire experimental period.

4. The plasma hexosamine was significantly increased within 24 hours post inoculation and remained so throughout the entire period of study.

5. The results obtained not only closely resemble those noted in rabbits treated with the partially purified streptokinase, but also in the animals treated with trypsin.

6. The histologic and hexosamine changes noted in these studies may be the result of either one or a combination of the following mechanisms: (a) the liberation of the proteolytic enzyme from its inhibitor (antitrypsin) by trypsin; (b) the direct transformation of the enzyme from its inactive state, plasminogen, to its active form plasmin, by streptokinase; or (c) the conversion of the inactive form, plasminogen, to its active state, plasmin, by a tissue activator liberated or increased in amount by the action of these inoculants.

REFERENCES


6. ———: The activation of plasminogen by chloroform. J. Gen. Physiol. 30: 149, 1946.


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