THE DIGESTION OF COLLAGEN IN BURNS*

JOSEPH M. MILLER, M.D.

Since the proteolytic enzymes in current use are not effective agents for the debridement of burns, attempts were made to find an enzyme or a combination of an enzyme and a modifier of the substrate which would digest burned skin. Skin contains a large amount of collagen (2). Therefore, an investigation of the digestion of collagen was made.

The plant proteolytic enzymes, papain, ficin, and bromelin can digest collagen in the presence of modifiers of the substrate (1). The modifying agents, urea, thiourea, and Duponol, when they acted upon collagen caused a grossly visible swelling of the collagen which was not evident when only the plant enzymes were present.

Thiourea appeared promising for use in combination with the plant enzymes. Its unit activity was high and its stability was good. Animal tests indicated, however, that thiourea had toxic systemic effects when it was employed locally.

METHOD

Thermal burns, which measured about 1 cm. by 2 cm., were produced on the shaved areas of the backs of anesthetized rats with a red-hot platinum loop until the site was charred. The rats were divided into groups of six. The first group was treated with an ointment which contained 10 per cent papain, 10 per cent urea, and 0.5 per cent chlorophyll in a hydrophilic base. The second group was treated with an ointment comprising 10 per cent papain, 5 per cent thiourea, and 0.5 per cent chlorophyll in a similar base. The third group was not treated. A fourth group of four rats which were not burned was maintained as a normal control. The ointments were applied once daily. At the end of 48 hours, three of the six animals treated with the thiourea ointment were dead. It was suspected and confirmed by autopsy examination that these animals had ingested some of the thiourea ointment. Therefore, the three remaining rats were placed in separate cages. Three additional animals were placed in the experiment in place of the three which had died. At the end of another 48 hours, one more of the original rats died having had a total of 4 days of treatment. Deaths did not occur in any of the other groups. At the end of nine days, the eschar was shed spontaneously from all of the treated rats and smooth pink skin was present at the site of the burn. Eschar was still present at the site of the injury in the six untreated animals. At the end of 15 days, the eschar on the burned areas was shed spontaneously in the last 2 of the untreated rats.

Duponol is an effective modifying agent to produce collagenase activity by the plant enzymes. This substance, however, has practical disadvantages for local use. The task of putting the amount of Duponol in solution which is required to provide collagenase activity by the plant enzymes is formidable.

Urea, therefore, appeared to be the most practical of the three modifiers which were used to prepare collagen for digestion by the plant enzymes. Experiments were conducted to determine if urea would denature ficin, bromelin, and papain and make them inactive. Each enzyme was placed in a solution of 6 molar urea for 24 hours. The enzymes were precipitated from solution by the addition of 95 per cent isopropyl alcohol, washed, and dried. The enzymes which were treated in this manner with urea did not differ in ability to digest beef powder from the enzymes which had not been treated (table 1). Pretreatment with urea did not make the enzymes capable of digesting collagen (table 2). Ficin and bromelin do not need sulfhydryl groups for their activation so cysteine was added only to the papain for its activation.

Ficin, bromelin, and papain have comparable proteolytic spectra. In the presence of water, however, ficin and bromelin become active and lose their digestive ability. Papain will retain its digestive ability in water unless activating substances are present.

This characteristic gives papain a practical flexibility which is desirable in a therapeutic digestant. Solutions and ointments which contain papain can be stored for prolonged periods of time. The urea in such solutions or ointments

Received for publication August 19, 1958.

* From the Surgical Service, Veterans Administration Hospital, Fort Howard, Maryland.

467
TABLE 1
The digestion of beef powder, 800 mg.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight of beef powder in mg. remaining after digestion for 2 hours at 37°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ficin, 10 mg.</td>
<td>358</td>
</tr>
<tr>
<td>Ficin, 10 mg., treated</td>
<td>346</td>
</tr>
<tr>
<td>Bromelin, 10 mg.</td>
<td>420</td>
</tr>
<tr>
<td>Bromelin, 10 mg., treated</td>
<td>417</td>
</tr>
<tr>
<td>Papain, 10 mg.</td>
<td>396</td>
</tr>
<tr>
<td>Papain, 10 mg., treated</td>
<td>405</td>
</tr>
</tbody>
</table>

TABLE 2
The digestion of collagen, 80 mg.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight of collagen in mg. remaining after digestion for 18 hours at 37°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ficin, 10 mg.</td>
<td>78.5</td>
</tr>
<tr>
<td>Ficin, 10 mg., treated</td>
<td>81.0</td>
</tr>
<tr>
<td>Bromelin, 10 mg.</td>
<td>79.3</td>
</tr>
<tr>
<td>Bromelin, 10 mg., treated</td>
<td>79.0</td>
</tr>
<tr>
<td>Papain, 10 mg., and cysteine, 3 mg.</td>
<td>81.3</td>
</tr>
<tr>
<td>Papain, 10 mg., treated, and cysteine, 3 mg.</td>
<td>79.2</td>
</tr>
</tbody>
</table>

does not affect the stability of the enzyme. It was decided, therefore, to restrict further testing to the combinations of papain, urea, and cysteine.

The combination of papain and urea without an activator for the enzyme will not digest collagen. Collagen is characterized by a high content of glycine, proline, and hydroxyproline, a very low content of tyrosine, methionine, and histidine, and an absence of cystine and tryptophane (2). Sulphydryl groups cannot be made available from these amino acids in collagen by urea for activation of the papain. When cysteine which contains sulphydryl groups is added to the combination of papain and urea, the digestion of collagen is prompt.

Sulphydryl groups are made available from many proteins, however, when they are acted upon by urea. For example, activating groups are present in beef powder, casein, and egg albumin. Urea exposes the sulphydryl groups for the activation of the papain. The presence of sulphydryl groups in the substrate makes the addition of cysteine unnecessary to insure proteolysis. This fact has been amply demonstrated clinically. An ointment containing papain and urea is uniformly active in infected wounds. Papain alone, however, is not always active in such wounds. It is inferred that, although wound substrates usually contain substances which could activate papain, urea may be required to expose these radicals.

Since the combination of papain and urea digests collagen when an activator is added, the digestion of collagen should occur in a mixed substrate, in which, with collagen, there are other proteins capable of furnishing sulphydryl groups. This concept was tested in vitro and found to be correct. Collagen, 48.1 mg., was suspended in 12 ml. of distilled water. The mixture was incubated for 18 hours at 37°C. The suspension was filtered through a number 50 Whatman filter paper and was washed to remove all soluble materials. The filter paper and any non-filterable material were then dried to a constant weight and weighed. The previously determined dry weight of the filter paper was deducted from this weight. The difference represented collagen which was not digested by the combination under test. The addition of either cysteine or beef powder to the combination of papain and urea produced effective digestion of the collagen (table 3). The urea acted upon the beef powder to produce sulphydryl radicals which were like those supplied by the cysteine.

It may be concluded, therefore, that the combination of papain and urea digests collagen in infected wounds which contain proteins other than collagen. In such cases the enzyme is activated by sulphydryl groups which are freed from these other proteins by urea. This fact is of considerable practical importance for with very few exceptions the activator for the enzyme is present in such wounds and merely needs to be released from its complex union in the proteins. In addition to digesting the other proteins in pus in an infected wound, the combination of papain and urea will also attack collagen. The ability to digest collagen removes a limitation which has been inherent in other proteolytic preparations which have been used locally.

The combination of papain and urea, however, will not digest collagen in the eschar in second and third degree burns of the skin. Sulphydryl groups are not freed from the skin by urea to activate the papain.

Tests with skin from a cadaver illustrate this point. One gram of skin was placed in 10 ml. of
DIGESTION OF COLLAGEN IN BURNS

Distilled water with various combinations of substances and incubated at 37°C for 20 hours (table 4). At the end of the period of incubation, the necessary substances were added as they were needed so that each test tube contained skin, 1.0 gram, papain 0.5 gram, urea 3.6 grams, and cysteine, 20 mg., and the volume was increased to 20 ml by the addition of distilled water. A blank tube which did not contain skin was also prepared. The solutions were mixed and filtered.

The filtrates were diluted five times with distilled water and the extent of digestion in 1 ml was measured in terms of optical density in the Beckman DU Spectrophotometer at a wavelength of 380 nm. The reading for the blank was 0.032. The solutions which contained the skin were read against the blank.

The extent of digestion was also measured by measurement of the amount of amino acids which were released during digestion by the formol titration method. The necessary substances were added as needed to make all of the solutions the same. A volume of 18.6 ml was titrated with a 0.1 normal solution of sodium hydroxide. The blank did not contain skin. The reading for the blank was 5.25 ml of a 0.1 normal solution of sodium hydroxide. The values shown are those with that for the blank subtracted.

Observation of the samples of skin showed varying amounts of digestion without any digestion of the epidermal layer. The dermal part of the skin appeared to have been entirely digested in the solution containing papain, urea, and cysteine. A consequential change of the skin did not appear in any of the other solutions although the combination of papain and urea caused considerable maceration. It was evident that all three elements of the combination, papain, urea, and cysteine, were necessary to produce material digestion of the undenatured dermal proteins.

Denaturation of collagen usually makes it more susceptible to proteolytic digestion. Samples of skin were charred over an open flame or boiled for 15 minutes. Solutions of papain and cysteine without urea produced significant although not complete digestion of the dermal tissue. The addition of urea to this combination effected more rapid and more complete digestion. The combination of papain and urea did not have consequential digestive ability. If denaturation of collagen by heat is sufficient, digestion by papain and cysteine can be effected without urea.

A comparable result was obtained when pieces of eschar from a patient with a third degree burn were treated with an ointment containing 10 per cent papaine, 10 per cent urea, and 0.5 per cent chlorophyll in a solution of a borate buffer at a pH of 6.2. The eschar softened and the underlying layers of tissue became mushy. Digestion, however, was not complete.

Keratin is extremely resistant to digestion by any of the enzymes. Papain, urea, and cysteine, alone, or in combination, did not affect the keratin.

Keratin has a high content of cystine (2). The harder the keratin and the more resistant to enzymes, the higher is the content of cystine.
Apparently with an increasing content of cystine, the amounts of cysteine and methionine decrease. The relation of nondisulfide sulfur to disulfide sulfur becomes smaller as keratinization becomes more complete. The disulfide bonds exist in cross linkages in the complex molecule. Such bonds are resistant to fission.

COMMENT

The fact that the plant proteolytic enzymes can be employed in combination with substances which modify the substrate suggests many interesting possibilities for the therapeutic use of these agents. The modifying substances markedly increase the digestive ability of the enzymes. Aside from the rapid and efficient lysis of other proteins, the most interesting result of combining plant enzymes and modifiers is the endowment of such combinations with the capacity to digest collagen.

The digestion of collagen in infected wounds is important. Collagen constitutes a significant portion of many tissues. The digestion and removal of this material along with fibrin and protein debris will lead to quicker healing of wounds.

In burns, the dermis, which forms the tough and impermeable portion of the eschar, is comprised principally of collagen. Agents which were capable of digesting collagen, therefore, would permit access of enzymes to the underlying tissues and allow treatment of these areas in the hope of providing more rapid healing.

There is little doubt that the dermal layer can be digested by the combination of papain, urea, and cysteine. These digestants do not, however, affect the keratin layer of the epidermis.

SUMMARY

A combination of papain, urea, and cysteine will digest collagen in the dermal portion of skin from a cadaver. All elements of the combination are essential for this activity. Urea does not inactivate the plant enzymes. It does denature the substrate, however, and makes sulfhydryl groups available for activation of papain if they are present and can be released from the substrate. If sulfhydryl groups cannot be released from the substrate, another substrate in the wound may provide them or cysteine can be added to the wound. Dermal tissue which is denatured by heat is moderately susceptible to digestion by the combination of papain, urea, and cysteine. The keratin layer of the epidermis is not affected by this combination. Since the plant proteolytic enzymes retain their activity in combination with substances which modify their substrate, they have a valuable therapeutic versatility. Papain has a particular advantage because it retains its activity in a solution and in an ointment and therefore the enzyme is well adapted for local use.

REFERENCES
