PRELIMINARY AND SHORT REPORT

THE DIGESTION OF COLLAGEN*

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For some years there has been interest in the discovery of enzymes which would digest collagen. Substances of this sort should be extremely useful since they should digest burn eschar and facilitate its removal from the burned area.

To date, apparently only one enzyme, which is derived from the *Clostridium histolyticum*, a collagenase, has shown promise of fulfilling this need. This substance has two main disadvantages as an agent for widespread use in debridement of burns. The commercial preparation of this material necessitates the construction of an isolated unit so that contamination of other pharmaceuticals would be avoided. The cost of the medication would rise accordingly. Such an expenditure is not justified since the enzyme will attack not only necrotic tissues but also living tissues.

Interest, therefore, exists in the discovery of a combination of substances which will lyse collagen, be innocuous to viable tissue, be prepared easily, and be made at a reasonable cost. This report describes a number of preparations which appear to meet these criteria. These combinations of substances have not been assayed clinically to learn their practical value. Their ability to digest collagen has been established and the factors of availability and cost are well known.

The preparations described are combinations of proteolytic enzymes with other substances. In every case the combination of substances has been found essential to obtain collagenase activity. The enzymes studied were the plant enzymes, papain, bromelin, and ficin. Trypsin is not practical to use, as it is attacked and rendered inactive by the substances which have been combined with the proteolytic enzymes.

A distinction has been made between papain, ficin, and bromelin. Papain is relatively stable in the presence of moisture, if activators are not present, and can be handled in a different fashion from that necessary with the other two enzymes. Papain appears to be less vigorous in action than the other two enzymes and requires more energetic treatment to endow it with collagenase activity.

In table 1, the various combinations in which papain exhibited collagenase activity are listed. In all tests of ability to digest collagen, the combination under examination was placed in solution in a test tube containing 80 mg. of collagen in 12 ml. of distilled water. The mixture undergoing digestion 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. In such an experiment, the content of moisture represents a variable of significant proportions. To eliminate this variable it is necessary to weigh the filter paper originally and after the test procedure under standardized conditions. This was done by drying to constant weight at 55°C, normalizing temperatures in a desiccator, and weighing, with the least interval possible between removal from the desiccator, and final weighing. This period was about eight to ten seconds. It was not felt desirable to dry the collagen which was to be the substrate for the digestion, since the increased temperature might alter the nature of the material. In these results the original moisture content of the 80 mg. of collagen remains a possible variable which may show up as a reduced weight of the final residue after digestion. An ample number of determinations have established, however, that the moisture content of 80 mg. of collagen does not rise above 5 mg. so that this variable at its highest level does not compromise the significance of the results obtained. In some instances the residue after digestion weighed more than the 80 mg. represented by undigested collagen which presumably was the only water insoluble substance employed. The additional weight is believed to consist of modifying substances which were adsorbed by the collagen.

Papain, in 2 molar solutions of thiourea, which is saturation, or in solutions of thiourea built to saturation with other soluble substances, has significant ability to lyse collagen. Papain in combination with saturated solutions of urea, or

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Received for publication January 7, 1958.

TABLE 1

Digestion Of Collagen, 80 mg., By Combinations Incorporating Papain

Ingredients	Weight of Collagen in mg. Remaining after Digestion for 18 hours at 37°C
None	78.1
Papain, 10 mg.	81.1
Papain, 10 mg., and urea, 12.96 gm. (18 molar)	78.2
Papain, 10 mg., and thiourea, 0.192 gm. (1 molar)	67.4
Papain, 10 mg., and Duponol, 2.4 gm. (saturated solution)	85.8
Urea, 12.96 gm. (18 molar), and cysteine, 3.0 mg.	81.2
Thiourea, 1.824 gm. (2 molar)	83.7
Thiourea, 0.912 gm. (1 molar), and cysteine, 3.0 mg.	78.4
Duponol, 2.4 gm. (saturation), and cysteine, 3.0 mg.	83.5
Papain, 10 mg., urea, 12.96 gm. (18 molar), and cysteine, 3.0 mg.	4.5
Papain, 10 mg., urea, 4.32 gm. (6 molar), and cysteine, 3.0 mg.	8.1
Papain, 10 mg., and thiourea, 1.824 gm. (2 molar)	2.1
Papain, 10 mg., thiourea, 0.912 gm. (1 molar), and cysteine, 3.0 mg.	18.2
Papain, 10 mg., Duponol, 2.4 gm. (saturated solution), and cysteine, 3.0 mg.	32.9
Papain, 10 mg., Duponol 1.2 gm., and cysteine, 3.0 mg.	3.7

TABLE 2

Digestion Of Collagen, 80 mg., By Combinations Incorporating Ficin Or Bromelin

Ingredients	Weight of Collagen in mg. Remaining after Digestion for 18 hours at 37°C
None	77.3
Ficin, 10 mg.	81.6
Urea, 4.32 gm. (6 molar)	83.2
Thiourea, 0.912 gm. (1 molar)	78.1
Duponol, 1.2 gm.	84.0
Bromelin, 10 mg.	78.7
Bromelin, 10 mg., urea, 4.32 gm. (6 molar), and cysteine, 3.0 mg.	73.4
Ficin, 10 mg., and urea, 4.32 gm. (6 molar)	13.3
Ficin, 10 mg., and thiourea, 0.912 gm. (1 molar)	6.5
Ficin, 10 mg., and Duponol, 1.2 gm.	8.3
Bromelin, 10 mg., and urea, 4.32 gm. (6 molar)	28.8
Bromelin, 10 mg., and thiourea, 0.912 gm. (1 molar)	44.8
Bromelin, 10 mg., and Duponol, 1.2 gm.	24.2

TABLE 3

Digestion Of Collagen, 80 mg., By Enzyme Of Clostridium Histolyticum, Lederle

Ingredients	Weight of Collagen in mg. Remaining after Digestion for 18 hours at 37°C
None	81.2
Enzyme of Clostridium histolyticum, Lederle, 10 mg.	31.2
Enzyme of Clostridium histolyticum, Lederle, 10 mg., and urea 4.32 gm. (6 molar)	85.6
Enzyme of Clostridium histolyticum, Lederle, 10 mg., and thiourea, 0.912 gm. (1 molar)	87.8
Enzyme of Clostridium histolyticum, Lederle, 10 mg., and Duponol 1.2 gm.	118.1

detergent, plus cysteine, likewise has significant ability to digest collagen. With urea, thiourea, or a detergent in less than a saturated solution, but with cysteine present, papain also has ability to digest collagen.

Ficin and bromelin in various combinations exhibited collagenase activity (table 2). These enzymes resembled each other in activity as they were evaluated by these tests. They differed in at least one respect. Ficin responded well to activation by cysteine, whereas bromelin was adversely affected by cysteine. Thus, although the enzymes differed in mechanism, their end-effects in these tests were similar. Ficin and bromelin, although they did not possess the ability to lyse collagen in themselves, showed significant such ability in combination with urea, thiourea, and Duponol. These enzymes did not require saturated solution of the modifying agents to digest collagen. They did not require the resence of cysteine. Actually, bromelin was adversely affected by cysteine.

The combinations described were significantly effective in digesting collagen. They were more active in this regard than the enzyme of

Clostridium histolyticum, Lederle* (table 3). All of the substances employed are in good supply and relatively inexpensive.

This is a preliminary communication. We intended to report later on the mechanism involved in the digestion of collagen by the combinations described to the extent that they become clear. Considerable clinical work will be required to establish the efficacy of the combinations described. It is hoped that this disclosure will interest investigators in this problem.

* The enzyme of the Clostridium histolyticum, Lederle, was supplied by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

SUMMARY

Combinations of proteolytic enzymes with other substances have been found to digest collagen. These combinations are effective, innocuous to viable tissue, easily prepared, and made at a reasonable cost.