Role of Granulocyte Collagenase in Collagen Degradation

Gerald S. Lazarus, MD, John R. Daniels, MD, Jane Lian, PhD and Mary C. Burleigh, PhD

INFILTRATION BY LEUKOCYTES in inflammatory conditions is frequently associated with collagen degradation. Resorption of collagen occurs during abcess formation, when large numbers of granulocytic leukocytes are present. Leukocytes have been shown to be responsible for the basement membrane degradation seen in such immunologic phenomenon as the arthus reaction and the arteritis of serum sickness.[1] Extracts of polymorphonuclear leukocytes are capable of digesting human renal basement membrane and causing damage to vessel walls in rabbit skin.[2] These observations imply that granulocytes have a mechanism for degrading collagen.

The mechanisms by which collagen is degraded have required elucidation, since collagen is quite resistant to degradation by most proteolytic enzymes. In 1962 Gross and Lapiere [3] described the first animal collagenase; this enzyme was found in the medium of tissue cultures of metamorphosing tadpole tails. Subsequently, collagenase was demonstrated in tissue culture fluids from: skin,[4-14] synovium from patients with rheumatoid arthritis,[15-19] synovial fluid from patients with rheumatoid arthritis,[20] involuting postpartum rat uterus,[21] bone of various species,[22-26] human gingiva,[27-29] crab hepatopancreas,[30] human middle ear cholesteotoma,[31] cornea [32-34] and carageenin granuloma.[35]

The study of collagenases requires some understanding of the biochemical characteristics of collagen. I will, therefore, review superficially some of the relevant aspects of collagen biochemistry. Tropocollagen molecules have a molecular weight of 300,000 and are 3000 Å in length and 15 Å in width.[36] These molecules are composed of three polypeptide chains, each of 100,000 molecular weight. The chains are referred to as α chains and they are characterized by the repeating glycine–X–Y triplet which is necessary for helix formation. There are

From the Strangeways Research Laboratory, Cambridge England, Department of Medicine, Albert Einstein College of Medicine, Bronx, NY, Department of Medicine, Stanford University Medical School, Stanford, Calif and the Department of Biochemistry, Boston University School of Medicine, Boston, Mass.

Dr. Lazarus is a Research Fellow of the Arthritis Foundation and Carl Herzog Fellow of the American Dermatological Association. His present address is: Section of Dermatology, Department of Medicine, Montefiore Medical Center, 210th St, Bronx, New York.

significant differences between constituent α chains, so that in most species at least two types are separable by chromatography.[36–38] These α chains may be covalently cross-linked through the lysyl residue at the fifth position from the A- or N-terminal end of the molecule, to form two-chain components (β), and three-chain components γ.[39] The cross-link area is further characterized by a lack of polyproline helix,[36] which makes this area especially susceptible to degradation by certain proteases which are not true collagenases.

Denaturation of collagen is the conversion of this rigid, tightly coiled, three-chain molecule to a random coil called *gelatin*. Collagen can be denatured by treating it with chemicals or by heating it to a sharply defined temperature. For solutions of salt-extracted rat skin collagen, this temperature is 37 C.[40] The possibility of denaturation of collagen solutions at physiologic temperature precludes their use in the study of collagenases. However, it is not generally appreciated that, even at temperatures as low as 25 C, collagen in solution is susceptible to degradation by several proteolytic enzymes.[41,42]

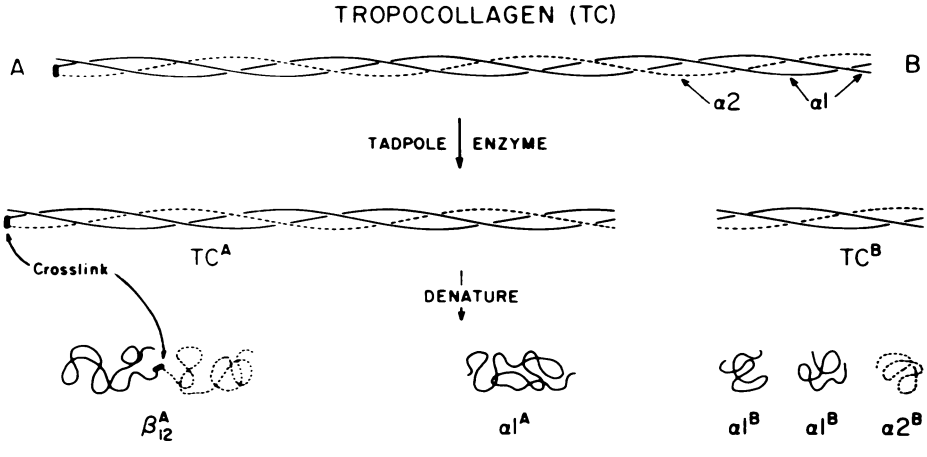
Individual collagen molecules secreted by fibroblasts aggregate into fibrils so that the molecules lie parallel to each other and overlap by one-quarter of their length. The organization into fibrils is governed by electrostatic forces, so that positive and negative charges on adjacent tropocollagen molecules neutralize each other.[43] Fibrils are stabilized further by intermolecular cross-links based on aldehydes derived from lysine and hydroxylysine.[44] Collagen fibrils are extremely stable; if they are not heated above their shrinkage temperature, constituent collagen molecules are degraded only by collagenases at 37 C.

Collagenases are enzymes capable of cleaving native collagen molecules through their characteristic helical portion. Almost all collagenases are also capable of degrading collagen molecules that have been stabilized into fibrils under physiologic conditions. Enzymes such as trypsin are not considered collagenases, since they only degrade an end of the native collagen molecule.[45] Finally, degradation of synthetic collagen peptides is not proof of collagenase activity; the purified collagenases do not degrade the synthetic collagen substrate, but associated peptidases are responsible for synthetic peptide hydrolysis.[46–48]

The enzyme found in the medium of tissue cultures of metamorphosing tadpole tails was the first collagenase described, and its mechanism of action has been extensively investigated.[3,47,49–53] Tadpole collagenase, under physiologic conditions of temperature and pH, cleaves the col-

lagen molecule three-quarters of the way from the N-terminal end (Text-figure 1).[51] The resulting N-terminal three-quarter-length product is referred to as TC^A and the C-terminal one-quarter-length product is designated TC^B. These products maintain their helical conformation in solution at 28 C and, as a consequence, the reaction is characterized by a fall in relative viscosity and no change in optical rotation.[49] The products can be induced to form SLS aggregates and can be studied electron microscopically,[49] or they can be evaluated by acrylamide gel electrophoresis.[52] The tadpole collagenase requires calcium, is inhibited by EDTA and cysteine and is unaffected by diisopropylfluorophosphate. Using an antibody to highly purified tadpole collagenase, Harper *et al* [53] have detected immunologically reactive, but enzymologically inactive, collagenase zymogen in tadpole homogenates.[53] In homogenates of human skin, Eisen *et al* [14] demonstrated the presence of enzymologically inactive collagenase by immunologic methods. They were able to restore enzymatic activity to their immunologically detected collagenase by chromatographically separating the collagenase from serum collagenase inhibitors in tissue homogenates. These data demonstrate that collagenase is present *in vivo* and could be of significance in connective tissue catabolism.

In our initial studies with human leukocytes we employed the radioactive, reconstituted collagen fibril assay system.[54] In this technic,



TEXT-FIG 1—Diagrammatic representation of the collagen molecule and the pieces resulting from digestion with tadpole collagenase. The minor helix of the individual chains is not shown; the major helix is not to scale. The letters A and B refer to the designations employed in electron microscopy to distinguish the ends of the molecule. In a sample of extractable collagen, some molecules would be crosslinked between α1 and α2, as shown; others between the two α1 chains and some not at all. The denatured products would than include α2A and βA11 in addition to those shown (From Kang *et al*, 1966 [51]).

radioactive collagen fibrils are prepared from a solution of highly purified ^{14}C-glycine-labeled acid extracted native collagen.[51] These fibrils are then incubated with the material to be tested at 37 C. After a suitable period of time, the fibrils are removed by filtration or centrifugation;[12,49] the amount of radioactivity in the clarified solution reflects peptide reaction products and solubilized collagen molecules. All experiments include assays of buffer blanks and trypsin controls, which indicate the extent of nonspecific proteolytic breakdown of collagen fibrils. We found that human granulocytes, prepared according to the methods of Cohn and Hirsch,[55] were capable of degrading 21% of our collagen fibrils. Activity was found primarily in the granule fraction of the leukocytes. Similar results were obtained with 99% pure granulocyte preparations from patients with chronic granulocytic leukemia. Normal human lymphocytes or rabbit alveolar macrophages had little, if any, collagenase activity.[54,56] Since our enzyme preparation was exceedingly unstable, the collagenase had to be purified.

During purification, it became obvious that, although our collagenase was capable of specifically cleaving the collagen molecule into TC^A and TC^B fragments (Figure 1), it had lost its ability to cleave reconstituted collagen fibrils. As a consequence, Dr. Daniels designed an assay which directly measured the formation of TC^B products.[58,59] He incubated a solution of radioactive collagen with the enzyme preparation at 25 C and terminated the reaction by adding urea. The material was then separated by acrylamide gel electrophoresis and stained with Coomassie blue. The segment of gel containing the TC^B fragment was removed, solubilized in H_2O_2 and counted. All kinetic experiments measured initial velocity and were performed at saturating substrate conditions.

Leukocytes were separated from red cells by centrifugation, sedimentation in dextran and hypotonic hemolysis. From this point all procedures were carried out at 4 C. The white cells were then washed in 0.34 M sucrose, homogenized and a pellet containing both nuclei and granules was collected. This pellet was sonicated and the supernatant from a 78,000*g* 60-minute centrifugation was collected.[59] The enzyme preparation was applied to a diethylaminoethyl cellulose column, at pH 8.5, and eluted with a linear salt gradient.[57] The fractions containing activity were pooled, concentrated by ammonium sulfate precipitation, and applied to a column of Bio-Gel A-0.5M and eluted with Tris buffer, pH 7.8, containing 250 mM NaCl.[59] The active fractions were pooled and used for study. This partially purified material was able to hydrolyze 335 pmoles of colla-

gen per milligram of enzyme protein at pH 7.8 and 25 C per hour. The exact purification of our enzyme was impossible to calculate, since the starting material was unstable during assay conditions. Its elution position from the agarose column, between bovine serum albumin and ovalbumin, suggested a Stokes radius compatible with a molecular weight of approximately 60,000. The enzyme preparation did not hydrolyze purified insulin A chain, insulin B chain or carboxymethylated bovine pancreatic ribonuclease.

The collagenase was able to effect a 65% reduction in relative viscosity without a detectable change in optical rotation.[57] This indicated that the overall helical structure of the TC^A and TC^B pieces had been maintained. Electron micrographs of SLS formed from reaction products revealed fragments of 2750 and 2150 Å which suggests that the electron microscopic site of cleavage was identical to that of the other human collagenase (Figure 2).[57] In addition, Bauer *et al*[10] have shown that there is a line of complete identity between granulocyte extracts and highly purified human skin collagenase when immunodiffusion is performed with an antiserum to purified human skin collagenase.

The enzyme showed maximal activity against native collagen between pH 7 and pH 8.[57] It was 50% inactivated after 80 minutes at 37 C. Hydrolysis was markedly effected by temperature, and there was a 50% increase in velocity when the temperature was raised from 21 to 25 C.[59] Compounds with free sulfhydryl groups, such as cysteine and D-penicillamine, were markedly inhibitory. The addition of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) or *p*-chloromercuribenzoate (PCMB) markedly activated collagenase activity.[59] In recent experiments with a 150-fold purified rabbit skin collagenase, 1 mM *p*-chloromercuribenzoate increased collagenase activity by a factor of 2.[60]

Incubation of denatured collagen or gelatin with partially purified granulocyte enzyme produced a complex pattern on acrylamide gel electrophoresis. The TC^B fragment was extremely prominent but multiple bands suggest that denaturation exposes new sites for enzyme action.[59]

There are several significant differences between granulocyte collagenase and other reported human collagenases. First, purification of the granulocyte enzyme results in loss of ability to degrade collagen fibrils (Figure 1); this implies that specific cleavage of the collagen molecule into TC^A and TC^B fragments does not guarantee dissolution of collagen fibrils. The mechanism by which granulocytes

degrade collagen fibrils must therefore involve additional proteolytic activity. This impression is strengthened by observations with crude granulocyte extracts. Incubations with crude material result in extensive fibril lysis and complex patterns on acrylamide gel electrophoresis (Figure 1). The second, somewhat unique, property of the granulocyte enzyme is that it is not inhibited by human serum. The α2-macroglobulins and α1-antitrypsin inhibitor have been shown to be capable of inhibiting most collagenases,[61] with the exception of the heavy synovial fluid collagenase[20] and the granulocyte enzyme. The addition of serum to the crude granule preparation inhibits fibril lysis and simplifies the acrylamide gel electrophoresis pattern to that seen with partially purified granulocyte collagenase (Figure 1). Such a phenomenon suggests that serum is inhibiting the nonspecific proteases which work in association with collagenase to effect fibril lysis. Similar results can be obtained by adding the inactivated postgranule supernatant fraction from human polymorphonuclear leukocytes and 1 mM diisopropylfluorophosphate. There are, therefore, two distinctive systems involved in granulocyte-mediated collagenolysis (Table 1).

The specific collagenase, which cleaves collagen into TC^A and TC^B pieces, is inhibited by EDTA and cysteine. The nonspecific proteolytic system is inhibited by human serum inactivated postgranule supernatant or cytosol and DFP; it is partially inhibited, as judged by simplification of the acrylamide gel electrophoresis pattern towards

Table 1—Differentiation of Collagenolytic Systems in Human Granulocytes by the Use of Inhibitors*

	Specific collagenase inhibition		Nonspecific protease inhibition
Cysteine	10 mM	+	0
EDTA	10 mM	+	0
Human serum	7 %	0	+
Inactivated post-granule supernatant	400 μg	0	+
DFP	1 mM	0	+
Soya bean trypsin inhibitor	250 μg	0	partial
TLCK	0.3 mM	0	partial
TPCK	0.3 mM	0	partial
Pepstatin	25 μg	0	0

* Incubations included 800 μg of collagen, 400 μg crude granule protein, and listed final concentration of inhibitor in 600 μl of 50 mm Tris pH 7.0, containing 15 mM $CaCl_2$ + 325 mM NaCl, 20 hours at 25C. Effects studied by acrylamide gel electrophoresis.

that found with purified granulocyte collagenase, by soya bean trypsin inhibitor, tosylphenylalanyl chloromethylketone (TPCK) and tosyllysyl chloromethylketone (TLCK). Barrett and Dingle have shown that pepstatin is a potent new inhibitor of acid proteinases, including cathepsin D.[62,63] Complete inhibition of the 6 units[64] of cathepsin D/10^9 leukocytes, in our preparations, by pepstatin did not influence collagen degradation. This is explained by the data of Harris *et al*[65] and our own,[66] which demonstrate that cathepsin D is only capable of cleaving the cross-link area of denatured collagen and does not hydrolyze the peptide chain.

The leukocyte postgranule supernatant fraction or cytosol does not inhibit the specific collagenase. Although cytosol itself is incapable of degrading collagen fibrils, its addition (750 μg/ml final concentration) to a 150-fold, purified, rabbit-skin collagenase preparation caused fibril lysis to double from 40 to 80% (Table 2).[60] This facilitation could be prevented by the addition of 1 mM diisopropyl flourophosphate. The cytosol, therefore, contains an enzyme which assists collagenase in degrading collagen fibrils. If this cytosol helper-enzyme is inactivated by incubation with 1 mM diisorpropyl fluorophosphate, or by heating to 75 C for 20 minutes, the addition of cytosol to crude granule preparations inhibits the nonspecific protease system, and a pattern of specific TC^A and TC^B pieces is seen on acrylamide gel electrophoresis (Figure 1). The cytosol, therefore, contains an inhibitor of the nonspecific protease system found in, granules, as well as an enzyme capable of facilitating collagen-degradation by specific collagenase. The nonspecific granule protease system, therefore, has striking similarities to the granulocyte elastase system described by Janoff.[2,67,68] It also has some characteristics in

Table 2—Effect of Human Granulocyte Cytosol on the Fibril Degrading Activity of Partially Purified Rabbit Skin Collagenase

Preparation	Fibril lysis (%)
Cytosol	4
Skin collagenase	44
Skin collagenase + DFP 1 mM	44
Skin collagenase + cytosol	80
Skin collagenase + cytosol + DFP 1 mM	41
Trypsin 25 μg	4

Incubations included 200 μg of reconstituted collagen fibrils, 12.5 μg of skin collagenase protein, 7.5 μg of cytosol protein, 1 mM DFP final concentration, in 300 μl of 50 mM Tris buffer pH 7.4 containing 5 mM $CaCl_2$ and 100 mM NaCl, 20 hours at 37 C.

common with the neutral proteinase found in rabbit leukocytes by Davies *et al.*[69]

The specific collagenase, which hydrolyses collagen to TC^A and TC^B fragments, is an essential part of the mechanism by which granulocytes degrade collagen. When crude granulocyte preparations are incubated with 1 mM *p*-chloromercuribenzoate, the partially purified granulocyte collagenase is activated and collagen fibril lysis is increased by 50 to 100%. Inhibition of collagenase by the addition of 10 mM cysteine or 10 mM EDTA decreases fibril lysis by approximately 50%.

Recently, we have used very fresh granule preparations, washed to remove cytosol inhibitor. These preparations have been more active than previous ones, and they were capable of degrading over 30% of our collagen fibrils. The addition of cysteine and EDTA in quantities sufficient to totally inhibit granulocyte collagenase inhibited fibril lysis by only 50%. Furthermore, acrylamide gel electrophoresis patterns, obtained from incubations of collagen solution with crude granulocyte preparations in which collagenase had been inhibited by EDTA and cysteine (pH 7.0, 25 C), revealed β to α conversion and multiple bands. This pattern indicates that the nonspecific protease has cleaved the cross-link area and partially degraded the collagen chain. These data suggest that the nonspecific protease system might be capable of limited, independent collagen hydrolysis. Such a possibility is strengthened by Janoff's observation that the granulocyte elastase complex can solubilize bovine achilles tendon collagen.[2] Further studies with purified enzymes should clarify the role of the nonspecific system in collagen degradation.

The mechanisms by which leukocytes degrade collagen are quite complex. The granule fraction of granulocytes contain a specific collagenase which cleaves collagen in solution into the characteristic TC^A and TC^B fragments, but is unable to solubilize collagen fibrils. The specific granulocyte collagenase is inhibited by EDTA and cysteine, but not by serum, cytosol or diisopropyl fluorophosphate. Granulocytes also contain a protease system which is inhibited by serum, inactivated cytosol and diisopropyl fluorophosphate. This protease(s) is capable of degrading specific products of collagenase activity, the TC^A and TC^B fragments, and may also be capable of limited independent collagen hydrolysis. Both specific collagenase and nonspecific protease are necessary for maximal collagen degradation. In addition, noncollagenase granulocyte proteases, such as Cathepsin D, might facilitate collagen catabolism by degrading the

matrix glycosaminoglycans and glycoproteins, thus expediting contact between the granulocyte collagenolytic system and collagen fibrils. The granulocyte, with its specific collagenase and its complement of other proteases, is admirably suited to the complex task of collagen catabolism.

Summary

The granule fraction of human granulocytes contains a specific collagenase which cleaves collagen molecules into characteristic TC^A and TC^B fragments. This specific collagenase is incapable of solubilizing collagen fibrils. Leukocyte granules also contain a second protease system capable of degrading specific products of collagenase activity and also may be capable of limited independent collagen hydrolysis. Both the specific collagenase and the nonspecific protease are necessary for maximal collagen fibril degradation.

References

1. Cochrane CG, Aikin BS: Polymorphonuclear leukocytes in immunologic reactions. The destruction of vascular basement membrane *in vivo* and *in vitro*. J Exp Med 124:733–752, 1966
2. Janoff A: Mediators of tissue damage in leukocyte lysosomes. X. Further studies on human granulocyte elastase. Lab Invest 22:228–236, 1970
3. Gross J, Lapiere CM: Collagenolytic activity in amphibian tissues: a tissue culture assay. Proc Nat Acad Sci USA 48:1014–1022, 1962
4. Fullmer HM, Lazarus GS, Gibson WA, Stam AC: Collagenolytic activity of the skin associated with neuromuscular disease. Lancet 1:1007–1009, 1966
5. Riley WB, Peacock EE: Identification, distribution and significance of collagenolytic enzymes in human tissues. Proc Soc Exp Biol Med 124:207–210, 1967
6. Eisen AZ, Jeffrey JJ, Gross J: Human skin collagenase. Isolation and mechanism of attack on the collagen molecule. Biochim Biophys Acta 151:637–645, 1968
7. Eisen AZ: Human skin collagenase: localization and distribution in normal human skin. J Invest Dermatol 52:442–448, 1969
8. Lazarus GS, Fullmer HM: Collagenase production by human dermis *in vitro*. J Invest Dermatol 52:545–547, 1969
9. Eisen AZ, Bauer EA, Jeffrey JJ: Animals and human collagenase. J Invest Dermatol 55:359–374, 1970
10. Bauer EA, Eisen AZ, Jeffrey JJ: Immunologic relationship of a purified human skin collagenase to other human and animal collagenases. Biochim Biophys Acta 206:152–160, 1970
11. Eisen AZ: Human skin collagenase: relationship to the pathogenesis of epidermolysis bullosa dystrophica. J Invest Dermatol 52:449–453, 1969
12. Lazarus GS: Collagenase and connective tissue metabolism in epidermolysis bullosa. J Invest Dermatol 58:242–248, 1972

13. Donoff RB, McLennan JE, Grillo HC: Preparation and properties of collagenases from epithelium and mesenchyme of healing mammalian wounds. Biochim Biophys Acta 227:639–656, 1971
14. Eisen AZ, Bauer EA, Jeffrey JJ: Human skin collagenase: the role of serum alpha globulins in the control of activity *in vivo* and *in vitro*. Proc Nat Acad Sci USA 68:248–251, 1971
15. Evanson JM, Jeffrey JJ, Krane SM: Human collagenase. Identification and characterization of enzyme from rheumatoid synovium in culture. Science 158:499–502, 1967
16. Evanson J, Jeffrey JJ, Krane SM: Studies on collagenase from rheumatoid synovium in tissue culture. J Clin Invest 47:2639–2651, 1968
17. Lazarus GS, Decker JL, Oliver CH, Daniels JR, Multz CV, Fullmer HM: Collagenolytic activity of synovium in rheumatoid arthritis. N Engl J Med 279:914–919, 1968
18. Harris JR, Evanson JM, Dibona DR, Krane SM: Collagenase and rheumatoid arthritis. Arthritis Rheum 13:83–94
19. Bauer EA, Eisen AZ, Jeffrey JJ: Studies on purified rheumatoid synovial collagenase *in vivo* and *in vitro*. J Clin Invest 50:2056–2064, 1971
20. Harris ED, Dibona DR, Krane SM: Collagenases in human synovial fluid. J Clin Invest 48:2104–2113, 1969
21. Jeffrey JJ, Gross JJ: Collagenase from rat uterus. Isolation and partial characterization. Biochemistry 9:268–273, 1970
22. Walker DG, Lapiere CM, Gross J: Collagenolytic factor in rat bone promoted by parathyroid extract. Biochem Biophys Res Commun 15:397–402, 1964
23. Kaufman EJ, Glimcher MJ, Mechanic GL, Goldhaber P: Collagenolytic activity during active bone resorption in tissue culture. Proc Soc Exp Biol Med 120:632–636, 1965
24. Fullmer HM, Lazarus GS: Collagenase in human, goat and rat bone. Israel J Med Sci 3:758–761, 1967
25. Fullmer HM, Lazarus GS: Collagenase in bone of man. J Histochem Cytochem 17:793–798, 1969
26. Shimizu M, Glimcher MJ, Travis D, Goldhaber P: Mouse bone collagenase: isolation, partial purification, and mechanism of action. Proc Soc Exp Biol Med 130:1175–1180, 1969
27. Fullmer HM, Gibson W: Collagenolytic activity in gingivae of man. Nature 209:728, 1966
28. Fullmer HM, Gibson WA, Lazarus GS, Bladen HA, Whedon KA: The origin of collagenase in periodontal tissues in man. J Dent Res 48:646–651, 1969
29. Fullmer HM: Metabolic hydrolysis of collagen, Metabolic Hydrolysis and Metabolic Conjugation. Vol 2. Edited by W Fishman. New York, Academic Press, Inc, pp 301–343, 1970
30. Eisen AZ, Jeffrey JJ: An extractable collagenase from crab hepatopancreas. Biochim Biophys Acta 191:517–525, 1969
31. Abramson M, Gross J: Further studies on a collagenase in middle ear cholesteotoma. Ann Otol Rhinol Laryngol 80:177–188, 1971
32. Itoi M, Gnädinger C, Slansky HH, Freeman MI, Dohlman CH: Collagenase in the cornea. Exp Eye Res 8:369–373, 1969

33. Slansky HH, Gnädinger MC, Itoi M, Dohlman CH: Collagenase in corneal ulcerations. Arch Opthalmol 82:108–111, 1969
34. Brown SI, Weller CA, Wasserman HE: Collagenolytic activity of alkali-burned corneas. Arch Opthalmol 81:370–373, 1969
35. Perez-Tamayo R: Collagen resorbtion in carageenin granuloma. Lab Invest 22:137–159, 1970
36. Piez KA: Soluble collagen and denaturation components, Treatise on Collagen. Vol 1. Edited by GN Ramachandran. New York, Academic Press, Inc, pp 207–243, 1967
37. Miller EJ: Isolation and characterization of a collagen from chick cartilage containing three identical α chains. Biochemistry 10:1652–1660, 1971
38. Miller EJ, Epstein EH, Piez KA: Identification of three genetically distinct collagens by cyanogen bromide cleavage of insoluble human skin and cartilage collagen. Biochem Biophys Res Commun 42:1024–1029, 1971
39. Pinnell SR, Martin GA: The cross linking of collagen and elastin: enzymatic conversion of lysine in peptide linkage to alpha-aminoadipic delta-semialdehyde (allysine) by an extract from bone. Proc Nat Acad Sci USA 61:708–716, 1968
40. Kühn K, Zimmerman BK: Renaturation of soluble collagen. IV. Regeneration of native collagen molecules from α subunits. Arch Biochem Biophys 109:534–541, 1965
41. Martin GR, Mecca CE, Piez KA: Environmental Variables in Oral Disease. Edited by SJ Kreshover, FJ McLure. Washington, DC, American Association for the Advancement of Science, p 155, 1966
42. Drake MP, Davison PF, Bump S, Schmitt FO: Action of proteolytic enzymes on tropocollagen and insoluble collagen. Biochemistry 5:301–321, 1966
43. Kühn K, Braumer K, Zimmerman B, Pikkarainen J: The formation and structure of insoluble collagen, Chemistry and Biology of the Intercellular Matrix. Vol 1. Edited by EA Balazs. New York, Academic Press, Inc, 1970, pp 251–273
44. Bailey AJ, Peach CM, Fowler LJ: The biosynthesis of intermolecular cross-link in collagen.[43] pp 385–404, 1970
45. Seifter S, Harper E: The collagenases, The Enzymes. Vol 3. Edited by PD Boyer. New York, Academic Press, Inc, 1971, pp 649–698
46. Heidrich HG, Prokopova D, Hanning K: The use of synthetic substrates for the determination of mammalian collagenase. Is collagenolytic activity present in mitochondria. Hoppe-Seylers Z Physiol Chem. 350:1430–1436, 1969
47. Harper E, Gross J: Separation of collagenase and peptidase activities of tadpole tissue in culture. Biochem Biophys Acta 198:286–292, 1970
48. Evanson JM: Mammalian collagenases and their role in connective tissue breakdown, Tissue Proteinases. Edited by AJ Barrett, JT Dingle. Amsterdam, North Holland Publishing, 1971, pp 327–345
49. Gross J, Nagai Y: Specific degradation of the collagen molecule by tadpole collagenase. Proc Nat Acad Sci USA 54:1197–1204, 1965
50. Nagai Y, Lapiere CM, Gross J: Tadpole collagenase: preparation and purification. Biochemistry 5:3123–3130, 1966
51. Kang AH, Nagai Y, Piez KA, Gross J: Studies on the structure of collagen using collagenolytic enzyme from tadpole. Biochemistry 5:509–515, 1966

52. Sakai T, Gross J: Some properties of reaction of tadpole collagenase with collagen. Biochemistry 6:518–528, 1967
53. Harper E, Bloch KJ, Gross J: The zymogen of tadpole collagenase. Biochemistry 10:3035–3041, 1971
54. Lazarus GS, Brown RS, Daniels JR, Fullmer HM: Human granulocyte collagenase. Science 159:1483–1485, 1968
55. Cohn ZA, Hirsch JG: The isolation and properties of the specific cytoplasmic granules of rabbit polymorphonuclear leucocytes. J Exp Med 112:983–1022, 1960
56. Lazarus GS: Studies on the degradation of collagen by collagenases, The Lysosomes. Vol 3. Edited by JT Dingle. Amsterdam, North Holland Publishing Co (In press)
57. Lazarus GS, Daniels JR, Brown RS, Bladen HA, Fullmer HM: Degradation of collagen by a human granulocyte collagenolytic system. J Clin Invest 47:2622–2629, 1968
58. Daniels JR, Lian J, Lazarus GS: Granulocyte collagenase preparation and kinetic studies. Clin Res 17:154, 1969
59. Daniels JR, Lian J, Lazarus GS: Granulocyte collagenase purification and kinetic studies. J Biol Chem (In press)
60. Burleigh PCM, Lazarus GS, Reynolds JE: Physiological role of rabbit skin collagenase in collagen degradation. Unpublished data
61. Eisen AZ, Bloch KJ, Sakai T: Inhibition of human skin collagenase by human serum. J Lab Clin Med 75:258–263, 1970
62. Barrett AJ, Dingle JT: The inhibition of tissue acid proteinases by pepstatin. Biochem J (In press)
63. Dingle JT, Barrett AJ, Poole AR, Stovin P: Inhibition by pepstatin of human cartilage degradation. Biochem J (In press)
64. Barrett AJ: Cathepsin D. Purification of isoenzymes from human and chicken liver. Biochem J 117:601–607, 1970
65. Harris ED, Locke S, Lin TY: Limited degradation of gelatin substrate by Cathepsin D. Connective Tissue Research, Vol 1. (In press)
66. Burleigh PCM, Lazarus GS, Reynolds J, Barrett AJ: The effect of lysosomal proteinases on collagen and gelatin. Unpublished data
67. Janoff A, Scherer J: Mediators of inflammation in leukocyte lysosomes IX. Elastinolytic activity in granules of human polymorphonuclear leukocytes. J Exp Med 128:1137–1151, 1968
68. Janoff A, Blondin J: Further studies on an esterase inhibitor in human leukocyte cytosol. Lab Invest 25:565–571, 1971
69. Davies P, Rita GA, Krakauer K, Weissmann G: Characterization of a neutral protease from lysosomes of rabbit polymorphonuclear leukocytes. Biochem J 123:559–569, 1971

Acknowledgments

We gratefully acknowledge the support of the Medical Research Council for MCB. We also thank C. C. Entwistle, MB, ChB, MRC Pathol, of the East Anglia Regional Transfusion and Immunohematology Center for providing us with human granulocytes. Drs. J. J. Reynolds, J. T. Dingle and A. J. Barrett provided encouragement and much useful discussion.

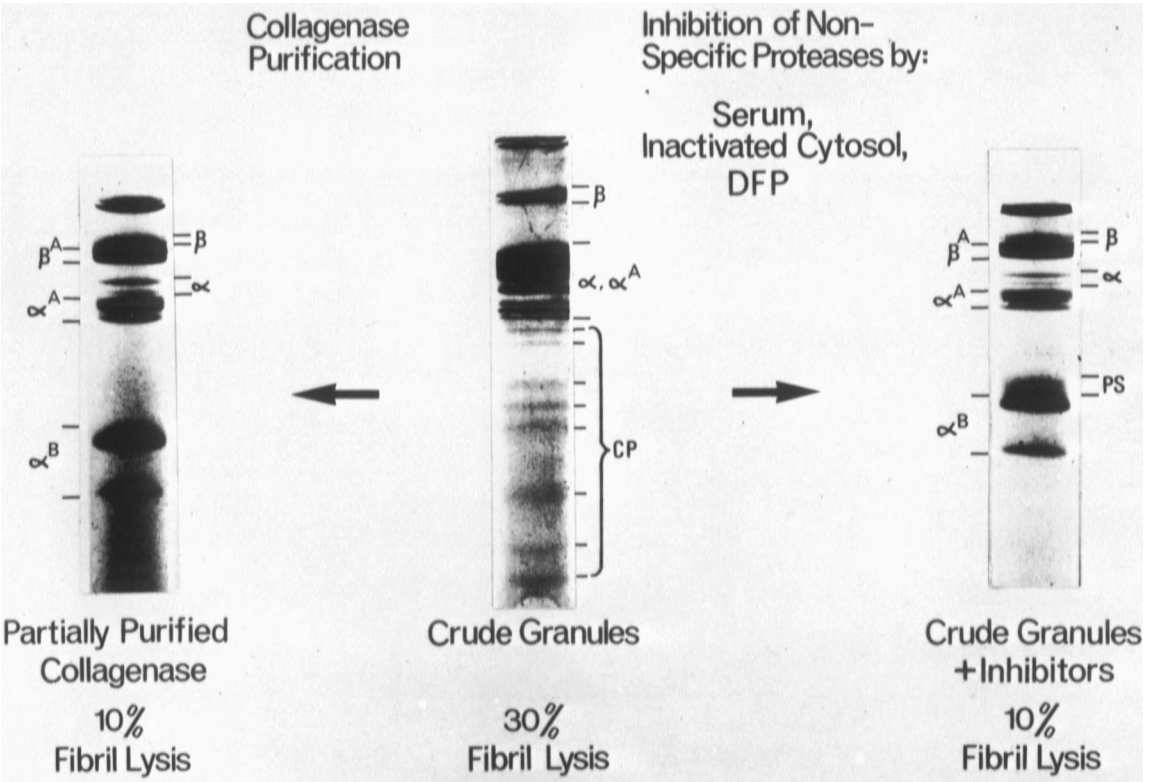


Fig 1—The mechanisms by which human leukocytes degrade collagen as studied by acrylamide gel electrophoresis and reconstituted collagen fibril assays. In the center is crude granulocyte extract, on the left partially purified granulocyte collagenase and on the right crude granule extract with either 7% human serum, 400 μg inactivated post-granule supernatant protein or 1 mM DFP. Percent fibril lysis is after 20 hours incubation at 37 C; the acrylamide gel electrophoresis patterns are after 20 hours at 25 C. α = monomeric chain; β = dimeric chain; *superscript A* = N-terminal three-quarters cleavage product; *superscript B* = C-terminal cleavage product; *PS* = protein smudge seen with serum and cytosol; *CP* = collagen peptides.

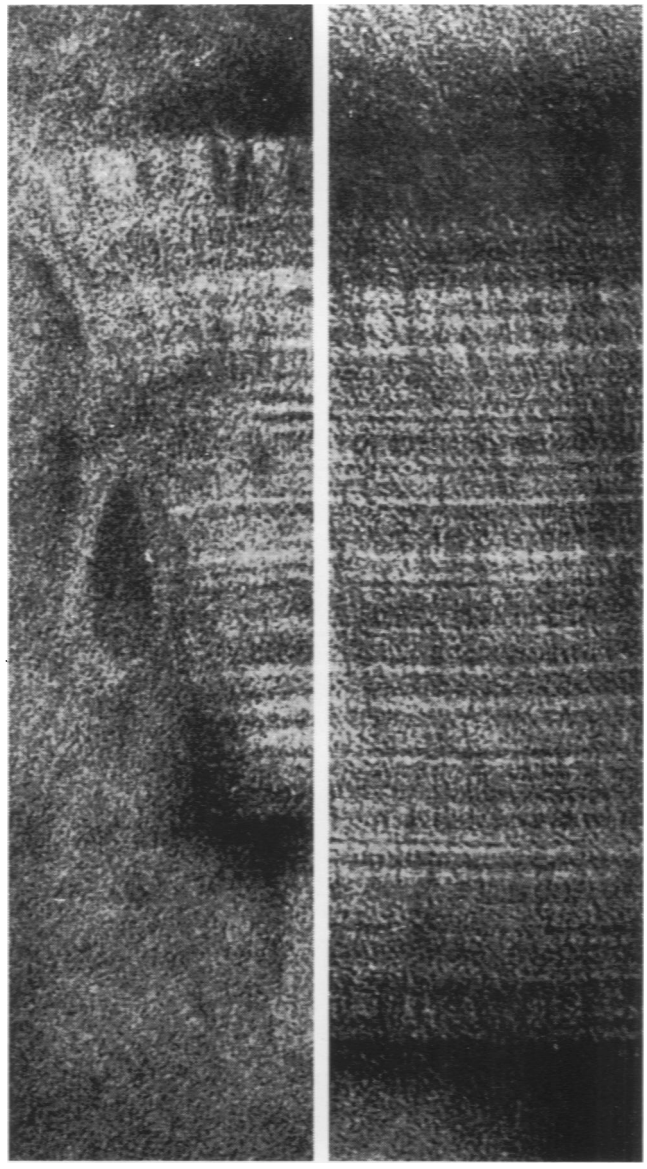


Fig 2—Comparison of SLS aggregates from collagen incubated with partially purified granulocyte collagenase (*left*) and heat-inactivated granulocyte collagenase (*right*). The SLS formed after incubation with active enzyme is missing the carboxy terminal one-quarter of the molecule (From Lazarus *et al*, 1968[57]).