# Human Granulocyte Elastase

## *Further Delineation of its Role in Connective Tissue Damage*

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THE PRECEDING PAPER by Dr. Lazarus and his colleagues [1] reviewed work on granulocyte collagenase and the collagen-degrading system of the neutrophil. A second fibrous protein of connective tissue, elastin, can be recognized as another potential target for neutrophil-mediated proteolysis, since it was found, in our laboratory, that human polymorphonuclear leukocytes (PMN) contain an elastase as well as the collagenase described before. The present paper deals with our studies of human granulocyte elastase. Initially, I will review, in summary fashion, some of the chief characteristics of the enzyme and its interaction with inhibitors. I will then present some previously unpublished preliminary findings which may offer an approach to further delineating the role of granulocyte elastase in connective tissue damage.

The first point to make is that the two known human granulocyte enzymatic activities (collagenase and elastase), directed against fibrous connective tissue proteins, can be separated by anion-exchange chromatography of leukocyte extracts, using methods previously described by Dr. Lazarus.[2] As he indicated, human leukocyte extract passed through diethylaminoethyl (DEAE)-cellulose in 0.01 M Tris-HCl buffer at pH 8.6 (4 C) separates cationic proteins, which elute from the resin with the starting buffer, from acidic proteins which, under these conditions, bind to the anion-exchanger. A subfraction of the bound proteins can be subsequently eluted with Tris buffer containing 0.045 to 0.075 M NaCl and 0.002 M $CaCl_2$, and was reported by Lazarus to contain the specific collagen-monomer cleaving enzyme of the starting extract.[2] This same fractionation step was utilized in our early experiments, except that discontinuous, rather than continuous, salt elution was employed. The resultant fractionation revealed hydrolytic activity directed against orcein-dyed and undyed bovine ligament elastin, *tert*-butyloxycarbonyl

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(*t*-BOC)-L-alanine-*p*-nitrophenyl ester (a moderately specific synthetic elastase substrate) and denatured hemoglobin in the cationic proteins of the leukocyte extract. None of these activities were detectable in the acidic proteins of the leukocyte extract eluted by increasing the concentration of NaCl. These results were reported earlier.[3,4]

Cationic elastase activity is first localized to PMN (as opposed to lymphocytes and monocytes) and then more specifically to the granule (lysosome-rich) fraction of the cells.[5] Table 1 summarizes the data from which these conclusions are drawn. Recently, other work has shown that human and rabbit alveolar macrophages [6] and human platelets [7] may be additional extrapancreatic sources of elastase-like enzymes in the body.

Enzymatic activity of PMN granules against dyed elastin is maximal at slightly alkaline pH,[5] decreases with increasing acidity but remains appreciable in the pH range associated with inflammatory exudates (gross measurements)—*ie*, at or slightly below neutrality.[8,9]

Human granulocyte elastin-digesting activity is partially suppressed by soybean inhibitor and salivary gland kallikrein-inactivator;[5] these properties readily distinguish it from porcine pancreatic elastase, which is not affected by these agents. Table 2 compares the inhibition profiles of the pig pancreatic enzyme and the human PMN agent and provides additional evidence of the nonidentity of these enzymes. The fact that 0.01 M $Na_2EDTA$ does not inhibit the PMN agent is especially significant, since this further distinguishes granulocyte elastase from granulocyte collagenase and from porcine

Table 1—Demonstration of Elastase Activity in Human PMN

| Enzyme source | Concentration protein tested (μg/ml) | Percent elastolysis |
|---|---|---|
| Trypsin | 25 | 0 |
| α-Chymotrypsin | 25 | 0 |
| Elastase | 25 | 100 |
| Human leukocyte homogenate | | |
| Granules | 500 | 100 |
| Nuclei | 500 | 15 |
| Microsomes and cell sap | 500 | 6 |
| Human leukocyte homogenate previously depleted of PMN by nylon-wool extraction | | |
| Granules | 500 | 7 |

100% arbitrarily selected as the amount of dye released from orcein-impregnated elastin by 25 μg of pancreatopeptidase E (3.4.4.7) under standard incubation conditions. (Further details of methods are given in the primary references cited in the text.)

Table 2—Effects of Inhibitors on PMN and Pancreatic Elastases

| Agent | Concentration | Percent inhibition of elastolysis* or esterolysis† by PMN granules | Percent inhibition of elastolysis* or esterolysis† by Pancreatopeptidase E (EC 3.4.4.7) |
|---|---|---|---|
| NaCl (M) | 0.15 | 0 | 70 |
| $Na_2EDTA$ (M) | 0.01 | 0 | 54 |
| SBTI (μg/ml) | 500 | 50 | 0 |
| Trasylol (u/ml) | 2000 | 50 | 0 |
| DPBB (M)† | 0.00025 | 0 | 50 |
| Human serum (%) | 0.5 | 0 | 55 |
| Human serum (%) | 2 | 10 | 100 |
| Human serum (%) | 5 | 25 | 100 |
| Human serum (%) | 10 | 57 | — |

* Substrate was orcein-dyed ligament elastin (all agents except DPBB).

† Substrate was t-BOC-L-alanine-p-nitrophenol.

Amounts of each enzyme were selected to give approximately equivalent elastolysis or esterolysis in the absence of inhibitor. (Further details of methods are given in the primary references cited in the accompanying text.) SBTI = soybean trypsin inhibitor; trasylol = salivary gland kallikrein inactivator; DPBB = 1-bromo-4-(2, 4-dinitrophenyl)-butan-2-one.

elastase. Until quite recently, difficulties in purifying human pancreatic elastase-like enzymes made it impossible to carefully compare factors responsible for the elastolytic activities of PMN and pancreas in the same species. However, some of the properties of a human pancreatic elastase-like esterase have now been described,[10] and, while many of its characteristics are shared by the PMN agent, the two enzymes differ radically in their isoelectric points. As will be demonstrated shortly, the isoelectric pH of human granulocyte elastase-like esterase, like that of porcine pancreatic elastase, is highly alkaline, whereas human pancreatic elastase-like esterase is reported to have a much lower isoelectric point.[10] Thus, the elastase-like esterase activity of human PMN is not likely to result from contamination of leukocyte preparations with a circulating elastase-like esterase derived from human pancreas.

Table 2 also shows that human granulocyte elastase is inhibited by serum, although higher concentrations of the latter are needed than are required for comparable inhibition of porcine pancreatic elastase. We [11] and Ohlsson [12] have recently demonstrated that the serum or plasma inhibition of human granulocyte elastase is largely due to the α1-antitrypsin component. The data from my laboratory suggest that, at their relative molar concentrations in serum, α1-antitrypsin is an important inhibitor of granulocyte elastase, whereas

α2-macroglobulin (plasmin inhibitor) appears to be less important (Table 3). In interpreting the data in Table 3 based on the inhibition of esterolysis by the two plasma inhibitors, it is worth noting that complexes of α2-macroglobulin with *pancreatic* elastase have recently been reported to retain esterase activity against low molecular weight substrates.[13] The demonstration of an interaction between granulocyte elastase and α1-antitrypsin [11,12] supports the speculation that this protease, as well as other neutral leukocyte proteases which form complexes with this inhibitor,[12] plays a role in connective

Table 3—Inhibition of Human Polymorphonuclear Leukocyte Elastase by Serum and Serum Fractions

Elastolytic Activity of Polymorphonuclear Leukocyte Granules*

| Serum type | TIC (mg/ml) | Concentration (%) | Elastolysis inhibition (%) |
|---|---|---|---|
| PiMM | 1.47 | 3 | 50 |
| | | 10 | 100 |
| PiMM | 1.25 | 5 | 50 |
| | | 7.5 | 75, 100 |
| PiZZ | 0.15 | 3 | 0 |
| | | 10 | 38 |
| PiZZ | 0.19 | 5 | 0 |
| | | 7.5 | 0 |
| PiZZ | 0.26 | 7.5 | 24 |

Esterolytic Activity of Polymorphonuclear Granules†

| Serum fraction | Concentration (mg/100 ml) | Esterolysis inhibition (%) |
|---|---|---|
| α1-Antitrypsin | 38 | 88 |
| α1-Antitrypsin | 25 | 84 |
| α1-Antitrypsin | 13 | 30 |
| α2-Macroglobulin | 23 | 28 |
| α2-Macroglobulin | 15 | 12 |
| α2-Macroglobulin | 8 | 0 |

* Elastin-orcein substrate; 2-hour incubation, pH 8.7, 37 C, 240 μg/ml granule protein per assay. TIC = trypsin inhibiting capacity.

† t-BOC-L-alanine-p-nitrophenol substrate; 5-minute incubation, pH 6.5, 25 C, 12 μg granule protein/ml per assay.

PiZZ sera were kindly supplied by Dr. Morton Galdston, New York University School of Medicine, New York, NY. Purified human α1-antitrypsin (Behringwerke AG) was supplied by Dr. Fritz Beller, New York University School of Medicine, NY. Purified human α2-macroglobulin was supplied by Dr. Frederick Miller, Health Sciences Center, State University of New York at Stony Brook, NY.

Reprinted from Janoff A.[11]

tissue degenerations (*eg*, of elastic fibers) associated with genetic states leading to deficiencies in serum α1-antitrypsin. One such possibility, currently under investigation, is familial emphysema, in which inherited abnormalities in α1-antitrypsin occur with unusually high frequency. Preliminary evidence favors the view that granulocyte elastase levels may be a second determinant in the clinical course of this disease.[14]

An interesting feature of our inhibition studies with granulocyte elastase (although perhaps of less clinical relevance than those involving α1-antitrypsin) concerns the finding of an inhibitor of the enzyme in the cytosol fraction (microsome-free supernatant) of the same cells.[15,16] Inhibition is relatively specific, in that esterolytic activity of trypsin and pancreatic elastase is unaffected under identical conditions. This specificity suggests that the intracellular inhibitor is distinct from α1-antitrypsin of human serum. Ion-exchange chromatography on carboxymethyl cellulose at pH 6.0 reveals that the inhibitor is an acidic component of cytosol. RNA can be ruled out, since the incubation of yeast RNA (which also inhibits granulocyte elastase in high concentrations) with pancreatic ribonuclease and spleen phosphodiesterase results in nearly complete degradation of the RNA with a concomitant loss of inhibiting capacity; identical enzymatic treatment of cytosol followed by prolonged dialysis does not lead to loss of its inhibiting capacity. On the other hand, Pronase destroys most of the inhibitory activity, as does 0.4 M trichloroacetic acid. However, inhibitory activity is not diminished by heating to 80 C for 20 minutes. Some of these data are reviewed in Table 4. Molecular sieving through Sephadex and Agarose gels and Diaflo membranes suggests inhibitory activity is associated with high molecular weight components of cytosol. It is tempting to speculate that intracellular inhibitors, of the type affecting granulocyte elastase, function to protect the cell against leakage of lysosomal proteases active at neutral pH. Leakage may occur from secondary lysosomes (*eg*, phagolysosomes) and could be accelerated in leukocytes engaged in phagocytosis. On the other hand, for several reasons,[16] it seems unlikely that the intracellular inhibitor would constitute an effective extracellular antagonist of damage to connective tissue elastin by human leukocyte elastase. Instead, as indicated earlier, serum inhibitors may play the more significant protective role in the extracellular environment. Recently, a several-times more potent inhibitor of the neutrophil elastase, with physicochemical properties

Table 4—Effects of Various Treatments Upon Inhibitor Activity of Leukocyte Cytosol*

| Material treated† (mg) | Treatment (concentration of agent) | Incubation time and temperature‡ | Loss of inhibition (%) | Loss of starting material§ (%) |
|---|---|---|---|---|
| Cytosol concentrate (0.85) | Pronase (10μg/ml) | 3 hr 37 C | 64 | 86 |
| Cytosol concentrate (0.85) | Pronase (10μg/ml) | 3 hr 37 C | 58 | 83 |
| Cytosol concentrate (0.85) | RNase (50μg/ml) and phosphodiesterase (0.34 U/ml) | 3 hr 37 C | 0 | NT |
| Yeast RNA (1.0) | RNase (50μg/ml) and phosphodiesterase (0.34 U/ml) | 3 hr 37 C | 100 | 85 |
| Cytosol concentrate (0.32) | Heat | 20 min 80 C | 0 | NT |
| Cytosol concentrate (0.85) | TCA (0.4M) | 1 hr 20 C | 100 | 96 |

* Inhibition of t-BOC-L-alanine-p-nitrophenol esterolysis by leukocyte granules.

† Represents protein in the case of cytosol concentrate.

‡ Except after heating, all treatments were followed by 48 hours of dialysis versus three changes of 0.01 M sodium phosphate-buffered saline (pH 7) at 20 C. In the case of tri chloroacetic acid (TCA) treatment, the precipitate was first discarded and the supernatant was dialyzed.

§ Loss of cytosol protein or yeast RNA to TCA precipitate or to dialysate after enzyme treatment.

NT = not tested.

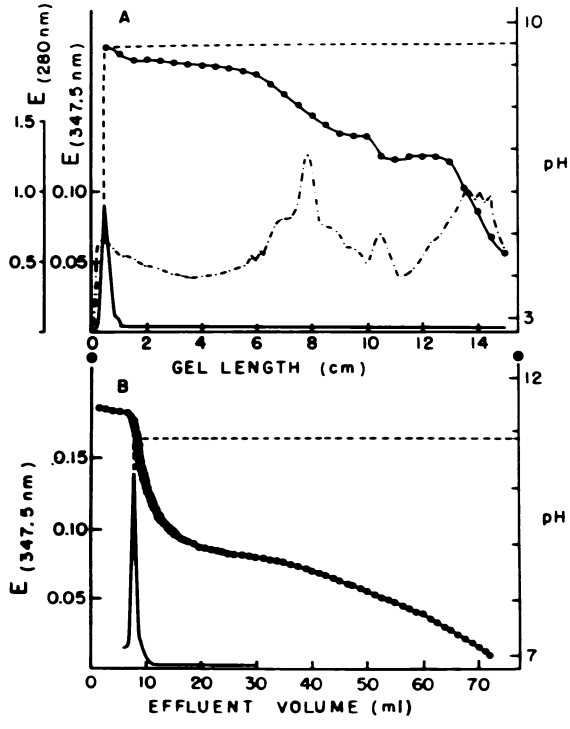
Reprinted from Janoff A, Blondin J. [16]

essentially similar to those described above, was demonstrated in cytosol fractions from human alveolar macrophages.[17]*

I now wish to shift the discussion to more recent observations which, although preliminary in nature, deal with a question of greater interest for this symposium—*ie,* delineating the role of human granulocyte elastase in connective tissue damage. If the cationic proteins of the leukocyte granule extract, separated by DEAE-chromatography as described earlier, are rechromatographed in phosphate-buffered saline (pH 7) on a 1.5 × 90 cm column of Sephadex G50, the proteins will be separated further into three fractions.[3] Hydrolytic activities at neutral pH against dyed-elastin (fivefold enrichment), denatured hemoglobin and *t*-BOC-L-alanine-*p*-nitrophenol are all restricted to the second (first retarded) peak. When this material is reconcentrated and again analyzed by iso-

*Folds JD, Welsh IRH, Spitznagel JK: Personal communication (and recent confirmatory data from our laboratory) suggest that the subcellular localization of the elastase inhibitor, which we found in PMN cytosol fractions, may in reality be the nucleus. This possibility is strengthened by virtue of the fact that many of the latter structures appear to be broken during homogenization of PMN by our technics.

electric-focusing in polyacrylamide gel,[18] it becomes apparent that the activity against *t*-BOC-L-alanine-*p*-nitrophenol focuses at an alkaline isoelectric point (9.5), approximately that exhibited by porcine (but not human) pancreatic elastase. At the same time, it is also evident that at least three other protein species are present in the Sephadex fraction (see Text-figure 1, panel A). This degree of heterogeneity renders it impossible to ascribe specific forms of connective tissue damage, by the fraction enriched in elastase, to any single enzyme, except that granulocyte collagenase can probably be considered to have been eliminated by the earlier anion-exchange step. The elastase-enriched Sephadex fraction vigorously degrades human renal basement membrane[3] and cartilage matrix proteoglycan[19] (as does pancreatic elastase, in the latter case), but these actions cannot be unequivocally ascribed to the contained elastase. In addition, whole granule extracts attack arterial walls[3,5] and human lung elastin;[20] such activities would appear to be logical reflections of the presence of elastase, but proof that this assumption is valid is still lacking. Moreover, Ohlsson[12] demonstrated that α1-antitrypsin forms two distinctly separate complexes with components




TEXT-FIG 1—Isoelectric focusing of leukocyte granule protein (Sephadex fraction) in acrylamide gel (*A*) and sucrose (*B*). Alanine *p*-nitrophenyl esterase activity measured as absorbance of free *p*-nitrophenol at 347.5 nm (——); protein measured as absorbance at 280 nm (–•–); pH values in the gel or effluent fractions (•); cathode (–); anode (+). (Reprinted from Janoff A, Basch RS).[18]

of human PMN granules, one of which corresponds to a complex with elastase and the other to a complex with a second and different neutral protease. Ohlsson's elastase behaves as a strongly basic protein, while his second granulocyte neutral protease behaves as an acidic protein at pH 8.6 (and may be eliminated along with granulocyte collagenase in the DEAE-chromatography step we routinely employ). Other recent work [21] argues that neutral proteolytic activity against denatured hemoglobin and elastin and esterolytic activity against *t*-BOC-L-alanine-*p*-nitrophenol are present in a single class of human PMN granules and cannot be separated by several physicochemical technics. Distinctions can be made, however, on the basis that neutral proteolytic activity against denatured hemoglobin requires cysteine-activation, while activities against elastin and *t*-BOC-L-alanine-*p*-nitrophenol do not.[21]

In order to approach the question of the precise role of granulocyte elastase in tissue injury mediated by PMN lysosomes, we recently adopted the use of specific elastase inhibitors. These compounds are chloromethyl ketone derivatives of a highly specific synthetic elastase substrate, N-acetyl-L-alanyl-L-alanyl-L-alanine-methyl ester, first described for pancreatic elastase by Gertler and Hofmann [22] and then for human PMN granules in our laboratory.[18] Rates at which this substrate is hydrolyzed by PMN granules and pancreatic elastase are compared in Table 5. The chloromethyl ketone derivatives of polyalanine esters were synthesized by Drs. James C. Powers and

Table 5—Hydrolysis of N-Acetyl-L-alanyl-L-alanyl-L-alanine Methyl Ester by PMN Granules and Pancreatic Elastase

| Enzyme | Amount tested (μg protein) | Hydrogen ion liberated* (μmoles/min) |
|---|---|---|
| Pancreatopeptidase E | 2.5 | 0.28 |
| | 5.0 | 0.55 |
| | 7.5 | 0.81 |
| | 10.0 | 1.14 |
| PMN granules | 16.0 | 0.08 |
| | 32.0 | 0.21 |
| | 50.0 | 0.46 |
| | 75.0 | 0.81 |
| | 100.0 | 1.12 |

* Assay conditions: pH 8.0, 25 C, substrate concentration = 0.004 M, reaction volume = 3.0 ml. (Further details of methods and materials are provided in the primary references cited in the text)

Data recalculated from Janoff A, Basch RS.[18]

Table 6—Inhibition of Hydrolysis of Synthetic Ester Substrates by N-Acetyl-L-Alanyl-L-Alanyl-L-Alanine Chloromethyl Ketone*

| Enzyme | Inhibitor concentration† (M) | Substrate | Percent inhibition |
|---|---|---|---|
| Trypsin | $1.6 \times 10^{-3}$ | BAPNA | 0 |
| Chymotrypsin | $1.6 \times 10^{-3}$ | GPAPNA | 7 |
| Pancreatic elastase | $1.6 \times 10^{-3}$ | t-BOC | 100 |
| Neutrophil granule extract | $1.6 \times 10^{-3}$ | t-BOC | 85 |

* Inhibitor-enzyme mixtures were preincubated 2 hours at 25C in 0.01 M sodium-phosphate-buffered saline, pH 6.5 and then compared with control enzymes which were incubated alone

† In preincubation mixture

BAPNA = benzoyl-arginine-**p**-nitroanalide, GPAPNA = glutaryl-phenylalanine-**p**-nitroanalide, t-BOC = t-BOC-L-alanine-**p**-nitrophenol

Peter Tuhy of the Georgia Institute of Technology, and through their kindness the following experiments were made possible.

The inhibitor selected for our initial tests was N-acetyl-L-alanyl-L-alanyl-L-alanine-O-$CH_2Cl$. It inhibits slowly, but irreversibly, by binding to elastase and must be preincubated with preparations containing the latter for several hours at room temperature. Table 6 shows the inhibition of three pancreatic serine esteroproteases and of PMN granules after preincubation with this agent. Inhibition was measured using synthetic ester substrates; it is clear that the chloromethyl ketone derivative is highly effective against pancreatic elastase and the elastase-like esterolytic activity of PMN granules while remaining essentially ineffective against trypsin and α-chymotrypsin, the two homologous pancreatic serine esteroproteases.

Table 7 shows the results of a similar inhibition study with the peptide chloromethyl ketone, except that inhibition was assayed using a protein substrate (casein). Two significant features of the

Table 7—Inhibition of Proteolysis by N-Acetyl-L-Alanyl-L-Alanyl-L-Alanine-Chloromethyl Ketone*

| Enzyme | Inhibitor concentration† (M) | Substrate | Percent inhibition |
|---|---|---|---|
| Trypsin | $0.6 \times 10^{-3}$ | Casein | 0 |
| Chymotrypsin | $0.6 \times 10^{-3}$ | Casein | 6 |
| Pancreatic elastase | $0.6 \times 10^{-3}$ | Casein | 88 |
| Neutrophil granule extract | $0.6 \times 10^{-3}$ | Casein | 45 |

* Inhibitor-enzyme mixtures were preincubated as described in Table 6

† In preincubation mixture

results bear attention. First, as had occurred previously with synthetic substrates, trypsin and chymotrypsin essentially remained uninhibited by the agent at the concentrations employed, while pancreatic elastase and PMN granules were affected. Second, despite the marked inhibition of alanine-*p*-nitrophenyl esterase activity of PMN granules by the peptide chloromethyl ketone, as shown in Table 6, the degree to which caseinolysis was inhibited by PMN granules was less pronounced. On the other hand, as expected, caseinolysis by pancreatic elastase was almost completely inhibited. This observation suggests that several neutral proteases capable of digesting casein are present in human PMN granules and that only one of these (that accounting for approximately half the total caseinolytic activity of the granules) is the elastase or, at least, is susceptible to inhibition by the chloromethyl ketone derivative of a specific elastase substrate. An interpretation such as this is consonant with Ohlsson's finding [12] of two neutral proteases in human PMN granules, each of which attack casein but only one of which attacks elastin.

Table 8 summarizes our experience, to date, with several different connective tissue components, representing potential targets of neutrophil granule proteases in tissue damage. These observations constitute a preliminary effort to delineate the action of granulocyte elastase in tissue injury, using the peptide chloromethyl ketone inhibitor. As shown in this table, the attack by PMN granules upon cartilage matrix proteinpolysaccharides and renal basement membrane was not significantly affected by preincubating the granule extracts with the elastase inhibitor. In the same experiment, aliquots of granule extract, which had been preincubated with the chloromethyl ketone, were tested separately and shown to have essentially no alanine-*p*-nitrophenyl esterase activity. As expected, mixtures of trypsin and inhibitor retained full tryptic activity against synthetic substrate and against basement membrane. The activity of pancreatic elastase on the alanine-*p*-nitrophenyl ester and basement membrane was abolished by preincubation with the inhibitor. Thus, at the very least, these results suggest that another neutral protease and/or other lysosomal enzyme(s) of the neutrophil granule, distinct from the granulocyte elastase, play the dominant role in degrading basement membrane and cartilage matrix proteinpolysaccharide. However, elastases are capable of attacking such substrates, and the granulocyte elastase may normally participate in a supplementary fashion during a neutrophil-mediated attack upon them.

Table 8—Inhibition of Digestion of Connective Tissue Components by N-Acetyl-L-Alanyl-L-Alanyl-L-Alanine-CMK*

| Enzyme | Amount (μg) | Inhibitor concentration† (M) | Substrate | Indices measured | Inhibition |
|---|---|---|---|---|---|
| Human PMN granule protein | 200 | $1.2 \times 10^{-3}$ | Rabbit articular cartilage | Loss of metrachromasia | None or slight |
| Trypsin | 25 | $7.3 \times 10^{-3}$ | Human renal basement membrane‡ | Release of HO-proline and sialic acid-containing fragments; starch gel electrophero-grams of digestion supernatants | None |
| Pancreatic elastase | 25 | $1.2 \times 10^{-3}$ | | | Complete |
| Human PMN granule protein | 400 | $7.3 \times 10^{-3}$ | | | None |
| Pancreatic elastase | 6 | $1.2 \times 10^{-3}$ | Rat aorta (0.04 mm thick rings) | Loss of elastica staining | Complete |
| Human PMN granule protein | 125 | $1.2 \times 10^{-3}$ | | | Complete |
| Pancreatic elastase | 25 | $1.6 \times 10^{-3}$ | Human lung elastin-enriched fraction§ | Solubilization of protein | Complete |
| Human PMN granule protein | 500 | $5.0 \times 10^{-3}$ | | | Complete |

* Inhibitor-enzyme mixtures were preincubated as described in Table 6

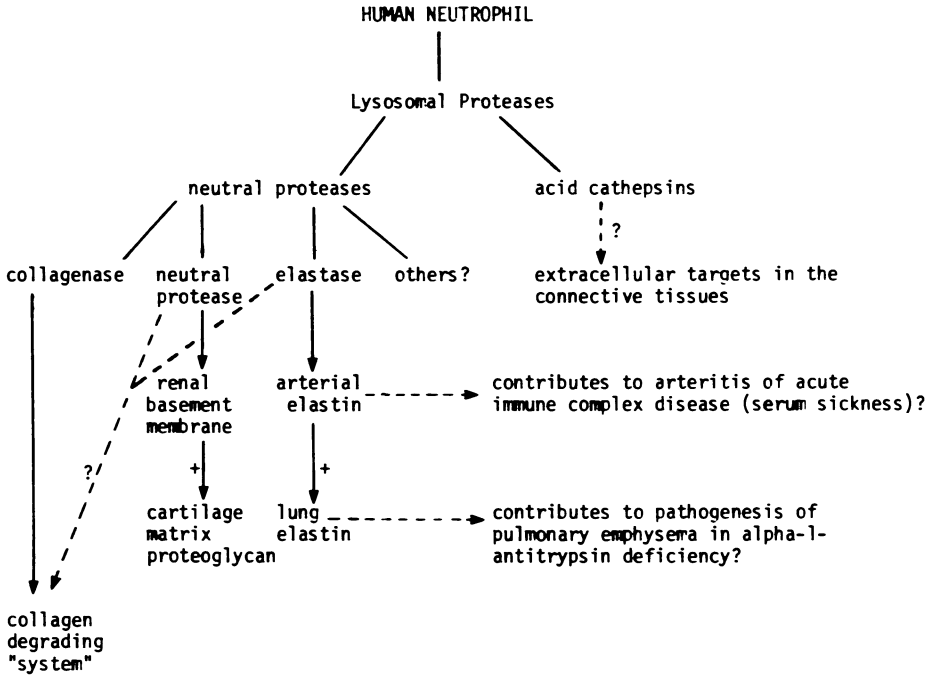
† In preincubation mixture

‡ Kindly supplied by Dr. Charles G. Cochrane

§ Preparation described in Janoff A, Sandhaus RA, Hospelhorn VD, Rosenberg R.[30]

When elastin-rich connective tissues were studied, a completely different pattern was observed (Table 8). PMN granules were completely prevented from digesting arterial wall and a connective tissue fraction from lung by pretreating the granules with the peptide chloromethyl ketone elastase-inhibitor, suggesting that granulocyte elastase probably plays a dominant role in neutrophil-mediated attack upon these connective tissue elements.

A tentative working hypothesis could hold that granulocyte elastase is a critical mediator in those inflammatory diseases or other neutrophil-exacerbated degenerative processes which affect elastic fibers. Suggested relationships between neutrophil proteases and connective tissue targets are schematically summarized in Text-figure 2. This schema will obviously be subject to considerable modification as work proceeds in this area. Additional studies are clearly called for to properly delineate the roles of granulocyte elastase and other neutrophil enzymes in mediating tissue damage,




TEXT-FIG 2—Possible interactions between human neutrophil proteases and selected connective tissue targets.

but until highly purified enzymes are available from human neutrophils, studies with specific inhibitors may provide one useful approach to the problem.

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