A Neutral Collagenase from Human Gastric Mucosa

By DAVID E. WOOLLEY, JOHN S. TUCKER, GERALD GREEN and JOHN M. EVANSON

*University Department of Medicine, University Hospital of South Manchester,
West Didsbury, Manchester M20 8LR, U.K.*

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1. Biopsy specimens of human gastric mucosa, maintained in culture for 7 days in the absence of serum, released a collagen-degrading enzyme into the medium. The yield of active enzyme reached a maximum after 2–3 days, and viable tissue, capable of protein synthesis, was essential for its production. 2. At 25°C the enzyme attacked undenatured collagen in solution, resulting in a 55% loss of specific viscosity and producing the two products TC_A and TC_B characteristic of neutral-collagenase action. 3. Electron microscopy of segment-long-spacing crystallites of these reaction products showed the exact cleavage locus of the collagen molecule to be between bands 43 and 44 (I-43). The larger TC_A and smaller TC_B products were fragments representing 77 and 23% respectively of the length of the collagen molecule. 4. Optimal enzyme activity was observed over the pH range 7.5–8.5 and a mol.wt. of approx. 38 000 was derived from gel-filtration studies. 5. The enzyme was shown to be inhibited by the human serum proteins α_2 -macroglobulin and a smaller component of mol.wt. approx. 40 000; α_1 -anti-trypsin was not inhibitory. 6. EDTA, 1,10-phenanthroline, cysteine and dithiothreitol all inhibited collagenase activity. 7. The gastric enzyme has properties similar to other well characterized collagenases, but differences exist with respect to its molecular size and the site of attack on the collagen molecule.

After the discovery of the first animal collagenase from resorbing tadpole tail fins by Gross & Lapiere (1962), neutral collagenases were shown to be produced by a wide variety of human and mammalian tissues (see reviews by Harris & Krane, 1974*a,b,c*) including rheumatoid synovium (Evanston *et al.*, 1968), skin (Fullmer *et al.*, 1966; Eisen *et al.*, 1968), neoplasms (Dresden *et al.*, 1972), cornea (Slansky *et al.*, 1968; Itoi *et al.*, 1969) and intestinal mucosa (Hawley *et al.*, 1970). Although the precise function of these enzymes in connective-tissue degradation is as yet not fully resolved, evidence is accumulating that they play a crucial role in the metabolism of collagenous matrix.

Since many pathological conditions of the upper intestinal tract such as peptic ulceration and invasive neoplasia must inevitably involve breakdown of the collagenous supporting tissue we have examined gastric biopsy specimens for a true neutral collagenase by using established tissue-culture techniques. Our findings indicate that gastric-mucosal tissue in culture produces an enzyme having the properties of a true neutral collagenase but differing in some respects from similar enzymes previously described.

Experimental

Materials

Dulbecco's modified Eagle's medium, single strength and without Phenol Red, was purchased from Wellcome Reagents Ltd., Beckenham, Kent,

U.K. Benzylpenicillin (Glaxo Laboratories Ltd., Greenford, Middx., U.K.), streptomycin sulphate (Dista Products, Liverpool, U.K.) and Nystatin (E. R. Squibb and Sons, Twickenham, Middx., U.K.) were the antibiotics used in the culture system. Disposable tissue-culture flasks (Falcon Plastics) were purchased from the Scientific Instrument Centre, Liverpool U.K. [¹⁴C]Glycine (90 mCi/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

For the collagenase assay disposable polypropylene micro-assay tubes were purchased from Gelman Hawkesley, Lancing, Sussex U.K. Bio-Solv solubilizer BBS-3 and butyl-PBD [5-(4-biphenyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole; Beckman Ltd., Glenrothes, Fife, Scotland, U.K.] were used with scintillation-grade toluene (BDH Chemicals Ltd., Poole, Dorset, U.K.). Ultrafiltration of culture medium was performed in the Amicon model 8-MC micro-ultrafiltration system by using PM-10 membranes (Amicon Ltd., High Wycombe, Bucks., U.K.). Sephadex G-200, G-150 superfine, G-75 superfine and Blue Dextran were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Electrophoresis-grade acrylamide, *N,N'*-methylenebisacrylamide and *N,N,N',N'*-tetramethylethylenediamine were purchased from Eastman Kodak Co., Liverpool, U.K. Coomassie Brilliant Blue R250 was purchased from R. A. Lamb, Alpertton, Middx., U.K.

Sodium dodecyl sulphate, *o*-toluidine reagent, ATP (sodium salt), puromycin aminonucleoside,

actinomycin D and the protein standards transferrin, ovalbumin, carbonic anhydrase and myoglobin were purchased from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K. Immunoplates for human α_2 -macroglobulin and α_1 -anti-trypsin were obtained from Hyland Laboratories Inc., Costa Mesa, Calif., U.S.A., and ICL Scientific, Fountain Valley, Calif., U.S.A., respectively. All other chemicals were AnalaR grade wherever possible and were purchased from BDH Chemicals Ltd., Poole, Dorset, U.K.

Tissue culture

Biopsy specimens of normal gastric mucosa, obtained at investigative gastroscopy with the approval of the Ethical Committee of the University Hospital of South Manchester and with the informed consent of the patients, were first washed with three changes of Dulbecco's modified Eagle's medium containing 50 units of benzylpenicillin and 50 μ g each of streptomycin sulphate and Nystatin/ml. The tissue specimens were then transferred to disposable plastic culture flasks containing 1.5 ml of Dulbecco's tissue-culture medium and incubated at 37°C in an atmosphere of O₂+CO₂ (95:5) for 7 days as described by Evanson *et al.* (1968). The culture medium was changed at 24 h intervals and stored at -20°C after adjustment to 20 mM-Tris/HCl (pH 7.6)/2 mM-CaCl₂. Medium with collagenase activity was concentrated by ultrafiltration at 4°C by using Amicon PM-10 membranes to facilitate the study of the properties of the enzyme.

Preparation of salt-soluble [¹⁴C]glycine-labelled collagen

Guinea pigs (16 days old and weighing approx. 225 g) were each injected intraperitoneally with 200 μ Ci of [¹⁴C]glycine in 1.0 ml of sterile 0.9% NaCl. Then 6 h later the animals were anaesthetized with ether and killed by a blow on the head; neutral salt-soluble collagen was extracted from the minced skins with 0.5 M-NaCl in the cold and purified by the method of Gross (1958). Acid-soluble collagen was obtained by extracting the residue, after two salt extractions with 0.1 M-acetic acid. The collagen preparations were freeze-dried and stored at -20°C until use. Solutions of collagen were prepared by dissolving the collagen in cold potassium phosphate buffer, pH 7.6 (*I* = 0.4 mol/litre), followed by dialysis against 0.4 M-NaCl at 4°C. The specific radioactivity of the collagen thus prepared was usually in the range 30 000–40 000 c.p.m./mg of collagen and the purity of the collagen was confirmed by disc electrophoresis in 5% polyacrylamide gels containing sodium dodecyl sulphate.

Collagenase assay with reconstituted collagen fibrils

Collagenolytic activity was assayed by measuring the release of soluble radioactive products from a pellet of thermally reconstituted [¹⁴C]glycine-labelled collagen fibrils by a modification of the method of Nagai *et al.* (1966). Assays were carried out in plastic micro-tubes of 400 μ l capacity, and a typical reaction mixture contained 25 μ l of collagen solution reconstituted at 37°C for 16 h and 5–100 μ l of enzyme solution diluted to 200 μ l with 50 mM-Tris/HCl buffer, pH 8.0, containing 10 mM-CaCl₂ and 0.17 M-NaCl. Each assay contained approx. 50 μ g of collagen and 2000 c.p.m. of ¹⁴C radioactivity. Duplicate assays together with appropriate controls were incubated at 37°C, usually for 8 h, and reactions were terminated by the addition of 10 μ l of a solution of 100 mM-EDTA adjusted to pH 8.0. The assay tubes were centrifuged at 20°C for 30 min at 20 000 *g* and a 100 μ l sample of the supernatant from each assay was dispensed into 10 ml of a scintillation mix consisting of 6.8 g of butyl-PBD, 50 ml of Bio-Solv solubilizer BBS-3 and 950 ml of toluene and counted for radioactivity in a Beckman model LS-230 liquid-scintillation spectrometer to 3% standard error. To check that denatured collagen was absent from the collagen preparation, control assays containing 5 μ g of trypsin were run with each series of assays. These trypsin controls seldom released more than 3% of the radioactivity in excess of the controls with buffer, and any collagen preparation that released more than 5% was discarded.

Glucose determination

To assess glucose uptake of biopsy specimens during the period of culture, glucose concentrations in the culture medium were measured daily by the method of Hultman (1959), by using *o*-toluidine reagent and measuring *E*₆₂₀. The glucose uptake of the tissue was expressed as a percentage of the initial glucose concentration of the culture medium.

Viscometry

Viscosity measurements were made by using 1 ml-capacity Ostwald viscometers with water flow times of 28 s at 25°C. The 1 ml reaction mixtures contained 50 mM-Tris/HCl buffer, pH 8.0, 0.4 M-NaCl, 10 mM-CaCl₂, 0.8 mg of collagen and gastric-mucosal collagenase or trypsin.

Disc gel electrophoresis

Electrophoresis in 5% polyacrylamide gels containing sodium dodecyl sulphate (Weber & Osborn, 1969) was used to examine collagen degradation products. Reactions between collagen and collagenase at 25°C were stopped by the addition of 4 M-urea,

1% sodium dodecyl sulphate and 1% dithiothreitol in 50mM-sodium phosphate buffer, pH 7.0, and the collagen products were denatured by heating at 50°C for 30 min. Electrophoresis of the samples was carried out at 6mA per tube and the gels were stained with Coomassie Brilliant Blue.

Electron microscopy

Segment-long-spacing crystallites were prepared by dialysis of 0.3 ml solutions of collagen or collagenase reaction products against 5 ml of 0.4% ATP (sodium salt) in 0.1M-acetic acid for 48 h at 4°C (Stark & Kuhn, 1968; Bruns & Gross, 1973). The crystallite suspensions were applied to Formvar-coated grids and stained with 0.1% phosphotungstic acid for 30 s and 0.01% uranyl acetate for 3 min at 4°C, each grid being washed with water after each stain. The grids were examined with an AEI 801A electron microscope operated at 80 kV and micrographs were taken at a direct magnification of $\times 100\,000$.

Molecular-weight estimation

The molecular weight of the enzyme was estimated by gel filtration at 4°C in a column (35 cm $\times$ 1.5 cm) of Sephadex G-150 (superfine grade). The eluting buffer was 20mM-Tris/HCl, pH 8.0, containing 0.17M-NaCl and 10mM-CaCl₂, and 1 ml fractions were collected. The column was calibrated with the protein standards transferrin (74000), ovalbumin (43000), carbonic anhydrase (29000) and myoglobin (17000). The void-volume and salt-exclusion values were determined by using solutions of Blue Dextran and potassium ferricyanide respectively. The column eluate was monitored for E_{280} and collagenase activity was determined by the ¹⁴C-labelled collagen-fibril assay.

Serum protein fractionation

Human serum was fractionated by gel filtration at 4°C in a column (90 cm $\times$ 2 cm) of Sephadex G-200. The eluting buffer was 20mM-Tris/HCl, pH 8.0, containing 0.17M-NaCl and 10mM-CaCl₂, and 2 ml fractions were collected. Further separation of the low-molecular-weight serum proteins was achieved in a column (50 cm $\times$ 0.9 cm) of Sephadex G-75 (superfine grade) by elution with the same buffer and collection of 0.66 ml fractions.

The serum proteins α_2 -macroglobulin and α_1 -anti-trypsin in the eluate fractions were determined immunologically by single radial immunodiffusion (Mancini *et al.*, 1965).

The proteinase assay system of Kunitz (1947), in which added trypsin degrades the substrate casein for 1 h at 37°C, was used to determine the inhibitory activity of α_1 -anti-trypsin.

Hydroxyproline and protein measurement

Hydroxyproline was measured by using a technique modified from that described by Woessner (1961) with internal hydroxyproline standards. Protein was determined by the Folin method of Lowry *et al.* (1951), with crystalline bovine serum albumin as a standard.

Results

Collagen-gel lysis

When biopsy specimens were placed on gels of thermally reconstituted collagen in tissue-culture medium and incubated at 37°C, lysis of the surrounding collagen was observed, indicating the release of a collagenolytic enzyme. In contrast, control biopsy specimens subjected to freezing and thawing before planting on the gel, produced no collagen lysis. Since a solution of the collagenolytic enzyme was not inactivated by freezing and thawing, this suggested that the released enzyme activity was dependent on the survival *in vitro* of the explanted tissue.

Microscopy

Light-microscope studies of representative specimens before culture showed the presence of only the mucous membrane or glandular layer of the gastric mucosa, comprising secretory epithelium and the lamina propria consisting of mesenchymal cells in loose connective tissues.

Tissue culture of biopsy specimens

Biopsies were cultured for 7 days, as described in the Experimental section, and the culture medium obtained each day was assayed for collagenase activity by using the ¹⁴C-labelled collagen-fibril method (Fig. 1a). The amount of glucose remaining in each day's medium, expressed as percentage glucose uptake, was shown to remain reasonably constant over the period of maximal collagenase production between days 2 and 4. The low values found for measurable enzyme activity on day 1 are characteristic of the pattern of collagenase yield by many tissues (Evanson *et al.*, 1968; Eisen *et al.*, 1970, 1971) and are probably explained by the initial washing-out of known serum inhibitors from the cultured specimens.

When culture flasks containing the same number of biopsy specimens were treated with puromycin or actinomycin D, agents which do not directly inhibit collagenase but do inhibit protein synthesis, relatively little collagenase activity was released during culture (Fig. 1b). Similarly, enzyme activity was difficult to detect in flasks containing biopsies which had previously been frozen and thawed. In contrast, the

Fig. 1. Release of collagenolytic activity from gastric mucosa in tissue culture

(a) Culture medium harvested on successive days of culture was assayed for collagenase activity (○) and glucose content (▲) as described in the Experimental section. (b) Comparative study of the release of collagenase activity. Cultures treated with puromycin (2.5 μ g/ml; □) or actinomycin D (2.5 μ g/ml; Δ) produced relatively little enzyme when compared with the untreated control cultures (○). Biopsy specimens which were frozen and thawed before culture also produced minimal enzyme activity (●). Each culture flask contained three tissue fragments of similar size.

untreated control flasks released significant amounts of collagenase activity, which followed the pattern noted with other tissues (Evanson *et al.*, 1968; Eisen *et al.*, 1970). As the cultures containing puromycin and actinomycin D continued to take up glucose, it is apparent that viable cells capable of protein synthesis are essential for the production and release of collagenase.

To examine the properties of the collagenase further, culture media which contained high enzyme activity were pooled and concentrated by ultra-filtration. As it is known that the neutral collagenases attack collagen monomers in solution at 25°C at one specific locus, resulting in a decrease of specific viscosity, we have examined the action of the gastric enzyme on collagen under these conditions.

Viscometry

Viscometric studies at 25°C showed that the addition of enzyme to a collagen solution results in a

Fig. 2. Effect of gastric-mucosal collagenase and trypsin on the viscosity of a collagen solution at 25°C

A portion (0.10 ml) of collagenase (○) or trypsin (50 μ g/ml, ▽) was added to 0.90 ml of a collagen solution (0.8 mg/ml). The viscosity changes were measured in a 1 ml-capacity Ostwald viscometer with a water flow time of 28 s at 25°C.

loss of specific viscosity, which at completion represented approx. 55% of the original value (Fig. 2). Similar studies with trypsin produced no more than a 3% loss in specific viscosity.

Disc gel electrophoresis

When the reaction had reached completion, as judged by no further fall in viscosity, a sample of the reaction mixture was taken for examination by disc gel electrophoresis in 5% polyacrylamide gels containing sodium dodecyl sulphate. Plate 1 shows that two products are released by gastric collagenase attack and these correspond approximately to $\frac{1}{4}$ - and $\frac{1}{2}$ -length portions of the collagen molecule, labelled TC_A and TC_B respectively in Plate 1(b). The two protein bands comprising the TC_A fragment represent the α_1 - and α_2 -chain components, which have different electrophoretic mobilities as shown in the control gel (Plate 1a).

Segment-long-spacing crystallites

Further elucidation of the exact cleavage locus along the collagen molecule was achieved by electron-microscopic examination of segment-long-spacing crystallites (Plate 2). (In the presence of ATP at low pH, collagen molecules or their undenatured fragments align in perfect transverse register to form banded crystallites which may be observed and measured by electron microscopy.) The larger TC_A fragments were generally observed as segment-long-spacing dimers forming N-terminal junctions, but the smaller TC_B crystallites were usually difficult to find. Accurate alignment of the banding patterns

EXPLANATION OF PLATE I

Disc gel electrophoresis of the reaction products of gastric-mucosal collagenase and collagen in solution at 25°C

The control sample without enzyme (a) and the reaction products taken from the viscosity experiment described in Fig. 2 (b) were equivalent to 50 μ g of collagen. Each was examined by disc gel electrophoresis in 5% polyacrylamide gels containing sodium dodecyl sulphate. F, buffer front.

EXPLANATION OF PLATE 2

Determination of the collagenase-mediated cleavage locus of the collagen molecule at 25°C from electron micrographs of segment-long-spacing crystallites of the reaction products

(1) The TC segment-long-spacing crystallite has bands 43 and 44, numbered according to Bruns & Gross (1973). Arrow indicates collagenase cleavage locus deduced from (2), as interband 43 (I-43). (2) The TC_A segment-long-spacing dimers show *N*-terminal junctions, N-N, with the newly formed *C*-terminal ends, designated C', represented by band 43. Bands 41 and 43 are labelled in each TC_A crystallite.

Fig. 3. Activity of gastric-mucosal collagenase as a function of pH

The buffer solutions of 50 mM-Tris/maleate (○) and 50 mM-Tris/HCl (●) contained 0.17 M-NaCl and 10 mM-CaCl₂. Enzyme activity at selected pH values was determined by the ¹⁴C-labelled reconstituted collagen-fibril assay.

shows that, according to the nomenclature of Bruns & Gross (1973), the point of cleavage occurs at interband 43. All the three clearly defined TC_A crystallites in Plate 2 show termination of the fragment at this band and confirmation of this cleavage site was obtained by examination of 14 other TC_A crystallites obtained by using two gastric enzyme preparations. Direct measurement of the length of the crystallites showed that the fragments TC_A and TC_B represent 77 and 23% respectively of the length of the collagen molecule. This specific cleavage locus for the gastric enzyme is close to but differs from that reported for other collagenases (Harper *et al.*, 1971; Leibovich & Weiss, 1973; Woolley *et al.*, 1975a), being located slightly closer to the carboxyl terminal end of the collagen molecule.

pH optimum

The pH-dependence of the enzyme is shown in Fig. 3; optimal enzyme activity was observed over a neutral pH range of 7.5–8.5 with no activity below pH 5.0.

Molecular-weight estimation

Preliminary estimation of the molecular weight of the enzyme by gel filtration in Sephadex G-150 (superfine grade) calibrated with protein standards of known molecular weight gave a value of approx. 38 000 (Fig. 4).

Enzyme-inhibition studies

The effect of various enzyme inhibitors on gastric collagenolytic activity was examined as shown in Table 1. The known collagenase inhibitors EDTA

Fig. 4. Estimation of molecular-weight of gastric-mucosal collagenase by gel filtration on Sephadex G-150 (superfine grade)

A concentrated collagenase preparation was applied to the column and eluted with 20 mM-Tris/HCl buffer, pH 8.0, containing 0.17 M-NaCl and 10 mM-CaCl₂, at a flow rate of 4 ml/h. E_{280} was monitored (—) and eluate fractions (1 ml) were collected and assayed for collagenase activity (○). A, B, C and D represent the V_e values for the protein standards transferrin, ovalbumin, carbonic anhydrase and myoglobin respectively. V_0 , void volume; V_t , salt exclusion volume. The mol.wt. of 38 000 for the enzyme was calculated from a plot of V_e/V_0 against log (molecular weight) of the protein standards.

and 1,10-phenanthroline produced 100% suppression of activity, suggesting that free metallic ions are essential for enzyme activity. Cysteine and dithiothreitol were also inhibitory, but to a smaller extent. *N*-ethylmaleimide had no inhibitory effect, suggesting that thiol groups are not involved in collagenase activity.

Whole human serum in various dilutions, as high as 100:1, produced total inhibition. After fractionation of human serum proteins by gel filtration in Sephadex G-200, two regions of enzyme inhibition were observed (Fig. 5). These corresponded to α_2 -macroglobulin, identified immunologically, and to a smaller serum component which eluted after α_1 -anti-trypsin. When the third protein peak from the Sephadex G-200 elution was concentrated by ultra-filtration, complete separation of α_1 -anti-trypsin from the smaller serum inhibitor was achieved by ion-exchange chromatography on Sephadex DEAE A-50 (Woolley *et al.*, 1975b). This confirmed that α_1 -anti-trypsin, identified immunologically and by trypsin inhibition, did not inhibit collagenase activity whereas the smaller serum protein did (Table 1).

Discussion

The demonstration that gastric-mucosal tissue has the capacity to produce a true neutral collagenase adds further support to the postulate that enzymes specifically capable of degrading undenatured

Fig. 5. Gel filtration of human serum on Sephadex G-200 and inhibition of gastric-mucosal collagenase activity

Serum (2ml) was eluted with 20mM-Tris/HCl buffer, pH8.0, containing 0.17M-NaCl and 10mM-CaCl₂, at a flow rate of 12ml/h. Fractions (2ml) were collected and each was examined for inhibitory activity (○, percentage of control activity) by adding 100μl of eluate to the collagenase assay mixture (see the Experimental section) before the addition of enzyme. α₂-Macroglobulin (α₂-M, ■) and α₁-anti-trypsin (α₁-AT, ▲) contents of the eluent fractions were determined immunologically. The horizontal bar denotes the fractions pooled for concentration. —, % Transmission at 254nm.

Table 1. Effect of various agents and serum proteins on gastric-mucosal collagenase

Collagenase (50μl) was used in each ¹⁴C-labelled collagen-fibril assay. The inhibitors were dissolved in 50mM-Tris/HCl/0.17M-NaCl/5mM-CaCl₂, adjusted to pH8.0. Control tubes containing 50μl of culture medium without enzyme were incubated for 8h at 37°C as described in the Experimental section. Each evaluation represents the mean of at least four assays after subtraction of control values and is expressed as a percentage of the fibril degradation produced by the enzyme alone.

Reagent added	Concn. in assay	Radioactivity (c.p.m.) released	Enzyme activity (% of control)
None		1400	100
EDTA	10mM	30	2
1,10-Phenanthroline	1mM	10	0
N-Ethylmaleimide	1mM	1390	100
Cysteine	2mM	840	60
	10mM	350	25
Dithiothreitol	2mM	420	30
	10mM	211	15
Protein added			
None	—	980	—
Human serum	2% (v/v)	0	0
α ₂ -macroglobulin	50μg/ml	15	1
α ₁ -Anti-trypsin	40μg/ml	1010	102
40000-mol.wt. serum protein	40μg/ml (total serum protein concn.)	30	3

collagen are ubiquitous in mammalian tissues. Their precise role in the pathophysiology of a wide variety of clinical conditions is not clear, but there is mounting evidence that in conditions such as rheumatoid

arthritis (Harris *et al.*, 1970) and corneal ulceration (Slansky & Dohlman, 1972; Berman *et al.*, 1973) destruction of collagenous tissues is probably the result of exaggerated collagenase action.

The gastric-mucosal enzyme described in the present paper has the essential features of a neutral collagenase, being Ca^{2+} -dependent and functional at neutral pH, but it differs in some noteworthy respects from similar enzymes derived from other animal tissues. At 25°C the gastric collagenase cleaves the tropocollagen molecule to produce the two typical TC_A and TC_B fragments, but examination of segment-long-spacing crystallites indicates that the specific cleavage site (I-43) is slightly removed from that (I-41) found for the tadpole (Harper *et al.*, 1971) and synovial enzymes (Leibovich & Weiss, 1973; Woolley *et al.*, 1975a). This finding could mean that the peptide bond, Gly-Ile, reported by Gross *et al.* (1974) to be the specific site of cleavage of α_1 chains by rabbit tumour and tadpole collagenase, is not that cleaved by the gastric enzyme, since only one Gly-Ile peptide bond is present in this region (α_1 -CB7) of the α_1 chains (Gross *et al.*, 1974). Thus the suggestion that animal collagenases may have a specific affinity for the unique amino acid sequence surrounding the Gly-Ile bond (Gross *et al.*, 1974) is not supported by the present findings, which suggest that the gastric enzyme recognizes a different peptide sequence or steric configuration of the collagen substrate. However, both cleavage loci (I-41 and I-43), although approx. 8 nm or 35 amino acid residues apart, are within the region considered by Leibovich & Weiss (1973) to represent a weak helical portion of the collagen molecule.

The mol.wt. of approx. 38000 for the gastric enzyme differs from that of other human collagenases. Our own comparative studies using skin, granulocyte and rheumatoid synovial enzymes gave mol.wt. values of 62000, 54000 and 33000 respectively, and no evidence was found for the existence of polymers or enzyme-protein complexes (Woolley *et al.*, 1973; D. E. Woolley, unpublished work). The significance of these variations in molecular size, determined by gel-filtration experiments, has yet to be resolved, but it is noteworthy that despite apparent size heterogeneity many of the human enzymes are immunologically cross-reactive, indicating a common molecular conformation (Bauer *et al.*, 1970; Woolley *et al.*, 1975c).

Inhibitory studies using serum protein fractions showed the expected inhibition by the α_2 -macroglobulin component, but with the lower-molecular-weight species the findings were somewhat unexpected. α_1 -Anti-trypsin, monitored immunologically and by its inhibition of trypsin activity, had no effect on the gastric collagenase, but a small protein of mol.wt. approx. 40000 which eluted close to α_1 -anti-trypsin when fractionated on Sephadex G-200, produced total suppression of enzyme activity. Other studies have shown that this protein also inhibits other human collagenases derived from rheumatoid synovium, skin and granulocytes (Woolley *et al.*,

1975b). The nature of this serum protein is unknown, but this finding of a small collagenase inhibitor presumably capable of free diffusion within tissues, in contrast with the large α_2 -macroglobulin, suggests a possible new factor in the regulation and control of collagenase activity in gastric, synovial and skin tissues.

The cellular source of the collagenase derived from the gastric mucosal specimens has yet to be determined. Light-microscopic examination of representative explants taken at time of maximal enzyme yield showed normal histological appearances of all cell types. Since both rabbit and human fibroblasts (Hook *et al.*, 1973; Werb & Burleigh, 1974; Bauer *et al.*, 1975) and also epithelial cells such as those of corneal origin (Slansky *et al.*, 1969; Dohlman, 1971) have been shown to produce a neutral collagenase, it is not yet possible to ascribe the elaboration of enzyme in gastric mucosa to any specific cell type.

In the study of collagen degradation, which has undergone rapid expansion in the past decade, little detailed attention has been directed towards the gastro-intestinal tract. The present observations that gastric mucosa has the capacity to produce collagenase make it necessary to explore the role of this enzyme in those lesions of the stomach, such as ulceration or neoplasia, which involve breakdown of gastric connective tissue.

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