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# Collagenase for Peyronie's Disease Experimental Studies

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**Summary.** This pilot study was designed to test the feasibility of using purified clostridial collagenase in the clinical management of Peyronie's disease. The basic properties of this agent are discussed. We studied its effect on Peyronie's plaque tissue by a quantitative in vitro assay utilising the liberation of free α-amino groups as an index of enzymatic collagenolysis. Tissue from three patients with Peyronie's disease was used. Tunica albuginea from a second group of three normal patients was studied in the same manner, and no selectivity for the collagen of Peyronie's plaques was identified. Utilising human pericardium as a uniform collagenous substrate, a simple dose-effect relationship was established, and the distribution characteristics of injected collagenase observed. Its effects on blood vessels and nerves in vivo was determined as well as the effects of collagenase on the histology of normal and diseased human tissue in vitro. A tentative dose for use in Peyronie's disease was established, which is discussed in light of existing toxicological data. The study was designed to test the feasibility of purified collagenase in the clinical management of Peyronie's disease. Data included detail plaque digestion and dose-effect relationships in vitro, as well as the histological effects on plaques, blood vessels, and nerves in vivo and in vitro. It is concluded that collagenase may warrant further clinical testing in the treatment of Peyronie's disease.

**Key words:** Peyronie's Disease, Clostrideal collagenase, Connective tissue, Collagen, Injection therapy, Scar dissolution.

## Introduction

While advances have been made in the surgical treatment of Peyronie's disease during recent years, specific therapy for the problem continues to be unsatisfactory. It is universally acknowledged that there is no medical or local therapy that provides predictable and lasting benefits for the patient with this distressing problem. Our goal was to investigate the feasibility of using a fibrolytic agent that could be injected into the plaque resulting in its softening with subsequent restoration of penile symmetry and function.

In this pilot study we sought to determine the in vitro biochemical activity of purified clostridial collagenase on Peyronie's plaques and to study its histological effects in vitro and in vivo. Several questions regarding this drug required answers. Is it active against the connective tissue comprising the pathological plaques? What is its activity on surrounding normal tissue? Can the extent of tissue digestion be controlled? What effect does this agent have on the histology of normal and diseased tissue? What constitutes an effective dose in the human lesion? Can valid extrapolation of animal $LD_{50}$ data be made prior to undertaking a clinical trial?

### *Collagenase*

Clostridial soft tissue infections have long been recognised as particularly destructive, with the capacity to spread easily through tissue planes. The observation that cell-free filtrates of clostridial cultures destroyed more connective tissue than muscle led to the eventual isolation of two collagenases from Cl. Histolyticum [7].

The specific collagenolytic properties of clostridial filtrates were first described in the 1940s. Mandl et al. were the first to isolate this collagenolytic fraction in 1953 [3]. Five years later they described in more detail its properties and mode of purification [3]. In 1964 it was found to consist of two distinct collagenase [4], a finding that has been confirmed by others [5, 6]. These enzymes have been named Clostridiopeptidase A and Collagenase 2. In more recent years other specific collagenases have been isolated from the tissues of vertebrates [7, 8]. Their precise role in the biology of vertebrate connective tissue is unknown, although they are thought to play a part in the remodeling of collagenous tissue and in the control of fibroplasia [9].

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Purified clostridial collagenase is available commercially in two forms, a chromatographically purified preparation containing both collagenases with trace amounts of an unidentified protease contaminant, and a lyophylised chromatographically-purified preparation of Clostridiopeptidase A. In contrast to the vertebrate collagenases which leave the tropocollagen molecule into two fragments, the bacterial collagenases act at many sites along the peptide chain, clipping short segments from each end [10]. Its biochemical properties make it well suited for our purposes [3]. It is active over a wide pH range with an optimum around 7.4. Native collagen is its only substrate; globular, soluble, and other structural proteins are unaffected. Enzyme activity is high at 37 °C. It is not subject to autodigestion and is not inhibited significantly in vivo. The purified preparation is quite stable with respect to time and its cost is reasonable. In experimental animals, toxic doses are far in excess of effective therapeutic doses [11, 12].

The approved clinical use of bacterial collagenase is currently limited to topical application for debridement of dermal ulcers and burn eschar [13]. Its effects on prolapsed intervertebral discs have been studied in humans by injecting the agent directly into the disc space [14]; in this group of patients, there were no significant untoward effects.

## Materials and Methods

All collagenase used in this study was the chromatographically purified preparation containing both collagenases described above (code CLSPA Worthington Labs). It was stored at −20 °C in lyophylised form until the day it was to be used, at which time it was suspended in 0.05 M potassium phosphate, 0.00036 M calcium chloride buffer at pH 7.4.

Tissue was obtained at operation without the use of cautery. In addition to samples of Peyronie's plaques from three different patients, several segments of normal tunica albuginea were obtained from patients without Peyronie's disease who were operated on for placement of penile prostheses. The tissue was quick frozen with dry ice and stored at −20 °C for approximately 10 days.

The frozen specimen were dessicated and dry weights recorded, The activity of collagenase was then determined on each specimen using a modification of Mandl's method [2]. Each tissue fragment was incubated at 37 °C for 48 h in 5 ml of a 0.02% solution of collagenase in buffer (400 units). Mild magnetic stirring was continued throughout the incubation. One-tenth of 1 ml of the supernatant was removed from each incubation tube at hourly intervals up to 5 h, then again at 24 and 48 h. Collagenase action was terminated in these 0.1 ml samples by addition of three drops of glacial acetic acid followed by lyophylisation. The residua obtained was stored at −20 °C. After 48 h incubation the plaque fragments were removed, washed in distilled water, dessicated, and weighed.

The extent of enzymatic collagenolysis over time was then assayed by determination of the free α-amino group content of each lyophylised aliquot by the colorimetric ninhydrin method [15]. The dried aliquots were suspended in 0.2 ml of distilled water, and the pH checked to ensure it was about 5. Two ml of ninhydrin reagent were added, and the mixture heated over steam for 20 min. After cooling, all samples were diluted with 5 ml of 50% ethanol. The absorbance was read at 600 nm in a Zeiss PMQII uv/vis spectrophotometer. High optical density samples were re-diluted and the readings corrected for the dilution factors. All optical density readings were then normalised for pre-digestion tissue dry weights. For controls, three pieces of plaque tissue and one piece of tunica albuginea were incubated in buffer without collagenase and subjected to the mechanical effects of magnetic stirring alone.

Fresh human pericardium was obtained at autopsy. After complete defatting it was frozen flat between layers of cellophane and stored at −20 °C. The tissue was thawed by soaking in the above buffer at 37 °C. "Intradermal" injections of differing strengths of enzymes were made in the centre of each piece. Six concentrations were used: 10 U, 30 U, 60 U, 100 U, 200 U, and 400 units each in 0.1 ml of buffer. A bleb was raised and the excess solution blotted off. Paper markers were placed to identify the injection sites. These tissue sheets were placed on filter paper in glass petri dishes and incubated 24 h at 37 °C. They were then transferred to 10% neutral formalin. Histological sections were taken through the injection site, then stained with hemotoxylin/eosin and Van Gieson's (a stain for collagen) [16]. Pericardial thickness at the centre of the sections and at the edges was determined as the mean of four measurements made in each area using a microscope with a micrometer ocular.

In an anaesthetised rat the left femoral neurovascular structures were exposed surgically. Two-tenths of 1 ml of a sterile solution containing 600 U of collagenase in buffer was infiltrated in the adventitial tissues surrounding them and the wound closed. 24 h later the rat was sacrificed and the block of tissue containing this area removed. Corresponding tissue from the contralateral side was removed as a control then fixed, sectioned and stained as decribed above.

The segments of human corpora cavernosa were excised at autopsy within 4 h of death. The specimens were divided and one-half reserved as a control. The other half was soaked in buffer for 1 h to normalise tissue pH. It was then injected in the dorsal midline with 0.2 ml of a 0.5% collagenase solution (400 U). Control and injected tissue was placed on filter paper in glass petri dishes and incubated at 37 °C for 24 h. The tissue was fixed and sectioned. It was stained with hemotoxylin/eosin, Van Gieson's stain, and elastic Van Gieson's stain.

A segment of freshly obtained Peyronie's plaque was divided into two equal parts. The matching faces of each piece were marked with mercurochrome. A volume of 0.1 ml of a 1.0% collagenase solution (400 U) was injected into one piece, and an equal volume of saline injected into the other. Both pieces were then incubated in the same manner as the corpora. At 24 h they were fixed in formalin and sectioned parallel to the marked faces. Slides were stained with hemotoxylin/eosin and Van Gieson's stain.

## Results

Although plaque and tunica tissue were incubated for 48 hours, ninhydrin readings demonstrated that most free α-amino groups were released in the first 24 h (Figs. 1 and 2). Substantial reductions in tissue dry weights were recorded and failed to demonstrate any selectivity of the enzyme for plaque collagen over tunica albuginea collagen. Of the three tunica specimens the maximum decrease in dry weight observed following digestion was 99%. For the plaque fragments incubated in collagenase and buffer the maximum loss of dry weight was greater than 99%, with a mean decrease of 88.6% for the three specimens. These figures were significant when compared to controls, tissue subjected to incubation and mechanical stirring without collagenase. The maximum reduction in dry weight under these conditions was 12% with a mean of 9.3% for the three plaque segments. The single tunica albuginea control showed a 17% reduction in dry weight. Serial ninhydrin reactivity for controls was not done.

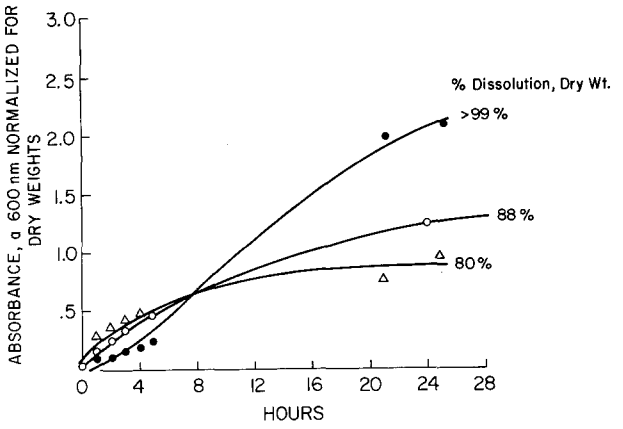



**Fig. 1.** Peyronie's plaques. The liberation of free amino acids from plaque collagen over time in three plaque fragments treated with collagenase. The percent loss in dry weight for each treatment is given. Three fragments incubated without collagenase underwent a mean reduction in dry weight of only 9.3%

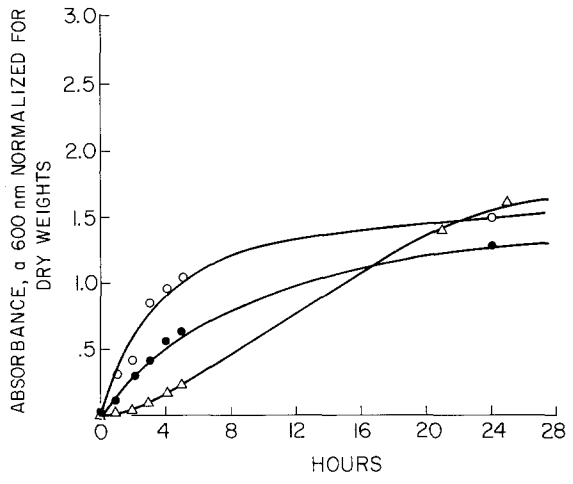



**Fig. 2.** Tunica albuginea. The liberation of free amino acids from tunica albuginea collagen over time in three fragments of normal tunica. Enyzmatic collagenolysis documented

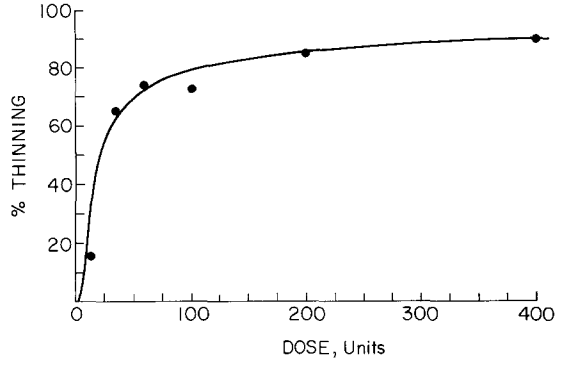



**Fig. 3.** Pericardial thinning. Curve generated to most nearly fit data generated by treatment of uniform pericardial substrate with collagenase injections. Demonstrates qualitative doese-effect relationship and suggests significant gross collagenolysis occurs in the 200–400 unit dose range

The increase in free α-amino groups that paralleled tissue weight loss implied that dissolution represented enzymatic collagen cleavage. Moreover, since the specificity of purified collagenase had been demonstrated [17], the above measurements could be used to provide an estimate of the biochemical composition of the plaques. It appeared that they are largely collagen.

In the piece of pericardium injected with 400 units of collagenase a grossly visible zone of clearing was noted in the fixed tissue centering around the injection site. Its diameter was not greater than that of the bleb raised at the time of injection. Lesser doses exhibited microscopic evidence of thinning. The radius of the thinned area did not change with dose to any extent, remaining approximately the size of the initial injection bleb. The percent of thinning derived from the centre and edge measurements of each piece was then plotted against dosage (Fig. 3). As expected it demonstrated that in a uniform substrate increasing the dose tended to increase the amount of lysis. The lack of any increase in the radius of lysis with increasing doage illustrated that the enzyme did not spread readily through tissue in vitro. It appeared to exert its effects while confined to the region of injection.

When the rat femoral canal was re-opened following collagenase injection a moderate hematoma was noted. This appeared to have come from small vessels, as no active bleeding was seen at the time the tissue was harvested. The microscopic sections confirmed that there was no damage to arterioles and arteries containing smooth muscle. Some small veins appeard to have ruptured. Although there was dissolution of the fibrous perineurium, no change in the nerve fibres was seen on the injected side when compared with the control (Fig. 4).

Definite collagenolysis occurred in the injected corpora within a 2 mm radius of the injection site. This was apparent in the sections stained with Van Gieson's technique and ranged from complete disappearance of collagen fibre bundles to vacuolisation and loss of their characteristic staining properties. The enzyme's specificity for collagen was shown in the sections stained for elastin fibres. There was excellent preservation of elastic elements in areas where collagen had been completely dissolved (Fig. 5).

In Fig. 6, a gross zone of lysis is seen dorsally in a complete penile cross section. Figure 7 is a higher magnification of a similarly injected specimen where there has been substantial loss of tunica albuginea collagen in the dorsal midline. At higher power the more superficially located vessels and nerves showed no histological evidence of injury or digestion.

The injected Peyronie's plaque fragment underwent considerable reduction in overall size. Microscopically the treated plaque showed widespread fraying and dispersal of collagen bundles compared to the dense compact collagen seen in the control tissue injected with saline (Fig. 8).

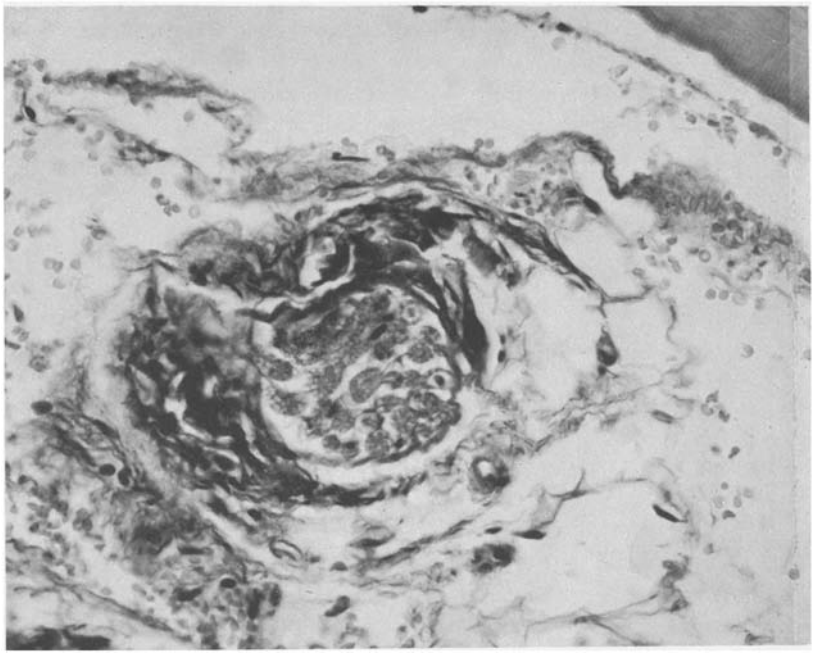


**Fig. 4.** Section of nerve demonstrates dissolution of collagenous perineurium (*lower right hand quadrant*) with histological preservation of nerve fibres within. No histological changes of myelinated fibres seen when compared with untreated controls

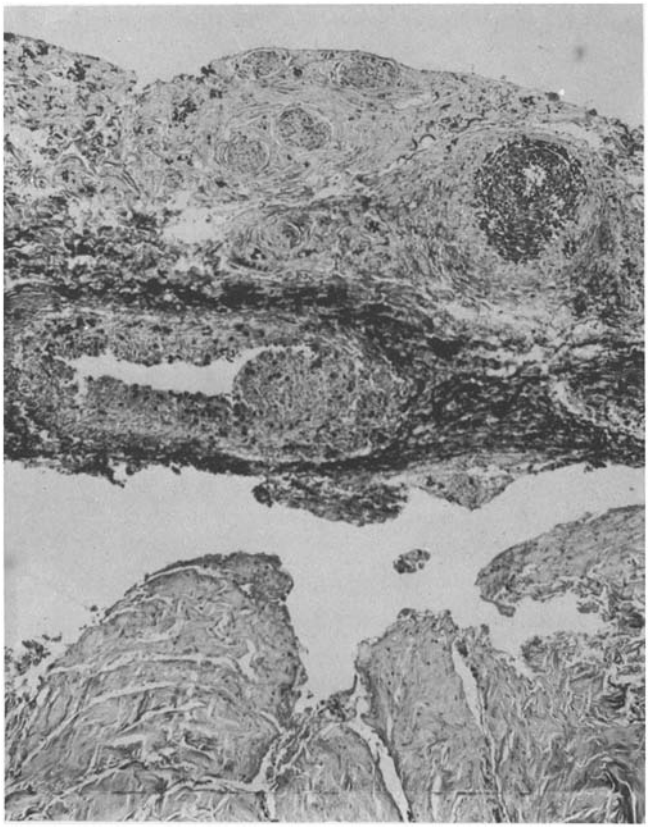


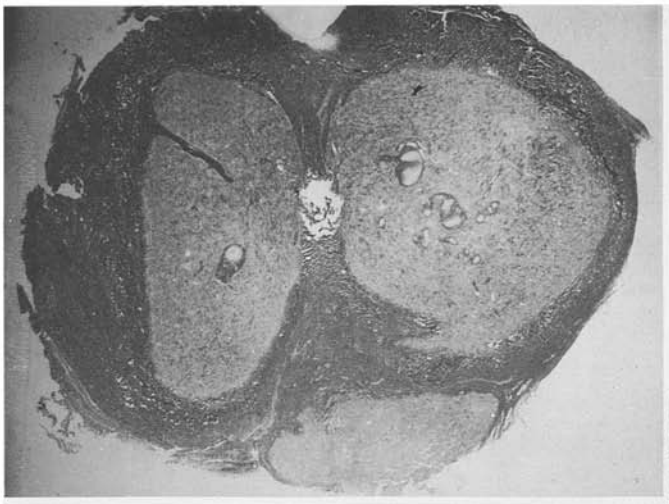
**Fig. 5.** Elastic fibres (*dark staining*) and superficial neurovascular structures preserved histologically above an area where extensive enzymatic collagenolysis has occurred. This is a close-up taken from the section of Fig. 7

## Discussion

Collagenase causes extensive dissolution in vitro of the tissue comprising Peyronie's plaques, as well as normal tunica albuginea. There are factors which may overcome this lack of selectivity and permit its clinical use in the treatment of Peyronie's disease.

Although the pericardial lysis assay cannot be interpreted quantitatively, it shows qualitatively that the extent of enzyme-induced collagenolysis is related to the dosage used. This experiment also showed that very little diffusion and spreading occur from the site of initial enzyme deposition, a finding noted by other investigators [18]. This anatomical confinement of digestion is related to the mucopolysaccharide "ground substance" which embeds the collagen in connective tissue but is not subject to the action of collagenase [19].

The specificity of collagenase for its substrate has other desirable consequences. Elastic tissue is preserved, a feature which may be particularly important in treating Peyronie's disease. Fragmentation of elastic fibres has been observed in this condition [20], which would make salvage of existing elastic elements mandatory for preservation of normal tissue mechanical properties. Vascular smooth muscle is not digested by this enzyme which protects all vessels except small venules. Collagen forms the bulk of their walls up to 0.2 mm, where a well developed circular coat of smooth muscle begins to appear. Although nerve fibre bundles are sheathed in a collagenous perineurium, the axons themselves are wrapped in myelin, a lipid not digested by collagenase. Rat femoral nerves treated in vivo with high doses of collagenase showed excellent histological preservation of fibres. Indeed collagenase has been used to selectively remove the connective tissue which surrounds nerves, isolating them for histologic study [21]. Garvin and Jennings studied the



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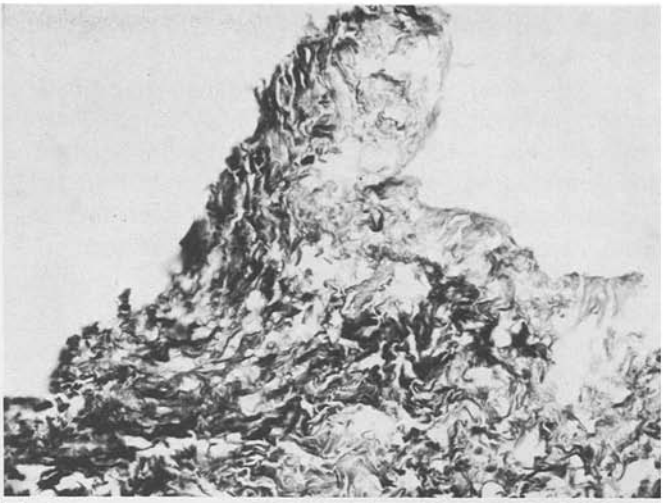

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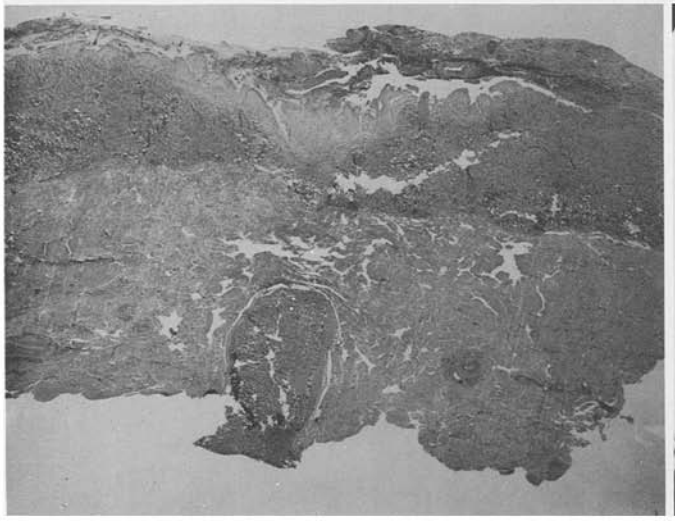

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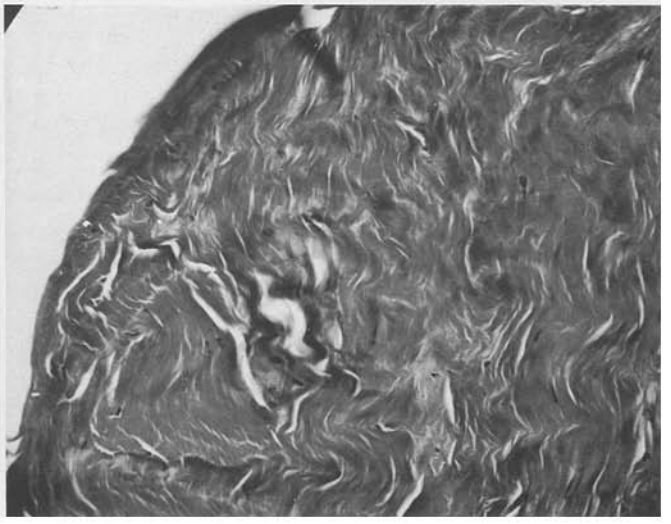

8B

**Fig. 6.** Dorsal enzymolysis defect in complete penile cross-section

**Fig. 7.** Injected dorsal tunica albuginea showing loss of staining for collagen in midline, above the intercorporal septum. Fig. 5 is higher magnification form this specimen

**Fig. 8A and B.** Collagenase and saline-injected plaque fragments, both at same magnification. A demonstrates effect of Collagenase injection on plaque histology

physiological effects of chymopapain on animal nerves in vivo [22]. Although chymopapain is a protease with a wider range of substrates than collagenase, they found it caused no change in threshold voltages for muscle twitch or sensory ending response. The abundant data on collagenase use for dispersion in cell culture techniques have shown it is not cytotoxic, and does not harm cell membranes [21].

The injected autopsy tissue demonstrated how localised collagenolysis could be produced. The size of the area dissolved in this model may not be completely equivalent to the effect of the same dose in vivo, however, The amount of collagen "dissolved" (that which lost its tinctorial qualities in Van Gieson's stain) in the autopsy material represents the collagen that had undergone extensive enzymatic cleavage. Adjacent collagen may undergo denaturation to a lesser degree. There are two factors which tend to limit unpredictable continuation of collagenolysis: as noted previously, the enzyme does not spread well through connective tissues. In addition, the duration of action is limited to approximately 24 h.

The administration of collagenase has immunological consequences [23]. With injections repeated after the development of antibody titres there would be a risk of anaphylaxis or other less serious hypersensitivity reactions. Also the presence of specific antibodies inhibits the enzyme [24]. However, if the time required to mount an antibody response is considered it is apparent that multiple injections could be given over a short (4 to 6 day) period. Repeated immunisation prior to the development of high antibody titres does not produce eventual titres significantly different from those expected with a single immunisation [25]. The classic amnestic response with amplified antibody production fol-

lowing a booster occurs only if the booster is given in the presence of substantial antibody titres.

From the effects observed in this pilot study, we estimate the dose required for an effect on Peyronie's plaques in vivo is in the range of 100 to 400 units as a 0.5% solution. This dose needs to be interpreted in light of the literature on collagenase toxicity. Garvin established an acute intravenous $LD_{50}$ in rats of 1,272 ± 156 units/kg for purified colstridial collagenase [12]. Although extrapolation of these figures to humans is probably inaccurate, it is useful for comparison. For the 70 kg man, an intravenous $LD_{50}$ of 89,040 units is obtained, which is in excess of the average effective dose for Peyronie's plaques by a factor of 356. Sussman and Mann found there were no adverse changes in red cell, white cell, or platelet counts in rats receiving 100 times the effective dose for discolysis [11].

The cause of death in Garvin's study was pulmonary haemorrhage and extensive tissue digestion. It seems that in humans systemic toxicity could be avoided with a high margin of safety. Local toxicity might be encountered as ecchymosis formation, due to the effects observed on small non-muscular venules. Extensive tissue necrosis would not be anticipated with the use of proper doses. Neural damage would not be expected, since we did not observe this histologically and none has been demonstrated physiologically after treatment with less specific proteases.

The actual effects of collagenase in patients with Peyronie's disease would be difficult to determine using animal or in vitro methods, since no adequate models for this condition exist. A clinical trial would be required. The authors hope this pilot study has furnished sufficient data to test intralesional collagenase in such a trial, since it has high activity against the plaques, a controllable local effect, and acceptable safety with regard to systemic and local toxicity.

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