

Fig. 1. Longitudinal profiles of the crystalloid (C) are seen in the extracellular sheath (E). M, muscle; G, gland cell. $\times 90,000$.

Fig. 2. Transverse section of crystalloid. $\times 270,000$.

Fig. 3. Transverse section of crystalloid with a locus (encircled) that shows a starlike substructure. $\times 200,000$.

270 nm in thickness. Normal ultrastructural examination shows an electron dense flocculent material that is apparently randomly dispersed on an electron lucent background. High power examination of areas where the dense material appeared clumped revealed inclusions that possessed an elaborate fine structure. When these inclusions were cut in the longitudinal plane, they presented

an image of narrow dark bands separated by wide light bands (center-to-center spacing ca. 14 nm) (Figure 1). Cross-sectioned inclusions sometimes showed an aggregate of densely outlined, electron lucent subcircular profiles (Figure 2), and, less often, a highly organized hexagonal formation was seen (Figure 3).

At the ultrastructural level, highly oriented connective tissue lattices, particularly those composed of collagenous elements, have been interpreted as aiding in the preservation of structural integrity in the face of compressive or shearing stress^{3,5}. This might well be the case in the brown recluse spider venom glands since both compressive and shearing stresses probably occur during the muscular contractions that expel venom from the gland. On the other hand, such structures have been reported as characteristic of forming elastic tissue⁶.

Extensive morphological and compositional analyses of the crystalloid have been undertaken in order to solve the numerous questions raised by this report^{7,8}.

Résumé. Nous décrivons des inclusions cristallines observées dans l'élytre extracellulaire des glandes venimeuses de *Loxosceles reclusa*.

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⁵ J. N. GOLDMAN and G. B. BENEDEK, Invest. Ophthalmol 6, 574 (1967).

⁶ M. D. HAUST and R. H. MORE, in *The Connective Tissues* (Eds. B. WAGNER and D. SMITH; The Williams and Wilkins Co., Baltimore 1967), p. 365.

⁷ This research supported by medical research funds of the Veterans Administration Hospital, Little Rock (Arkansas, USA).

⁸ We wish to express our gratitude to Messrs A. M. FIELDS and R. PATTERSON for their technical assistance.

Collagenase Associated with Macrophage and Giant Cell Activity

Collagenases and collagenolytic activity have been demonstrated in association with a number of animal and human tissues. These studies have been extensively reviewed by EISEN et al.¹. Phagocytosis of collagen by macrophages is known^{2,3}, but not the occurrence of a collagenase in association with these cells.

We wish to report the presence of a collagenase apparently produced by macrophages and giant cells in talc-induced granulomas. Foreign body granulomas were induced by injecting 1 ml of a sterile 2% suspension of talc (USP) in saline into the middle gluteal muscle of Long-Evans rats. The rats were killed after 7, 14, 21 and 28 days. The area of the granuloma was removed, and well-defined granulomas 1.5–2 cm in diameter were located and excised. 1 to 2 mm³ from the interior of the granulomas were placed on guinea-pig skin-extracted collagen gels produced by the method of GRILLO and GROSS⁴. After 3 days' incubation, lysis of some gels was

observed. This lysis was more extensive with the 14-day explants. The lysate containing the collagen degradation products was separated by disc acrylamide gel (7.5%) electrophoresis at pH 4.7. The original skin-extracted collagen in Tyrode's solution was treated similarly as a control. The electrophoretograms showed definite cleavage of the collagen molecule, with a pattern of cleavage products comparable to those obtained previously from dermis, bone, synovial tissues, and other sites¹ (Figure 1).

PÉREZ-TAMAYO⁵ has reported collagenolytic activity associated with carrageenin granulomas, which he believed

¹ A. Z. EISEN, E. A. BAUER and J. J. JEFFREY, J. Invest. Dermat. 55, 359 (1970).

² D. BRANDES and E. AUTON, J. Cell Biol. 41, 450 (1969).

³ P. F. PARAKKAL, J. Ultrastruct. Res. 29, 210 (1969).

⁴ H. C. GRILLO and J. GROSS, Devel. Biol. 15, 300 (1967).

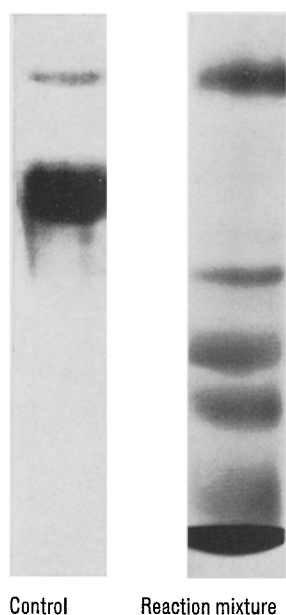


Fig. 1. Disc acrylamide gel electrophoresis patterns of control, and granuloma collagen lysate with several new degradation products. 14-day-old granuloma, 3-day incubation of tissue on collagen gel.

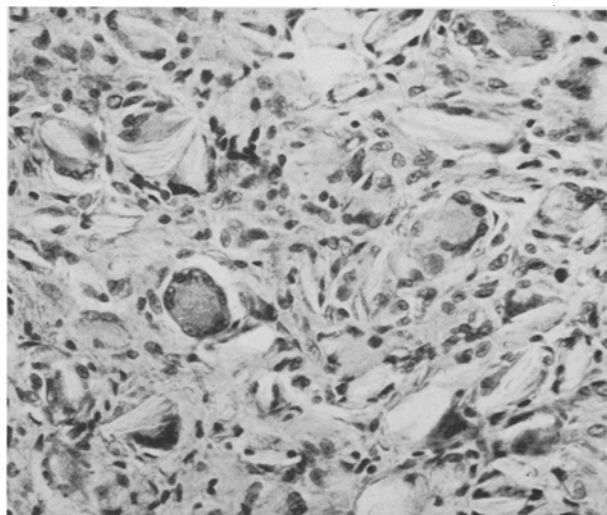


Fig. 2. Granuloma, photomicrograph of central area showing talc, macrophages and giant cells. H.E. $\times 205$.

to be associated with fibroblast proliferation. Periods of higher collagen lysis occurred with increased fibroblast populations. However, in our studies maximum lysis occurred at 14 days, when histological examination showed a nearly exclusive macrophage and giant cell population (Figure 2), with only occasional fibroblasts at the periphery of the lesion. Enzyme histochemical examination of cryostat sections of tissue (macrophages and giant cells) surrounding the explants demonstrated intense acid phosphatase (pH 5.2) and aminopeptidase (pH 5.5 and

6.8) activity⁶. From these observations, it is believed that a collagenase was associated with the highly lytic enzyme-active macrophage and giant cell population in the central areas of the talc granulomas.

This preliminary observation is part of a continuing study which will be reported more extensively at a later date.

Zusammenfassung. Ein kollagenolytisches Enzym wurde in Talkgranulomen und im Zusammenhang mit Makrophagen sowie Riesenzellen gefunden und elektrophoretisch charakterisiert.

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⁵ R. PEREZ-TAMAYO, Lab. Invest. 22, 137 (1970).

⁶ T. N. SALTHOUSE, Expl. Surg. Med., in press (1971).

Interaction of Daunomycin with Nucleic Acids: Effect of Photoirradiation of the Complex

The biological activity of daunomycin is believed to be related to its ability to interact with the 'primer' DNA^{1,2}, thus inhibiting not only DNA-dependent RNA synthesis^{3,4}, but also DNA duplication⁴. Photodynamic activity of daunomycin against some DNA and RNA viruses has been described^{5,6}.

Daunomycin, a fluorescent glycosidic antibiotic of the anthracycline group, could sensitize photodynamic effects⁷. Preliminary irradiation (UV and visible) experiments of its DNA complex indicated that a stable combination of daunomycin with DNA took place. Various methods were used to test the stability of the photoirradiated complex: dialysis, gel filtration, solvent extraction and enzymatic digestion. We report here studies on the effects of UV-irradiation of daunomycin-nucleic acid complexes.

Materials and methods. In a typical experiment, a solution of calf thymus DNA ($5 \times 10^{-3}M$ of DNA phosphorus)

¹ E. CALENDI, A. DI MARCO, M. REGGIANI, B. SCARPINATO and L. VALENTINI, Biochim. biophys. Acta 103, 25 (1965).

² A. DI MARCO, in *Antibiotics* (Eds. D. GOTTLIEB and P. D. SHAW; Springer-Verlag, Berlin 1967), Vol. 1, p. 190.

³ D. WARD, E. REICH and I. H. GOLDBERG, Science 149, 1259 (1965).

⁴ G. HARTMANN, M. GOLLER, K. KOSCHEL, W. KERSTEN and H. KERSTEN, Biochem. Z. 347, 126 (1964).

⁵ M. A. VERINI, A. M. CASAZZA, A. FIORETTI, R. RODENGI and M. GHIONE, G. Microbiol. 16, 55 (1968).

⁶ A. SANFILIPPO, G. SCHIOPPACASSI, M. MORVILLO and M. GHIONE, G. Microbiol. 16, 49 (1968).

⁷ J. D. SPIKES, in *Photophysiology* (Ed. A. C. GIESE; Academic Press, New York 1968), Vol. 3, p. 33.