

Fig. 3. Enlarged view of Figure 2. Note MAO activity of some perikarya and their surrounding neuropil in the dorsolateral cell group of the anterior horn. $\times 150$.

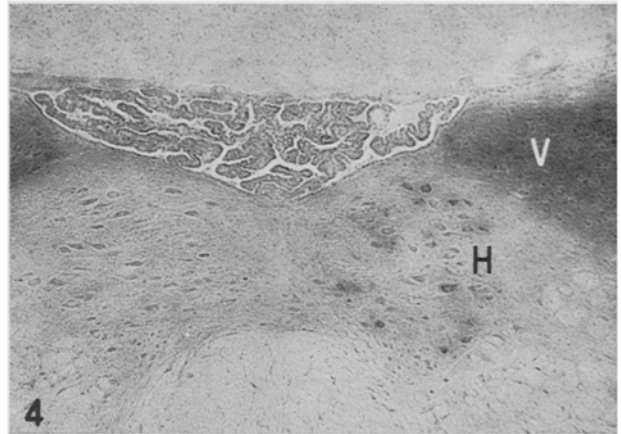


Fig. 4. Cross section of the dorsal part of the medulla oblongata stained for MAO. 20 days following section of left hypoglossal nerve. Most nerve cells and surrounding neuropil of the ipsilateral hypoglossal nucleus (H) showed moderate activity. Strong activity is seen in the dorsal nucleus of the vagus nerve (V). $\times 60$.

the reacting nerve cells and deafferented posterior horn might be activated in the metabolism of amines. Similar finding was reported by BARRON and TUNCBAY⁹, that glial cells in the anterior and posterior horns showed marked increase of thiamine pyrophosphatase following brachial plexectomy. TOHOYAMA et al.¹⁰ also obtained similar results on thiamine pyrophosphatase activity of the glial cells in the spinal cord and medulla oblongata following neurotomy. Although one of the functional meanings of MAO is the cross-link formation of collagen (BORNSTEIN et al.¹¹, PAGE and BENDITT¹², etc.), we are unaware whether it is applicable to the perikaryon and neuroglia under regeneration and degeneration. Hence, further study would be necessary for the relation of MAO increase and increased production of neuro- and gliofilament in the central nervous tissues.

Zusammenfassung. Es werden Veränderungen der MAO-Aktivität im Rückenmark oder in der Medulla oblongata

von Ratten beschrieben, die durch die Sektion des N. ischiadicus oder des N. hypoglossus verursacht wurden.

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⁹ K. D. BARRON and T. O. TUNCBAY, *J. Neuropath. exp. Neurol.* 23, 368 (1964).

¹⁰ M. TOHOYAMA and T. MAEDA, *Annls. Histochem.* 17, in press (1972).

¹¹ P. BORNSTEIN, A. H. KANG and K. A. PIEZ, *Proc. natn. Acad. Sci., USA* 55, 417 (1966).

¹² R. C. PAGE and E. P. BENDITT, *Biochemistry* 6, 1142 (1967).

Crystalloid Inclusions in the Connective Tissue of Spider Venom Gland

Crystalline inclusions have been seen in many types of cells and in nearly all compartments of the cell^{1,2}. However, we have seen few reports concerning the presence of non-mineralized crystalline inclusions in the matrix of connective tissue. JAKUS³ has demonstrated the gridlike orientation of collagen units in Descemet's membrane, and HAUST⁴ has reported the presence of lattice like profiles in forming elastic tissue. The present report deals with lattice like profiles that are located within the extracellular sheath of venom glands from the brown recluse spider, *Loxosceles reclusa*.

Venom glands were removed from the spiders, sliced into 1 mm thick blocks, fixed in a phosphate-buffered 3% glutaraldehyde solution for 3 h, rinsed in a phosphate-buffered 7% sucrose solution, and postfixated in 2% buffered osmium tetroxide. The blocks were dehydrated in methanol and soaked in propylene oxide prior to being embedded in epoxy resin. Thin sections were stained with

combinations of potassium permanganate, uranyl acetate, and lead citrate before being observed in an Hitachi model HU-11B electron microscope.

A brown recluse spider possesses 2 venom glands that are located on either side of the midline in the anterior-superior area of the cephalothorax. Each gland is enveloped with several muscle layers. The extracellular sheath is interposed between the deepest muscle layer and the basal surface of the secretory epithelium. It averages ca.

¹ D. W. FAWCETT, *The Cell* (W. B. Saunders, Philadelphia 1966), p. 319.

² Z. HRUBAN and M. RECHCIGL JR., *Int. Rev. Cytol. Suppl.* 1, 63 (1969).

³ M. A. JAKUS, *J. biophys. biochem. Cytol.* 2 (Suppl.), 243 (1956).

⁴ M. D. HAUST, *Am. J. Path.* 47, 1113 (1965).

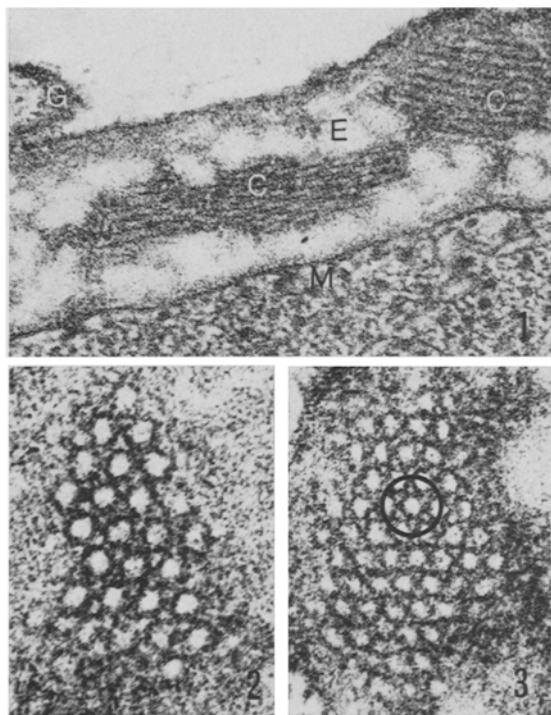


Fig. 1. Longitudinal profiles of the crystalloid (C) are seen in the extracellular sheath (E). M, muscle; G, gland cell. $\times 90,000$.

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).