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Histochemistry of the Tissue Capsule Surrounding Intradermal Mites, *Hannemania* spp. (Acarina: Trombiculidae) in New Mexico Amphibians

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The first occurrence of larval intradermal mites, *Hannemania* spp., from New Mexico amphibians was recently reported (Duszynski and Jones, 1973, Int. J. Parasit. **3:** 531–538). These parasites are always encapsulated by host connective tissue in the dermis of their host and the capsules are grossly visible below the epidermis. Basic histochemical procedures were used to help characterize and give us a better understanding of the structure and formation of this tissue capsule.

Infested skin was removed from live animals which had been pithed and pieces were either fresh-frozen in an Ames Lab Tek cryostat (–20 C) or chemically fixed in Bouin's fixative, FAA, 10% aqueous (v/v) acrolein (Duszynski and Jones, loc. cit.), or freeze-substituted in absolute ethanol after fixation in liquid nitrogen (N_2) for 3 min (ibid.). Tissue fixed in Bouin's and FAA was embedded in 56 to 58 C paraffin and sectioned at 7 to 9 μ. Tissues fixed in 10% acrolein, or in liquid N_2, were embedded in a monomer plastic and in paraffin, respectively, and sectioned as reported elsewhere (ibid.). Frozen material, embedded in OCT compound, was sectioned in the cryostat at 7 to 12 μ. In addition to routine staining of paraffin-embedded material (H & E), the sectioned material was subjected to the techniques listed in Table I. The usual controls were used in the PAS, lipid, and phosphatase determinations (Humason, loc. cit.).

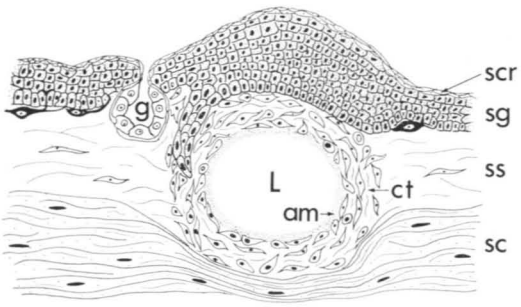

FIGURE 1. Diagram of section of amphibian skin showing tissue capsule formed by the host in response to presence of a *Hannemania* sp. chigger. Entire capsule located within the stratum spongiosum of the dermis. Note acanthosis of epidermal cells above the capsule, compression and distortion of the stratum compactum directly beneath the capsule, and proliferation of host cell fibroblasts forming outer layer of the capsule. scr = stratum corneum and sg = stratum germinativum, the 2 layers of the epidermis; ss = stratum spongiosum and sc = stratum compactum, the 2 layers of the dermis; ct = outer stratum of host connective tissue cells; am = amorphous, anucleate innermost layer of the capsule; L = lumen of the capsule where the chigger lives; g = a skin gland.

The histology of the skin of various amphibians is similar and the major layers of the dermis and epidermis are illustrated diagrammatically (Fig. 1). Duszynski and Jones (loc. cit.) elaborated on the work of Hyland (1961, Exp. Parasit. **11:** 212–225) and characterized the host-formed tissue capsule within the dermis of *B. punctatus* infested by *Hannemania*. Observations on the capsule surrounding these chiggers in other amphibians (*A. tigrinum*, *H. arenicolor*, *R. pipiens*) showed that histologically it is identical in all hosts examined. This capsule consists of two layers (Fig. 1), an outer stratum of host connective tissue cells and an inner, amorphous, noncellular layer which is less well understood, but which is partially characterized here (Table I).

The accumulation of host cell fibroblasts and histiocytes in the outer layer of the capsule is a response on the part of the host to isolate the parasite from host tissue and probably to effect repair function. Tests for carbohydrates, connective tissue, DNA–RNA, and protein were all positive in this layer as would be expected, but lipids could not be demonstrated here. The main reaction product for alkaline phosphatase was found to occur in granular form in the cells of this layer with a reaction in the form of a few granules also associated with the inner layer. Gold and Gould (1951, Arch. Biochem. Biophys. **33:** 155) and Washburn (1955, J. Invest. Dermatol. **24:** 537–544) reported that the concentration of alkaline phosphatase increases as new connective tissue

TABLE I. *Staining reactions of the 2 layers of the tissue cyst which surrounds chiggers,* Hannemania *spp., within the dermis of various amphibians (all techniques are from Humason, 1972,* Animal Tissue Techniques, *3rd ed., Freeman, San Francisco, unless cited otherwise).*

	Stain or substrate	Embedding media used	Host skin	Tissue capsule layers: Outer layer	Tissue capsule layers: Inner layer
Carbohydrate	Alcoholic PAS	paraffin	*Ambystoma tigrinum*	+	+
Connective tissue	Mallory's[1]	plastic, paraffin	*Bufo punctatus*	+	–
DNA–RNA	Feulgen	paraffin	*A. tigrinum, B. punctatus*	++	±
	toluidine blue[2]	plastic	*A. tigrinum, B. punctatus*	++	–
Protein	Acid fuchsin	plastic	*A. tigrinum, B. punctatus*	+	±
	bromphenol blue	paraffin	*A. tigrinum, B. punctatus*	+	++[3]
Lipid	oil red O	OCT compound	*A. tigrinum, B. punctatus* *Hyla arenicolor, Rana pipiens*	–	+
Acid phosphatase	sodium glycerophosphate	OCT compound	*R. pipiens*	±	+
Alkaline phosphatase	paranitrophenol phosphate	OCT compound	*R. pipiens*	+	±

++ = Very strongly positive.
+ = Positive.
± = Weakly positive or variable.
– = Negative.

[1] From Weesner, 1960, General Zoological Microtechniques, Williams and Wilkins, Baltimore.

[2] From Sidman et al., 1961, Stain Tech. **36:** 279–284.

[3] The increased intensity here may be an artifact of technique. Paraffin sections fixed in Bouin's exhibited this phenomenon, while paraffin sections fixed in FAA and monomer sections generally showed even staining intensity in both layers.

is formed during wound healing in rats. Also, this enzyme is apparently bound by freshly precipitated collagen fibers (ibid.). Acid phosphatase was slightly visualized in this layer and the acellular inner layer gave a very strong reaction for that enzyme. The presence of an acid hydrolase such as acid phosphatase as a marker for lysosomal activity has been reported by de Duve (1963, Ciba Foundation Symposium on Lysosomes, Little and Brown, Boston) and Novikoff (1963, ibid.) and the degeneration of cells due to the action of acid hydrolases is a well-known phenomenon (Brown and Millington, 1968, Histochemie **12:** 83–94). This observation plus the striking decrease in DNA–RNA concentration in the innermost capsule layer, i.e., the layer in proximity with the parasite, indicates a general metabolic breakdown of the cells in this area. We can speculate on how this cellular breakdown might occur. Acid phosphatases are membrane-bound and exert no influence on the cell until that membrane is altered. Therefore, some agent is important in the disruption of these membranes and the subsequent release of acid hydrolases. Perhaps the action of parasite metabolites serves as an accessory mechanism in initiating this process. Cryostat-sectioned material stained with oil red O shows large amounts of lipid in this inner capsule layer. These may be remnants of host cell membrane which are the end products of parasite secretions initiating host cell autolysis through alteration of those lysosomal membranes.

Some of the techniques used in this study were learned while one of us (DWD) was a participant in the National Science Foundation sponsored Short Course on Histochemistry held in the Department of Biology, Vanderbilt University, Nashville, Tennessee, under the direction of Dr. B. J. Bogitsh. His assistance in this regard is sincerely appreciated.

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