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HISTOLOGICAL CHANGES ASSOCIATED WITH TRICHODINID INFECTIONS IN THORNY SKATES, *Raja radiata* DONOVAN

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Abstract: Histological changes in the wall of the copulatory sac of normal adult female thorny skates, *Raja radiata,* were compared with those naturally infected with *Trichodina oviducti.* The parasites were associated with extensive defoliation of the copulatory epithelium and in some instances had penetrated the submucosa resulting in petechiae. An excessive exudate that appeared at the vent was made up of mucus, sloughed cells and parasites. It is speculated that shedding of the copulatory epithelium may be due to a hyaluronidase-like enzyme. Furthermore, the restriction of *T. oviducti* to adult skates may be dependent on the presence of certain mucopolysaccharides that are very low or absent in immature skates.

INTRODUCTION

Trichodinid ciliates have been reported to cause damage to the gills of fish.[5] In heavy infestations, there is hypersecretion of mucus, erosion or proliferation of the branchial epithelium and occasional hemorrhage. *Trichodina oviducti,* a parasite of skates, *Raja radiata* and *R. ocellata,* inhabits the copulatory sac of adult females, whereas in adult males, it may be found in the seminal groove of the claspers and sometimes in the vent.[8] Experimental evidence suggests that the parasite is venereally transmitted. In females, it was reported to cause shedding of the copulatory epithelium. The present study investigated the histological changes associated with natural trichodinid infections in female thorny skates, *R. radiata.*

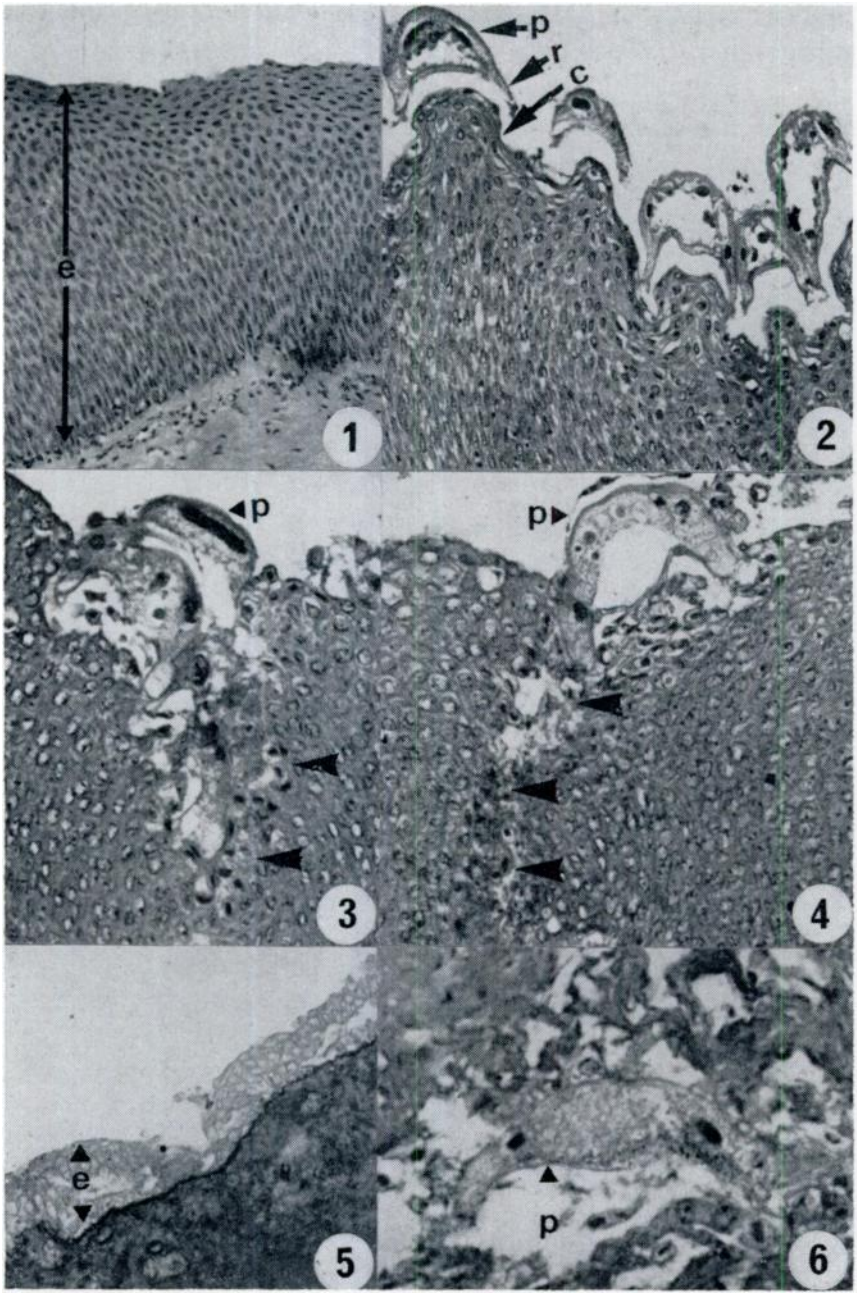
MATERIALS AND METHODS

Adult female thorny skates were obtained from coastal areas adjacent to St. John's, Newfoundland, Canada. The wall of the copulatory sac, adjacent to the oviducts, was removed and pieces of tissue fixed in Zenker's fluid, Gomori's 1-2-3, (one part formalin, two parts saturated mercuric chloride and three parts water) and sea water Bouin's (75 ml saturated picric acid in filtered sea water, 25 ml formalin and 5 ml glacial acetic acid). The tissues were processed according to standard histological methods and sections cut at about 7 μm. They were stained routinely with Harris' hematoxylin and eosin and Mallory's triple stain. In addition several staining techniques were utilized to demonstrate particular features. These include the periodic acid Schiff (PAS) test for carbohydrates; PAS after salivary and diastase digestion for glycogen; Alcian blue (pH 2.5) for acid mucopolysaccharides and Nile blue (formol-calcium; frozen sections) for neutral fats.[7]

RESULTS AND DISCUSSION

Structure of the Wall of the Copulatory Sac Uninfected Adults

The wall of the copulatory sac has longitudinal folds extending from the vent to the opening of the oviducts. It is made up of stratified epithelium several layers in thickness and subtended by a basement lamina (Fig. 1). Beneath the latter is a layer of connective tissue with some elastic fibers, containing numerous blood vessels. This is in turn surrounded by a layer of circular muscle.



Legend of Plates

Photomicrographs of sections through the wall of the copulatory sac of thorny skates stained with hematoxylin and eosin (Figs. 1-4, 6) or xylidine ponceau (Fig. 5).

Fig. 1. Section from uninfected adult skate. e = epithelium. x 125

Fig. 2. Trichodinids (P) attached to the epithelial surface. Note where the rim (r) of each parasite makes contact with the host's epithelium, a cleft (c) is produced. x 125

Fig. 3. Parasites (p) penetrating the epithelium. Note pycnotic nuclei (arrows) of host's cells. x 200

Fig. 4. A trail (arrows) of damaged cells lies ahead of the parasite (= p) without evidence of **T. oviducti** in this region. x 200

Fig. 5. Eroded epithelium (e) as a result of a heavy infestation. (e = epithelium). x 125

Fig. 6. Parasite (p) that has penetrated into the submucosa. Note damage to host tissue. x 400

In the superficial layers of the epithelium, the eosinophilic cytoplasm contains vacuoles and pycnotic nuclei, whereas in the deeper layers the nuclei are larger and vesicular and their cytoplasm is basophilic. The apical portions of the cells of approximately one-third of the epithelium are intensely PAS positive and this reaction is not abolished by prior amylase digestion. There is, however, some trace of glycogen. Upon staining with alcian blue, an area comparable in thickness to the PAS zone stains blue indicative of mucoproteins and acid mucopolysaccharides. Since glands are absent in the copulatory sac it is likely that the cells which constitute the PAS-alcian blue zone serve a secretory function by producing a mucoid material that covers the epithelial surface.

Infected Adults

Defoliation of the epithelial layer was related to the intensity and stage of the infection. In light to moderate infections, (Fig. 2), there was excessive sloughing of epithelial cells, sometimes to the extent that there was little or no evidence of the mucopolysaccharide region. The epithelial surface appeared sculptured with elevations that conformed to the adhesive discs of the parasites (Fig. 2). In many instances, there was evidence of sloughing in deeper layers opposite the margins of the adhesive discs, suggestive of a chemical rather than mechanical damage (Figs. 3 and 4).

The changes in acute trichodiniasis were associated with hemorrhage in the mucosa, desquamation of the superficial epithelium and an increased mucoid secretion. The epithelial wall was considerably reduced in thickness and in some areas when large numbers of parasites were present it was almost denuded (Fig. 5). Parasites were located in the mucoid exudate, while others were observed either in close contact with the epithelial wall or in the submucosa (Fig. 6). In the latter case considerable destruction of the tissue occurred including the rupture of blood vessels and appearance of petechiae. Sloughed cells were ingested by the parasites (Fig. 3). Occasionally, there was evidence of an inflammatory response in areas where the epithelium had been eroded. There was only one occurrence of a secondary infection in which bacteria were microscopically identified in denuded areas but no attempts were made to routinely demonstrate possible bacterial or viral infections.

The destruction caused by *T. oviducti* is comparable to that induced by other trichodinids.[5] The skeletal denticles which support the adhesive disc of each ciliate are covered by a thin membrane and do not make contact with the host's tissue.[4,5] The adhesive disc in fact is comparable to a 'sucker' and is used for attachment. As its rim appears to be the only part of the parasite in contact with the copulatory epithelium, some damage could be expected (vide, Fig. 2). How-

ever, it would not account for the erosion that was apparent in the deep areas of the copulatory sac epithelium without evidence of trichodinids in these regions. Since physical damage by *T. oviducti* is unlikely, probably a chemical substance is secreted (or excreted) to induce shedding of the copulatory epithelial cells. It is known that some tissue invading organisms gain entry by virtue of histolytic enzymes which cause exfoliation of superficial epithelia.[6] Hyaluronidase, an enzyme that acts on mucopolysaccharides, performs such a function and is known in a variety of microorganisms including protozoa. Of special interest is its occurrence in *Trichomonas vaginalis*, a parasite that is venereally transmitted. In acute trichomonad infections, this enzyme has been detected.[1] Since mucopolysaccharides were detected in the copulatory sac epithelium, it is speculated that possibly a similar if not identical enzyme(s) is involved in the exfoliation in skates infected with *T. oviducti*.

Histochemical tests have failed to demonstrate the presence of neutral fats or fatty acids in the wall of the copulatory sac of uninfected adult skates. A strong PAS reaction occurred in about a third of the epithelial layer but most of this neutral mucopolysaccharide was not glycogen since it was resistant to diastase digestion. There was also evidence of acid mucopolysaccharides in this zone. In contrast to mammals which store large amounts of glycogen in the liver, elasmobranchs convert most of their excess carbohydrates to lipids and only traces of glycogen are stored in the liver. A preliminary analysis of skate blood (poled blood from three adult females) revealed that 54% of the total carbohydrates are reducing sugars of which 8.5 mg% is glucose (D. H. Shaw, unpublished data). Mammalian blood, on the other hand, contains considerably higher levels (human, 90mg%) of glucose.[2] It is therefore conceivable that the storage products in the wall of the copulatory sacs of adult skates are different from their mammalian counterparts. The relationship between *T. oviducti* and the copulatory epithelium in skates might not therefore be as simple as is the case between *Trichomonas vaginalis* and the vagina of its host—a situation in which establishment of an infection is glycogen dependent. Although attempts to establish *T. oviducti* in subadult female skates, following injection of 17B estradiol, were unsuccessful, it is probable that the factor or factors responsible for establishment of the infections are associated with maturity since to date, a) examination of 1262 *R. radiata* has revealed natural infections only in adults (44% of 716 fish were infected) and b) experimental transmission of the parasite to subadult skates is transitory and short-lived (Khan, unpublished data). Moreover, as the mucopolysaccharide zone which is prominent in the wall of the copulatory sac of adult skates is considerably reduced in juvenile fish (Khan, unpublished data) possibly one or more of its components, necessary for establishing an infection, is low or absent in subadults.

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