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PARASITIC CASTRATION IN *FISSURELLA CRASSA* (ARCHAEOGASTROPODA) DUE TO AN ADULT DIGENEA, *PROCTOECES LINTONI* (FELLODISTOMIDAE)

MARCELO E. OLIVA

Instituto de Investigaciones Oceánologicas, Facultad de Recursos del Mar, Universidad de Antofagasta, casilla 170, Antofagasta Chile

Specimens of Fissurella crassa *(Archaeogastropoda) from Ilo, southern Perú, are infected with the adult stage of the digenetic trematode* Proctoeces lintoni *(Fellodistomidae). The histopathological analysis of the male and female gonads show a strong effect of the parasite on the structure and function of these organs.* P. lintoni *live unencysted in the gonads, and the main mechanical damage is originated by the action of a well developed acetabulum. Chemical actions of parasitic secretions may also be involved. The infected gonads show altered structure and the gametogenic processes is aborted. There is no evidence of hemocytic response, but leucocite infiltration is evident at least in male infected gonads. An increased content of polysaccarides is evident in infected gonads.*

Key words: *Fissurella crassa* – *Proctoeces lintoni* – Mollusca – Digenea – parasitic castration

The key hole limpets *Fissurella* spp. (Mollusca: Archaeogastropoda) are found parasitized in the gonads, by the adult of the digenetic trematode *Proctoeces lintoni* Siddigi & Cable 1960. *F. crassa* shows a prevalence of infection of over 80%, mean intensity of 14 and a maximum reported of 107 (Oliva & Díaz, 1988). These values, in an infectious processes that affect the gonads, may decrease the reproductive success of the host population.

The ability of members of *Proctoeces* to develop and reach sexual maturity in the mollusc host is well known (Cheng, 1967). The effects of *Proctoeces* spp. can be lethal or can originate partial or total castration (Bray & Gibson, 1980; Matshkevsky, 1985). The phenomenon of parasitic castration due to digenetic trematodes can be defined as the total or partial reduction of gamete formation (Pearson & Cheng, 1985). All articles about parasitic castration due to digeneans, are related with the action of larval worms. In this paper, a first approach to the histopathological effects produced by an adult digenea on the reproductive success of marine gastropods is given.

MATERIALS AND METHODS

The key hole limpets were hand collected from the upper level of the rocky shores, near Ilo, southern Perú; 33 specimens, ranging from 55 to 60 mm were obtained. The size of the molluscs ensured that all of the sample would be parasitized (see Oliva & Diaz, 1988). After collection, specimens were dissected, the gonads were isolated and fixed in 10% hot formalin. Latter they were processed according to traditional histological techniques. Samples were embedded in parafin, serially sectioned (5 μm) and stained with haematoxylin-eosin, Van Gienson tricromic or SPA (Schiff Peryodic acid). Parasites of other molluscs were also collected and fixed alive, to study the intestinal content of the worms.

RESULTS

Normal Histology – Male: the testes are surrounded by a fibro-collagen conective tissue, which in turn is covered by a single epithelial tissue layer. The testes are internally, composed of a large amount of tubules, the center of each of which contains a haemolymphatic duct (Fig. 1). The direction of the

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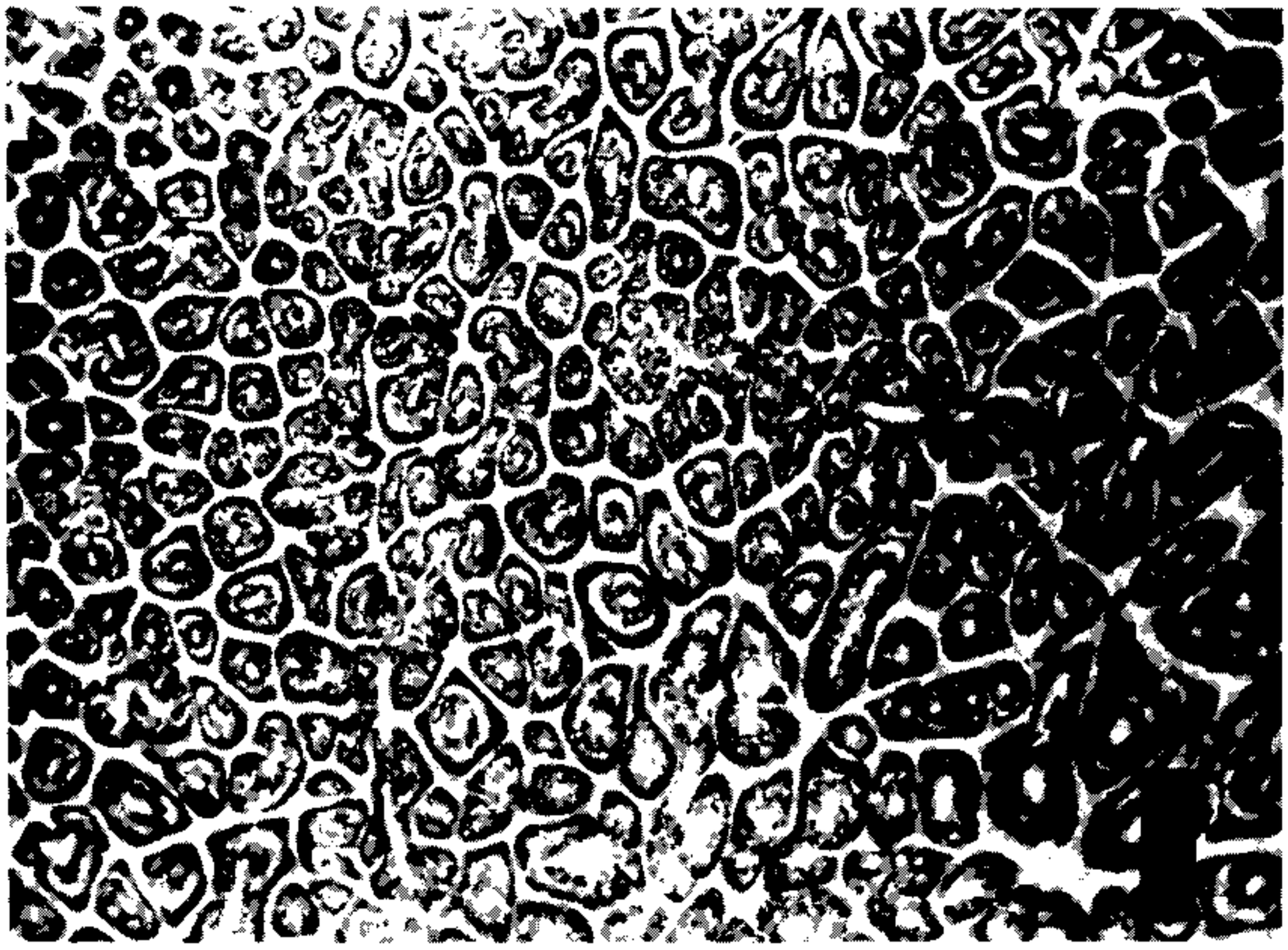

Fig. 1: *Fissurella crassa*. Non-infected male gonad. Ft: fused tubules. (bar = 0.56 mm).

gametogenic process is from the center of the tubules to periphery. The intertubular space is occupied by muscular tissue. The tubules can be fused, but only moderately in healthy gonads. *Female:* the surrounding tissue of the female gonads is like that of the male. The fibro-collagen tissue give rise to internal projections into the gonads, these projections can be branched and form septa, that in turn, originate compartments which contain sexual cells in different developmental stages (Fig. 2).

Histopathology – The main mechanical damage is caused by the attachment of the fluke by its acetabulum, to the inner surface of the gonadal covering tissue. This attachment causes the tissue destruction (Fig. 3). Associated to the mechanical action of the acetabulum, there is a disorganization, not only in the gonadal structure, but also in the functioning of the gonads. Thus, the affected gonadal tissue is not functional and a parasitic castration becomes evident. The process that affects the keyhole limpet is defined as a regression, because the gonadal tissue, at the end of the castration process, is sexually indistinguishable.

Structural disorganization – Male: the disorganization implies an alteration in the distribution of the tubules, with an apparent decrease in their number. The fused tubules increase notoriously and appear without a defined distribution pattern. The diameter of the haemolymphatic duct also increase and the intertubular material is partially lost. The width of the fibrocollagen conective tissue in parasitized gonads is wider than in healthy ones, and shows internal projections into the gonads. The thickeness of the wall of the haemolymphatic duct also increases. The altered tubules show scattered haemolymphatic cells, that infiltrate the wall of the haemolymphatic tubules. Moreover, the germinal cells are not associated with the walls of the haemolymphatic vessels, and are considerably displaced toward the periphery of the tubules, reaching the medial zone (Fig. 4). Furthermore, the gametogenic process is aborted. *Female:* as in males, the fibro-collagen tissue is wider in the infected than in the non infected zone. There are inner projections, but these shows an enlargement in the distal zone, and a structure like the altered male tubules is formed. There is no evidence of leucocitary infiltration in the female gonad.

Functional disorganization (regression) – Three phases can be defined or recognized in the regression process: I. *Initial regression:* the main characteristic of this step, is that sexual identity of the gonadal tissue remains evident. The gametogenesis is not absolutely aborted, but altered. The presence of mature gametes is evident, and the degree of disorga-

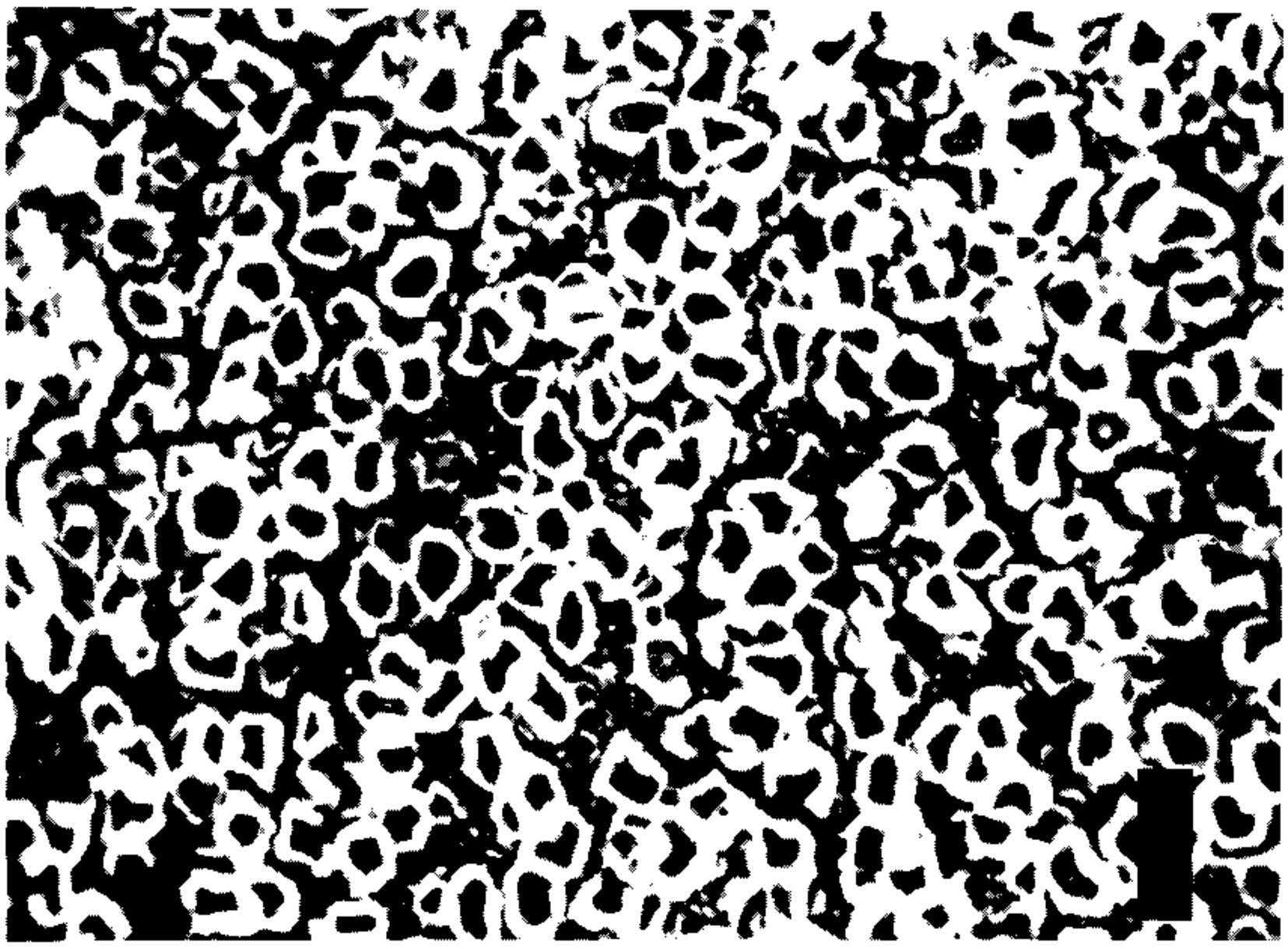

Fig. 2: *Fissurella crassa*. Non-infected female gonad (bar = 0.56 mm).

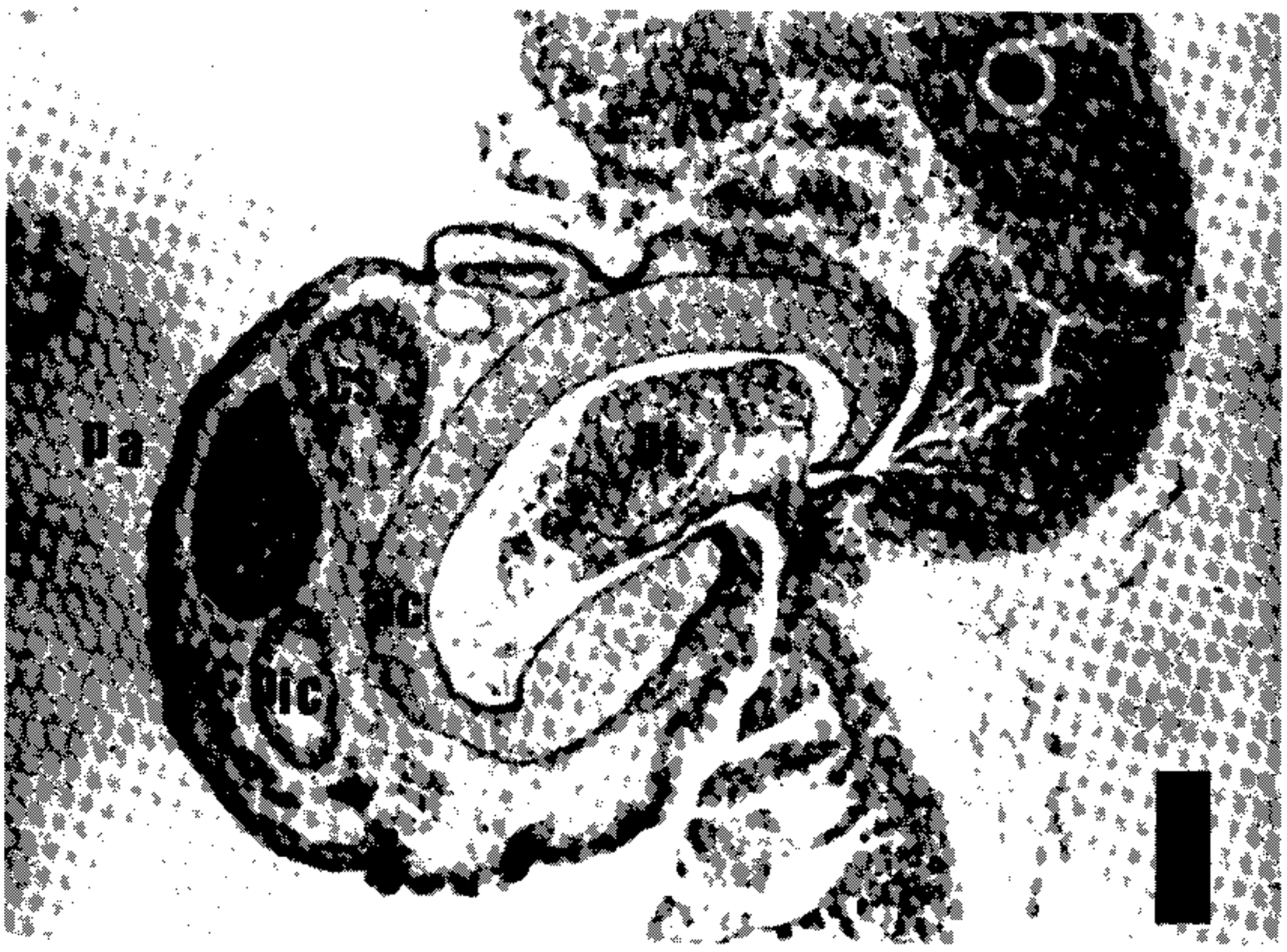

Fig. 3: *Fissurella crassa*. Infected female gonad. Oo = Oocite; Pa = Parasite; Ac = Acetabulum; Pic = Parasite intestinal caeca; Cs = Cirrus sac; Ov = Ovary of the parasite; Gt = Gonadic tissue directly affected by the acetabulum. Note the structural disorganization, compare with healthy gonad (Fig. 2) (bar = 0.28 mm).

nization is low. The gonadal wall show enlargement and there is an increase in polysacharides when compared with healthy gonads. II. *Advanced regression:* it is not possible to define the sexual identity of the gonads. The presence of germinal and support cells is associated to fibro-collagen tissue of haemolymphatic vessels of males, only. The same description applies to the enlarged portion in the distal end of gonadal wall projections in female gonads. The gametogenesis process is inhibited and no celular duplication occurs. The polysaccharide content is higher than in the initial phase. III. *Total regression:*

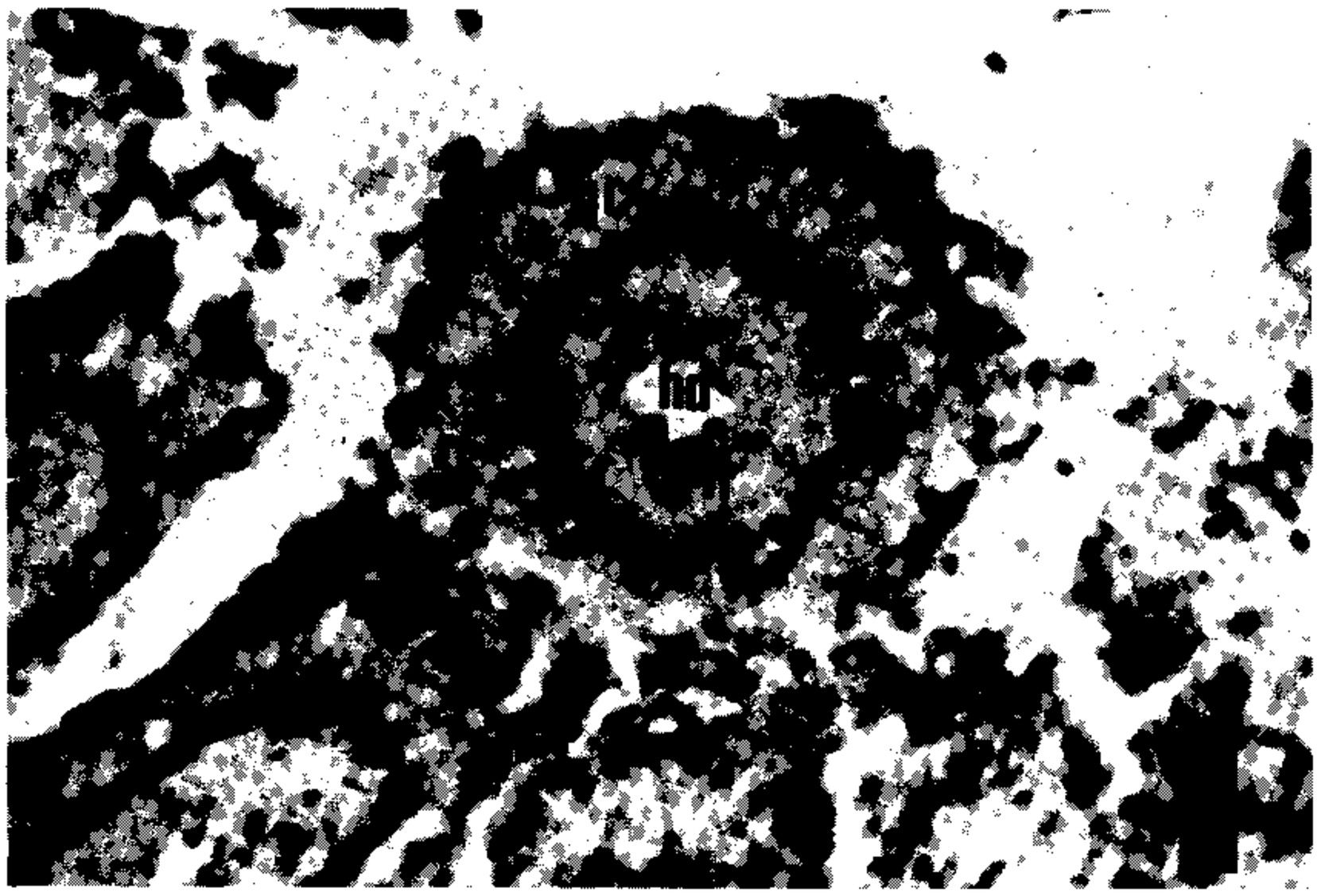

Fig. 4: infected male: structure of a tubule. Li = Leucocitary infiltrate; Gc = germinal cells; Hd = Haemolymphatic duct (bar = 0.03 mm).

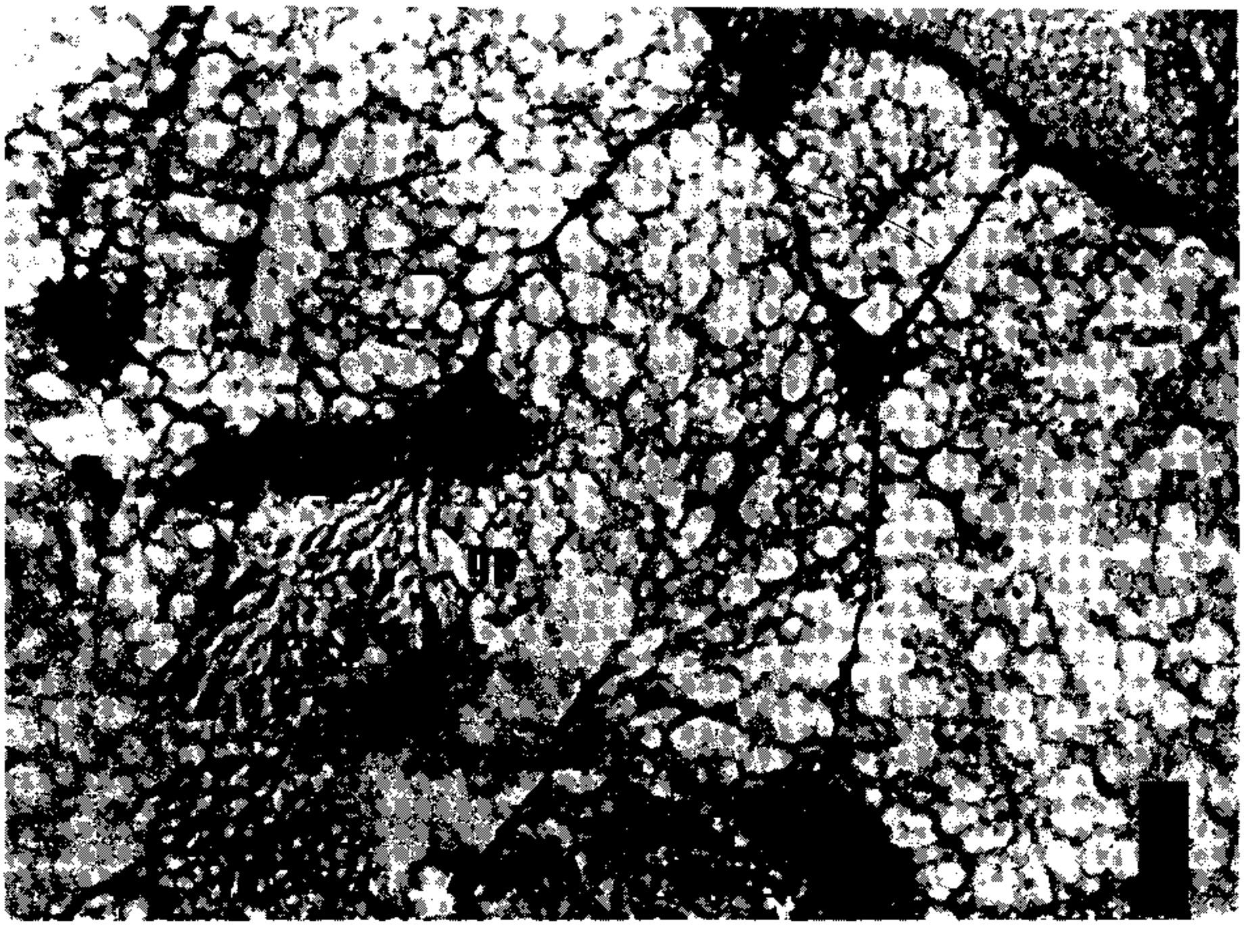

Fig. 5: absolutely altered gonad, sex unknown. Note the reticular structure of the altered gonad and the granular pigment (Gp) of unknown origin (bar = 0.01 mm).

the gonadal zones affected are well delimited by a fibro-collagen membrane. The area surrounded by the membrane, shows several compartments, different in size and shape. The wall of each compartment is a fibrous conective tissue with cells that have a prominent nucleoli with enlarged and smooth nuclear membrane. The contained cells my be germinal cells considerably altered. The compartments are characterized by containing a reticular and eosinophilic material with a high content of polysacharide. The altered condition of the

gonads is similar in both males and females, and the sex of the host remains indistinguishable. Moreover, the presence of a brown granular pigment is evident (Fig. 5).

DISCUSSION

The parasitic castration of molluscan host by digenetic trematodes, is known since 1873, and was first described by Mc-Crady. More recently, many investigators have reported this phenomenon (see Sullivan et al., 1985; Pearson & Cheng, 1985, for a review of the effect on reproduction, and Souza, 1983; Minchella, 1985, for a review of the effects on host life history variations in response to parasitic castration). All the known reports on parasitic castration, are related to larval stages of digenetic trematodes, but not by the action of an adult trematode. To the best of our knowledgment, the castration of *F. crassa* by *P. lintoni,* is the first report of parasitic castration due to an adult digenetic trematode on a gastropod host.

Sullivan et al. (1985) gave a hipothetical mechanism by which the parasite may inhibit the host gametogenesis. They considered primary and secondary effects. The first one, are related with an effect on the gonads, the latter, on the host physiology and indirectly on reproductive success. The damage originated by *P. lintoni* can be considered as primary, because the altered gonadic tissue are those related with the parasite. Mechanical effects are easy to see in the attachment of the acetabulum to the inner surface of the gonads (Fig. 3). This damage has not been previously described, because it is the consequence of the action of a well developed sucker, that is found only in the adult digenean. Thus, to the mechanical effects on the gonads, proposed by Sullivan et al. (1985), a new one, originated by the sucker action of digenetic trematodes must be added. Ingestion is another mechanical effect that can cause parasitic castration. The ingestion of gonadal (including gametes), lymphatic and/or epithelial cells by parasites, has been reported by Cooley (1962), who claimed that the rediae of *Parorchis acanthus* actively ingested gonadal cells of the gastropod *Thais haemastoma.* A similar picture is described by Crews & Esch (1986) for *Helisoma anceps* infected with *Halipegus occidualis.* Sullivan et al. (1985) said that there is no evidence of host cell ingestion by rediae of some digenetic trematode parasites of *Ilyanassa obsoleta.* Ingestion of host cells by members of *Proctoeces,* has been detected by Winstead & Couch (1981) in infective processes on *Crassostrea virginica* and by Turner (1985) in infected *Ischadium recurvum* by *P. maculatus.* Finally, George-Nascimento & Quiroga (1983) claim that *P. humboldti* (= *P. lintoni* see Oliva & Zegers, 1988) make use of host gametes as food. We were unable to find gametes or gonadal tissue in the intestine of *P. lintoni* in either: worms studied from serial section of gonads or parasites isolated and fixed alive in hot formalin.

The structural and functional disorganization of the gonads, can not be considered as a consequence of the mechanical damage alone but chemical damage must be considered, as well. The characteristics of the regression closely resemble those described by Sullivan et al. (1985), and specifically can be described as: 1) Gonads devoid of gametic precursor cells and/or gametes, but if gamete precursors are present, they are altered and are not functional, and 2) the gametic precursors are present, but they are unable to develop and gametogenesis is stopped in this phase. In both situations, the disorganization and non functioning cells are found in tissues near of the parasite. Thus, total regression and castration occur only is these tissues, but the rest of the gonad is functional, but only if a small number of parasites is present. Furthermore, Le Breton (1979) claimed that the duplication of germinal cells is stimulated by a mitogenic factor that is secreted by the ganglionar brain, and Nassi (1979) pointed out that parasites can play a role in the interference of the synthesis or secretion of gonadotropic hormones, that are elaborated in the central ganglia and are believed to control gametogenesis in molluscs. Thus, the presence of germinal cells that are unable to duplicate and develop, can be a consequence of the blockage and/or the inactivation of such a mitogenic factor, due to the interference of the gonadotropic synthesis by a metabolic secretion of the parasite. This hypothesis is reinforced by the data of Pearson & Cheng (1985) who found a gametogenesis-inhibiting factor in extracts of daughther sporocysts of *Zoogonus lassius.*

The presence of a granular pigment, similar to those found by Sullivan et al. (1985) is apparent in gonads that are in total regression. This material remain of unknown nature and, in our case, is associated with a reticular material and high polysacharide level.

The response of the key hole limpets to the infection in not clear. There is no evidence of a significant hemocytic response or inflamation and encapsulation is not evident. But, there are no clear responses in molluscs to infections originated by metazoans. Thus, a hemocytic response is evident in *Ischadium recurvum* infected with *P. maculatus,* as has been demonstrated by Turner (1985), but is not evident in *C. virginica* infected with *Proctoeces* sp. (Winstead & Couch, 1981). Huffman & Fried (1985) found that living rediae and sporocysts of *Sphaeorodiotrema globulus* do not originate an hemocytic response, but the ocurrence of dead rediae did provoke a hemocityc response. Cheng et al. (1983) found a very special situation, they said that "two specimens (of the snail *Ilyanassa obsoleta*) infected with *Zoogonus lassius* that had been collected in 1981, both contained numerous hemocytic capsules in their tissues, whereas neither of two snails infected with the same parasite, but collected in 1982, showed any evidence of a cellular response". The nature of the high variability in the hosts, in terms of hemocytic response at both, the intra and interspecific levels, remain unknown. A possible explanation of the absence of hemocytic response in *F. crassa* infected with *P. lintoni* can be due to the inability of the mollusc to recognize the parasite as foreign material.

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