RESEARCH NOTE

Unusual Histological Findings in *Biomphalaria glabrata* with High Degree of Resistance to *Schistosoma mansoni* Miracidia

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Resistance to *Schistosoma mansoni* miracidial infection in susceptible snails is probably always partial, some sporocysts escaping destruction and giving rise to cercariae, albeit in small numbers and with delayed shedding (C Pereira de Souza et al. 1995 *Rev Inst Med Trop S Paulo 37*: 201-206). Amebocyte proliferation and formation of encapsulating lesions around developing larvae are a hallmark of snail resistance (CT Pan 1965 *Am J Trop Med Hyg 14*: 931-976).

Strongly resistant *Biomphalaria glabrata* were observed through successive generations during an investigation on genetic influences in resistance. Several snails from an F-15 generation were experimentally exposed to *S. mansoni* miracidia. They were kept at a temperature of 25±2°C. Checking for cercarial shedding was made one month following miracidial exposure and weekly thereafter for those which were negative. Three months afterwards some snails (Group 2) started eliminating a few cercariae (approximately ten, compared with one-hundred in non-resistant control snails - Group 1), while others shed none (Group 3). Five snails from each group were then submitted to histological examination after Bouin's fluid fixation, paraffin embedding and hematoxylin-eosin staining of multiple histological sections. Highly susceptible Group 1 snails exhibited sporocysts in several stages of development, with widespread distribution in several organs, but without signs of tissue reaction. Group 2 snails presented the histological findings usually expected in such cases (WL Newton 1952 *J Parasitol 38:* 362-366). There were foci of multiplying sporocysts at different stages of differentiation, including almost mature cercariae, in several organs and tissues. These foci were seen side by side with areas exhibiting strong proliferation and infiltration of amebocytes, which often accumulated and formed granuloma-like or encapsulating structures containing disintegrating sporocysts in their centers.

Group 3 snails, that completely failed to eliminate cercariae after three months of exposure, did not exhibit *S. mansoni* sporocysts in histological sections, but, instead, presented an unusual and striking picture of amebocyte reactivity. This basically consisted of focal and diffuse proliferation of amebocytes, accompanied by an expansion of the extracellular matrix, which sometimes simulated the process of fibrosis seen in vertebrate tissues. The diffuse amebocyte reaction was conspicuous in the albumen gland of all the five cases examined and also appeared associated with thickening and increased basophilia (calcification) of the amorphous secretion material accumulated in the ducts and acini of the albumen gland which were lined with disintegrating epithelial cells (Fig. 1). A collar of amebocyte proliferation and matrix thickening was frequently seen around the ducts and tubes, especially at the external coat of the digestive tract (esophagus and intestines) and around the acini of the nidamental gland. A few scattered areas of focal thickening of the stroma appeared amongst the digestive glands and in the ovo-testis (Fig. 2). Focal reactions frequently assumed a granuloma-like appearance. Encapsulating lesions were especially prominent in the tubular and sacular portions of the kidneys (Fig. 3). They were also seen within the connective tissue bordering the mantle cavity, but were rare in the foot and other areas of the anterior portion of the snail. These reactions were formed by amebocytes, fibrils and fibers concentrically disposed around a central area in which a few small, irregular basophilic granules were sometimes seen (Fig. 4). The possibility that such granules represented sporocyst remnants cannot be ruled out. Such changes in the absence of parasitic structures seem unusual. Although the possibility of self-cure has been mentioned in *S. mansoni*-infected snails (K Lie et

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al. 1980 *Ann Trop Med Parasitol 74*: 157-166, CHJ Schutte 1975 *South African J Sci 71:* 8-20), a histological picture of amebocyte reactivity in the apparent absence of sporocysts has not hitherto been described.

The present findings suggest that a strong immune response can occur in such snails during the course of infection, assuming that our Group 3 snails achieved total clearance of the parasites after sporocysts migrated throughout the snail tissues.

Control experiments were performed to investigate the possibility of other causes for the changes described. Intact laboratory raised snails from both Belo Horizonte, State of Minas Gerais and Feira de Santana, State of Bahia, three months of age or older, were histologically examined to see whether "old age" or some environmental factors could be responsible for the histological changes observed in our Group 3 snails. All these control snails exhibited an essentially normal histology.

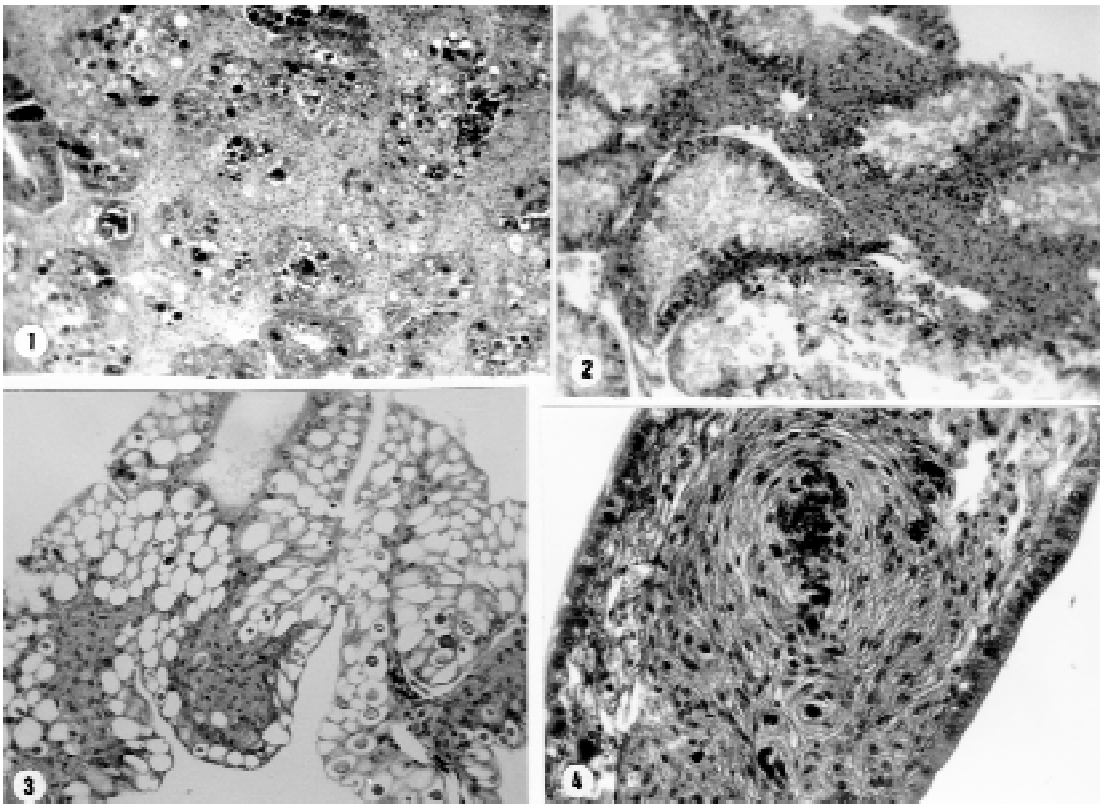

Fig. 1: diffuse proliferation of amebocytes causing expansion of the interstitial tissues and dissociation of the functional structures of the albumen gland. Secretory material in the ductular lumen is thick and calcified. Hematoxylin & Eosin, 100 X. Fig. 2: focal stromal thickening in digestive glands of *Biomphalaria glabrata*. Hematoxylin & Eosin, 160 X. Fig. 3: tubular region of the kidney showing the characteristic clear cells and focal areas of stromal thickening, with amebocyte proliferation, but no parasitic structures. Hematoxylin & Eosin, 250 X. Fig. 4: granulomatous reaction encapsulating a dark granular material found in the mantle collar. Hematoxylin & Eosin, 400 X.