STRUCTURE AND FUNCTION OF THE CALCIUM CELLS OF THE FRESHWATER PULMONATE SNAIL LYMNAEA STAGNALIS

by

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SUMMARY

The morphology and function of the calcium cells of Lymnaea stagnalis were studied. These cells contain concretions consisting of an organic matrix in which inorganic material, mainly CaCO₃, is deposited. The organic matrix consists of protein, carbohydrate and lipid, synthesized by the Golgi apparatus. It appeared that deposition of CaCO₃ is an active process; CaCO₃ is released from the concretions into the haemolymph via "pores" in the plasma membrane of the calcium cells. Experiments on snails exposed to water equilibrated with 10% CO₂ in air strongly suggest that calcium cells function in pH homeostasis.

INTRODUCTION

In molluscs solid calcium salts are not only present in the hard parts such as shell, operculum and epiphragm, but also in the soft body components (e.g. WATABE et al., 1976). In bivalves, for example, calcareous deposits occur in the gill lamellae and the mantle (NUMANOI, 1939; ISTIN & GIRARD, 1970); in gastropods such deposits are present in the connective tissue (KAPUR & GIBSON, 1968; TIMMERMANS, 1969; TOMPA & WATABE, 1976), the foot muscles (LITTLE, 1965) and the digestive gland (ABOLINS-KROGIS, 1970; BURTON, 1972). In the latter organ the calcium salts are located in special cells of the digestive gland epithelium (ABOLINS-KROGIS, 1970; WALKER, 1970; BURTON, 1972). With respect to the other calcium storing tissues it is not clear whether the salts are located intra- or extracellularly (LITTLE, 1965; TIMMERMANS, 1969; ISTIN & GIRARD, 1970; RICHARDOT, 1976), most likely due to the fact that extensive histological and ultrastructural studies are lacking. Several hypotheses have been forwarded as to the function of the calcareous deposits in the soft body parts. Firstly, it has been suggested that calcium salts are stored for shell repair and operculum or epiphragm formation (ABOLINS-KROGIS, 1961; ISTIN & GIRARD, 1970). Secondly, the salts have been considered as metabolic waste products, especially of protein catabolism (KNIPRATH, 1975). Finally, the deposits are thought to be an anion pool, mainly of carbonates, which might play a role in buffering tissue fluids (KRIJGSMAAN, 1928; BURTON, 1970, 1976). Experimental work to elucidate the functional significance of the calcium deposits has not been carried out.
Preliminary studies in our laboratory have shown that in the connective tissue of the freshwater snail *Lymnaea stagnalis* calcium deposits are located intracellularly, in calcium cells (SMINIA, 1975). The present study deals with the morphology, distribution and function of these cells.

**MATERIALS AND METHODS**

For the investigations adult, laboratory-bred specimens of *Lymnaea stagnalis* (shell height 25–35 mm) were used.

**Morphology.** For light microscopy snails were fixed in Sörensen buffered Baker’s formol (pH 7.4). Serial paraffin sections of 7 μm thickness were stained by various histological and histochemical techniques (PEARSE, 1968). For the demonstration of calcium salts the metal substitution technique of Von Kossa (PEARSE, 1968) and the chloranilic acid method (CARR et al., 1961; CHAPLIN & GRACE, 1976) were applied. Control sections were treated with di-Na-EDTA. For electron microscopy pieces of connective tissue of various parts of the body were fixed in either a freshly prepared mixture of glutaraldehyde (final conc. 0.8%) and OsO₄ (final conc. 1%), buffered at pH 7.2–7.4 with a Na-veronal-acetate/HCl buffer (2 hrs, 4°C), or in a Sörensen buffered 2% glutaraldehyde solution (1 hr, 4°C), followed by washing overnight in the same buffer and post-fixation in a Sörensen buffered 1% OsO₄ solution (2 hrs, 4°C). For the demonstration of calcium at the ultrastructural level both the potassium pyroantimonate (SIMSON & SPICER, 1975) and the ammonium oxalate method (KoHNICK, 1969) were used (controls were treated with the Ca-chelator EDTA). Ultrathin Epon sections, cut on a Reichert ultramicrotome, were stained with 1% aqueous uranyl nitrate and Reynolds’ lead citrate and examined in a Philips EM300 electron microscope.

**Enzyme histochemistry.** Reactions for the demonstration of alkaline phosphatase and carbonic anhydrase were carried out. Histochemical procedures and incubation media as recommended by GEYER (1973) were used. For light microscopy cryostate sections (thickness 8 μm) of connective tissue fixed for 2–6 hrs at 4°C in Sörensen buffered Baker’s formol or glutaraldehyde (2%, pH 7.2) were employed. For ultrahistochemistry the tissues were fixed for 1 hr in Sörensen buffered glutaraldehyde or paraformaldehyde (2%, pH 7.2). After rinsing in the buffer 100–200 μm thick sections, made on a Tissue Chopper, were incubated.

**Isolation and chemical characterization of calcium salt concretions.** For the isolation of calcium concretions samples of the connective tissue layer