Studies on the Ovotestis of the Slug Agriolimax reticulatus (Müller)

2. The Epithelia

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Summary. The follicle cells, nurse cells and germinal epithelia, which are closely associated with the oocyte of Agriolimax reticulatus (Müller) during its development in the ovotestis, have been studied using light and electron microscopy. The various secretory, digestive and phagocytic activities of these cells have also been investigated using electron cytochemical tests for oxidisable polysaccharide, acid phosphatase and electron-opaque tracer molecules. The oocyte lies initially between the germinal epithelia and a layer of nurse cells but, as oocyte vitellogenesis proceeds, it becomes encapsulated by a layer of follicle cells. Both the follicle and the nurse cells are active in secretion and digestion and contain Golgi apparatus, granular endoplasmic reticulum and acid phosphatase-rich digestive vacuoles. The significance of these activities is discussed in relation to oocyte vitellogenesis, secondary envelope formation and the digestion and recycling of cellular material.

Key words: Ovotestis (Agriolimax reticulatus) – Follicle cells – Phagocytosis – Cytochemistry – Ultrastructure.

Introduction

The oocytes of many species are surrounded by a layer of cells, generally known as the follicular epithelium. These cells are initially squamous shaped but in some cases they may subsequently become cuboidal and then columnar (Anderson, 1974). A number of roles have been ascribed to the follicle cells: by acting as a selectively permeable barrier, the epithelium may control the immediate microenvir-
ment of the oocyte and hence its ontogeny. In a number of species the follicle cells are synthetically active and appear to contribute to the secondary membranes and also to the yolk of the oocyte.

The follicle cells from a variety of Molluscan species have been studied using the electron microscope: namely, Amphineura (Anderson, 1969b; Selwood, 1968, 1970); Cephalopoda (Bottke, 1974; Richard and Dhainant, 1973); Gastropoda (Bottke, 1972; Taylor and Anderson, 1969). In all these species the follicle cells are synthetically active and possess well developed Golgi apparatus and arrays of rough endoplasmic reticulum. However, the assumption made by a number of light microscopists (Lankester, 1875; Vialleton, 1888; Yung Ko Ching, 1930; Bolognari, 1976) that their synthetic activity is directly involved in oocyte vitellogenesis has not been confirmed. This conclusion is based on the observation that the oocyte does not incorporate exogenous macromolecules by pinocytosis (Anderson, 1969b; Bottke, 1973, 1974; Richard and Dhainant, 1973; Taylor and Anderson, 1969). In the species of Amphineura studied, the follicle cell is involved in the secretion of secondary envelopes (Anderson, 1969b; Selwood, 1968, 1970).

In the present study, on the slug *Agriolimax reticulatus* (Mü), ultrastructural and cytochemical investigations on the synthetic and digestive activities of the follicular epithelium and associated cell types during oogenesis will be described.

**Methods and Materials**

**Electron Microscopy**

Ovotestes from adult slugs, which had been freshly collected from local parks and gardens, were rapidly dissected out and fixed for 1 h at 0–4°C in 3% phosphate buffered glutaraldehyde, pH 7.2. They were then washed overnight in 0.2 M buffer before being post-fixed in 1% osmium tetroxide (Millonig, 1961) at 0–4°C for 1 h. Ovotestes were subsequently dehydrated in a series of alcohols and routinely embedded in Araldite. Ultrathin sections were cut on a LKB III ultramicrotome and mounted on carbon-coated, copper grids. Sections were stained with uranyl acetate (Gibbons and Grimstone, 1960) and lead citrate (Reynolds, 1963). The grids were examined either in an A.E.I. E.M.6 or a Philips 300 electron microscope operating at 80 kV.

**Demonstration of Oxidisable Polysaccharide (Bowen et al. 1975)**

Details of the method used are described elsewhere (Hill and Bowen, 1976). After fixation in 3% glutaraldehyde, ovotestes were oxidized in 4% chromic acid for 40 min., impregnated in ammoniacal silver nitrate solution for 20 min. and fixed in 30% formalin for five minutes. The ovotestes were subsequently post-fixed in 1% buffered osmium tetroxide and routinely prepared for electron microscopy. As a control, ovotestes were impregnated in the silver solution without prior oxidation in chromic acid.

**Demonstration of Acid Phosphatase**

Acid phosphatase activity was demonstrated using the paranitrophenyl phosphate method of Ryder and Bowen (1975).