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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (2): 322-325, 2007
ISSN 1028-8880

Gross Morphology and Ultrastructural Study of Albumen Gland of the Land Snail *Archachatina marginata ovum* (Pfeiffer) (Pulmonata: Achatinidae)

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Abstract: The gross morphology of the albumen gland of the edible land snail *Archachatina marginata ovum* (Pfeiffer) was studied by measurement of the shell length of the snails and wet weight of albumen gland of specimens collected from wild. Additionally, the structure of the albumen gland was studied by light and electron microscopy. The gland is large during the breeding season and there is no correlation between its weigh and the shell length of the snail. The albumen gland is made up of tubules which consist of closely packed single layer of elongated columnar secretory cells arranged radially around a central lumen. The secretory cells have large nuclei, secretory granules and are densely packed with rough endoplasmic reticulum. The secretory cells of the albumen gland produce secretions which are massively stored in their cytoplasm. Two types of secretions were identified, small droplets and large globules. The large globule was so extensive that the diameter of the cell was greatly reduced. TEM shows that the secretory globules contain fine morular granules characteristic of glycogen in several cell types and probably β-glycogen.

Key words: Albumen gland, ultrastructure, Achatinidae, *Archachatina marginata ovum*, secretory cells

INTRODUCTION

Although the biology of the reproductive systems of pulmonate gastropods has been well studied (Lusis, 1961; Ghose, 1963; Rigby, 1965; Runham and Laryea, 1968; Breckenbridge and Fallil, 1973), very few of the previous studies have investigated the fine structure of the albumen gland. The albumen gland is a female accessory sex gland found in the reproductive tract of pulmonate snails (Mukai *et al*., 2004) which supplies the nutritive fluid in which the embryo develops (Baynes, 1973). The fluid secreted by the albumen gland, known as albumen (Bayne, 1967) or perivitelline fluid (Mukai *et al*., 2004) constitutes a major part of the eggs of pulmonate snails, for example, in *Strophocheilus oblongus*, a south African giant snail, the egg which weighs 1.0-2.9 g contains 72% water, 18% albumen gland and 10% shell. The perivitelline fluid provides the developing embryo with protein and galactogen and determines the number of eggs in the egg mass (de Jong-Brink *et al*., 1983; Boyle and Yoshino, 2000).

The tubules of the albumen gland empty their secretion into the albumen canal within the gland. The albumen canal and the hermaphrodite duct join to form the fertilization sac (Kugler, 1965). The fertilized eggs receive yolk from the albumen gland and then pass into the uterus where the yellow egg shell is added before they move to the oviduct and out through the genital opening (Emberton, 1985). When, the albumen gland cease secreting fluid, the ovulated oocytes that remain in the hermaphrodite duct and carrefour are reabsorbed in the spermatheca (Boyle and Yoshino, 2000).

The present study will describe the morphology of the albumen gland of *A. marginata ovum* using light and electron microscopes.

MATERIALS AND METHODS

Snail collection: Thirty two sexually mature specimens of *A. marginata ovum* of shell length 110-190 mm were collected randomly by hand picking from a site at Iguobazuwa village, near Benin City in the rainforest area of southwest Nigeria (lat. 06° 19^{1} N: long. 05° 36^{1} E). They were brought to the laboratory within 24 h of collection. Each snail was weighed to the nearest 0.1 g on an electric balance and measured with a caliper to the nearest 0.1 mm.

Gross morphology of the albumen gland: The shell was broken carefully, the snail was removed, the entire reproductive system was dissected and the albumen gland taken out immediately and weighed. The relationship between the shell length and weight of albumen gland was statistically analysed using correlation coefficient and regression analysis (Dytham, 2004).

Light microscopy: Pieces of the albumen gland were fixed in aqueous Bouin for light microscopy. After fixation the tissues were dehydrated in graded ethanol and embedded

in paraffin wax. Serial sections were stained in Heindenhains iron haematoxylin and counter stained in alcoholic eosin.

Electron microscopy: Small pieces of the albumen gland were fixed overnight in 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer with 5% sucrose (pH 7.8) at 4°C (Glauert, 1982; Reid, 1982). Fixed tissues were rinsed in several changes of sodium cacodylate buffer and post-fixed in 1.0% osmium tetroxide in the same cacodylate buffer for 2 h at room temperature. The tissues pieces were then dehydrated in graded ethanol and embedded in araldite via propylene oxide. Ultrathin sections were cut with glass knives using a Reichert OM U3 ultramicrotome and picked on copper grids and stained sequentially in uranyl acetate and Reynolds' lead citrate (Reynolds, 1963). The sections were examined on a Philip 400T electron microscope.

RESULTS

Gross morphology: The albumen gland is large in *A. marginata ovum* and during the breeding season in mature snails it is lemon yellow and translucent. It lies across the apical spermoviduct and it is connected to it by thin connective tissue strands. Considerable variability occurred in the weight of the albumen gland (Fig. 1). Thus for any given date, individual snails of the same population may differ widely in their reproductive development and size of the albumen gland. There was a no relationship between the shell length and weight of albumen gland ($r^2 = 0.11$, $p = 0.06$, $p > 0.05$).

Mature albumen gland: A mature and secreting albumen gland is made up of tubules observed in light microscopy as closely packed single layer of elongated columnar apocrine secretory cells radially arranged around a central lumen (Fig. 2a). The tubules are separated from each other by a thin connective tissue sheath which contains blood spaces and is continuous with the connective tissue that binds the gland peripherally. The intercellular spaces between the tubules were dilated and there were several elongated cisternae of granular endoplasmic reticulum located parallel to the convoluted plasma membrane.

The cell boundaries of the secretory cells are distinct and the nuclei are basally placed (Fig. 2a). The cells produce secretions which are massively stored in the cytoplasm (Fig. 3a and b) and the secretions appear as large polygonal granules in light microscope (Fig. 2a).

TEM shows that the secretory cells include a large nucleus (Fig. 2b); the cytoplasm is densely packed with cisternae of rough endoplasmic reticulum (Fig. 2b, 3a and b). Two types of secretions were identified in the

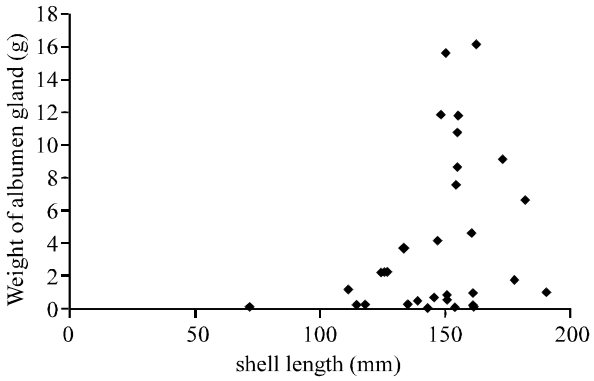

Fig. 1: Scatter diagram showing the relationship between the wet weight of albumen gland and shell of *A. marginata ovum*

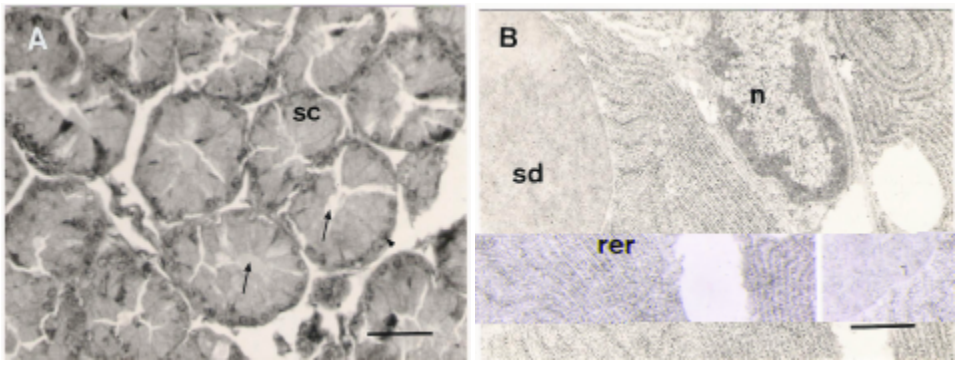

Fig. 2: (A). Light micrograph showing transverse section of a mature albumen gland of *A. marginata ovum.* Abbreviation: sc, secretory cell; lumen, arrow; nucleus, arrow head. Scale bar = 25 μm. (B) TEM of a mature albumen gland of *A. marginata ovum.* Abbreviations, n, nucleus; rer, rough endoplasmic reticulum; sd, secretory droplet. Scale bar = 0.3 μm

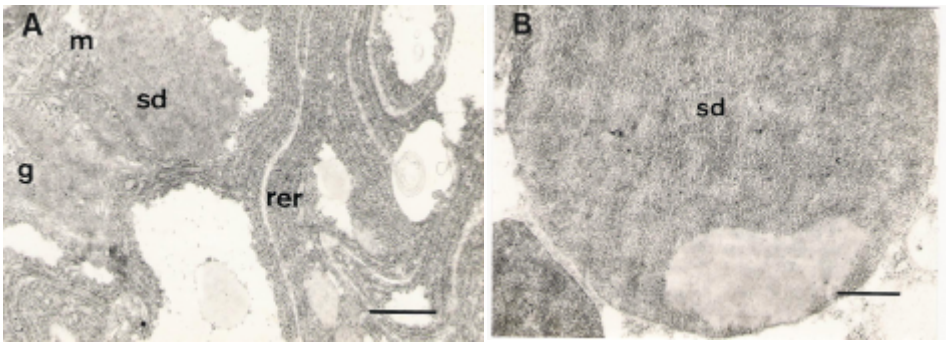

Fig. 3: (A) TEM of a mature albumen gland of *A. marginata ovum*. Abbreviations: g, Golgi complex; m, mitochondrion; rer, rough endoplasmic reticulum; sd, secretory droplet. Scale bar = 0.4 μm. (B) TEM of a large secretory droplet in the albumen gland of *A. marginata ovum*. Abbreviation: sd. Secretory droplet. Scale bar = 0.2 μm

cytoplasm, small droplet (0.7±0.03 μm in diameter) and large globules (3.0±0.4 μm in diameter). The large globule was so extensive that it filled the entire cytoplasm. The secretion contains fine morular granules (Fig. 2a and 3) characteristic of glycogen.

DISCUSSION

The size of the albumen gland in pulmonates increases with sexual maturation and it is a determinant of the maximum number of eggs that can be produced at any one time (Tompa, 1984; Gomez, 2001). In juvenile *A. marginata ovum*, the albumen gland is very tiny and creamy white, small white, flaccid and spent in post reproductive stage and recently spawned but prior to egg-laying, the albumen gland is lemon yellow and translucent (Egonmwan, 2004). In this study, no correlation was observed between the shell length of *A. marginata ovum* and the weight of the albumen gland; there is a tendency of the weight of the albumen gland to increase or decrease during egg-laying. In the pulmonate snails, *Limicolaria flammea*, with a defined annual cycle, breeding almost through out the year (Egonmwan, 2004) and *Triodopsis tridentata tridentata* (Emberton, 1985) that exhibit regular reproductive cycle and live for less than two year, the growth of the albumen gland follows the maturation of the gonad and there is no seasonal reduction in the weight in mature snails. In *A. marginata ovum* which lives for five years or more (Plummer, 1975) and has a defined breeding season; the growth of the albumen gland is cyclical and seasonal. No gametes were observed in the albumen duct of *A. marginata ovum* as reported for *Succinea putris*, an opisthobranch mollusc (Rigby, 1965).

The secretion produced by the albumen gland of gastropods, known as perivitelline fluid (Kugler, 1965; Morishita *et al*., 1998; Mukai *et al*., 2004) is high in polysaccharide, galactogen and protein which provide nourishment to the embryo during development (Horstman, 1956; Kugler, 1965) and hatchlings (Hunger and Horstman, 1968). In the freshwater snail *Helisoma duryi*, the major secretory protein of the albumen gland was identified as a native glycoprotein of 288 kDa (Mukai *et al*., 2004). Just before egg laying in the species, the albumen gland is enlarged and in *Helix pomatia*, the polysaccharide content increases markedly and the total polysaccharide of the snail is composed of 85% galactogen (Nieland and Goudsmit, 1969). The analysis of albumen gland protein in egg masses at different stages of development showed that the amount steadily decreased during embryogenesis (Mukai *et al*., 2004).

ACKNOWLEDGMENTS

I wish to thank Dr. Bayo Otitoloju (University of Lagos) for advice on statistical analyses and Yisa Ibrahim for the microphotography.

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