SOME ULTRASTRUCTURAL STUDIES ON THE EXCRETORY BLADDER OF *PODOCOTYLE STAFFORDI* MILLER, 1941, (DIGENEA)

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CONTENTS

1. SYNOPSIS

The ultrastructure of the excretory bladder of *Podocotyle staffordi* Miller, 1941, a digenean parasitic in the gut of the flounder *Platichthys flesus* (L.), is described. Crystalline 'excretory corpuscles', not previously recorded in adult digeneans, are shown to be associated with the bladder epithelium. An attempt is made to explain their formation by suggesting that they might have an osmo-regulatory function. This is related to the location of this parasite in the gut of a marine teleost, a region of variable osmolarity.

2. INTRODUCTION

Very few papers have been written on the ultrastructure of the excretory bladder of adult digeneans. Pantelouris and Threadgold (1963) studied the excretory system of *Fasciola hepatica* L. and Erasmus (1967) the reserve bladder of *Cyathocotyle bushiensis* Khan. The reserve bladder of the latter is a modified excretory bladder, and therefore does not conform to the normal digenean morphology. No research appears to have been done on the excretory bladder of digeneans parasitic in the gut of marine teleosts. This group is of special interest because these parasites encounter an environment of varying osmolarity, and thus may have an osmoregulatory problem lacking in the gut-parasites of other vertebrates. The ultrastructure of the excretory bladder of *Podocotyle staffordi* Miller, 1941, from the flounder *Platichthys flesus* (L.) has therefore been examined in an attempt to compare it with that of digeneans from other vertebrates reported on by other workers, and to see if such a study could shed any light on the ability of these parasites to withstand such variations in the osmolarity of their environment.

3. MATERIAL AND METHODS

Specimens of *P. staffordi* were obtained from flounders from the Ythan estuary, Aberdeenshire. These specimens were fixed, immediately after removal from the host, in 1 per cent osmic acid in sodium phosphate buffer at pH 7·2 for 1 hour. They

were dehydrated in ethanol, embedded in Epon 812 (Luft, 1961), and polymerised at 60°C for 48 hours. Transverse sections of the whole worms were cut on a Huxley ultra-microtome and mounted on uncoated grids. The sections were then stained for 30 minutes in a 5 per cent solution of uranyl-acetate in 50 per cent ethanol, followed by lead citrate (Reynolds, 1963) for 10 minutes. They were then examined under an A.E.I. E.M. 6B electron microscope at 60 kV.

4. OBSERVATIONS

The bladder-wall of *P. staffordi* consists of a single layer of epithelial cells. These cells are about 0·6 μm in thickness, except in the region of the nucleus where they reached 2 μm. The cells contain a large nucleus, rough endoplasmic reticulum, free ribosomes, mitochondria and three types of possible secretory granule. The endoplasmic reticulum and mitochondria are especially preponderate in the region of the nucleus (plate 1B). The three types of possible secretory granule (plates 1A & E) are as follows: type A, very granular, electron dense, about 150 nm in diameter, surrounded by a membrane and may be discoid; type B, round, slightly or non-staining, 50 nm in diameter, and occur close together in large numbers, often appearing to join and form long parallel canals (plate 1A); and type C, osmophobic or containing a slightly granular material, 200–300 nm in diameter, and occasionally appearing to release their contents into superficial vacuoles (plate 1C). Occasionally tubular or sheet-like invaginations of the inner plasma-membrane can be seen to pass deep into the cell (plate 1B): these are similar to the structures recorded by Davis *et al.* (1968) in the gastrodermal cells of *Haematoloechus medioplexus* Stafford, 1902. From the luminal surface of these cells project microrugae (leaf-like processes similar to those found in the gastrodermis of digeneans) which have bent over and anastomosed either with each other or with the outer plasma-membrane to form superficial vacuoles (plates A, B, D & E). Inside many of the superficial vacuoles are large crystalline corpuscles formed in concentric layers (plate 1A). These 'excretory corpuscles' vary in size to as much as 7 μm in diameter. Much of the luminal surface of the bladder is covered by superficial vacuoles.

Beneath the basal plasma-membrane is a basement membrane and a wide region of interstitial matrix (plates 1 A, B, D & E). Beneath these are layers of circular and longitudinal muscle (plate 1B), which are frequently interspersed with what appear to be cytoplasmic connections between the bladder epithelium and the sub-epithelial cells. Type B and C secretory granules can be seen passing along these connections (plates 1 A, D & E).

The bladder epithelium does not give a positive reaction to the modified coupling azo dye method of Pearse (1960) for alkaline phosphatase activity, but the excretory ducts do.

5. DISCUSSION

The presence of 'excretory corpuscles' in the excretory bladder of digeneans has been noted by Erasmus (1967) in the metacercariae of *Cyathocotyle bushiensis* and by Martin and Bils (1964) in the metacercariae of *Acanthoparyphium spinulosum*

Johnston, but were not mentioned by Pantelouris and Threadgold (1963) in their study of the excretory system of *Fasciola hepatica*. Erasmus (1967) noted that these corpuscles were soon lost when the metacercariae entered the definitive host. Their presence, therefore, in adult Digenea does not appear to have been recorded previously. Their suggested function in metacercariae, according to Erasmus (1967), is for carbon dioxide fixation and the storage of phosphates. This is similar to some of the suggested functions of calcareous corpuscles in cestodes (von Brand *et al.*, 1965). The ultrastructure and formation of the corpuscles from the metacercariae of *A. spinulosum* was described by Martin and Bils (1964). The presence of these corpuscles in adult *P. staffordi* is not unique, as I have also found them similarly positioned in *Hemiurus ocreatus* (Rud.), a totally unrelated digenean from the gut of the flounder.

The question therefore arises, why, in respect of the 'excretory corpuscles', are *P. staffordi* and *H. ocreatus* different from the species studied by other authors? In adult *C. bushiensis* the bladder-epithelium is associated with lipid excretion, but there was no evidence for the presence of lipids in the bladder-epithelium of *P. staffordi*. According to Erasmus (1967) lipid excretion was not necessarily associated with the microrugae; but he did not attempt to postulate a function for these structures, though at the electron microscope level he did show that they possessed alkaline and acid phosphatase activity. The apparent lack of alkaline phosphatase activity in the bladder-epithelium of *P. staffordi* could be due to a less sensitive technique. With regard to the 'excretory corpuscles', the difference, if carbon dioxide fixation is a function, between the aerobic conditions in the gut of the flounder and those in the gut of a duck, the definitive host of *C. bushiensis*, must be very small, as in both cases the oxygen tension is very low (my own preliminary experiments; Crompton *et al.*, 1965). This cannot be directly responsible for the corpuscles therefore. As mentioned above, one significant difference is that digeneans parasitic in the gut of marine teleosts are subject to an environment of varying osmolarity. This variation is caused by the need of marine teleosts to swallow sea-water. The osmolarity of the body-fluids of *P. staffordi* are probably hypotonic to that of the gut, as when food is not present in the gut it moves back to the regions of the gut with the lowest osmolarity (MacKenzie and Gibson, 1970), and it appears to survive longest *in vitro* in an environment with a lower osmolarity than is normally found in the flounder gut (unpublished results). The parasites may, therefore, have to absorb water or actively lose salts in order to keep the osmolarity of its body-fluids down. These corpuscles may be a means to that end, in addition to having a carbon dioxide fixing function. It is possible to imagine the mixing of two salts in the superficial vacuole in such a way that the solubility product of one of the salts produced in the mixture is exceeded, especially when, as shown by MacKenzie and Gibson (1970), the concentrations of magnesium and calcium ions in the flounder gut are built up to a high level as water and the monovalent ions are absorbed from the swallowed sea-water (crystals free in the lumen of the flounder gut have often been observed). That such a mechanism could occur is supported by an analysis of the chemical composition of the 'excretory corpuscles' of the metacercariae of *A. spinulosum* by Martin and Bils (1964), which showed a high calcium

carbonate content, and of the calcareous corpuscles of cestodes (von Brand *et al.*, 1967), which have a high magnesium, calcium, carbonate and phosphate content. In addition, it is difficult to imagine how else these corpuscles could be formed without postulating the active uptake of water. It is therefore possible to postulate, on the above evidence, that these 'excretory corpuscles' might be formed thus:

(1) vacuoles containing salts such as sodium carbonate (fixed carbon dioxide) are formed in the sub-epithelial cells, and are passed to the bladder epithelial cells via the cytoplasmic connections (suitable vacuoles, such as the type C secretory granules, are visible in these cells);

(2) the superficial vacuoles are formed by anastomosis of the microrugae with the outer plasma-membrane of the cell and with each other, thus entrapping fluid from the lumen which may contain calcium and magnesium ions;

(3) the contents of the secretory vacuoles are released into the superficial vacuoles (what appear to be type C secretory granules releasing their contents are shown in plate 1C);

(4) the salts in the superficial vacuoles mix, and, as the solubility products of salts such as magnesium and calcium carbonate are soon reached, they crystallize out of solution;

(5) further growth of the corpuscles could be obtained by the release of more secretory vacuoles into the superficial vacuoles and by anastomosis of further microrugae with the wall of the superficial vacuole (this, and variations in the solubility products in different salts, might account for the concentric nature of the corpuscles);

(6) once these salts have precipitated the osmolarity in the superficial vacuole will fall and water can be re-absorbed by the cell via the outer plasma-membrane and microrugae by osmosis (at the base of the bladder epithelial cells vacuoles, perhaps type B secretory granules, might transport this water to the sub-epithelial cells);

(7) eventually the microruga around the corpuscle breaks away from the epithelial cell, and the corpuscle passes through the excretory pore.

The parasite, therefore, may be able to use these corpuscles to lose fixed carbon dioxide and unwanted ions and, at the same time, reclaim water from the lumen of the excretory bladder. If such a mechanism is involved, it is probably evolved from the carbon dioxide fixing corpuscles of the metacercariae, which, due to the presence of high concentrations of calcium and magnesium ions in the gut of marine teleosts, have been retained in these adult digeneans.

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PLATE 1

The ultrastructure of the excretory bladder of the adult *Podocotyle staffordi*.

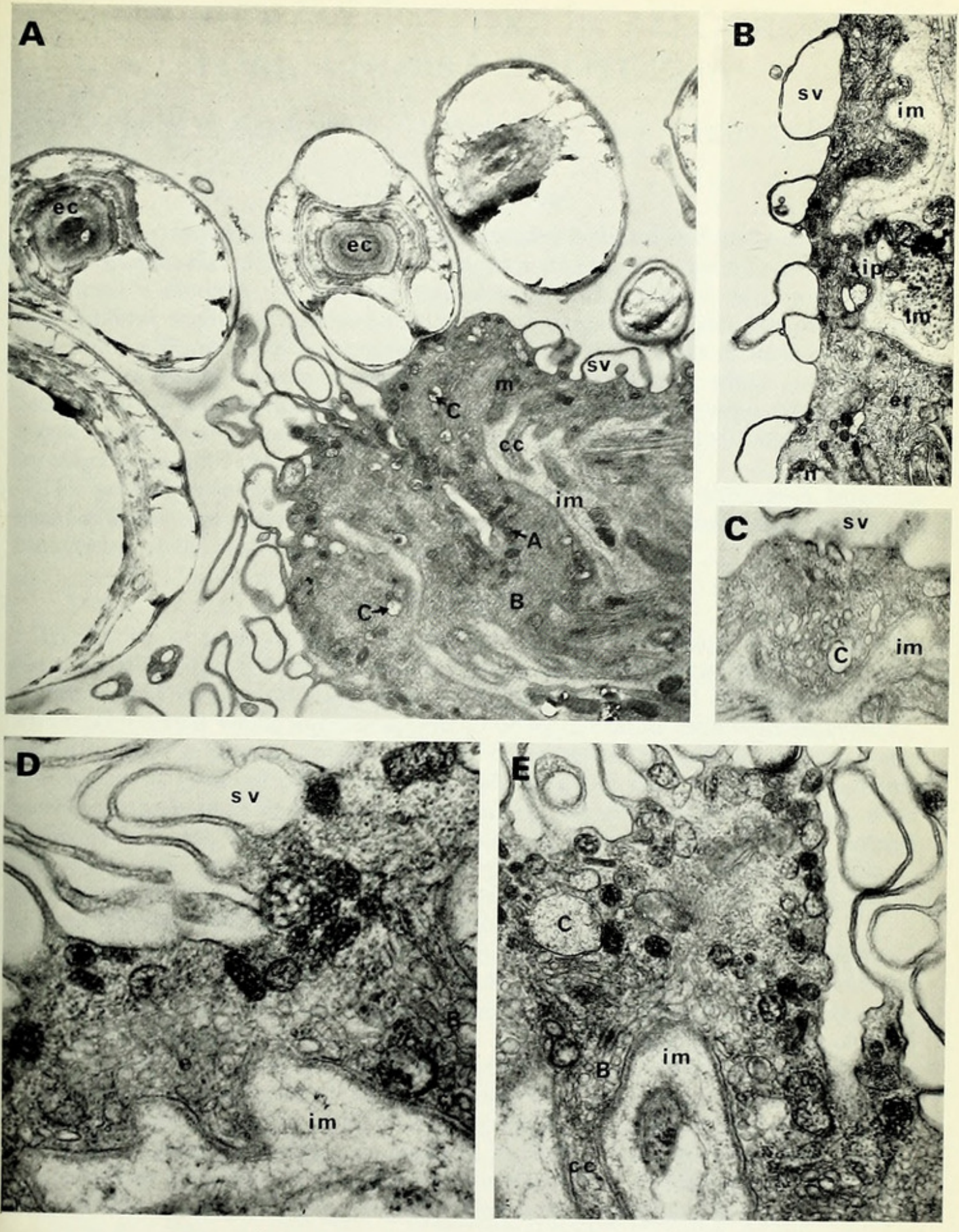
A. Section of bladder epithelium showing 'excretory corpuscles'. (× 8000).

B. Section of bladder epithelium showing that the cells are thicker in the region of the nucleus. (× 10 000).

C. Possible release of secretions from bladder epithelial cells. (× 17 000).

D. & E. Projections of bladder epithelial cells possibly connecting sub-epithelial cells. (× 31 000).

(A, secretory granule; B, secretory granules; C, secretory granule; cc, cytoplasmic connection; ec, 'excretory corpuscle'; er, endoplasmic reticulum; im, interstitial matrix; ip, invaginations of plasma-membrane; lm, longitudinal muscle; m, mitochondrion; n, nucleus; sv, superficial vacuole).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