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The Macropodid Oesophagus IV*. Observations on the Protozoan Fauna of the Macropodid Stomach and Oesophagus

D. L. Obendorf

Department of Veterinary Paraclinical Sciences, University of Melbourne, Veterinary Clinical Centre, Princes Highway, Werribee, Vic. 3030; present address: Mt Pleasant Laboratories, Tasmanian Department of Agriculture, P.O. Box 46, Launceston South, Tas. 7250.

Abstract

Holotrich ciliates are recorded from the stomach and oesophagus of 10 species of herbivorous macropod (*Macropus agilis*, *M. dorsalis*, *M. eugenii*, *M. fuliginosus*, *M. giganteus*, *M. parryi*, *M. parma*, *M. rufus*, *Wallabia bicolor* and *Thylogale billardierii*). No flagellated or entodinomorph protozoa were found. Small amoeboid protozoa are also recorded from the stomach and oesophagus of *M. giganteus*, *M. rufus* and *W. bicolor*. The findings are discussed in relation to the environment of the macropodid stomach.

Introduction

Although there are several reports of pathogenic coccidia (Winter 1959; Barker *et al.* 1963*a*, 1963*b*, 1972) and amoeba (Roberts *et al.* 1973) from the gut of macropods, there are few records concerning the naturally occurring ciliate protozoans of the macropodid stomach. Moir *et al.* (1956) first reported the presence of ciliate protozoans in the stomach content of adult quokkas, *Setonix brachyurus*. In subsequent studies of the microbial flora inhabiting the gut of pouch-dependent *S. brachyurus*, Yadav *et al.* (1972) stated that these ciliate protozoa were first detected in the stomach when joeys began to ingest plant material. Similar ciliates were also found in the stomach of grazing red kangaroos, *Macropus rufus* (Harrop and Barker 1972) whilst Dellow (1979) determined that the predominant ciliates detected in the stomachs of six species of macropodine were holotrichs.

Studies concerning the digestion of herbivorous macropods have demonstrated several similarities with the ruminant herbivores (Tyndale-Biscoe 1973). The sacculated stomach of quokkas was shown to contain a large proportion of bacteria, as well as protozoa (Moir 1968). In this study, ciliate protozoans are reported from the stomach and oesophagus of numerous herbivorous macropods. In addition, some observations on the protozoa located in the oesophagus are presented.

Materials and Methods

Several species of wild kangaroos, wallabies and potoroos were collected from various natural habitats. The stomach and oesophagus of these animals were fixed in 10% (v/v) formol–saline and 2·5% (v/v) glutaraldehyde in 0·1 M phosphate buffer (pH 7·4), and subsequently processed for light and transmission-electron microscopy as described by Obendorf (1984*a*). Samples of fresh stomach content were also collected from three tammar wallabies, *Macropus eugenii*, which originally came from a wild population on Kangaroo Island, S.A., and had been subsequently maintained in outdoor enclosures. They grazed the available pasture and the perennial herbaceous shrubs growing in their enclosures. However, their diet was supplemented with freshly cut lucerne, grass and commercially prepared livestock pellets. Only adult grazing wallabies were selected for these studies.

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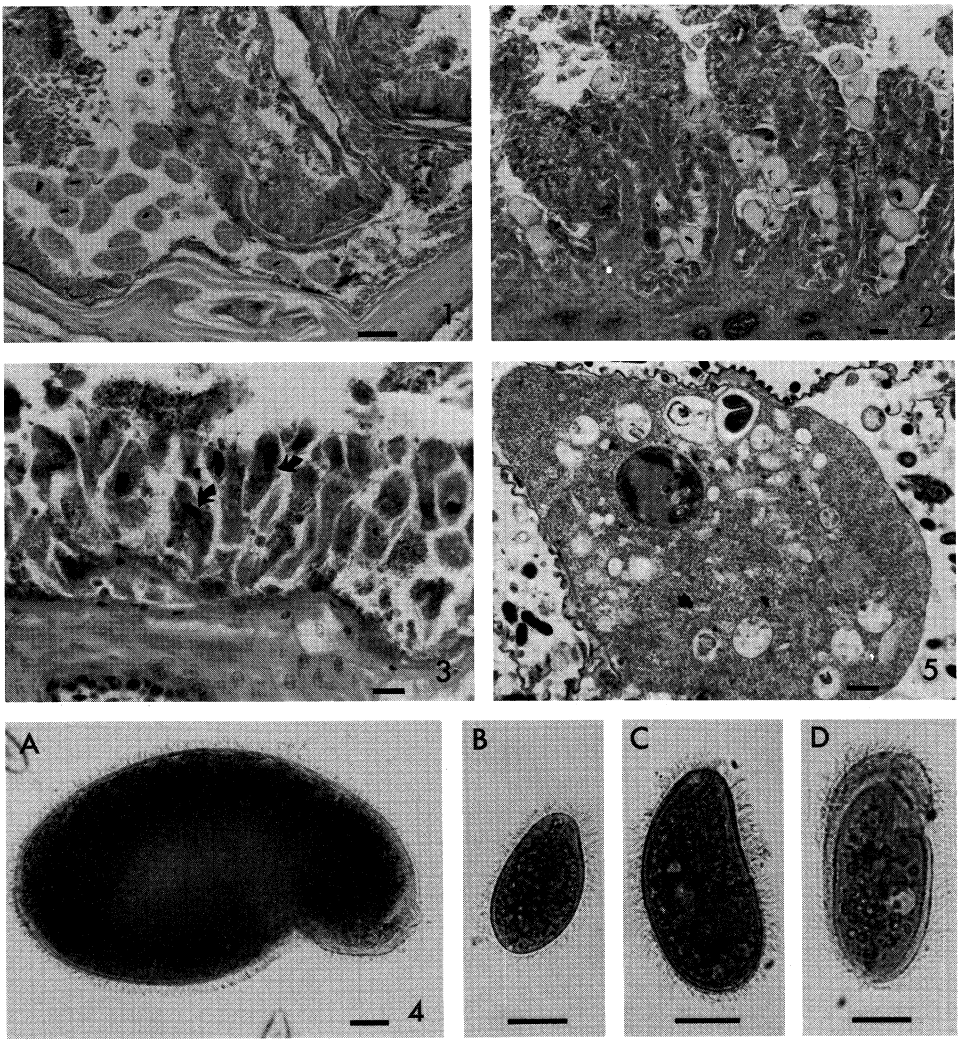

Fig. 1. Transverse section through the middle thoracic oesophagus of *Macropus giganteus* showing holotrich protozoa intermingled with the bacterial plaque and desquamating epithelial cells. Haematoxylin and eosin. Scale line = 0·1 mm.

Fig. 2. Transverse section through the distal thoracic oesophagus of *Macropus agilis* showing numerous holotrich ciliate protozoa between the oesophageal papillae. Haematoxylin and eosin. Scale line = 0·01 mm.

Fig. 3. Transverse section through the distal thoracic oesophagus of *Macropus dorsalis* showing numerous holotrich ciliates (arrows) in close proximity to, and at right angles with, the oesophageal epithelium. Haematoxylin and eosin. Scale line = 0·01 mm.

Figs 4*A*–4*D*. Four types of holotrich ciliate removed from the oesophagus and stomach of *Macropus eugenii*. Scale lines = 0·01 mm.

Fig. 5. Transmission-electron micrograph of the small amoeba in the oesophagus of *Macropus rufus*. This protozoa has no cilia and is lodged between two residual cell membranes of ruptured epithelial cells. Vacuoles containing bacteria can be seen within its granular cytoplasm. Scale line = 1 μm.

Light-microscopic observations of the fresh content from the diverticulum and blind sac regions of the proximal stomach compartment, the sacculated and non-sacculated regions of the middle stomach compartment, the gastric pouch and the pylorus were carried out as soon as possible after the animals had been killed. Samples (1 g) of stomach content from each stomach region containing motile protozoa were fixed in 2·5% (v/v) buffered glutaraldehyde. These stomach samples were washed through an 80-mesh sieve (900 squares/cm^2) using 0·9% (w/v) NaCl.

The washings were centrifuged for 10 min and the sediment made up to a known volume. Known aliquots from each sample were stained with Sedi-Stain (Clay-Adams Parsippery, New Jersey, U.S.A.), and examined with a light microscope. The protozoans present were identified and classified according to appearance and size. Total and differential counts were calculated from a mean of three separate counts conducted on each stomach sample. The washings of the oesophagus and the stomach content from the proximal stomach regions from several other species of macropods were similarly processed. The protozoans present were examined; however, no counts were made in these specimens.

Results

Ciliate protozoa were found in the stomach and oesophagus of *Macropus agilis*, *M. dorsalis*, *M. eugenii*, *M. fuliginosus*, *M. giganteus*, *M. parryi*, *M. parma*, *M. rufus*, *Wallabia bicolor* and *Thylogale billardierii*. With the exception of one type of protozoan, all the ciliates were holotrichs. No flagellates or entodinomorph protozoa were found in any of the stomachs or oesophaguses examined. Based on the appearance and size of the holotrichs, several morphologically different types were recorded. Within any one macropodid specimen, the ciliates found in the oesophagus were similar to those found in the content of the proximal stomach. In the giant kangaroo species (*M. rufus*, *M. giganteus*, *M. fuliginosus*) the holotrichs were located between the longitudinal mucosal folds of the oesophageal lining (Fig. 1), whilst in the species of wallaby (*M. agilis*, *M. dorsalis*, *M. eugenii*, *M. parryi*, *M. parma* and *W. bicolor*) they were commonly found between the oesophageal papillae (Fig. 2). In most oesophaguses examined, few ciliates were present, but occasionally many were found closely associated with the lining of the oesophagus. In one *M. dorsalis* the numbers of ciliates apposed to the surface epithelium was so large that the holotrichs assumed unconventional elongated shapes. These ciliates were closely packed into a compact layer with their cytostomes directed towards the epithelium (Fig. 3). Indeed, the bacterial plaque normally adherent to the lining of the oesophagus was replaced by the layer of holotrichs. Bacteria were present within vacuoles in the endoplasm of these ciliates.

In the stomach, ciliates were found amongst the pieces of plant tissues. In several macropods few protozoa were found in the stomach, those present being located primarily in the proximal stomach compartment. In *M. eugenii*, ciliates were found in the highest numbers in the diverticulum and blind sac regions of the proximal stomach compartment. Three of four distinct types of holotrich ciliate in numbers ranging from $7 \cdot 8 \times 10^5$ to $1 \cdot 9 \times 10^6$ per gram of stomach content were recorded from these two stomach regions. In the middle compartment comprising the sacculated and non-sacculated stomach regions, the number of holotrich ciliates was considerably lower ($6 \cdot 8 \times 10^4$ to $3 \cdot 4 \times 10^5$ per gram). Within the same animal, the number of ciliates present in either region of the middle stomach compartment was similar. No motile protozoa were found in the gastric pouch (acid-secreting) or pyloric regions.

Ciliates resembling *Isotricha* spp. and measuring 137·5–267·5 by 95·0–147·5 μm (range of 25) were the largest protozoans found in the stomachs of *M. eugenii* (Fig. 4*A*). Three smaller holotrich forms resembling *Dasytricha* spp. (Figs 4*B*–4*D*) were always seen in greater numbers than the *Isotricha*-like ciliates irrespective of the stomach region. No protozoa were found in the oesophagus or stomach of two southern potoroos, *Potorous tridactylus*.

A small amoeboid protozoan measuring 6–12 by 6–8 μm (range of 25) was occasionally found in the stomach and oesophagus of *M. rufus*, *M. giganteus* and *W. bicolor*. This

protozoan had no cilia or flagella and was always found closely associated with the surface layers of the squamous epithelium and the adherent bacterial populations. Electron micrographs showed that these protozoans were located within pockets created between the cell membranes of partially desquamated epithelial cells, and intermingled with remnant squamous cell membranes. Vacuoles containing degenerate bacteria were commonly seen within these protozoa (Fig. 5).

Discussion

Holotrich ciliates were the predominant protozoans found in the upper alimentary tract of macropods. In *M. eugenii*, these ciliates were principally found in the diverticulum and blind sac regions of the proximal stomach compartment. Fewer protozoans were found in the oesophagus and middle stomach compartment. Dellow (1979) reported numbers of $1 \cdot 5 \times 10^4$–15×10^4 per gram of macropodine stomach content with the highest counts always recorded from the proximal stomach compartment with numbers decreasing progressively along the middle stomach compartment. It is known from ruminant studies that holotrich ciliates are sensitive to acidity (Purser and Moir 1959; Hungate 1966), proliferating in neutral to slightly alkaline environments (pH 7·5–8·5) suitably enriched by the presence of freshly ingested food containing soluble carbohydrates (Hungate 1966).

The proximal stomach compartment of macropods does provide such an environment. The pH of this compartment is near neutrality (Moir *et al.* 1956; Mykytowycz 1964; Dellow and Hume 1982) and the saliva entering from the oesophagus has good buffering capacity (Forbes and Tribe 1969). The absence of motile protozoa in the gastric pouch and the pylorus of *M. eugenii* is understandably due to the acid secretion (pH 2·6–5·1) which takes place in the fundic glands of the gastric pouch (Smales and Mawson 1978).

The presence of small numbers of ciliates in the lumen of the oesophagus of the macropodid species examined may be attributed to the practice of merycism, or regurgitation, of stomach contents into the oesophagus—a process reported to occur in numerous herbivorous kangaroos and wallabies (Mollison 1960; Barker *et al.* 1963*a*). In one case, however, large numbers of ciliates were found in close apposition with the oesophageal mucosa, with concurrent loss of the adherent bacterial plaque and the presence of bacteria within protozoa. These findings suggested that the protozoa were feeding directly on the bacteria adherent to the oesophageal lining and may use this environment for growth and replication. Protozoa are not the only organisms which ingest adherent populations of bacteria in the macropodid oesophagus. Several species of nematode inhabiting the oesophagus of certain macropods have been shown to similarly attach to the lining and feed on the adherent bacteria (Obendorf 1984*b*).

Bacterial ingestion by ciliate protozoans isolated from the forestomach of ruminants has been reported (White 1969; Coleman and Hall 1974). The importance of the engulfed bacteria to the nutrition and metabolism in the ciliate and the contribution of this process to the overall digestion of the host ruminant has yet to be established (Coleman 1975). In ruminants under conditions particularly favourable to protozoa, bacterial ingestion by these organisms has been suggested to cause a significant reduction in the number of bacteria present in the reticulorumen (Coleman 1975). Moir *et al.* (1954, 1956) found similar numbers of protozoans in the stomach content of quokkas, but again of relatively few morphological types. The reticulorumen of sheep and cattle and the colon and caecum of horses, by contrast, support larger and more morphologically diverse populations of ciliates (Levine 1973). Dietary factors imposed on wild populations of herbivorous macropod may account for the low numbers of protozoans in the stomach and oesophagus of several animals. The absence of protozoans in the stomach and oesophagus of *Potorous* sp. may be related to their omnivorous and insectivorous feeding habits (Guiler 1971).

The small amoeboid protozoan found in the stomach and oesophagus of three species of macropod had features in common with members of the genus *Entamoeba*. Several species of entamoeba have been recorded from the oral cavity of vertebrates and *Entamoeba bovis* has been recorded from the rumen of healthy cattle (Levine 1973). Gastric amoebiasis has been reported in the wallaroo, *M. robustus* (Roberts *et al.* 1973); trophorozoites measuring 10–20 μm were seen within the necrotic mucosa of the stomach while cyst forms were seen in the faeces of the wallaroos. The amoeboid protozoans found in this study were restricted to the surface layers of the stratified squamous epithelium of the oesophagus and stomach with no evidence of invasion into deeper epithelial layers.

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