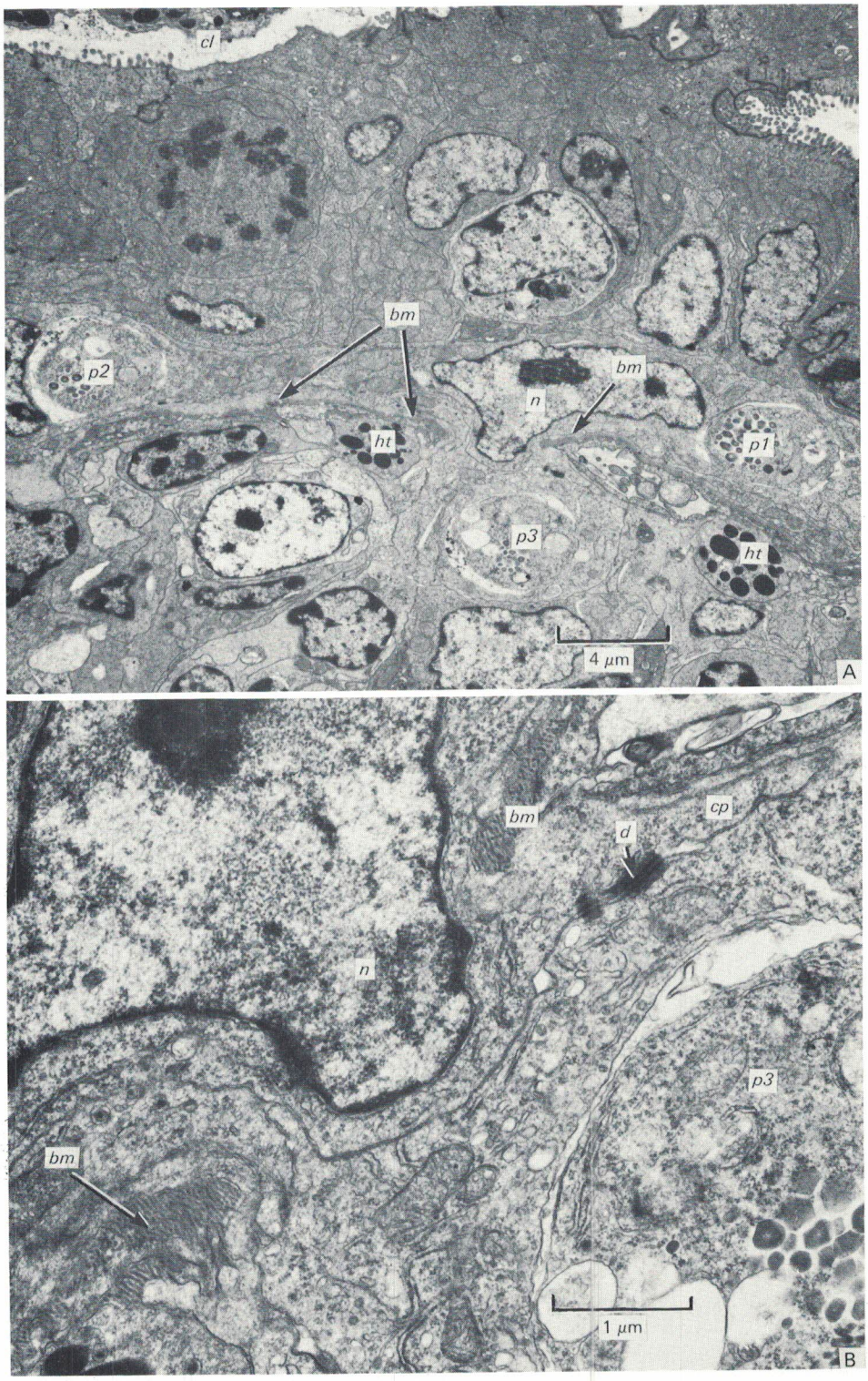
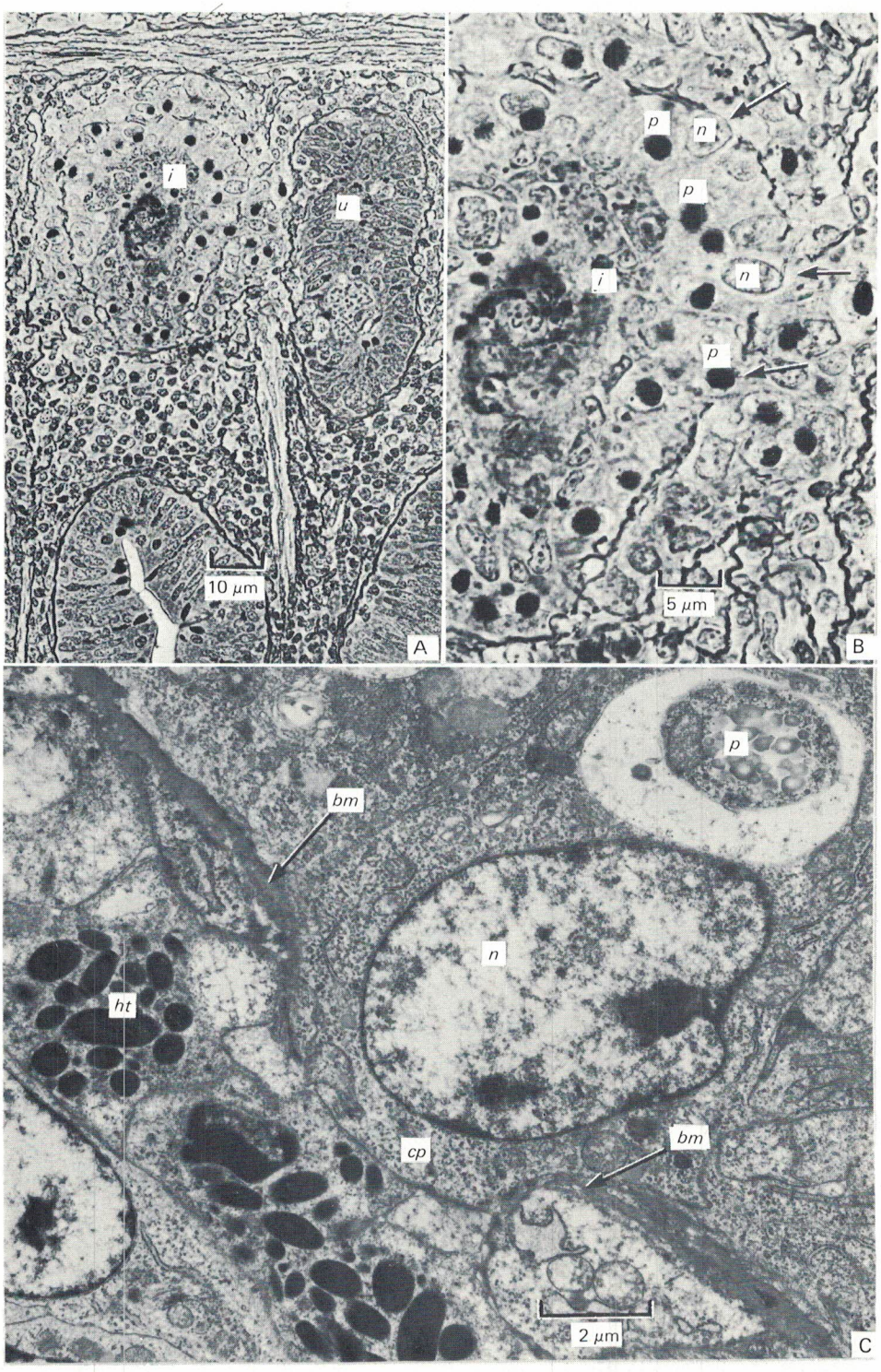


M. A. FERNANDO AND OTHERS



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EXPLANATION OF PLATES

Plate 1

Electron micrographs of chicken caecum infected with *Eimeria tenella*.

A. At 60 h p.i. heterophils (*ht*) are found on both sides of the basement membrane (*bm*) of the crypt and within its lumen. The crypt epithelial cells are rounded and disorganized and many of them contain 2nd-stage trophozoites in supranuclear parasitophorous vacuoles (*pv*).

B. At 84 h p.i. giant cells containing developing 2nd-stage schizonts are joined by desmosomes (*d*) and tight junctions (*tj*).

Plate 2

Sections of chicken small intestine 67 h after infection with *Eimeria necatrix*.

A and B. Low power (A) and high power (B) light micrographs of 2 μm methacrylate sections stained by silver impregnation for reticulin fibres and with PAS. Note the disrupted fibres of the basement membrane of the infected crypts (*i*) compared with the intact basement membrane of the adjacent uninfected crypt (*u*). Parasites (*p*) are densely stained by PAS and in (B) a parasite and host-cell nuclei (*n*) are seen projecting through breaks (arrows) in the basement membrane.

C. Electron micrograph to show a cytoplasmic process (*cp*) of an infected cell followed by its nucleus (*n*) protruding through a gap in the basement membrane (*bm*). The parasite is still within the confines of the crypt. Note heterophils (*ht*) outside the crypt.

Plate 3

A. Electron micrograph of the small intestine 120 h after infection with *Eimeria necatrix*. A host cell with a large nucleus (*n*) harbours a mature second generation schizont (*p*) and also encloses the remains of phagocytosed erythrocytes and leucocytes (arrows).

B. Electron micrograph of the caecum 60 h after infection with *E. tenella*. A cytoplasmic process (*cp*) of a crypt epithelial cell containing a parasite (*p*) protrudes through a gap in the basement membrane (*bm*) into the lamina propria (*lp*).

intact and partly digested erythrocytes and leucocytes and it is possible that the products of their digestion could be of use to the parasites.

Clearly, the mechanisms of invasion are, at present, poorly understood and further work on the influence of coccidial parasites on the behaviour of infected epithelial cells could conceivably contribute to our understanding of them. However, although enterocytes infected with *E. necatrix* or *E. tenella* resemble tumour cells in many respects, as noted by Tyzzer (1929), they do not divide and their invasiveness is clearly much more limited. They are rarely found deeper in the intestinal wall and there are no records of their presence in the peritoneal cavity, blood or lymphatic streams, or other organs.

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surface. After a few min, 0·5–1 mm slices were taken from the caecal wall and primary fixation was continued *in vitro*. After post-fixation with 1% osmium tetroxide the tissue slices were immersed in 0·25% aqueous uranyl acetate for 1 h, dehydrated in ethanol, transferred into epoxypropane and embedded in Araldite. Thin sections were stained consecutively with alcoholic uranyl acetate, lead citrate and a mixture of aqueous uranyl acetate with potassium permanganate.

The results of examination of tissues taken at 60 and 84 h after the inoculation of oocysts are presented.

RESULTS

At 60 h p.i. (*E. tenella*) and at 65 h p.i. (*E. necatrix*) many 1st-stage schizonts had released their merozoites and epithelial cells contained early second generation trophozoites within small supranuclear vacuoles. In some crypts the infected epithelial cells retained both their columnar shape and regular relationship with neighbouring cells. However, the terminal web was disrupted and the apical microvilli were irregular, short or absent in contrast to the normal appearance of the brush border of neighbouring unparasitized cells. In silver-stained methacrylate sections the basement membranes of infected crypts appeared similar to those of uninfected crypts. Infected crypts, unlike nearby uninfected ones, were surrounded by numerous heterophils (Pl. 1A) some of which had penetrated the basement membrane and, in severely affected crypts, were found in the lumen.

At later times (67–70 h p.i.) several morphological changes had occurred in infected crypts. In those in which most of the epithelial cells were infected both the basement membrane and the architecture of the crypt were disrupted (Pl. 2A, B). The basement membrane was displaced and contained gaps which allowed direct communication between the lamina propria and the lumen. The infected cells were swollen and rounded and the relationship between neighbouring cells was disturbed. Basal processes from some of these cells protruded through the gaps in the basement membrane (Pls 2C and 3B) and sometimes made contact with similar infected cells in the lamina propria. In less heavily infected crypts (*E. necatrix*), 2 layers of epithelial cells were often seen; the outer harbouring parasites, and the inner uninfected, as noted by Tyzzer *et al.* (1932) and Stockdale & Fernando (1975). The basement membrane in these crypts was less disrupted but, again, infected cells were seen projecting through breaks in the membrane (Pl. 4A); some were seen to be connected by desmosomes with infected cells outside the crypts (Pl. 4B). The nucleus, covered by a narrow layer of cytoplasm, seemed to lead the way followed by the parasite within the parasitophorous vacuole. At this stage infected cells were largely devoid of microvilli and were less osmiophilic than uninfected crypt epithelial cells.

By 72 h (*E. necatrix*) and 84 h (*E. tenella*) p.i. crypts were completely disrupted and no longer recognizable in severely affected areas, where only a thin layer containing groups of parasitized cells mixed with inflammatory cells and extravasated erythrocytes covered the sub-mucosa. Traces of the stroma and vascular elements of the lamina propria persisted locally. Although the cells which enclosed second generation trophozoites and early schizonts were giant cells, sometimes exceeding 25 μm in diameter and with much enlarged nuclei, they retained some

E. tenella) and of Tyzzer, Theiler & Jones (1932, *E. necatrix*) who believed that merozoites of the first generation invaded crypt epithelial cells and that the infected cells subsequently migrated into the lamina propria. Stockdale & Fernando (1975), however, confirmed the findings of Tyzzer *et al.* (1932) but their interpretation of the origins of the cells harbouring second generation schizonts in the lamina propria depended upon the limited resolution of the light microscope.

In this paper we corroborate the descriptions of Tyzzer (1929), Tyzzer *et al.* (1932) and Stockdale & Fernando (1975) by presenting ultrastructural evidence to show that the first generation merozoites of *E. necatrix* and *E. tenella* enter crypt epithelial cells, that a few hours later these infected cells leave the crypts through breaks in the basement membrane and that further development with nuclear division of the schizonts takes place after the infected cells reach the lamina propria.

MATERIALS AND METHODS

Eimeria necatrix

The Guelph strain of *E. necatrix* and 4-week-old White Leghorn chickens, raised coccidia free, were used throughout. Sixteen chickens were each infected with 1×10^6 sporulated oocysts and 2 were killed every hour from 65 to 72 h post-infection (p.i.). Four chickens were each infected with $1{\cdot}5 \times 10^5$ sporulated oocysts and 2 were killed at 96 and 100 h p.i.

A section of the small intestine, approximately 5 cm proximal to Meckel's diverticulum, was fixed for electron microscopy in a fixative containing 3% glutaraldehyde and 2% paraformaldehyde in 0·1 M phosphate buffer, pH 7·2. While in the fixative, the tissue was cut into pieces measuring approximately 1 mm^2. Selected pieces were post-fixed in 1% osmium tetroxide, dehydrated in acetone and embedded in Spurr's resin. Sections of 1 μm thickness were stained with methylene blue and examined with a light microscope. Gold or silver sections, stained with 2% uranyl acetate and Reynold's lead citrate, were examined with an electron microscope.

The rest of the small intestine was fixed in 10% buffered formalin. Longitudinal sections measuring 1 cm, taken from a location 10 cm and 20 cm proximal to Meckel's diverticulum, were embedded in methacrylate and sections of 2 μm thickness were cut on an ultramicrotome. The sections were stained with haematoxylin and eosin or with Gordon and Sweet's silver impregnation method for reticulin fibres (Culling, 1974) and counterstained with PAS.

Eimeria tenella

For experiments on *E. tenella*, 7-week-old S.P.F. (coccidia-free) unsexed Light Sussex chickens (HPRS strain) were used. A total of $2{\cdot}5 \times 10^7$ sporulated oocysts were inoculated into the crop and 4 chickens were killed at each of 4 different time-intervals. Under pentobarbitone anaesthesia, one horn of the caecum was removed and fixed in Bouin's fluid for paraffin embedding. The other was fixed for electron microscopy, commencing fixation *in situ* by injection of a fixative containing 2·27% glutaraldehyde in 0·2 M collidine buffer (pH 7·2) into the lumen via a syringe and hypodermic needle. Fixative was also applied to the external