Parasitology (1983), **86**, 391–398
With 5 plates

Invasion of chicken caecal and intestinal lamina propria by crypt epithelial cells infected with coccidia

M. A. FERNANDO[1], A. M. LAWN[2], M. ELAINE ROSE[2]
and M. A. AL-ATTAR*[1]

[1]*Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada NIG 2W1*
[2]*Houghton Poultry Research Station, Houghton, Huntingdon, Cambs. PE17 2DA*

(*Accepted* 4 *November* 1982)

SUMMARY

The development of second generation schizonts of *Eimeria necatrix* and *E. tenella* was studied with the electron microscope. Invasion of the crypt epithelial cells by merozoites of the first generation schizonts caused changes in the morphology of the infected cells and stimulated their migration into the lamina propria through breaks which appeared in the basement membrane of the crypts. Second generation schizonts developed in the lamina propria within these crypt cells whose epithelial origin was confirmed by their interconnection by desmosomes and tight junctions and by their possession of characteristic microvilli. A comparison is made between this invasion of the lamina propria by parasitized cells and invasion of connective tissue by malignant epithelial cells; the possible mechanisms involved are discussed.

INTRODUCTION

Second generation schizonts of *Eimeria necatrix* and *E. tenella* develop in cells within the lamina propria of the chicken small intestine and caecum respectively. The identity of the cell in which these schizonts develop has been extensively discussed (Long, 1970; Bergmann, 1970; Lee & Long, 1972; Fernando & Stockdale, 1974; Stockdale & Fernando, 1975). Lee & Long (1972) stated that the first generation merozoites of both these parasites 'invade connective tissue cells between the glands and give rise to the large second generation schizonts'. In a study on the pathogenesis of *E. tenella*, Bergmann (1970) noted that cells parasitized by second generation schizonts had the functional and morphological characteristics of macrophages but that, in the formation of cell clumps joined by desmosomes, they resembled epithelial cells. However, as Long (1970), Bergmann (1970) and Lee & Long (1972) pointed out, the growth of the large schizonts of these two parasites alters the morphology of the parasitized cell to such an extent that it may be difficult, if not impossible, to recognize it in the later stages of development of the schizonts.

These discussions largely ignored the classic descriptions of Tyzzer (1929,

* Present address: Department of Pathology and Forensic Medicine, College of Veterinary Medicine, University of Baghdad, Al-Ameria, Iraq.

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

protruding through them, as described by us in both *E. necatrix* and *E. tenella* infected crypts, are a frequent finding in invasive epithelial tumours and even in pre-neoplastic states such as human laryngeal papilloma (Sugar, 1968).

Invasion of the lamina propria by crypt cells might be an indirect or direct effect of parasitization. We cannot simply assume, however, that the stimulus for invasion is the breakdown of the basement membrane since uninfected crypt cells do not move out of disrupted crypts. We have therefore, to infer that the coccidial parasite initiates invasion directly. Mareel (1980) speculates that, in general, invasion results from a failure in the regulation of cellular activities. This could be due to defects in production of regulating substances, but Mareel (1980) suggests that most of the evidence points to a defective responsiveness of the malignant cells to these substances.

Other alterations in the basement membrane observed in invasive epithelial tumours include thinning, absence or alteration of hemidesmosomes, presence of atypical materials and multilayering (Ozello & Santipak, 1970; Hashimoto, Yamanishi & Dabbous, 1972; Hashimoto, Yamanishi, Maeyens, Dabbous & Kanxaki, 1973; Ozello, 1974; Rubio & Biberfeld, 1975; McNutt, 1976; Alroy & Gould, 1980). None of these changes was seen in infected crypts and crypt cells lack hemidesmosomes.

One can only speculate as to the causation of the breakdown of the basement membrane prior to, or during, migration. McNutt (1976) and Woods & Smith (1969) have suggested that the inflammation associated with precancerous lesions may be responsible. They found that the passage of inflammatory cells from the dermis into the epidermis caused extensive destruction of the basal lamina, and the heterophils seen around coccidia-infected crypts prior to basement membrane breakdown and epithelial cell migration might play a similar role. It seems more probable that the parasitized epithelial cells themselves are responsible for the defects in the basement membrane. As this structure is considered to be, at least in part, synthesized by the epithelial cells (Kefalides, 1975), two possible mechanisms for its breakdown have been postulated: (1) active destruction by epithelial cells and (2) loss of ability of the epithelial cells to make basement membrane (Mareel, 1980). The slow turnover of basement membrane observed in rats (6 weeks in the colon) and man (Walker, 1972*a*, *b*) suggests that malfunction of synthesis could not explain the loss of basement membrane in our study since only 2–4 h elapsed between infection of crypt epithelial cells and their migration into the lamina propria. We do not know whether cells infected with second generation schizonts of *E. necatrix* and *E. tenella* produce lytic factors *in vivo* but, if they do, these enzymes could, at least partly, be responsible for the breakdown of basement membrane and the massive destruction of adjacent uninfected tissue which we observed during the development of these parasites. Studies carried out *in vitro* provide some evidence for the production of a cytolytic factor by the parasite, or parasitized host cell, which can also affect unparasitized cells (Urquhart, 1981*b*).

In addition to locomotion during invasion and the production of lytic factors, cells involved in tumour formation are phagocytic (Mareel, 1980). This property was also shown by the parasitized cells described here, confirming the observations of Tyzzer (1929), Tyzzer *et al.* (1932) and Bergmann (1970). The cells contained

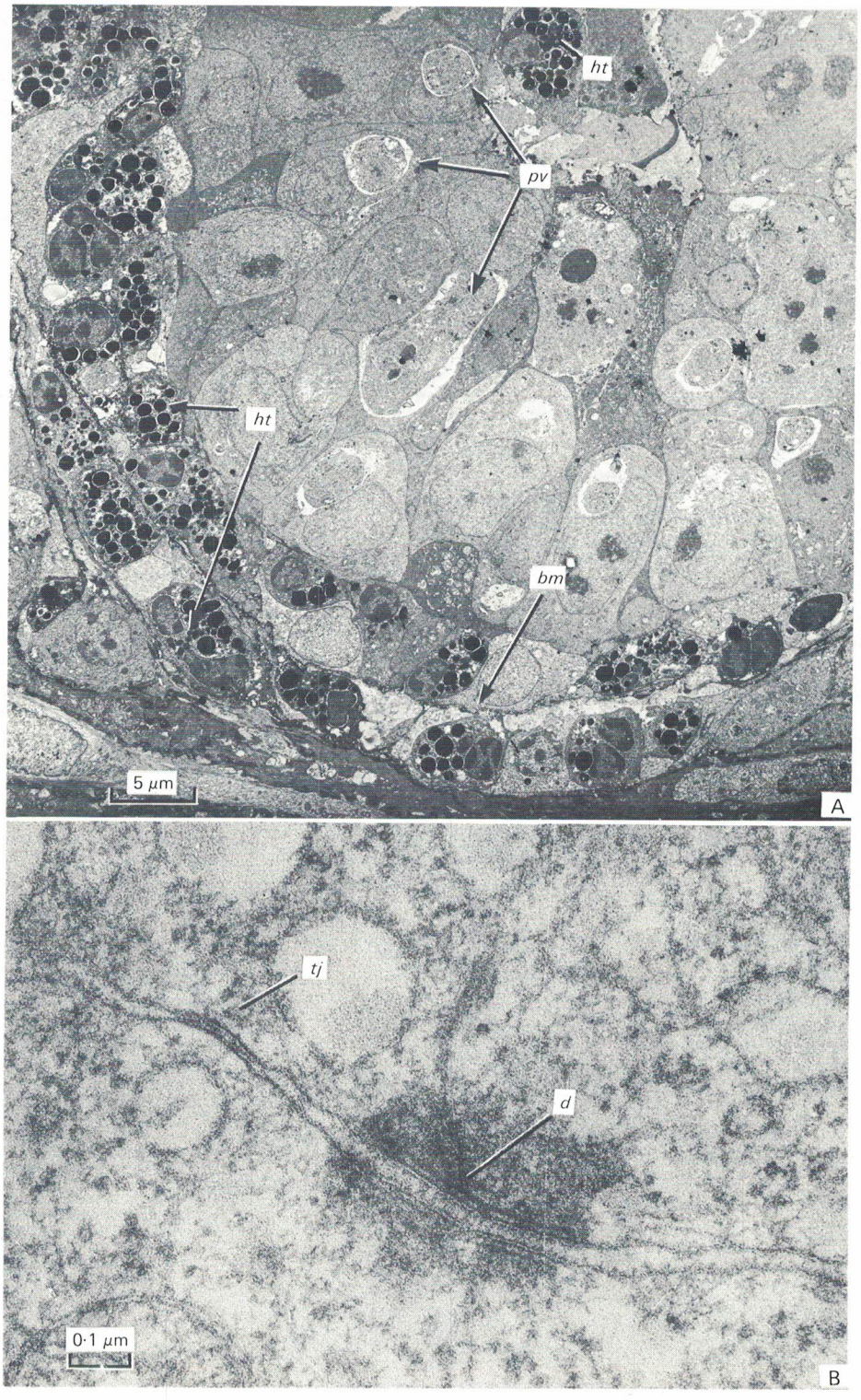
PLATE 4

Electron micrographs of small intestine 67 h after infection with *Eimeria necatrix*.
A. The nucleus (*n*) of a crypt epithelial cell harbouring a parasite (*p1*) protrudes through a gap in the basement membrane (*bm*). Two other parasites are visible, one (*p2*) remains within the crypt and the other (*p3*) is outside. *ht*, Heterophils; *cl*, crypt lumen.
B. Higher magnification of (A) shows a desmosome (*d*) connecting the cytoplasmic process (*cp*) of an infected cell, which is still within the crypt, and another which is outside the confines of the crypt basement membrane (*bm*). *p3*, Parasite as in (A) above.

PLATE 5

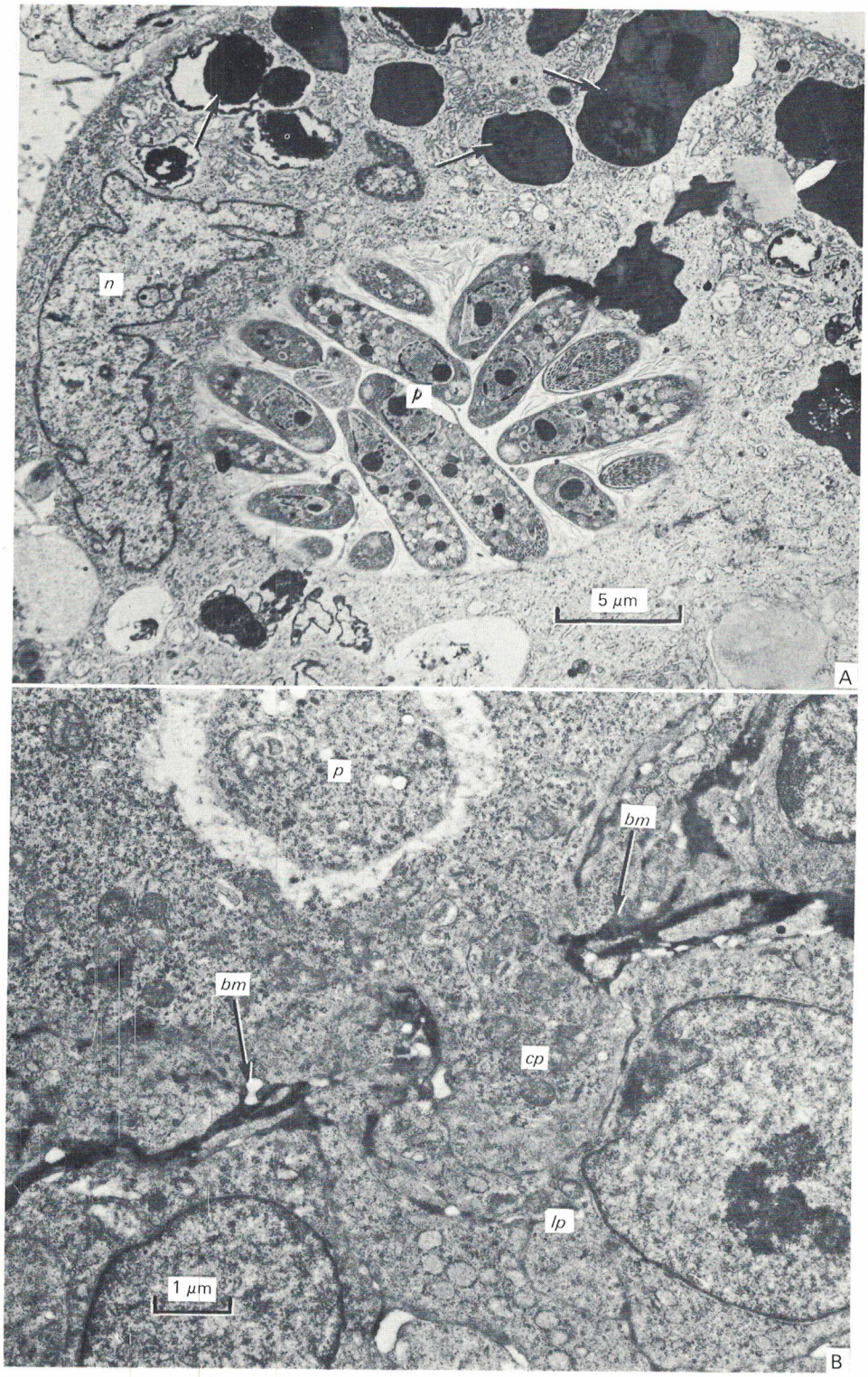
Electron micrographs of caecum 84 h after infection with *Eimeria tenella*.
A. Three giant cells containing schizonts (*p*) are visible and two contain ingested erythrocytes. One carries microvilli (*mv*) on its surface.
B. The microvilli contain core fibres (*cf*) and are coated with glycocalyx (*gc*), as are those of crypt epithelial cells.

Printed in Great Britain

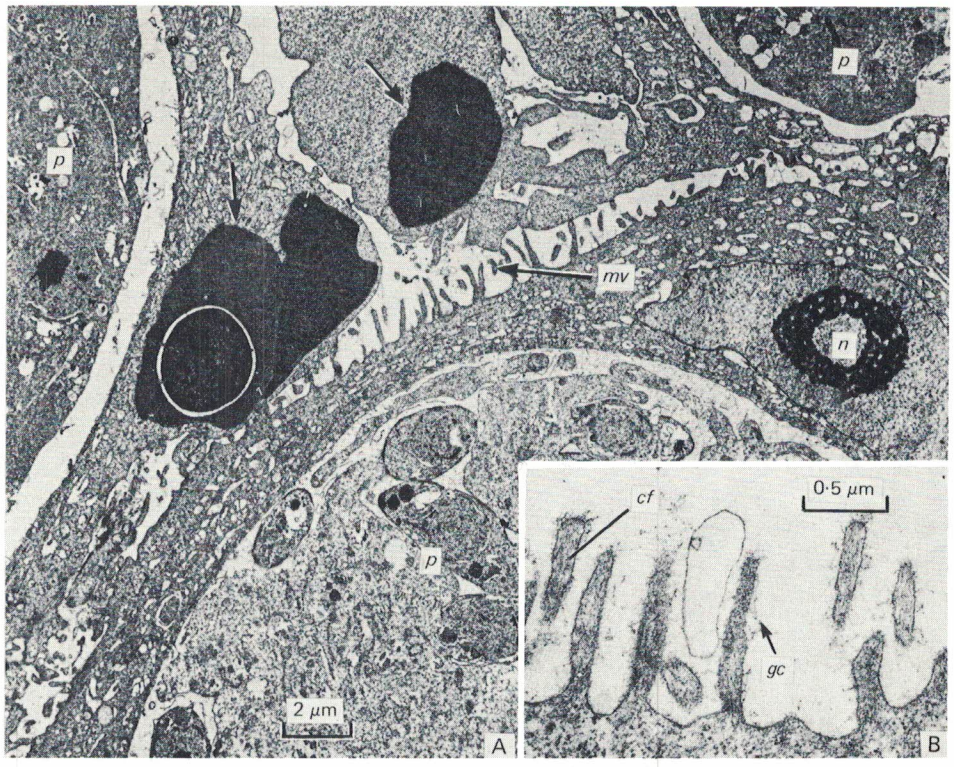


M. A. FERNANDO AND OTHERS

(*Facing p.* 398)



M. A. FERNANDO AND OTHERS


p
p
mv
n
p
2 μm
A
cf
0·5 μm
gc
B