ULTRASTRUCTURAL STUDIES OF INTESTINAL CAPILLARIASIS *CAPILLARIA PHILIPPINENSIS* IN HUMAN AND GERBIL HOSTS

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INTRODUCTION

Intestinal capillariasis, caused by infections with *Capillaria philippinensis* (Chitwood *et al.*, 1964, 1968), exhibits a sprue-like syndrome in man with symptoms of borborygmi, abdominal pain, diarrhoea, malabsorption, steatorrhoea, fluid and electrolyte loss and a marked protein-losing enteropathy (Dauz *et al.*, 1967; Singson, 1969). Although a number of clinical studies have been done (Whalen *et al.*, 1969; Watten *et al.*, 1972), the mechanism of this parasitic enteropathy has not been well clarified. Light microscopic observations on the intestinal mucosa of biopsy and autopsy materials have revealed large numbers of eggs, larvae and adult worms in the glands and adjacent tissues but with no appreciable inflammatory cell reaction (Canlas *et al.*, 1967; Uylangco *et al.*, 1968; Fresh *et al.*, 1972) Changes in villous structures have also been reported and these may be important in causing dysfunction of the epithelial cells. On the other hand, it has also been suggested that villous changes may be due to pre-existing diseases such as tropical sprue, rather than parasitic infections (Jeffries *et al.*, 1969; Klipstein, 1968).

Experimental studies recently have shown monkeys (*Macaca* sp.) and Mongolian gerbils (*Meriones unguiculatus*) (Cross *et al.*, 1972, 1973) to be susceptible to *C. philippinensis*. Not all monkeys, however, developed infections and even in those passing large numbers of eggs over long periods of time, characteristic symptoms of intestinal capillariasis were not observed. The gerbil, however, has been found to be highly susceptible with infections leading to loss in body weight, decreased blood levels of protein and potassium, and eventual death (unpublished report).

Although light microscopic studies have been done on tissues from humans with capillariasis philippinensis, no information is available concerning the subcellular morphology of such tissues. The present study was therefore undertaken to determine whether electron microscopic observations of intestinal tissues from humans and gerbils could further clarify our understanding of the pathological mechanisms involved in this parasitic enteropathy.

MATERIALS AND METHODS

Human intestinal mucosa : Patients with moderate to severe diarrhoea and borborygmi, and with *C. philippinensis* eggs in their stools,

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The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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were seen at the Southern Ilocos Sur Emergency Hospital in Tagudin, Ilocos Sur, Philippines. Peroral jejunal biopsy was performed on four patients under fluoroscopic observation using a Crosby capsule (Hartman *et al.*, 1959) and the mucosal specimens obtained were divided into two portions. One portion was fixed in 10% neutral formalin for conventional histopathological observation and the other was cut into one c.mm samples, immersed into cooled 3% cacodylate-buffered glutaraldehyde solution and subsequently processed for electron microscopic examination as outlined below.

Gerbil intestinal mucosa : *C. philippinensis* larvae recovered from the intestinal tract of experimentally infected fish (Cross *et al.*, 1972) were given to four gerbils by stomach tube. Thirty to 40 days after infection the animals were killed and portions of the jejunum removed and fixed in 10% neutral formalin for histological observation. Another portion of the intestinal tissue was sliced into 1-2 c.mm samples, fixed in 3% cacodylate-buffered glutaraldehyde solution and processed for electron microscopy.

Preparation of the tissue specimens for electron microscopy: The small cubes of the mucosal tissues, after one-hour prefixation in the 3% cacodylate-buffered glutaraldehyde solution, were postfixed in 1% osmium tetroxide at 4°C for 1 hour and then dehydrated in graded ethanols. After the specimens were embedded in epon 812 (Luft, 1961), sections one micron thick were cut with glass knives using a Porter-Blum ultramicrotome. The sections were stained with toluidin-blue and examined by light microscopy for parasitic invasion of the glands and lamina propria. Once parasitic loci were found, thin sections of 500 Å to 800Å were cut and mounted on unsupported copper grids. The sections were then stained with uranyl acetate and lead citrate, and examined with a Hitachi-11E electron microscope.

OBSERVATIONS

Light Microscopy

Human Jejunum : The changes in jejunal mucosa were similar to those described by other investigators (Canlas *et al.*, 1967; Uylangco *et al.*, 1968; Fresh *et al.*, 1972). The nematode was usually found in the glands and their adjacent lamina propria where infiltrating mononuclear cells were noted. Denudation of epithelium from the surface of the villi was often seen where the parasites were located in the subjacent glands and lamina propria. Lymphatic distension was pronounced in the lamina propria and on many occasions the parasites were lying in close proximity to the dilated lymphatic vessels (Fig. 1). Neither worms nor eggs were observed in the submucosa beyond the laminae propria.

Gerbil intestine : In epon-embedded sections stained with toluidin-blue the villi of jejunal mucosa were apparently intact except the sites where the parasites were approaching and penetrating the mucosal surface. The spaces between villi were greatly widened where eggs, larvae and adult worms were located. At this site of association with the villus epithelium, the parasite produced a compression on the brush border. An area in the epithelium was also found (Fig. 2) where the parasite appeared to be penetrating through the villus epithelium into the core of the villus. The epithelial cells around the entering worm appeared to be compressed and demonstrated increased staining density.

Electron Microscopy

Human jejunum : The fine structures of both absorptive and crypt epithelium adjacent to the parasitic infection revealed great deterioration of the cells. The surface epithelium showed evidence of abnormal extrusion and shedding of cells. At times the surface epithelial cells were found in remarkably

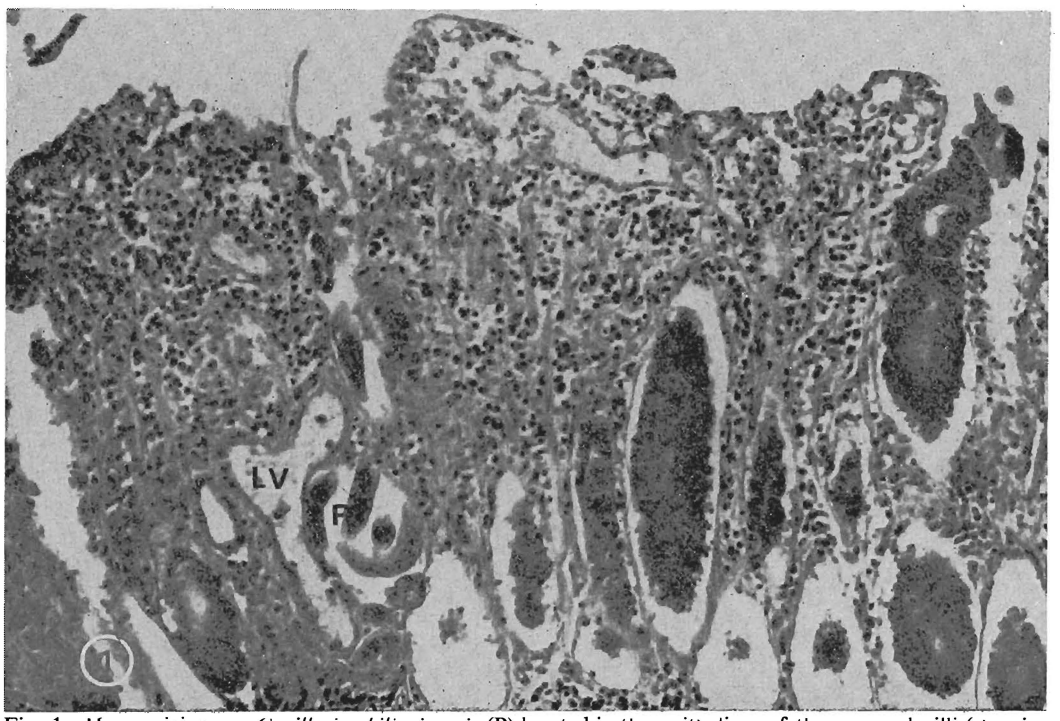

Fig. 1—Human jejunum. *Capillaria philippinensis* (P) located in the epithelium of the mucosal villi (showing denudation of the epithelial cells). Note the parasite lying in close proximity to a distended lymphatic vessel (LV). Hematoxylin-eosin stain, X120.

regressive change involving 3 to 4 cells and the parasite was observed adjacent or subjacent to these altered cells (Fig. 3). The microvilli were scant and disorganized. There was extensive cytoplasmic vacuolization and mitochondrial degeneration. The dominating vacuoles, each with a prominent unit membrane, varied in size, were irregular in shape, and their contents demonstrated little or no electron density. The nuclei showed either karyorrhexis or pyknosis with irregular contours. A striking and perhaps the most significant alteration of the epithelial cells was a complete loss of adhesion specialization of typical intestinal epithelium. There was a remarkably widened space between the lateral membranes of adjacent cells, which appeared as a large gap interrupted with membranous processes bridging between the two appositional lateral surfaces and appeared as cytoplasmic clefts within the cells (Fig. 4). This seemed to form large channels around the epithelial cells from the basal lamina toward the apex of the cells.

Gerbil jejunum : The ultrastructure of villus epithelial cells appeared partially eroded with the formation of microulcers and at times a parasite was located near the surfaces of epithelial cells (Fig. 5). Occasional red blood cells and cytoplasmic fragments were also seen in close proximity to the parasite.

As observed in sites where the parasites came into contact with the brush border, usually adjacent to a goblet cell, there was a concave surface in the villus epithelium caused by compression by the parasite. The apparent attachment of the worm onto the microvilli led to degeneration of the apical cytoplasma by condensation and obliteration

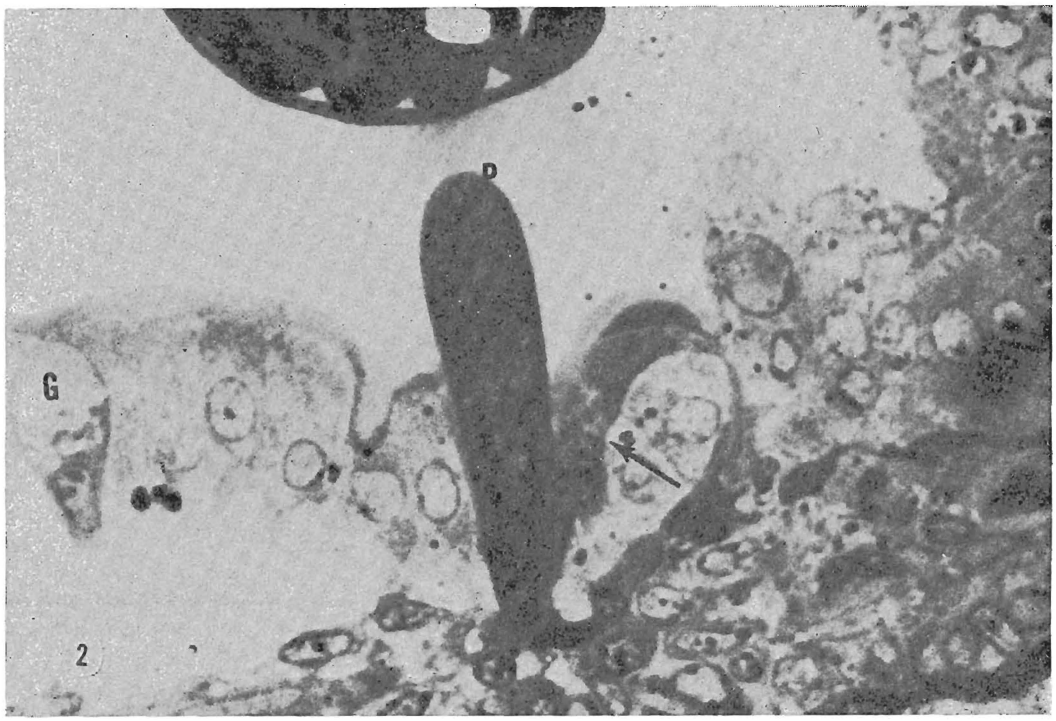

Fig. 2—Gerbil jejunum. A penetrating site of a parasite through the villus epithelium to the basal lamina. Note compression of the epithelial cells at the right side by the parasite (P) with increased density of the cells (arrow). Several inflammatory cells are also present around the tip of the parasite. A goblet cell (G) is observed at the left side far from the parasite. Epon-embedding section, toluidin-blue stain, X 800.

of the cytoplasmic structures; however, the distant part of the cytoplasm was less affected. Fig. 6, which magnifies by electron microscopy the feature in Fig. 2, shows a larval form of the parasite appearing to penetrate the mucosa, A thick layer of electron-dense homogeneous material was observed along the oral tip of the parasite. The basal lamina which seemingly acts as a barrier against invasion, and which lies directly beneath the tip of the parasite, was partially broken down and several lymphocytic and plasma cells appear to have accumulated below this damaged site. In another section of a penetrating parasite the adjacent epithelial cells opposite the cuticle pore showed dissolution and disruption of the plasma membranes and edematous loosening of the cytoplasmic components (Fig. 7). Other sections showed compressive degeneration of the cells with the appearance of large vesicular spaces within the cytoplasm (Fig. 8). The compressive and lytic actions of the parasite apparently only affect the adjacent epithelial cells; the cells distant from the parasites were for the most part intact.

DISCUSSION

Although the complete sequence of invasion of the intestinal mucosa by *C. philippinensis* was not observed, the present findings suggest that the direct pathological effects of the parasite on the epithelium is associated with two events: (1) The approximation of the nematode to the villus surface and possible

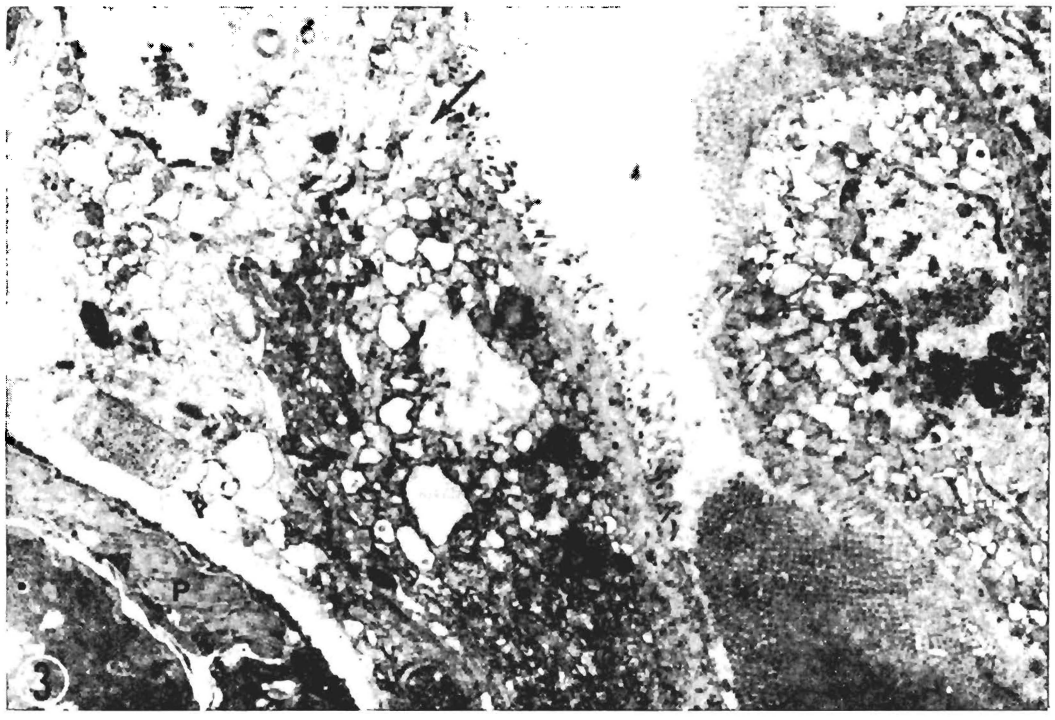

Fig. 3—Human jejunum. The pronounced degenerative changes of the epithelial cells with a subjacent parasite (P), in cross-section. Note the widened intercellular space between two cells (arrow). X 7,500.

Fig. 4—Human jejunum. Epithelial cells of the crypts of Leiberkuhn showing remarkably widened spaces between the lateral membranes of adjacent cells (arrows). Note also the scanty rough-surfaced endoplasmic reticulum (ER) and the occurrence of cytoplasmic clefts (CC). X 12,500.

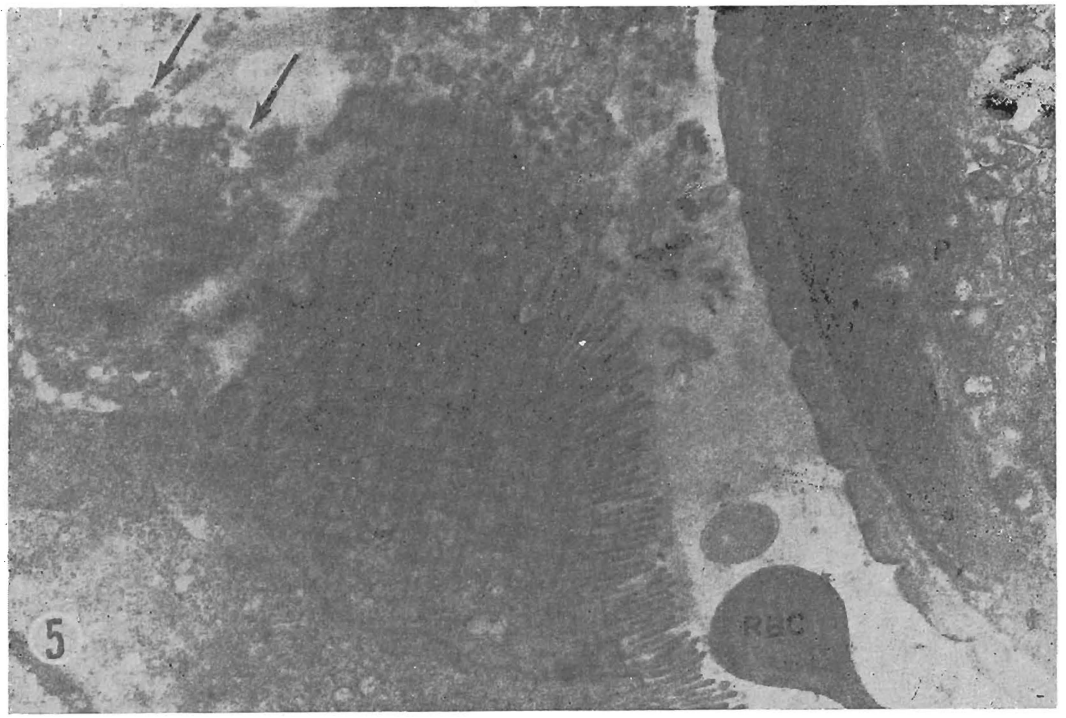

Fig. 5—Gerbil jejunum. Villus epithelial cells adjacent to a parasite in the intestinal lumen showing partial erosion of a cell with the formation of a microulcer in the epithelium (arrows). Note the sloughing out of the cytoplasmic debris in a space between the epithelial surface and the parasite. X 12,500.

elaboration of certain proteolytic substance may be responsible for the development of microulcers. (2) The direct penetrating force of the invading worm may further rupture mucosal cells or induce massive pressure on the in-situ epithelium that subsequently could introduce disturbances of circulation in the mucosal wall resulting in cellular injury and consequently in cellular dysfunction. The feature of local circulatory disturbance was evident in light microscopic observations such as congested capillaries, distended lymphatics and remarkable interstitial edema (Fresh *et al.*, 1972), especially with proximity of the parasites to lymphatic vessels in the lamina propria.

The resultant lesions in the intestinal mucosa with either ulceration of the epithelium or compressive degeneration of the cells may account in part for malabsorption and loss of fluids, proteins and electrolytes. The cellular dysfunction was particularly demonstrable in the human jejunal epithelium by widespread separation of the cells (Fig. 4). The adhesion specialization of normal intestinal epithelium (Farquhar and Palade, 1963) has a function to act as a barrier between tissue fluids in the intracellular spaces and the fluid content of the bowel, sealing one from the other (Toner *et al.*, 1971). Ballooning of the interepithelial spaces would result in continuous outflow of tissue fluid into the intestinal lumen causing intractable diarrhoea and progressing to eventual death. On the other hand, the intestinal lymphagiectasis observed in the affected mucosa may have been associated with the leakage of lymph containing chylomicrons, plasma proteins, and small lympho-

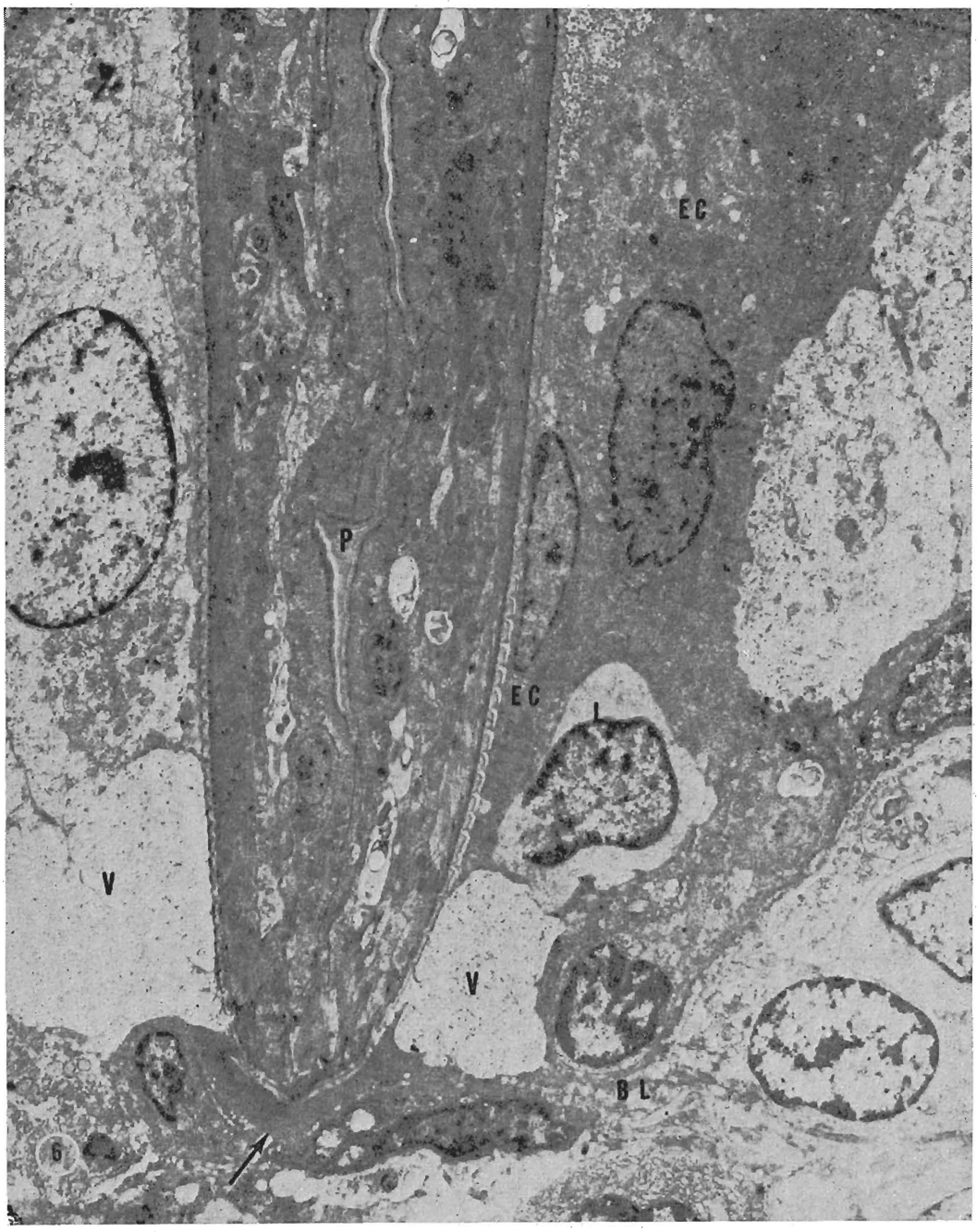

Fig. 6—Electron microscopic view of the penetrating site of the parasite in gerbil jejunal epithelium. Note a thick layer of electron-dense homogeneous secretory or excretory material (arrow) around the oral tip of the parasite in connection with two large vesicles (V) at both sides of the nematode. The basal lamina (BL) immediately below the oral tip of the worm appears deteriorated. At the right side of the parasite, the epithelial cells (EC) are compressed with increased compactness of the cytoplasmic organelles. A lymphocyte (L) is seen between these cells. X 7,500.

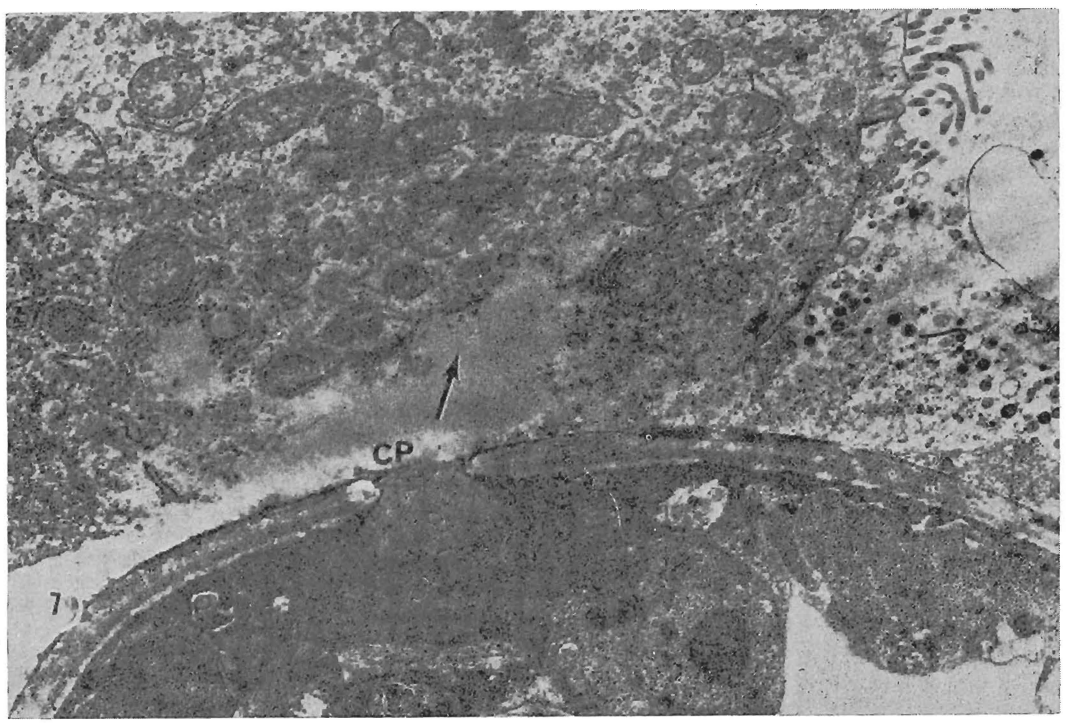

Fig. 7—Gerbil jejunum. Cross-section of *C.philippinensis* appearing to penetrate the jejunal epithelium. Note the cuticle pore (CP) of the parasite and dissolution of the plasma membrane and cytoplasmic organelles of the epithelial cell (arrow) opposite the cuticle pore. X 12,500.

cytes into the intestinal lumen (Jeffries *et al.*, 1969). Other factors which may contribute to the cause of diarrhoea are the mechanical and chemical irritation produced by the parasites on the intesinal wall (Marcial-Rojas, 1971). The condition is further compounded by the massive numbers of worms that are able to develop in the host (Cross *et al.*, 1970, 1972; Fresh *et al.*, 1972; Cross *et al.*, 1973).

The pathogenic action of an intestinal parasite depends upon its ability to invade the mucosa from the lumen; however, the nature of the intestinal epithelium and its basal lamina as barriers to intraluminal microorganisms has not been defined. At the sites of apparent attachment and penetration by *C. philippinensis* goblet cells were often observed in the vicinity of the worm entrance but actual penetration of the parasite into goblet cells was not observed. These cells are known to secrete mucin to protect the surface of epithelium. Possibly, the penetrating site of epithelium was preconditioned with preceding degradation of mucin by either intestinal bacteria (Hoskins and Zamcheck, 1968) or the secretory substances from the parasite. Once the nematode enters the epithelium it seems to meet another resistance from the basal lamina which is composed of an electron dense layer of fibrillar structures associated with collagenous fibrils. It appears that the parasite was able to break through the basal lamina and reach the lamina propria by either mechanical force or action by lytic substances secreted by the parasite. This substance (s) could possibly enable the parasite to penetrate the walls of blood vessels and enter the liver via portal tracts, however the nematode has only been found in the human liver on one occasion (Fresh *et al.*, 1972).

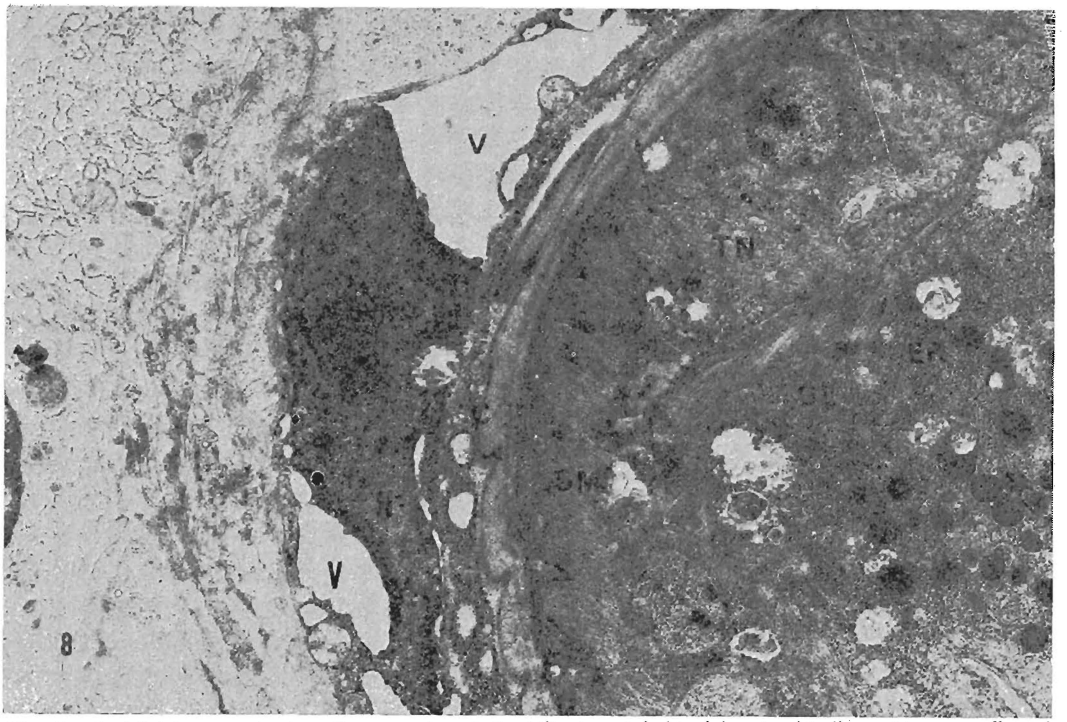

Fig. 8—Gerbil jejunum. An invading parasite of *C. philippinensis* in the jejunal epithelium. Note an affected cell showing pronounced degeneration with a deformed nucleus (N) and large vesicles (V) in the cytoplasm. The ultrastructure of the parasite features cuticle (C) composed of the triplelayered outer membrane and internal granular layer, the striations (arrow) in the media layer, the somatic musculature (SM) with tubular network (TN). Note also coelomocytes containing endoplasmic reticulum (ER), many apparent secretory granules (SG) and fenestrated membranes (FM). X 12,500.

SUMMARY

Jejunal biopsies from patients with intestinal capillariasis and sections of jejunum obtained at necropsy from experimentally infected Mongolian gerbils were examined by electron microscopy, The ultrastructure of human tissues showed a complete loss of adhesion specilization and widespread separation of the epithelial cells and it is suggested that these changes may be responsible for the loss of protein, fluids and electrolytes from tissue spaces into the intestinal luman. Electron microscopy of the sequence of attachment and penetration of the parasite into the jejunal mucosa of the gerbil showed that fine structural changes of the mucosa are not only the result of direct mechanical compression, but may also be the consequence of a lytic substance(s) originating from the parasite. The lesions are characterized by induction of microulceration of the epithelium and compressive degeneration of the cells. Large cystic intracellular spaces and disruption of the basal lamina observed adjacent to invading larvae may also be due in part to lytic substances released by the parasite.

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