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ELECTRON MICROSCOPIC CHANGES IN COLON IN EXPERIMENTAL SWINE DYSENTERY

By

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TEIGE, JON jr. and KNUT NORDSTOGA: *Electron microscopic changes in colon in experimental swine dysentery.* Acta vet. scand. 1979, *20*, 224—237. — Colonic lesions in experimental swine dysentery were studied electron microscopically. Changes indicative of stasis were commonly observed in the microcirculatory vessels of lamina propria. Early lesions in epithelial cells included sparse, short and irregular microvilli, swollen and degenerated mitochondria, and swelling and vesiculation of endoplasmatic reticulum. Numerous large spirochaetes were observed in these locations: a) in the crypts, b) free (i.e. not membrane bound) in cytoplasm of damaged epithelial cells, and c) in cavities, around vessels of lamina propria. It is suggested that stasis, and resultant disturbances in microcirculation in early developmental stages of swine dysentery, may play a pathogenetic role in the development of the necrotic colonic lesions. Finally, it is discussed whether a mechanism related to Sanarelli-Shwartzman reaction is implicated in the development of colonic lesions in swine dysentery.

electron microscopy; swine dysentery; spirochaetes; colitis; stasis; erythrocytes; thrombosis; Shwartzman reaction.

Swine dysentery is a condition which has received considerable attention during recent years, especially after an aetiological association with the "large spirochaete" (Treponema hyodysenteriae) was reported. Recent reports include microbiological investigations, inoculation experiments, parenteral immunization, and patho-morphological studies based on light microscopy and transmission and scanning electron microscopy (*Kennedy & Strafuss* 1976, *Songer et al.* 1976, *Hughes et al.* 1977, *Schleicher* 1977, *Glock et al.* 1978, *Harris et al.* 1978, *Teige* 1978).

In previous reports we have presented evidence indicating a predisposition for the disease in vitamin E/selenium deficient pigs (*Teige et al.* 1977, 1978). We have further described the

light microscopic colonic changes associated with experimental swine dysentery (*Teige*), and reported ultrastructural alterations in colonic epithelial cells in vitamin E/selenium deficient pigs (*Teige & Nafstad* 1978). The present paper discusses the ultrastructural changes in colon in swine dysentery, with special reference to a) vascular lesions, b) the morphology of damaged enterocytes and c) the location of the large spirochaete within the affected colonic wall.

MATERIAL AND METHODS

Quite advanced degenerative alterations in colonic mucosa of dysenteric pigs were expected, and it was not considered justifiable to try to distinguish between animals fed different diets; the investigation is based on 6 pigs from which a large number of specimens from animals referred to as pigs 1, 3, 5, 9 and 13 were examined; details on the experimental schedule are given in a previous paper (*Teige et al.* 1977). An additional pig was also inoculated. This animal was for 5 weeks prior to inoculation given a vitamin E deficient diet similar to that used in our first experiment (*Teige et al.* 1973), and the resulting deficiency from this diet was post-mortem evaluated by scattered massive liver necroses, yellow fat disease and muscular and myocardial degeneration. After inoculation with minced colon from dysenteric pigs all animals developed signs of dysentery; they were killed 3 days (the additional pig 6 days) after diarrhoea had developed. Necropsies were performed immediately after killing; pieces of colonic mucosa were fixed in 3 % glutaraldehyde, dissolved in Millonig's phosphate buffer and post-fixed in 2 % buffered osmic acid. The material was embedded in Araldite after dehydration in acetone. Semithin sections for light microscopy were stained with Toluidine blue. Overt necrotic areas were avoided for ultrathin sections; these were stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiscope I A. Characteristic lesions, repeatedly observed, were photographed. Sections from the biopsies, collected prior to inoculation (*Teige & Nafstad* 1978), served as controls.

RESULTS

Semithin sections disclosed that small vessels in lamina propria were distended and plugged with tightly packed red cells,

which at times seemed to be haemolyzed. This phenomenon was frequently observed also in areas where epithelial cells did not exhibit obvious damage (Figs. 1—2).

Electron microscopic examination revealed that the minute vessels of lamina propria were, as a rule, considerably distended; they frequently contained closely packed red cells which sometimes had undergone disintegration. Accumulations of platelets and polymorphonuclear leucocytes were also relatively commonly observed within the dilated vessels, together with a fibrin-like material; characteristic fibrin periodicity was, however, not observed. At times the material accumulated within the distended small vessels seemed to form occlusive "microthrombi". The endothelium was frequently severely damaged (Figs. 3—6, 8).

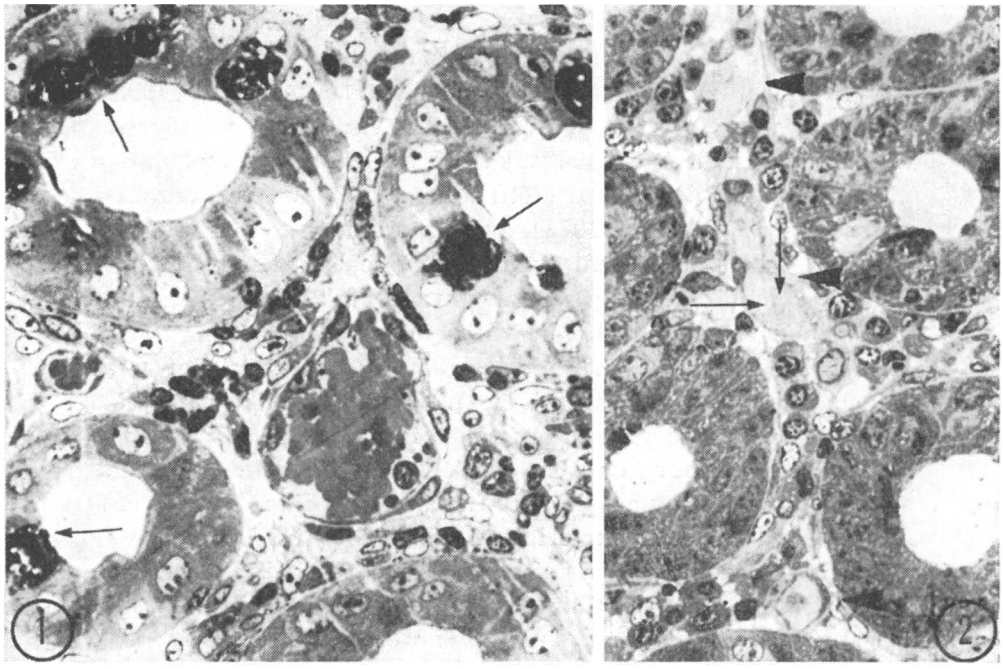


Figure 1. Photomicrograph of colon; a distended blood vessel is occluded by a mass which in great parts seems to consist of conglutinated red cells. There are incipient infiltration of mononuclear inflammatory cells in lamina propria and early degenerative changes in epithelial cells. Some goblet cells are indicated by arrows. Toluidine blue staining, × 160.

Figure 2. Photomicrograph of colon; epithelial cells are relatively well preserved; some dilated vessels (spear heads) contain a debris in which outlines of necrotic erythrocytes may be discerned (arrows). Toluidine blue staining, × 160.

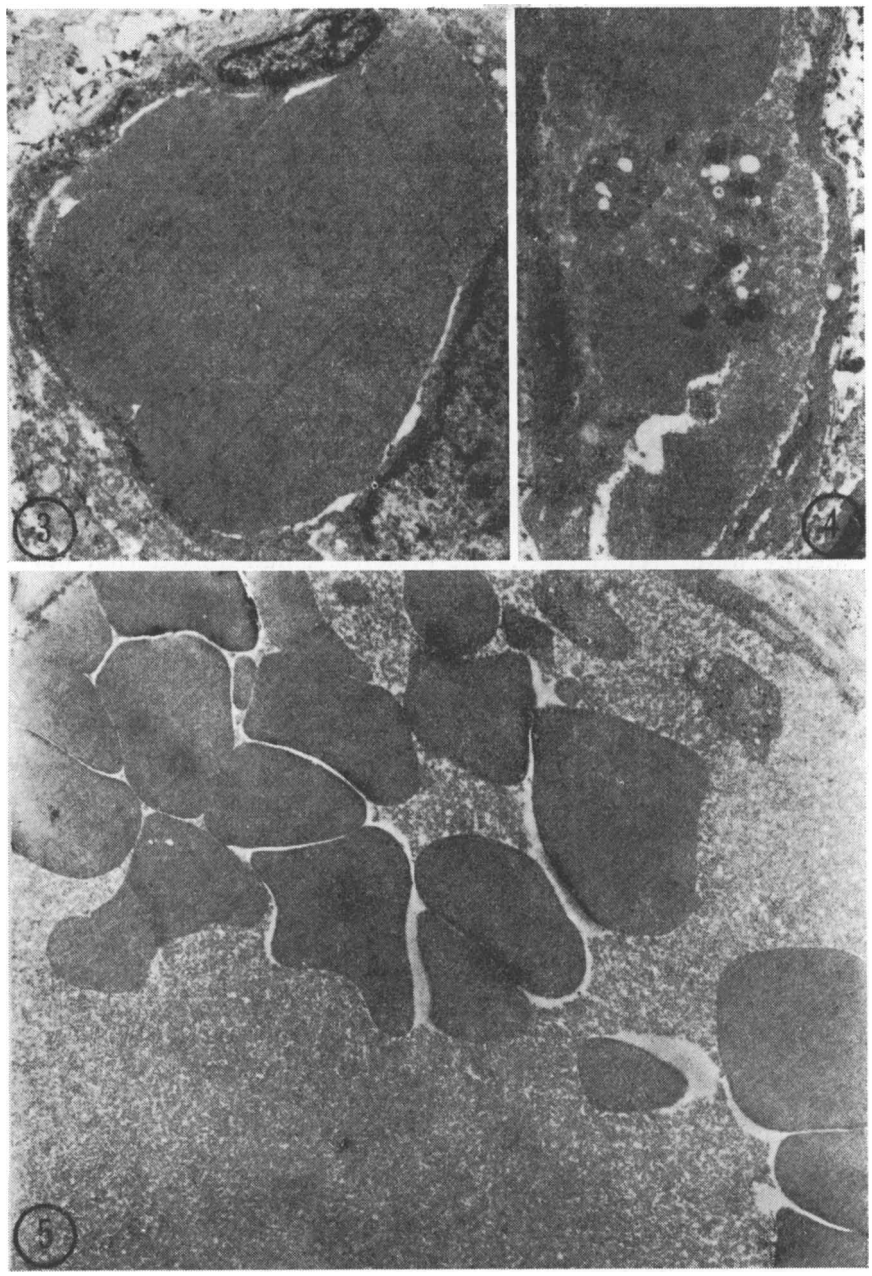


Figure 3. Low power electron micrograph of vessel in lamina propria of colon of dysenteric pig; the vessel is occluded by closely packed red cells. × 7000.

Figure 4. Low power electron micrograph of accumulation of red cells and platelets within a vessel in lamina propria of colon. × 7000.

Figure 5. Electron micrograph of part of a distended vessel in lamina propria; the vessel contains red cells, platelets and a relative electron dense material which possibly represents haemoglobin. × 9000.

Colonic enterocytes were in various stages of disintegration. Common observations in cells which were not completely necrotic included reduced number and shortening of microvilli, loosening of intercellular attachments, disruption of cell membranes towards the luminal surface, swelling and degeneration of mitochondria, and dilatation and vesiculation of endoplasmatic reticulum (Figs. 10, 12).

Microorganisms with morphological characteristics identical to T. hyodysenteriae, were readily observed in the following locations: a) in the crypts, b) free within the cytoplasm of damaged epithelial cells, and c) in accumulations around vessels in lamina propria; in the latter site they frequently seemed to be situated in cavities, but in the damaged tissue it was not always possible to decide whether these areas represented dilated lymphatics or simply were necrotic foci. The organisms had an external diameter about 0.27—0.30 μ, including the envelope, the diameter of the protoplasmic cylinder being 0.20—0.23 μ; as a rule 10—12 axial fibrils were visible in transverse sections, as rounded structures between the protoplasmic cylinder and the envelope (Figs. 7, 9, 11, 13).

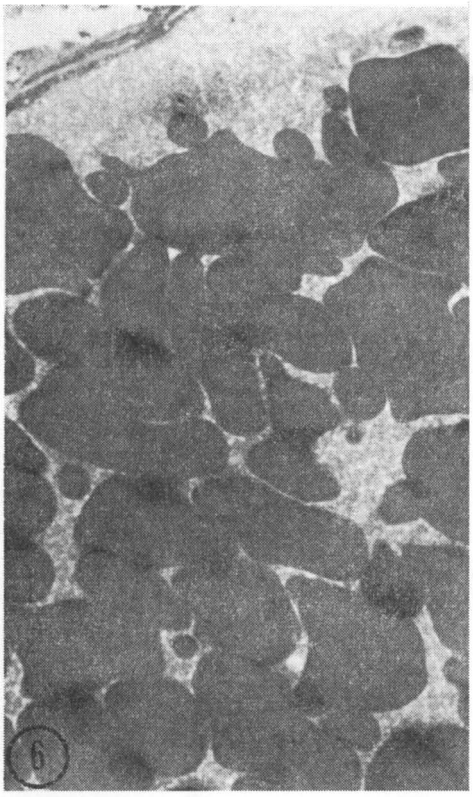
DISCUSSION

The electron microscopic alterations in colonic epithelial cells, described in this investigation, correspond in great parts to descriptions given by others in dysenteric pigs (*Taylor & Blakemore* 1971, *Glock et al.* 1974, *Schleicher* 1977). Reduced number and irregularity of microvilli, together with incipient degenerative alterations in cytoplasmic organelles have, however, also been described in vitamin E/selenium deficient pigs, independent of swine dysentery (*Teige & Nafstad* 1978); a possible importance of these early changes in colonic epithelial cells, as a predisposing factor in swine dysentery, remains to be elucidated.

Figure 6. Low power electron micrograph of part of a vessel in which there is accumulation of red cells; several cells are apparently undergoing fragmentation. × 3000.

Figure 7. Electron micrograph of spirochaetes accumulated in a necrotic area adjacent to a blood vessel. × 5250.

Figure 8. Electron micrograph of part of a blood vessel which contains red cells, a fibrin-like material, partly degranulated platelets and a debris of uncertain origin; spirochaetes are visible upper most to the left. × 9000.



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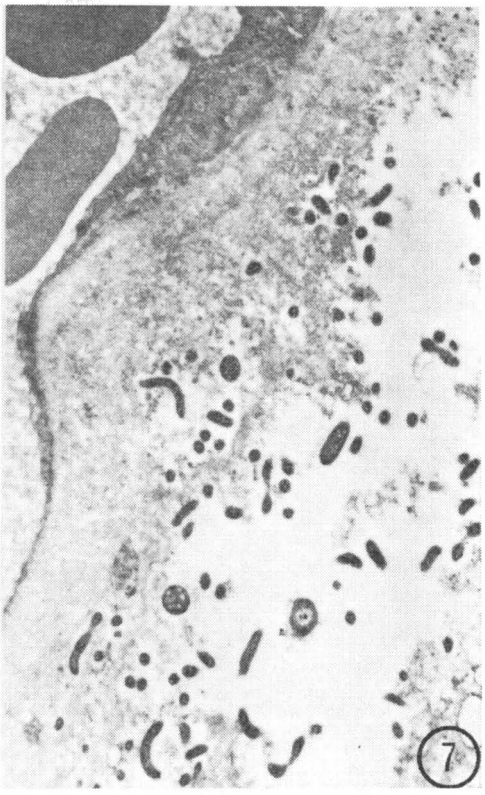

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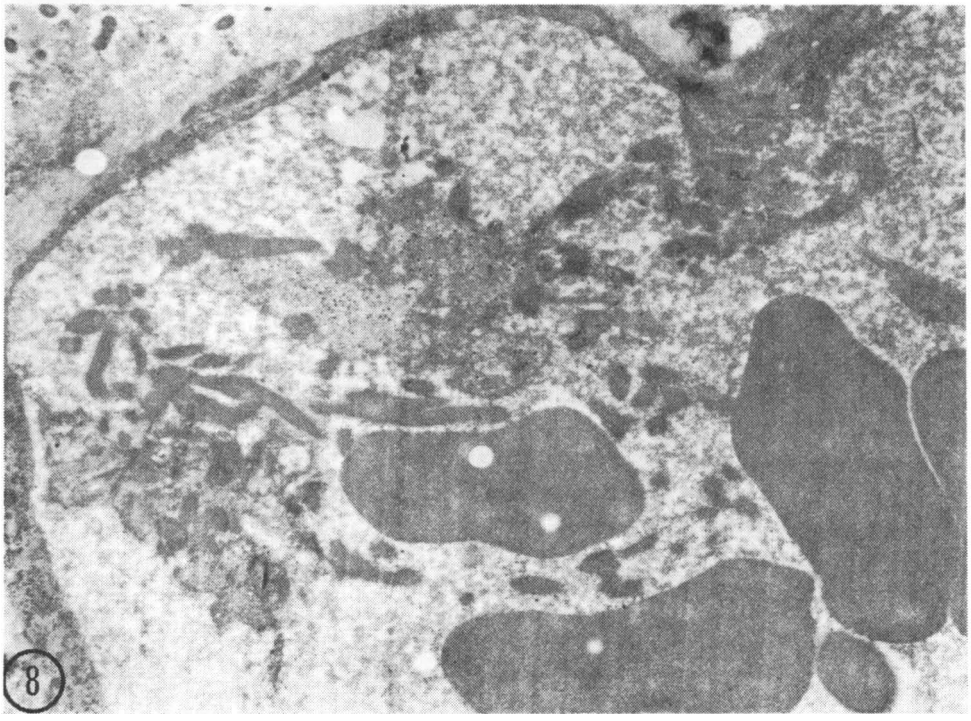

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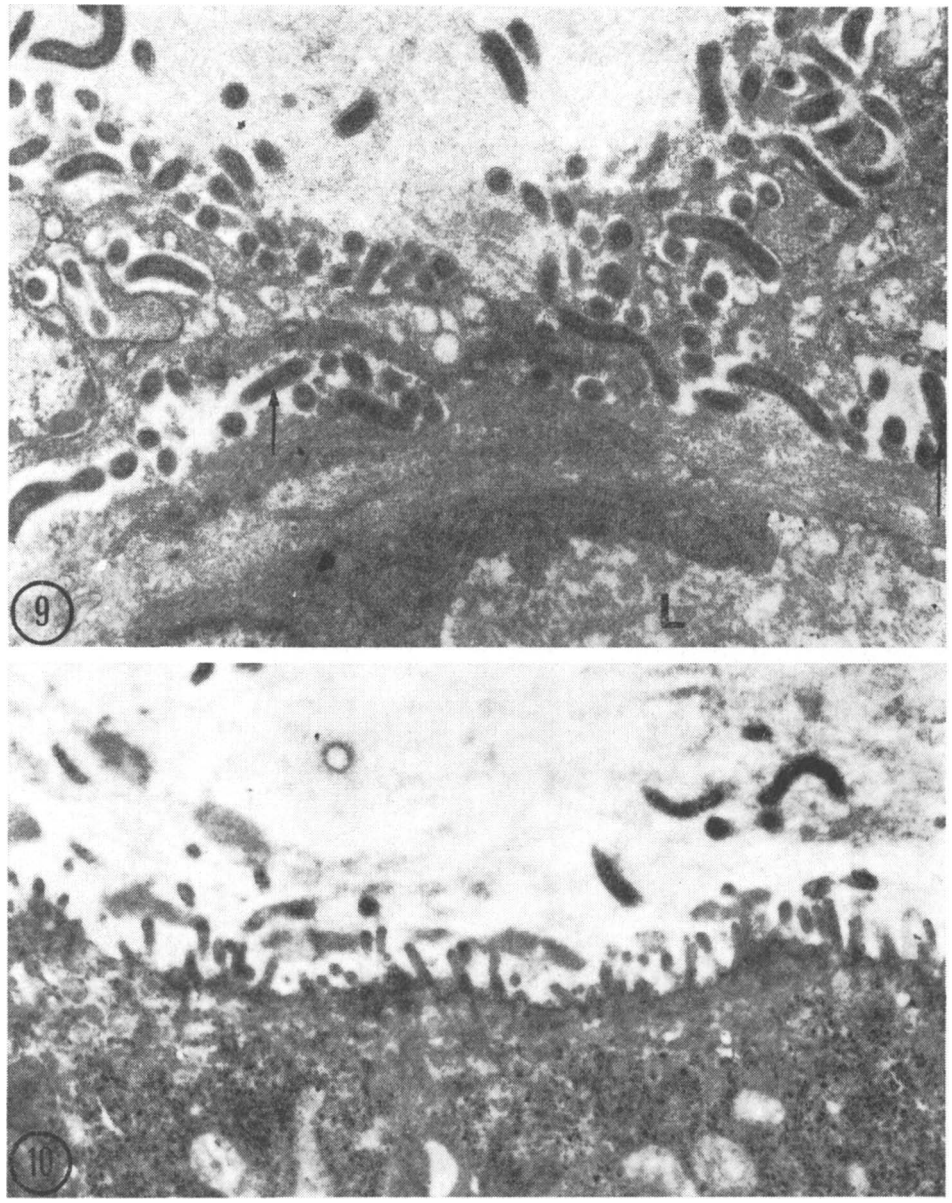
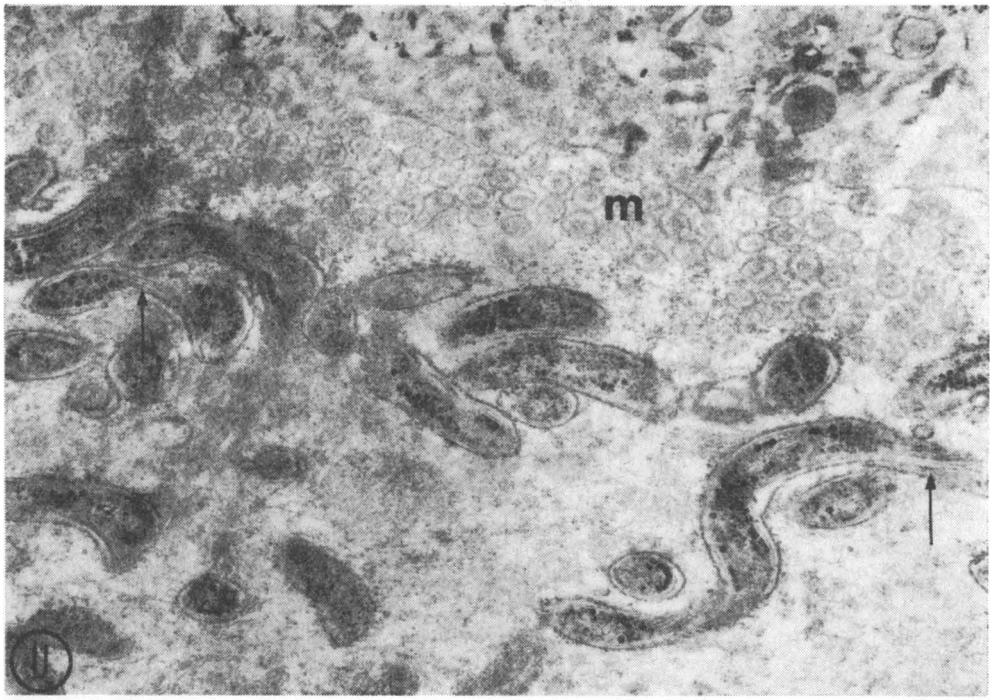


Figure 9. Electron micrograph of numerous spirochaetes (arrows) within a partly destroyed colonic epithelial cell; L is vascular lumen. × 18 000.

Figure 10. Electron micrograph of an epithelial cell with sparse and irregular microvilli; spirochaetes are visible in colonic crypt. × 15 000.



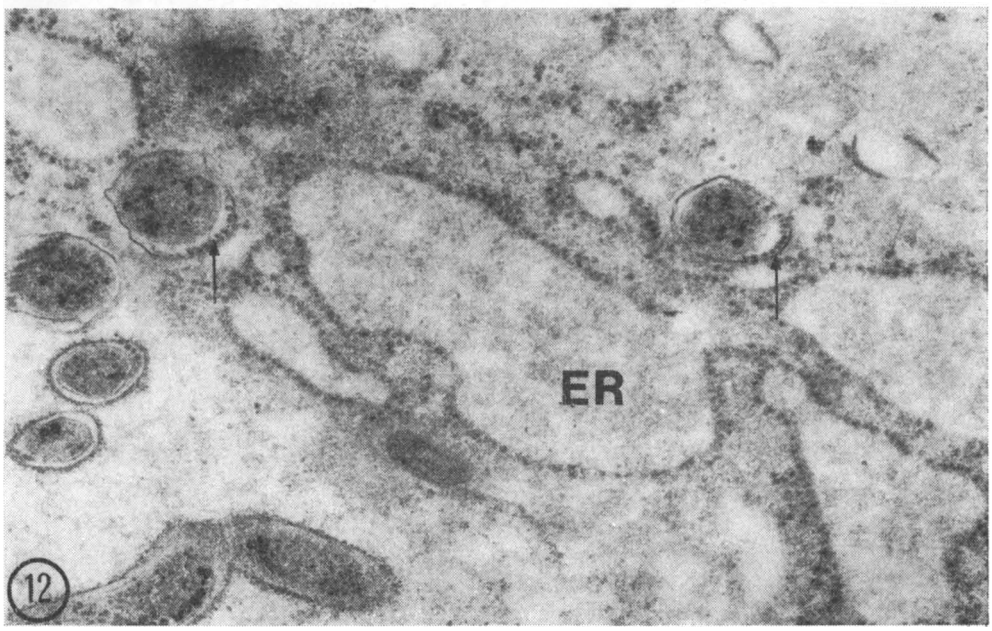


Figure 11. Electron micrograph of spirochaetes in colonic crypt; arrows indicate fibrils of axial filaments, m is microvilli. × 30 000.

Figure 12. Electron micrograph of a colonic epithelial cell in early disintegration, invaded by spirochaetes; arrows indicate axial filaments in transverse sections of spirochaetes; ER is swollen endoplasmatic reticulum. × 48 000.

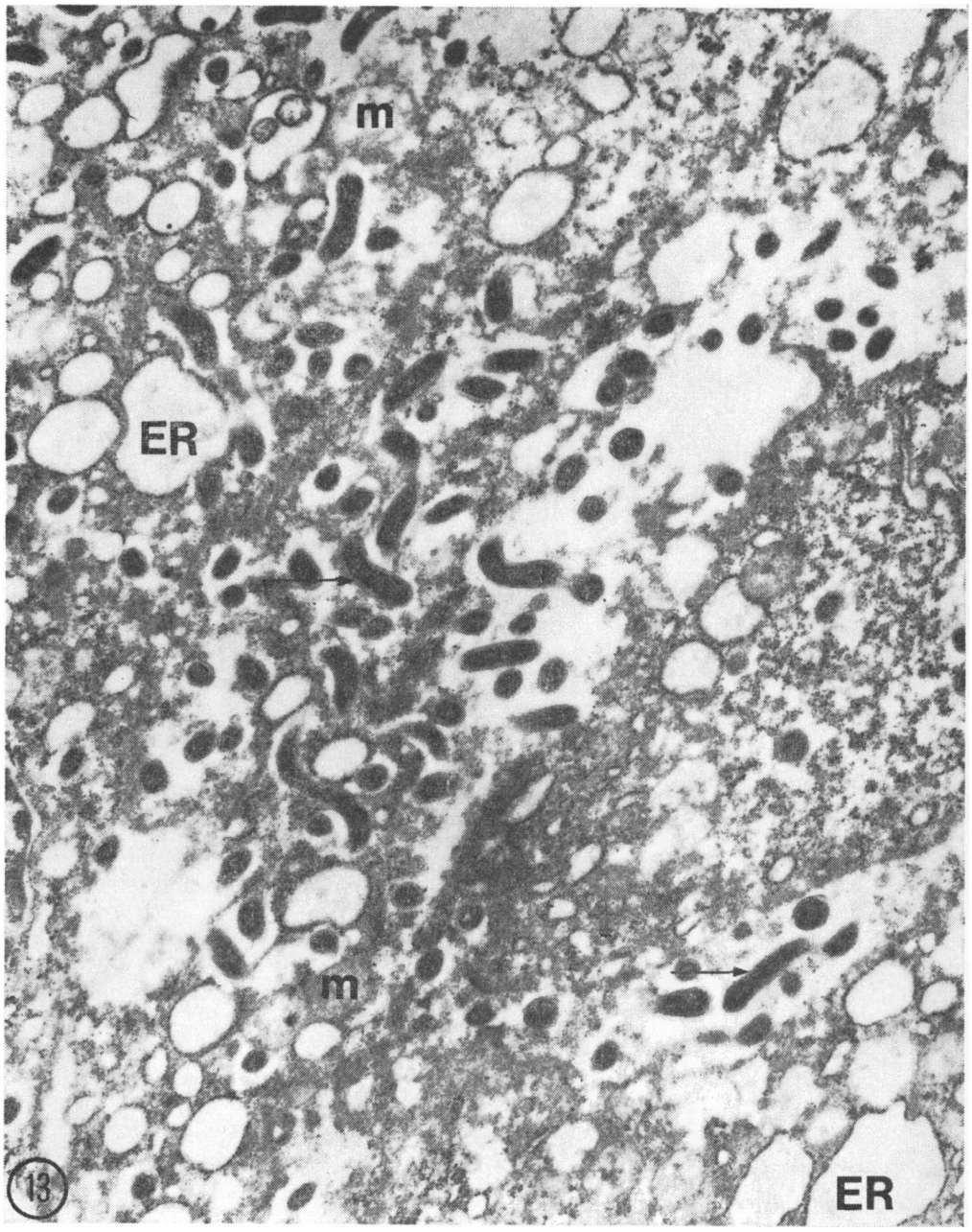


Figure 13. Electron micrograph of a colonic epithelial cell invaded by spirochaetes (arrows), ER swollen endoplasmatic reticulum, m degenerated mitochondria. × 48 000.

Swine dysentery is clinically characterized by loose, mucohaemorrhagic stool and dehydration; the mechanism by which fluid is lost is, however, incompletely understood, as is the role of T. hyodysenteriae as an entero-pathogen. It seems generally accepted that an increased exudation to intestinal lumen occurs during the development of swine dysentery (*Callinan & Russell* 1975, *Hughes et al.* 1976, *Moon* 1978). Increased permeability of vessels and intestinal mucosa, hypersecretion and malabsorption are among the major factors responsible for loose stool. Occurrence of fibrin and red cells in intestinal lumen indicates defects in membrane permeability severe enough to permit fibrinogen and erythrocytes to accumulate in intestinal lumen, in areas where endothelial and epithelial cells have been ruptured or destroyed (*Moon*). Fibrinous exudate on the mucosal surface and blood in the colonic contents are frequently present in dysenteric pigs, even in relatively early developmental stages, indicating that profund injury of intestinal mucosa is an early event in this entity.

The occlusion of minute vessels by closely packed and disintegrating red cells may be interpreted as a morphological expression of stasis (*Nordstoga* 1974), and the probable occurrence of stasis in the early developmental stage of swine dysentery could be thought to constitute an important factor in the pathogenesis of the disease, as hypoxia in areas adjacent to occluded vessels could be a major pathogenetic factor as to the necrotic lesions in the colonic wall. Spirochaetes were not observed in vessel walls, in microthrombi or within vessel lumina, and the significance and importance of their perivascular occurrence are obscure.

It is well documented that pure culture of T. hyodysenteriae, given orally, does not result in swine dysentery in gnotobiotic pigs, while several reports deal with successful transmission experiments in conventional pigs. It thus seems obvious that factors other than spirochaetes are involved in the pathogenesis of swine dysentery. Most authors seem to hold the view that there may be a synergism between spirochaetes and other microbes (*Glock & Harris* 1972, *Meyer et al.* 1974, 1975, *Callinan & Russell, Fernie et al.* 1975, *Brandenburg et al.* 1977, *Harris et al.* 1978). Patho-morphological colonic lesions, almost identical to swine dysentery may, however, be produced in pigs by intravenous

injection of a mixture of viable cells of Salmonella cholerae-suis and disintegrated cells of certain types of Gram negative bacteria; this treatment is followed by acute septicaemia, but in contrast to animals injected with pure culture of S. cholerae-suis alone, such pigs develop advanced colonic lesions (*Nordstoga & Fjølstad* 1970). It seems probable that this special response depends on the endotoxin content of the material injected. This observation agrees with the investigation of *Sanarelli* (1924), who recognized intestinal epithalaxia in rabbits in association with a corresponding experimental model; his investigation is regarded as the first recognition of the generalized Shwartzman reaction, also known as Sanarelli-Shwartzman reaction (*McKay* 1965). It is therefore possible that mechanisms related to Shwartzman phenomenon are operative in the development of swine dysentery, as also suggested previously (*Nordstoga et al.* 1968, *Espinasse* 1973, *Nordstoga* 1973, *Teige* 1978). As pigs with a nutritional deficiency caused by a high proportion of unsaturated fat in the feed are predisposed to the generalized Shwartzman reaction (*Teige et al.* 1973), it seems further possible that the influence of dietary factors on experimental swine dysentery may, at least in parts, be explained on this basis.

The experimental renal Shwartzman lesions are, classically, produced by bacterial endotoxins (*McKay, Sharp* 1977, *Watanabe & Tanaka* 1977). Endotoxin may probably be absorbed from the intestine when the intestinal mucosal barrier is disturbed (*Walker* 1978). Hence, it is also possible that endotoxins, of intestinal origin, act synergistically with spirochaetes in the development of fulminant swine dysentery. This could explain the resistance of gnotobiotic pigs.

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SAMMENDRAG

Elektron-mikroskopiske forandringer i kolon ved eksperimentell svinedysenteri.

De pato-anatomiske forandringer i kolon ved eksperimentell svinedysenteri ble studert i elektronmikroskopet. I de små blodkar i stratum proprium ble det påvist forandringer som tydet på stase. De ultrastrukturelle forandringene i epitelcellene besto i relativt tidlige stadier av redusert antall, forkortelse og deformitet av microvilli; mitokondriene var oppsvulmet og degenererte, og det oppsto større og mindre blæredannelser i det endoplasmatiske reticulum. Spirochæter, i stort antall og med distinkte strukturer, ble påvist a) i kryptene i kolon, b) fritt (ikke membran-bundet) i degenererte epitelceller, og c) i lommedannelser omkring blodkar i stratum proprium. Det antydes at stase inntrer på et relativt tidlig stadium i utviklingen av svinedysenteri, og at de nekrotiske forandringene som etter hvert utvikles kan ha direkte sammenheng med stase. Det diskuteres også hvorvidt forandringene i kolon ved svinedysenteri kan tenkes å være utviklet over en patogenetisk mekanisme av typen Sanarelli-Shwartzman's reaksjon.

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