Acta vet. scand. 1976, *17*, 223—232.

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REGIONAL ILEITIS IN PIGS

MORPHOLOGICAL AND PATHOGENETICAL ASPECTS

By

Lennart Jönsson and *Kjell Martinsson*

JÖNSSON, LENNART and KJELL MARTINSSON: *Regional ileitis in pigs. Morphological and pathogenetical aspects.* Acta vet. scand. 1976, *17*, 223—232. — Twenty-seven weaned pigs with a wasting appearance were investigated. From a morphological point of view the pigs were divided into three groups. In the group of pigs with macroscopical signs of regional ileitis, affected tissue showed substantial epithelial proliferation, and electron microscopic studies revealed the presence of microorganisms within the cytoplasm of the epithelial cells. There was a loss of, or only faint, enzymatic activity of alkaline phosphatase in the mucosal epithelium. In another group of five pigs there was no, or only slight, light microscopical signs of regional ileitis but presence of intracellular microorganisms. The enzymatic activity of the ileal epithelium was low. Low enzymatic activity of the ileal epithelium was observed in a third group of wasting pigs, which had no histological or electron microscopical signs of regional ileitis.

pigs; regional ileitis; wasting syndrome; alkaline phosphatase; intracellular microorganisms.

Regional ileitis or intestinal adenomatosis in pigs was first described in 1931 by *Biester & Schwarte*. Since then only a few reports on the disease have been presented, and not until the last few years has interest been focused on regional ileitis. In Sweden the disease is generally observed some weeks after weaning, and affected pigs show poor growth rate and a wasting appearance (*Martinsson et al.* 1974).

The pathological changes are usually confined to the terminal part of the ileum, starting at the ileo-cecal junction. In some cases the cecum and/or spiral colon are also affected (*Rowland & Rowntree* 1972, *Martinsson et al.*). The wall of the affected areas of the intestine is thickened and the mucosa is thrown into deep folds and often has a "cobblestone character". In most cases the

mucosal thickening is combined with a hypertrophy of the muscle coats (*Emsbo* 1951). Necrotic areas of the mucosa are sometimes observed.

The histopathological examination reveals a muscular hypertrophy and a proliferation of epithelial cells which causes a thickened mucosa (*Emsbo, Rowland & Rowntree, Martinsson et al.*). A prominent finding is an epithelial downgrowth of the Lieberkün crypts into the deep parts of the intestinal wall. Presence of "metastases" of crypt cells in the ileal mesenterial lymph nodes is reported by *Emsbo* and *Nielsen* (1971). In the affected mucosa heavy infiltrations with lymphocytes, histiocytes, fibroblasts and eosinophils are observed.

Recently, microorganisms have been observed at electron microscopy in the ileal epithelium of diseased pigs (*Rowland & Lawson* 1974, *Martinsson et al.*). *Lawson & Rowland* (1974) identified these intracellular organisms as Campylobacter sputorum subp. mucosalis.

Analysis of blood serum from diseased pigs reveals low levels of albumin (*Nielsen* 1971, *Martinsson et al.* 1974) and very low levels of alkaline phosphatases (*Martinsson et al.* 1974). The zinc content of serum also seems to be low (*Martinsson & Ekman* 1974).

The aim of this investigation is to study the ileum of pigs with regional ileitis, and wasting piglets without the disease, by electron microscopic and histochemical methods. The absorptive capacity of the ileal epithelium from diseased pigs is also investigated.

MATERIAL AND METHODS

Animals

Twenty-seven weaned crossbred pigs of Yorkshire and Swedish Landrace, 10—12 weeks old, were obtained from four different herds. All the pigs showed a wasting appearance with a poor growth rate as a common feature (Fig. 1). They were weaned at about six weeks. According to the owners, only few of the pigs had occasional diarrhea before or after weaning. Ten normal pigs of the same age were used as controls.

Experimental procedure

The pigs were anesthetized by intravenous injections of Nembutal®. During anesthesia the abdomen was opened and tissue

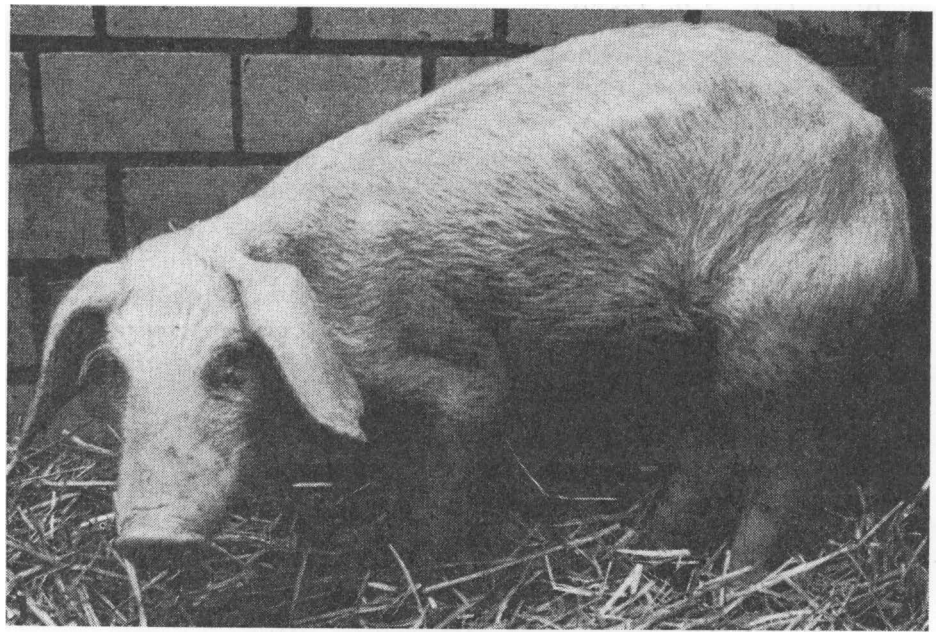

Figure 1. A 10 week old pig with regional ileitis and a typical wasting appearance.

samples were removed for electron microscopy and histochemical analysis.

Light microscopy

Specimens of jejunum, ileum and large intestine were fixed in neutral formol solution and embedded in paraffin. All sections were routinely stained with hematoxylin-eosin. Selected samples were also stained with periodic acid Schiff (PAS), Gram, Giemsa, Ziehl-Neelsen and toluidine blue. Unfixed cryostat sections were used for demonstration of enzyme activity of alkaline phosphatase (diazonium method).

Electron microscopy

Specimens were taken from the jejunal and ileal mucosa and were cut into 0.5 mm cubes. These cubes were fixed in glutaraldehyde, followed by osmium tetroxide, and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a Philips RA 201 electron microscope.

Pinocytosis of ileal epithelium

Three pigs with clinical and hematological signs of regional ileitis were used in this experiment. The pigs were anesthetized with Nembutal® intravenously and the abdomen was opened. Two hundred mg of a 5 % solution of Dextran Blue (Pharmacia Fine Chemicals, Uppsala, Sweden) per kg bodyweight was injected into the jejunum, and the abdomen was then closed. After 36 hrs. the pigs were euthanised and necropsied. Regional ileitis was confirmed at macroscopic and microscopic examination. The ileal epithelium was examined for pinocytosis of the macromolecular substances (Dextran Blue) as described by *Martinsson & Jönsson* (1975).

For bacteriological examination the methods of *Lawson & Rowland* (1974) were used.

RESULTS

Gross pathology

The animals were in a poor condition with a thin appearance. Pathological changes, which varied widely in severity, were observed in the intestine and in the mesenteric lymph nodes. The most affected cases showed obvious changes in the intestinal mucosa, muscular layer and on the serosal surface. The least affected cases showed slight mucosal changes, which were found only at histological examination.

The lesions were confined mainly to the ileum, but in advanced cases the changes were even noted in the aboral part of the jejunum. The affected parts of the intestine were thickened and rigid. The thickening of the intestinal wall was due to muscular hypertrophy and hyperplasia of the mucosa.

The mucosa was folded longitudinally and transversely, a picture similar to that of Johne's disease (Fig. 2). In some cases there were patches of ulcerations covered with necrotic debris and pseudomembranes (Fig. 3). The Payer's patches were enlarged and often protruded into the lumen like long broad bands. The serosa of the ileum and the mesenterium was edematous and thickened. In one pig the intestinal changes were complicated by an ileo-cecal invagination.

The regional lymph nodes showed a moderate degree of hyperplasia. In a few cases lymphoreticular hyperplastic areas of

about 1 cm in diameter were observed in the cecum. No other pathological changes were observed except slight enzootic pneumonia in some of the pigs.

Histopathology

The ileum of the control animals was lined with a simple epithelium of columnar type and numerous globlet cells. The villi were regular but the depth of the intervening crypts was variable.

The histopathological changes of pigs with clinical signs of regional ileitis were characteristic in advanced cases (Fig. 4). The villous pattern was absent except in the mildly affected areas. The mucosa presented a smooth, flattened, domed surface. The epithelial cells were tall, columnar in shape and eosinophilic. They were immature and lacked their protective mucous coat. Globlet cells were few. The crypts were elongated, branched and tortuous. They were often dilated and formed retention cysts filled with polymorphonuclear leucocytes and cell debris (Fig. 5). The histological picture had, in fact, an adenomatous appearance.

The lamina propria showed infiltration of mononuclear cells which, to some extent, replaced the glandular tissue. The submucosa exhibited prominent patches of lymphatic tissue, which frequently penetrated to the mucosal propria. The submucosa also showed inflammatory cells extending to the muscular layer, which was hypertrophied. There were infiltrations of mononuclear cells and fibrosis in the subserosal layer. The regional mesenteric lymph nodes showed a picture of unspecific chronic lymphadenitis.

Histochemistry

The activity of alkaline phosphatase in the control pigs was strongest in the epithelial cells of the apical part of the villi. The crypt cells, on the other hand, were non-reactive (Fig. 6). The vessels were strongly positive. The enzymatic activity of the ileal epithelium of the pigs with regional ileitis was diminished or absent. In some areas there was a faint activity in the apical parts of the villi (Fig. 7). The enzymatic activity of wasting pigs free from regional ileitis was diminished but higher in comparison with the diseased animals (Fig. 8).

Electron microscopy

Intracellular microorganisms were observed in the intestinal epithelium of affected parts in 21 cases. In 16 of these cases there were evident macroscopical and light microscopical signs of regional ileitis, whereas in five pigs no macroscopical and no, or very slight, light microscopical changes were observed. In six wasting pigs no macroscopical or light microscopical changes and no intracellular microorganisms were observed. The microorganisms were not observed in the control animals.

The microorganisms were most abundant just beneath the terminal web and were often surrounded by small unstained zones. No host membranes were observed lining these halo-like zones. The membranes of organisms varied considerably, and in a few cases they were detected in the gland lumina. The microorganisms were elongated and curved with rounded extremities. Their length varied between 1.5 and 2 μ. On cross section, they were round and measured 0.25—0.30 μ in diameter. A distinct cell wall surrounded the individual organism, and the internal structure was dense and osmiophilic (Fig. 9).

Similar microorganisms were not observed in the normal parts of the jejunum in pigs with regional ileitis or in control animals.

The epithelial cells of the changed intestine were elongated with a narrow apex and a basal nucleus. They were usually similar to more or less undifferentiated crypt cells. The microvilli were few and irregularly shaped. In the more mature cells the microvilli were abundant and of normal shape. The cells contained round electron-dense bodies in the apical region (Fig. 10), presumably secretory granules.

At bacteriological examination it was not possible to isolate the intracellular microorganisms.

Pinocytosis of ileal epithelium

The pigs which were injected with Dextran Blue into the jejunum were euthanised and necropsied 36 hrs. later. There were no signs of epithelial pinocytosis of the macromolecules. Most of the dye was found in the ingesta of the colon and rectum.

From a morphological point of view it was possible to divide the present wasting pigs into three groups:

1. Sixteen pigs with macroscopical and microscopical changes typical for regional ileitis. In this group, intracellular microorganisms were regularly observed.
2. Five pigs free from, or with only slight, light microscopical signs of regional ileitis but with intracellular microorganisms.
3. Six pigs free from macroscopical and microscopical intestinal lesions. No microorganisms were observed at electron microscopy in these animals.

DISCUSSION

The pigs in the present investigation had one property in common: namely, the poor growth rate after weaning. The gross pathology and the histopathology of the affected intestine in developed regional ileitis showed the same major changes as those described by earlier observers (*Emsbo* 1951, *Nielsen* 1955, *Dodd* 1968, *Nielsen* 1971, *Rowland & Rowntree* 1972, *Rahko & Saloniemi* 1972). The adenomatous structure of the immature hyperplastic epithelium in the present study seemed to be the result of a regenerative process following a destruction of the intestinal epithelium.

Two main forms of regional ileitis are reported to exist: the muscular type and the mucosal type. In the first type, both the circular and the longitudinal layers of the muscularis are hypertrophied. The second type is characterized by a diffuse hyperplasia of the mucosa together with a muscular hypertrophy. *Nielsen* (1955) suggested that regional ileitis should be reserved for cases with both muscular and mucosal lesions; cases with only muscular hypertrophy should be classified as "muscle hypertrophy of ileum".

Nielsen (1955) was of the opinion that there is an initial muscular hypertrophy which produces a rigidity of the wall and a narrowing of the lumen. He supposed that this circumstance lowered the vitality of the intestinal wall, thus leaving the mucosa vulnerable to toxins and bacteria present in the lumen.

In the present investigation there was no proof that a primary muscle hypertrophy will give rise to a secondary mucosal involvement. The pigs of the second group were the most interesting animals in a pathogenetical respect. Intracellular microorganisms were observed in the ileal epithelium of these pigs without any, or with very slight, histopathological changes,

suggesting regional ileitis. There was no evidence of muscular hypertrophy, which indicates that the mucosal changes are primary and muscle hypertrophy secondary.

The histochemical analyses of alkaline phosphatase showed diminished or no activity of the ileal epithelium in cases of regional ileitis. The enzyme activity of the ileal epithelium from pigs of group 3 was also decreased, but in the controls the enzyme activity was high. The significance of these findings is difficult to explain. The low activity of alkaline phosphatase in regional ileitis can be explained by the characteristic proliferation of immature epithelial cells. The low enzymatic activity of ileal epithelium in group 3 probably indicates a subcellular disturbance which may interfere with the development of regional ileitis.

Pinocytosis of macromolecules is observed in pigs up to an age of three weeks (*Moon* 1972, *Martinsson & Jönsson* 1975). In the present material there was no pinocytosis of the ileal epithelium. Absorption of microorganisms via a reawakened pinocytosis was considered unbelievable, as is the hypothesis that organisms have been present in the epithelium since the time when active pinocytosis still existed. The replacement time of ileal epithelium in pigs three weeks of age is only two-four days (*Moon* 1971).

The morphology of the intracellular microorganisms showed the same appearance as those identified by *Lawson & Rowland* (1974) as Campylobacter (Vibrio) sputorum subsp. mucosalis. As the microorganisms were regularly observed in pigs of the first and second groups but not in group 3, the following hypothesis of pathogenesis is proposed. Pigs which develop signs of regional ileitis show a slower growth rate than normal pigs. This may to some extent be caused by a malabsorption syndrome after weaning due to a subcellular disturbance. Subsequent invasion of microorganisms into the intestinal epithelium causes epithelial proliferation with hyperperistaltic activity and muscular hypertrophy. Consequently the pigs of group 2 should represent early stages of regional ileitis. A malabsorption syndrome in pigs after weaning has been investigated by *Kenworthy & Allen* (1966).

It is concluded from the present investigation that there may be two factors for the development of regional ileitis:

a. Predisposing moment, which lowers the vitality of the intestinal mucosa.
b. Intracellular invasion of certain microorganisms from the lumen of the intestine.

Several authors have reported that different forms of gastrointestinal disorders predispose regional ileitis. Thus *Biester & Schwarte* (1931) described regional ileitis in three pigs from a group recovering from enteritis. The same investigators also succeeded in transmission experiments on four pigs. *Emsbo* was of the opinion that the ileal mucosa was prone to trauma because of the slow passage of ingesta through this part of the intestine. The author supposes that a high bacterial count in the ileum may cause unspecific hyperplasia of the lymphoid tissue in the intestinal wall with narrowing of the lumen and stasis of the ingesta resulting in mucosal inflammation and muscular hypertrophy.

Nielsen (1971) reported that regional ileitis often develops after occurrence of diarrhea at the time of weaning. It is also suggested that proliferative hemorrhagic enteropathy may be an acute form of intestinal adenomatosis (*Rowland & Rowntree, Rowland & Lawson* 1975). In Sweden it is a common experience that regional ileitis is more often seen in certain herds than in others. It has also been observed that different forms of diarrhea are not a regular observation previous to outbreak of regional ileitis. In most cases it seems to be a wasting syndrome of the pigs which predisposes the disease.

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SAMMANFATTNING

Morfologiska och patogenetiska synpunkter på regional ileit hos grisar.

Författarna har undersökt 27 grisar med typiskt "pelle"-utseende. Ur morfologisk synpunkt indelades de i tre grupper. I gruppen med makroskopiskt påvisbar regional ileit sågs adenomatösa slemhinneproliferationer. Vid elektronmikroskopisk undersökning avslöjades intracytoplasmatiska mikroorganismer i epithelcellerna. Aktiviteten av alkaliskt fosfatas var låg eller saknades helt i slemhinneepithelet. I andra gruppen förekom intracellulära mikroorganismer men endast svaga histologiska tecken på regional ileit. Enzymaktiviteten i epithelcellerna var låg, liksom i tredje gruppen. I sistnämnda grupp förelåg varken ljusmikroskopiska eller elektronmikroskopiska förändringar tydande på regional ileit. Ur patogenetisk synpunkt diskuteras två faktorer som förknippas med sjukdomens uppkomstmekanism. Den ena är predisponerande gastrointestinal rubbning, den andra är intracellulär förekomst av mikroorganismer.

(Received March 24, 1976).

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Figure 2. Longitudinal section of the ileum showing a hyperplastic longitudinally and transversely folded mucosa.

Figure 3. Hyperplastic mucosa with a necrotic area in the aboral part of the ileum (arrows).

Figure 4. Histologic section of proliferating ileal mucosa at regional ileitis. Note the absence of villous pattern, downgrowth of crypts (arrows) and the cellular reaction in the mucosa and the submucosa. HE, × 45.

Figure 5. Section showing proliferation of tall epithelial cells. Some crypts are dilated, containing polymorphonuclear leucocytes and cell debris. HE, × 105.

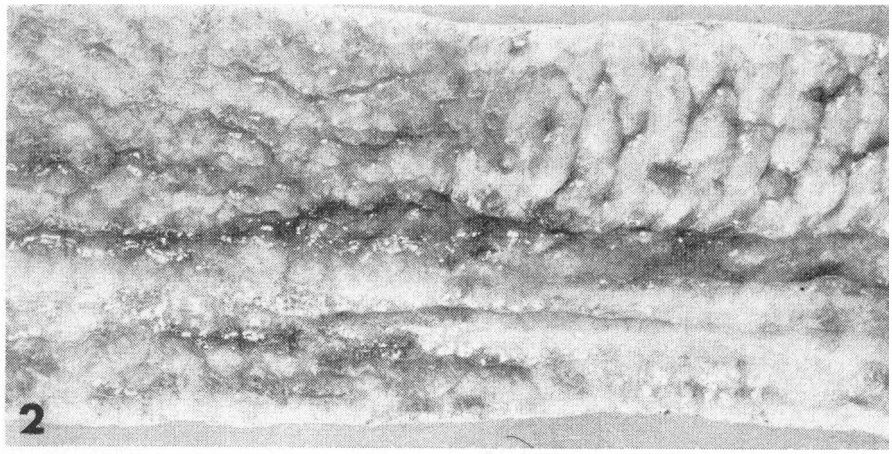
Figure 6. Alkaline phosphatase activity in the mucosa of normal pig ileum. Note the strong reaction in the villous epithelial cells and lack of reaction in crypt epithelium. × 105.

Figure 7. Alkaline phosphatase activity in the mucosa of ileum at regional ileitis. Only faint activity is observed in the apical part of the mucosa (arrows). × 105.

Figure 8. Alkaline phosphatase activity in the mucosa of ileum from wasting pigs without signs of regional ileitis. A slight activity is demonstrated in the villous epithelium (arrows). × 105.

Figure 9. Electron micrograph from upper portion of ileal mucosa at regional ileitis. Immature epithelial cells with poorly developed microvilli and numerous longitudinally and transversely sectioned organisms (arrows) within the cytoplasm and in the intestinal lumen. × 6000.

Figure 10. Apical portion of several immature epithelial cells of the mucosa at regional ileitis. Note the numerous intracytoplasmic organisms (large arrows) and the membrane-bounded granules of different sizes presumably representing secretory material (small arrows). × 10,000.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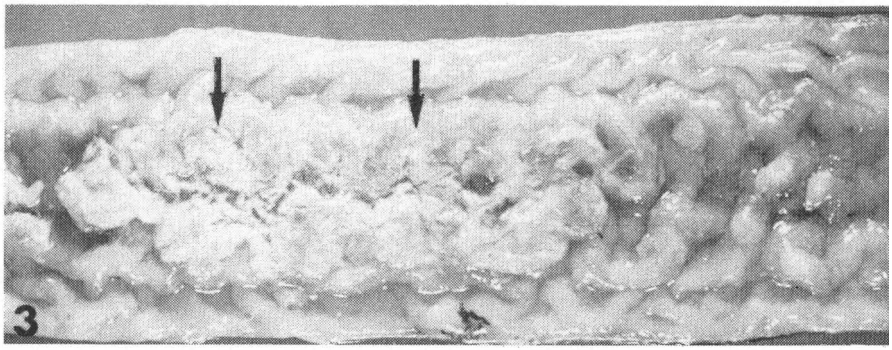
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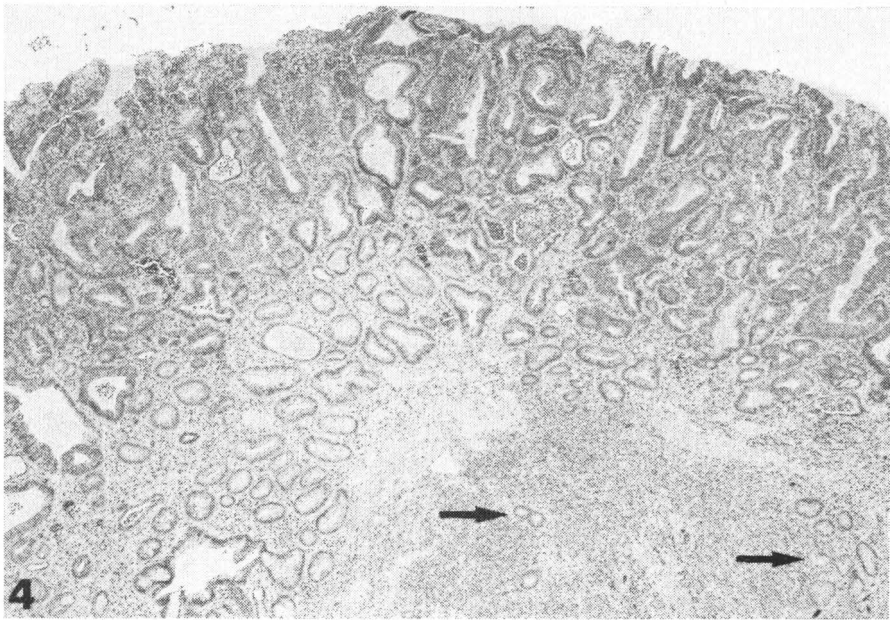
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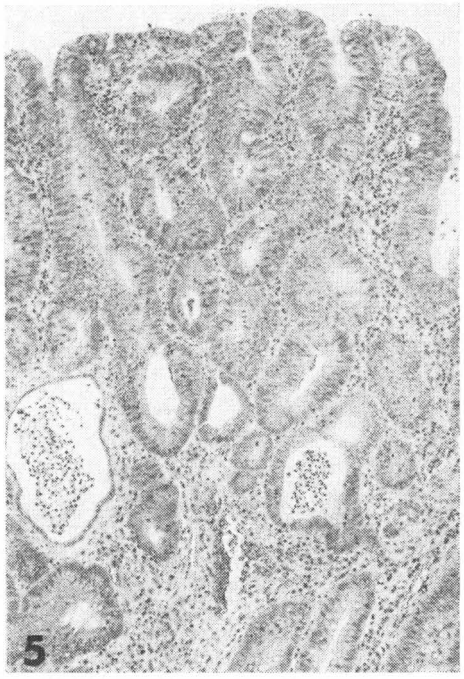
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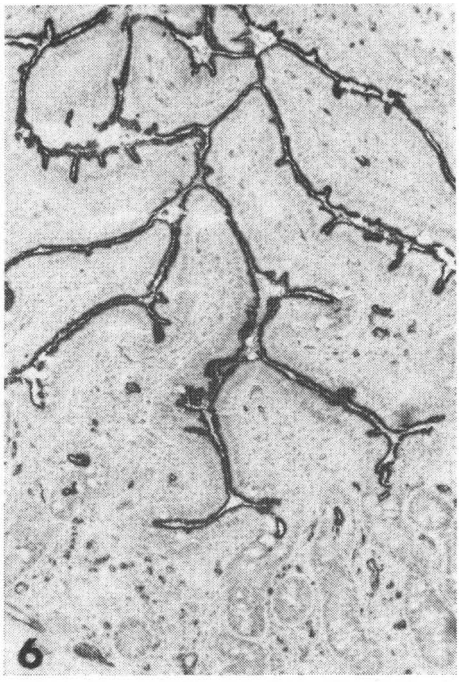
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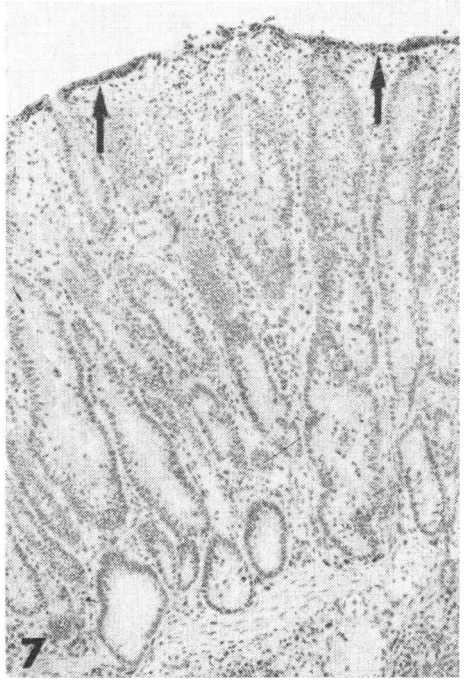
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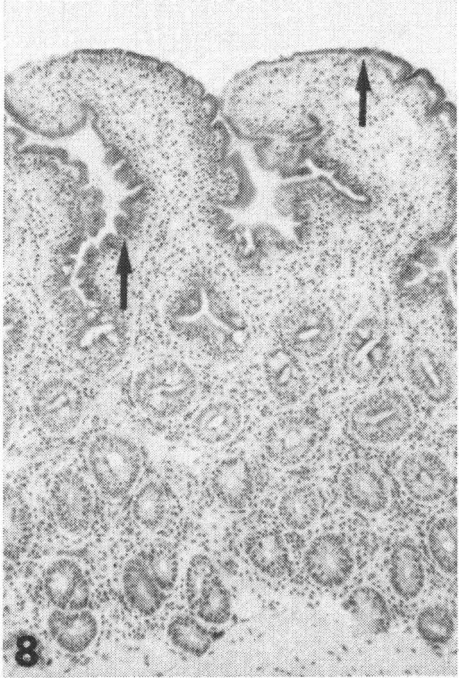
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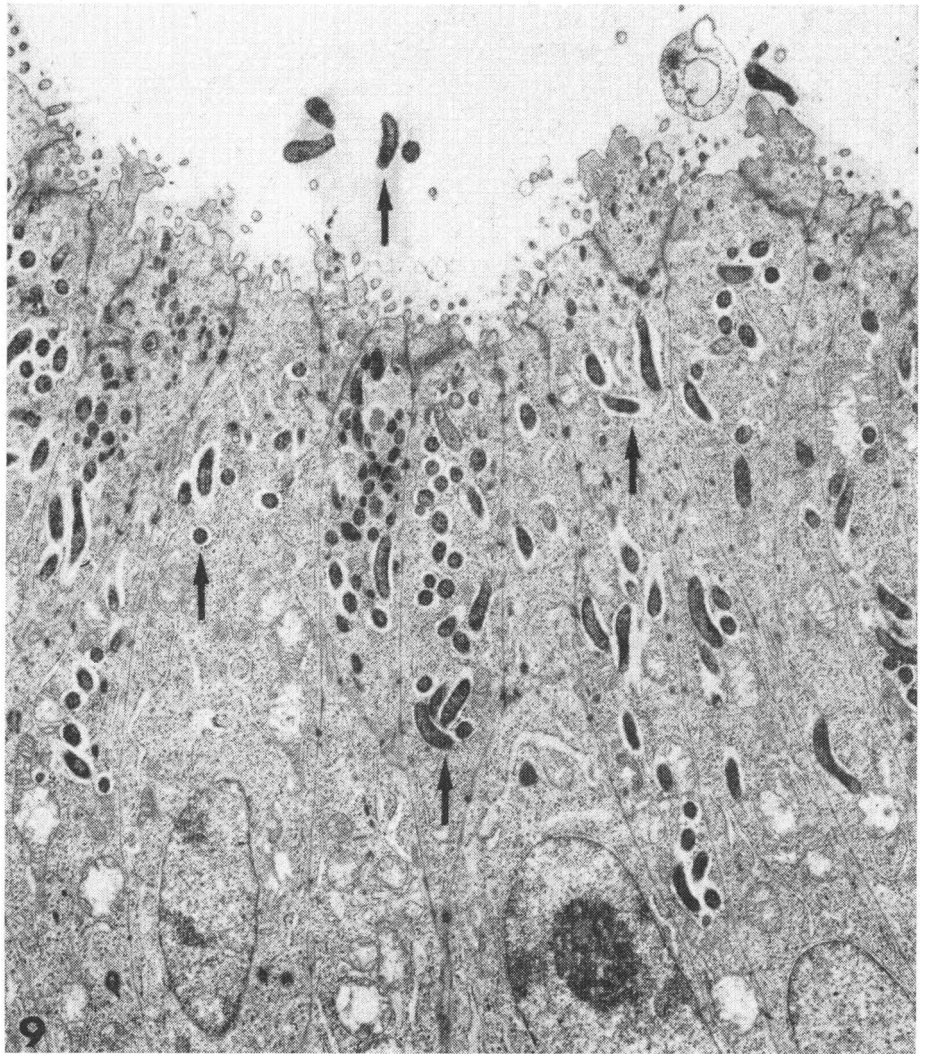

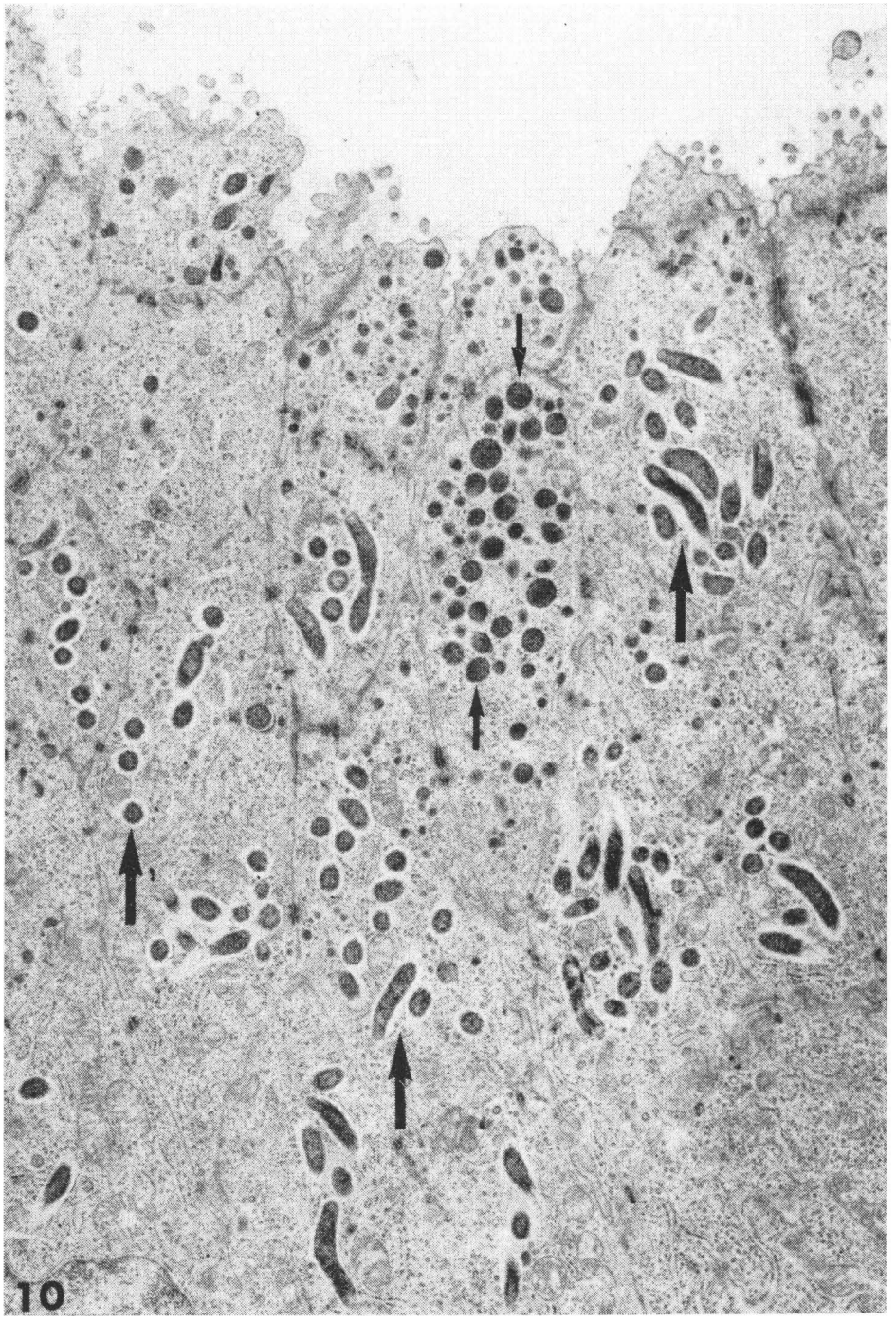
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