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PATHOLOGICAL CHANGES INDUCED BY MULTICEPS ENDOTHORACICUS IN THE INTESTINE OF DEFINITIVE HOST

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Abstract. The adult cestode *Multiceps endothoracicus* penetrates inside the intestine of its definitive host (*Vulpes corsac*) into the layer of Lieberkühn's crypts, sometimes up to stratum compactum mucosae. On day 130 p.i., the pathological changes at the site where the strobila penetrated deep into the mucosa are characterized by pressure atrophy of villi and Lieberkühn's crypts, devastation of parts of Lieberkühn's crypts, scattered haemorrhagiae and diffuse inflammatory infiltration of mucosa more marked in the vicinity of scolex. Eosinophiles prevail in the inflammatory infiltrate and plasmocytes are also present in some places. Proliferative changes are slight.

This paper is a part of studies on the morphology of larvae and adults of *Multiceps endothoracicus* and it is a continuation of the previous paper by Hulínská and Lavrov (1981). We assume that the description of the mode of attachment of this cestode in the intestine of its definitive host and the description of pathological changes induced by the parasite will contribute to a better knowledge of the mechanism of cestode attachment and of the pathogenicity of this species.

MATERIAL AND METHODS

A male of a larger group of corsacs (*Vulpes corsac*) was experimentally infected with two larvae of *Multiceps endothoracicus* obtained from great gerbil (*Rhombomys opimus*). The corsac was killed on day 130 p.i. and immediately dissected. The small intestine (jejunum with strobilae) was fixed in 4% glutaraldehyde in 0.1M cacodylate buffer for 5 days at 4 °C and then divided into small parts. Some of the excisions were postfixed in 1% OsO_4 in 0.1M cacodylate buffer and embedded into Epon 812, the others were postfixed in Baker's fixative and embedded in paraffin or gelatine. Paraffin sections were stained with haematoxylin-eosin, trichrome after Masson, Van Gieson's and Giemsa's methods and Weigert's method for elastica.

The following histochemical methods were used (intended for yet another purpose):
Detection of mucosubstances. Natural: PAS reaction combined with acetylation and desacetylation. **Acid:** Alcian blue, pH 2.6, with methylation and demethylation; Mowry's modification of AB-PAS.
Detection of proteins. SH groups: DDD with control blockade with N-ethylmaleimide. **SS groups:** DDD combined with thioglycolic acid; PFA-AB with Alcian blue control, pH 0.2; lugol aldehyde fuchsine reaction.
Detection of aminoacids. Tyrosine: Morel-Sisley diazotization reaction. **Tryptophan:** DMAB. **Tyrosine, tryptophan, histidine:** tetrazonium coupled reaction.
Detection of lipids. Sudan black B; acid hematein; Fettrot 7B. **Detection of phospholipids.** Luxol — fast blue.

Ultrathin sections were made with Reichert UOM2 ultramicrotome, contrasted with uranylacetate and lead citrate (Reynolds' solution) and examined in JEM 100 B electron microscope.

RESULTS

During the dissection of the experimental corsac, 43 mature strobilae were found in the jejunum (Plate I, Fig. 1). Some of them could be easily released from the mucosa, but most of them (34) were deeply embedded in the mucosa by their scoleces and even some parts of the proglottid chain, and were firmly attached. The attachment of the cestode in the mucosa of small intestine could be observed at histological

examination. The cestodes were immerged by their scoleces and some proglottids among the villi which were thus pressed (Plate III, Fig. 2). The scoleces were at different depths of the mucosa; sometimes they were localized at the base of villi, i.e., at the level of the opening of Lieberkühn's crypts, other times they were situated directly in the layer of Lieberkühn's crypts (Plate III, Fig. 1). In some cases the scolex was very deep in the mucosa and reached the layer of compact connective tissue, stratum compactum, which is characteristic of the small intestine of Canidae (Plate II, Fig. 1). The villi of intestinal mucosa situated near the parasite were narrow, their epithelium exhibited the signs of atrophy and was lacking in some places (Plate III, Fig. 2). The cellular reaction in the zone where the body of the cestode was in contact with changed villi was usually very slight or even absent. In a more distant vicinity, however, the villi were normal or slightly swallen. The capillaries of their stroma were full of blood and the stroma was more cellular. In some villi, there was a strong inflammatory cellulization in the stroma. There were even places in which the gland opening at the base of villi was dilated in form of a lacuna and partly filled with cells of the exudate. Connective tissue scars were visible in the vicinity. Also in the layer of Lieberkühn's crypts the glands were evidently pressed by the body of the parasite. The lumen of surrounding crypts was narrowed, even slit-like and their epithelium was flattened. The glands disappeared in some places and there remained only dense stroma, the fibrous structures of which ran in parallel with the body of the cestode. This relatively narrow zone of immediate contact passed to a mucosa in which the connective tissue of the propria between the crypts was edematous and with marked inflammatory infiltration (Plate III, Fig. 4). Blood capillaries were here dilated and congested and plasmorrhagia and haemorrhagia was observed. The crypt wall was damaged mechanically in some places, because during the penetration of the large cestode into the mucosa not only a single crypt was dilated, but several neighbouring glands were devastated. Therefore the cells of the exudate were found also in the lumen of the crypts.

In the inflammatory infiltrate prevailed eosinophiles (Plate IV, Fig. 3) which difusely infiltrated the mucosa and markedly accumulated in some sites. Near the deeply embedded scolex they formed a compact layer between the bottom of the crypts and compact layer, which was slightly concave and attenuated at the site were the scolex adhered to it (Plate II, Fig. 1). However, the inflammatory infiltration of the connective tissue between the crypts was observed even in a more distant vicinity, where a larger number of lymphoid cells were also present. The exudate thus contained also lymphocytes and particularly a large number of plasma cells forming small clusters in some zones of villus stroma and between the crypts (Plate IV, Fig. 1). The mast cells, conspicuous mainly after staining for acid mucopolysaccharides and present in deep layers of the mucosa, did not increase in number. The propria contained also a small number of small macrophages, slightly activated fibroblasts, activated endothelium of capillaries and small foci of accumulated histiocytes and connective tissue of hyaline appearance (Plate IV, Fig. 4).

Histochemical reactions revealed certain deviations in the secretory activity of goblet cells of villi and Lieberkühn's crypts in the immediate vicinity of the scolex and strobilae embedded in the mucosa. The reaction to mucus was there weaker and the mucus markedly disappeared (Plate III, Fig. 3). In the reactions for neutral mucopolysaccharides and for aminoacids tyrosine, tryptophan and histidine the fibrilar structures of stroma became more conspicuous in the sites where the epithelium of villi and intestinal glands was damaged and which adhered to the body of the parasite.

Electron microscopical examination of the contact zone between the cestode and host tissue did not provide any surprising results. It confirmed the participance of plasma cells in the infiltration; their endoplasmic reticulum was dilated in form of sacs and parts of cytoplasm with a secretion were separated at the periphery of cells (Plate IV, Fig. 2). There occurred few activated fibroblasts and even spherical or slightly elongate foci of a matter with very fine fibrils arranged in parallel or in whirls (Plate IV, Fig. 5). These foci probably corresponded to hyaline foci in the propria observed already in the light microscope.

DISCUSSION

The pathological changes in the mucosa of intestine and the general unfavourable effect of cestode infection on the host organism are related, in addition to other factors, with the intensity of infection, character of the parasite (mobile or sessile), configuration of its scolex and mode of attachment (Smyth 1969, Vaucher 1971). It is generally known that serious disease of Canidae can be caused by cestodes which usually infect the definitive host in large numbers, as *Echinococcus granulosus* or *Taenia multiceps* (syn. *Multiceps multiceps*) (Joest 1919, Nieberle and Cohrs 1961). It was reported that marked changes occur in the intestine of host infected with *Dipyllidium caninum*, as this parasite penetrates by its well developed rostellum up to the layer of Lieberkühn's crypts (Joest 1919). A massive infection with *Multiceps endothoracicus* is quite possible, because a single infective polycephalic larva can produce usually a large number of these cestodes. The reaction of the infected tissue to the presence of cestode may be various, from a catarrhal inflammation up to necrosis and abscess (Rees 1967). However, as it is generally reported, the cellular reaction is relatively slight even in the species the scolex of which penetrates up to Lieberkühn's crypts (*E. granulosus*). This fact is sometimes explained as a great regeneration ability of intestinal mucosa (Rees 1967).

The pathological changes, which were observed in the mucosa of infected intestine 130 days after experimental infection with *M. endothoracicus*, have the character of a catarrhal inflammation. They are characterized by pressure atrophy of villi and Lieberkühn's crypts and inflammatory infiltration with the participance of eosinophiles and plasma cells in the site of attachment. Remarkable is the localization of scolex in the deepest layers of propria which shows a developed penetration ability of this cestode. This seems to be associated with the shape and functional ability of its rostellum, as it is known in cestodes with a developed and long retractile rostellum also in other hosts than carnivores (Šlais 1961). In such cases the traumatic effect is great, as it was observed (haemorrhagia, devastation of crypts), though a great damage of the mucosa can be induced in some species even by suckers with strong musculature (Vaucher 1971). The markedly extruded rostellum with hooks embedded in Lieberkühn's crypts, as it was described by Smyth (1969) in *E. granulosus* and by Šlais (1961) in *Aploparaksis furcigera* and *Hymenolepis parvula* from ducks, was not found in our experiment. In spite of this we assume that during the penetration into deeper layers of mucosa, the glands, their parts, or even groups of glands were devastated, as it is indicated by the inflammatory exudate in the lumen of preserved crypts and that the armed rostellum participated in the penetration and traumatization of the tissue (Plate I, Fig. 2). However, some of *M. endothoracicus* specimens are only slightly attached and are situated only in superficial layer of mucosa in massive infection, as they could be easily removed from the mucosa or were spontaneously released from the host after its death. This was indicated by the changes in sites of gland openings among the villi which corresponded to posttraumatic reparation

of mucosa by connective tissue. The attachment of cestodes in the superficial layer of mucosa may be only temporary before the penetration into lower layers (may be in another site) and may manifest a competition between the specimens of the same species in massive infection.

In the cestodes, the scolex of which was situated in the deepest layers of mucosa, no marked production of connective tissue or zone of granulation tissue forming a barrier between the parasite and the surrounding tissue was observed around the scolex. On the contrary, a marked presence of eosinophiles and plasma cells on the inflammatory infiltration of the infected mucosa indicates a florid process in the vicinity of insertion of the tapeworm even in the late period of infection and probably also a local irritation of the surrounding tissue to the immune response. Also the fresh haemorrhagia in the propria suggests the activity and mobility of the parasite. Relatively small foci of connective tissue, partly of hyaline appearance, seem to be a residue after an inadequate replacement of destroyed parts of Lieberkühn's crypts, and according to their ultrastructure, they may be of amyloid character. The pressure of the parasite on the adjacent villi and intestinal glands results in the loss of epithelium which is manifested in the decrease or disappearance of their function. It is evidenced particularly by the much lower content of mucin in the goblet cells. The above described changes suggest that in massive infection, the function of the small intestine can be rather significantly affected.

ПАТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ, ВЫЗЫВАЕМЫЕ ЦЕСТОДОЙ *MULTICEPS ENDOTHORACICUS* В КИШКЕ ОКОНЧАТЕЛЬНОГО ХОЗЯИНА

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Резюме. Взрослая цестода *Multiceps endothoracius* проникает в кишке окончательного хозяина (*Vulpes corsac*) в слой либеркюновых крипт, иногда до самого stratum compactum mucosae. Через 130 дней после заражения патологические изменения в месте проникновения стробилы в слизистую характеризованы атрофией давления ворсинок и либеркюновых крипт, рассеянными геморрагиями и дифузной воспалительной инфильтрацией слизистой, более выраженной вблизи сколекса. В воспалительном инфильтрате преобладают эозинофилы, местами встречаются плазматические клетки. Пролиферативные изменения небольшие.

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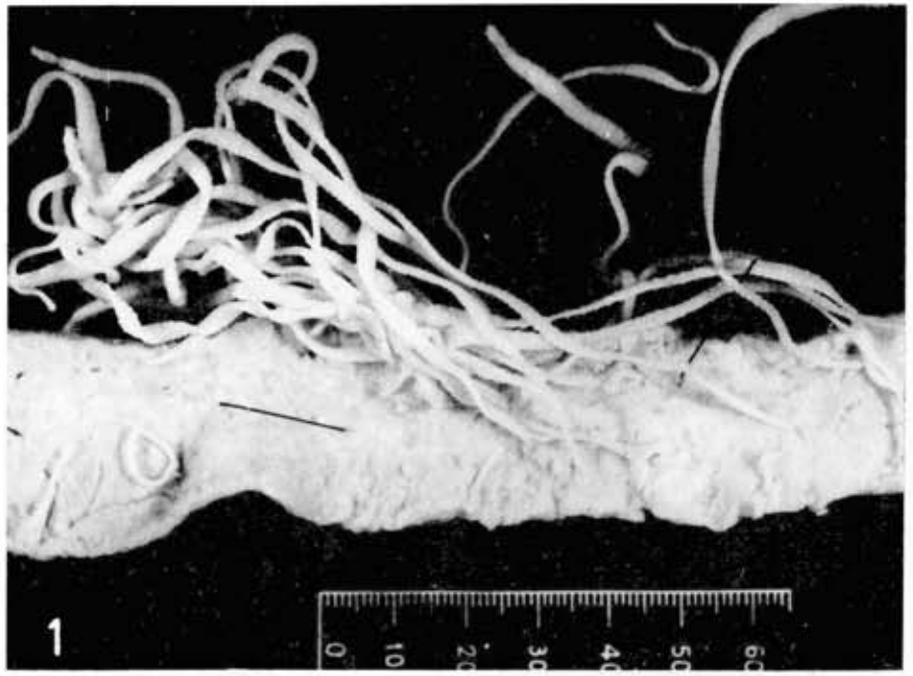

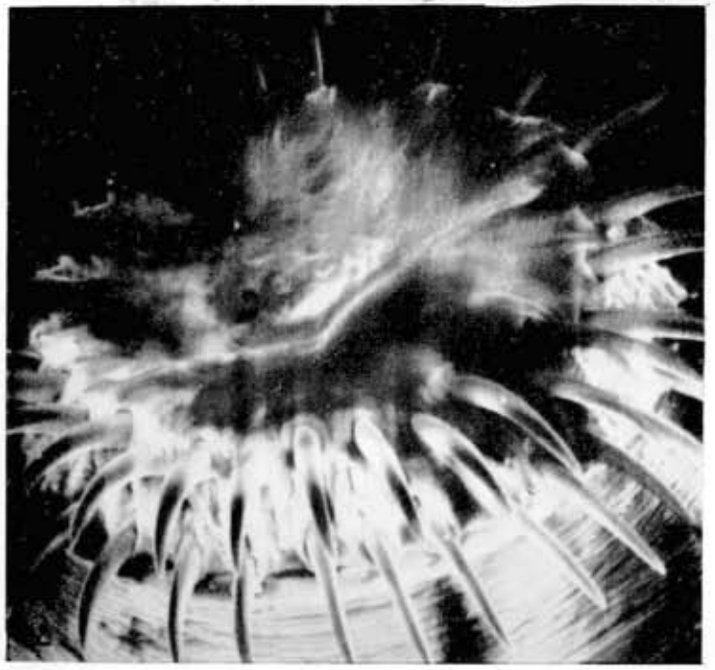

Fig. 1. A part of jejunum with attached specimens of *M. endothoracicus*. **Fig. 2.** Scolex of *M. endothoracicus* with evaginated rostellum (× 1,080).

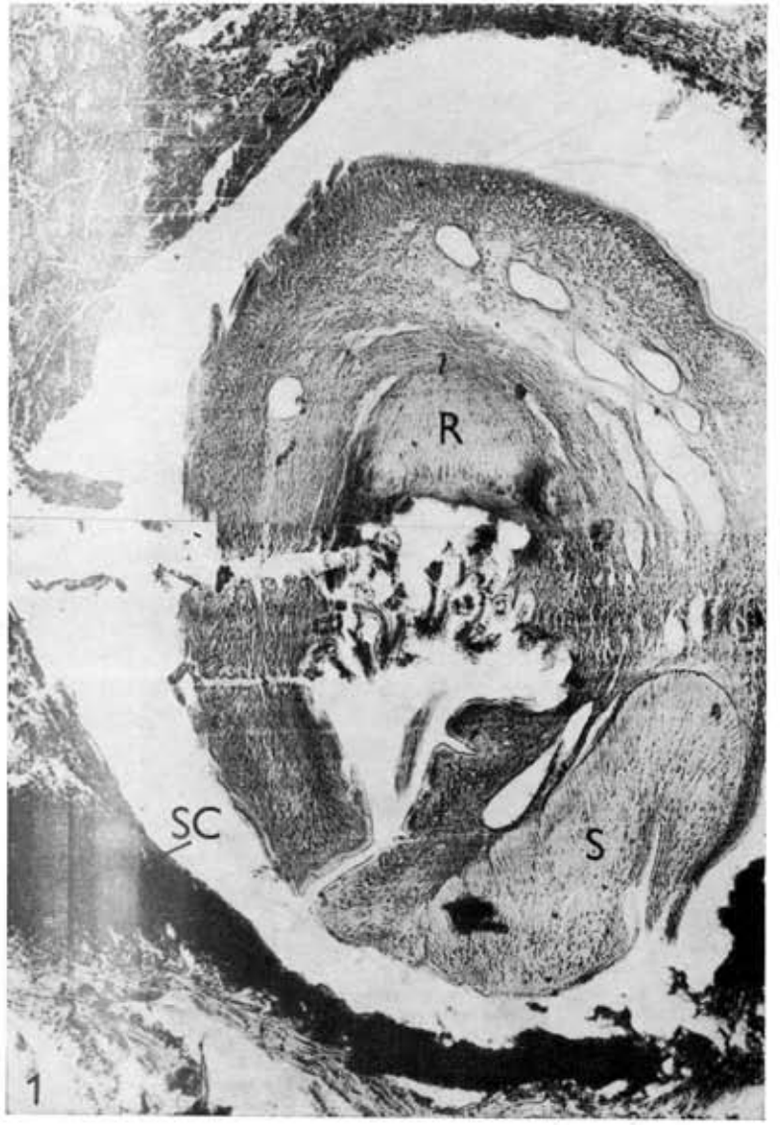

Fig. 1. Scolex of *M. endothoracicus* in deep layers of propria. Connective tissue among Lieberkühn's crypts (CL) with inflammatory infiltration, stratum compactum (SC) markedly attenuated at the site of scolex adherence. Sucker (S), rostellum (R). Van Gieson (× 135).

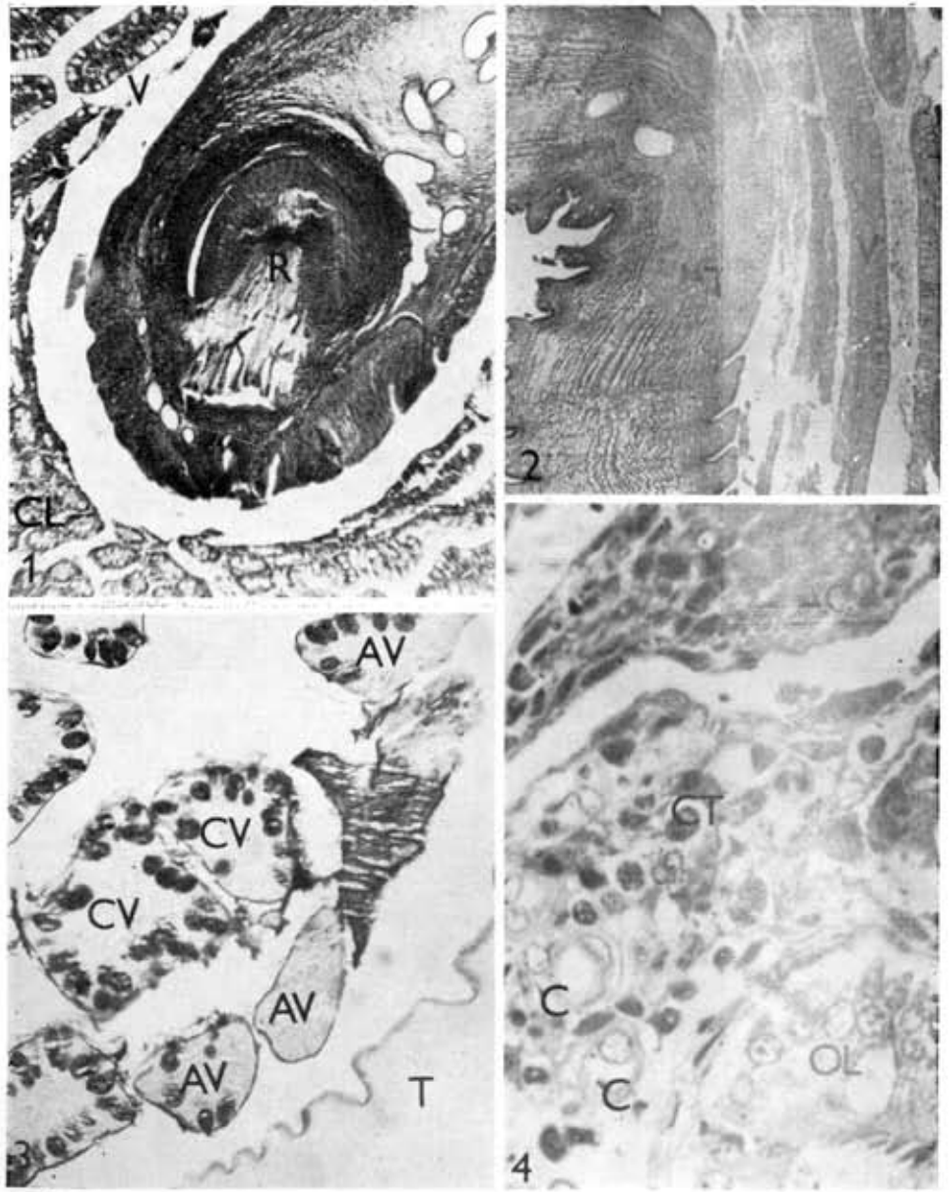

Fig. 1. Oblique section through scolex with invaginated rostellum (R) penetrating through villi (V) into middle parts of the layer of Lieberkühn's crypts (CL). Trichrome after Masson (×7.0). **Fig. 2.** Part of strobila (T) among villi of intestinal mucosa (V). Conspicuous compressive (pressure) atrophy of adjoining villi. Mallory (×55). **Fig. 3.** Transverse section through villi of intestinal mucosa (CV) near strobila (T). Mucus production ceases in atrophied hypo- to nonfunctional villi (AV). Lugol-aldehyde fuchsine reaction (×220). **Fig. 4.** Detail of edema and inflammatory infiltration of connective tissue (CT) among Lieberkühn's crypts (OL). Capillaries (C), residue of atrophied crypt (AC). Semi-thin section, toluidine blue (×660).

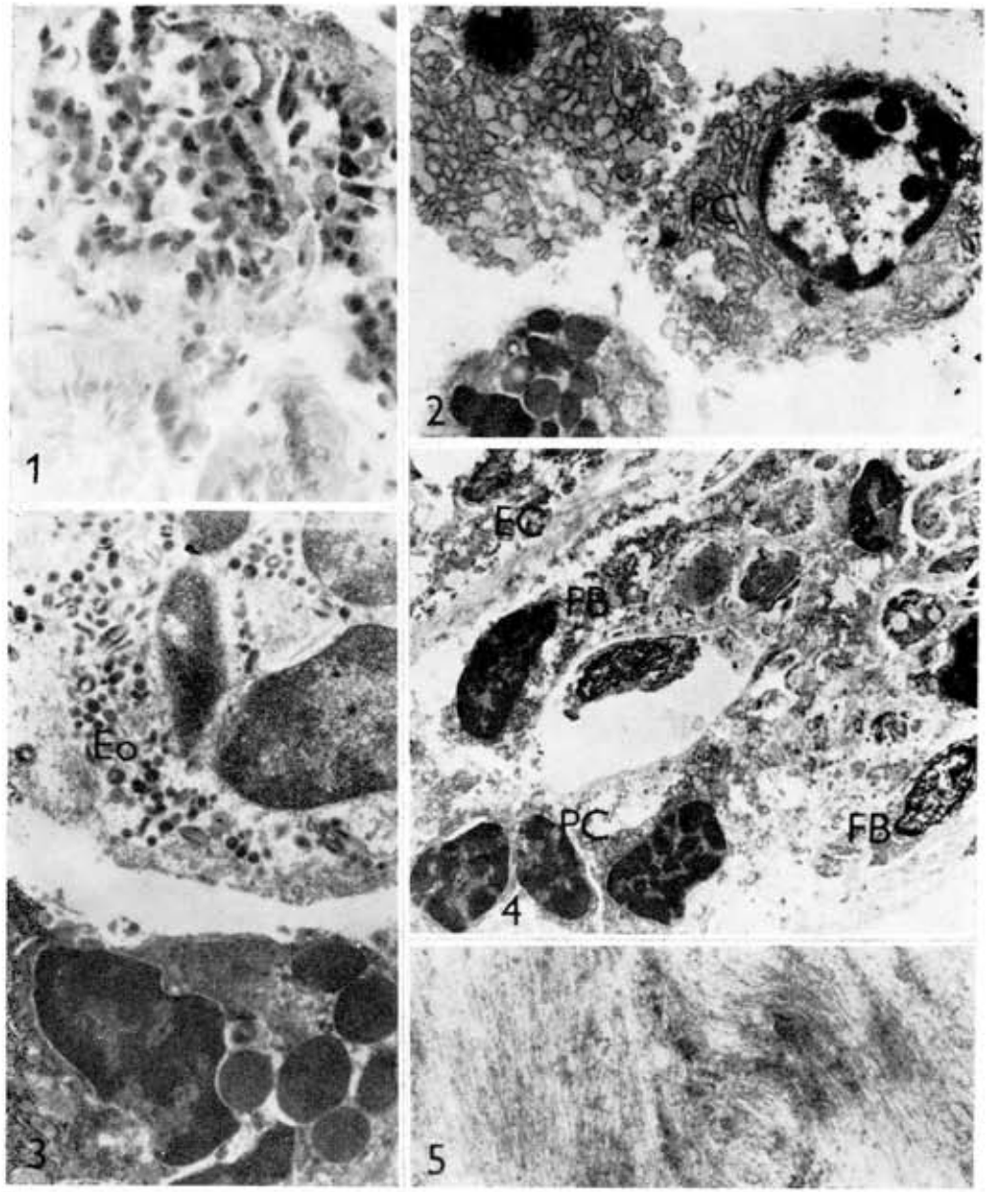

Fig. 1. Cluster of plasma cells in the stroma of villi. Haematoxylin-eosin (×420). **Fig. 2.** Detail of plasma cells (PC) (×7,800). **Fig. 3.** Detail of an eosinophile (EO) from cellular infiltrate of stroma of propria (×11,500). **Fig. 4.** Section through stroma among crypts. Plasma cells (PC), slightly activated fibroblasts (FB), dystrophic cells of epithelium of crypts (EC) (×5,000). **Fig. 5.** Detail of finely fibrillar structure of "hyaline" substances in the stroma of propria (×19,500).