Mucosa-associated lymphoid nodules in the large intestine of young calves: A morphological study with special regard to lymphoepithelial relationship and epithelial ultrastructure

by

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1. INTRODUCTION

In recent years, the role of the common mucosal immune system in the development of local, secretory immunity and in the induction of systemic tolerance has been intensely investigated and discussed. A functionally important part in the mucosal immune system is played by the mucosa associated lymphoid follicles, which have been extensively described in the small intestine. In addition to their beneficial roles as "collectors" of antigens and as "amplifiers" of the immune response (Waksman and Ozer 1976), they may also be the channel of entrance for infection (Owen 1983).

Mucosa associated lymphoid follicles in the large intestine were described in many animal species (Carlens 1928; Biswal et al. 1953; Rooney 1956; Grau and Walter 1958; Hebel 1960; Schofield and Cahill 1969; Atkins and Schofield 1972; Scott 1979; Nickel et al. 1982; Bland and Britton 1984) and in humans (Kealy 1976a; Burbige and Sobky 1977; Laufer and DeSa 1978; Kelvin et al. 1979; Kennedy et al. 1982; Watanabe et al. 1983). Their participation in the common mucosal immune system, their relationship to the local mucosal immune system in the small intestine and their role in the pathogenesis of diseases has not yet been well established.

In the bovine species, aggregated lymphoid tissue (ALT) is present as solitary lymphoid follicles throughout the large intestine. In addition patches are seen at the ileocecal entrance (ICE) and in the proximal colon (Carlens 1928; Rooney 1956; Grau and Walter 1958; Hebel

1960; Nickel et al. 1982). Histologically the lymphoid follicles form units, which were termed lymphoglandular complexes (Bautzmann 1948). These complexes are characterized by epithelial diverticula, which extend into lymphoid follicles in the submucosa.

In his original description of pathological alterations in bovine virus diarrhea (BVD), Ramsey (1956) reports severe typhlitis, colitis and proctitis in cattle. Typically focal, chronic, cystic colitis occurs 10 to 20 cm distal to the ileocecal entrance. This is the same site, where the colonic lymphoid patch in the proximal colon of cattle is located. In this segment of colon, cysts of 1 - 4 mm diameter are present in the submucosa and the epithelium is ulcerated. Colitis with lesions restricted to this area has also been reported in calves infected with Cryptosporidium sp. (Pohlenz et al. 1984b). In pigs changes in the discrete lymphoglandular complexes were seen in treponema infection (Ferguson et al. 1980), niacin deficiency (Dunne et al. 1949), salmonellosis (Lawson and Dow 1966) and diarrhea and runting of unknown etiology (Williams and Bertschinger 1974). In humans lymphofollicular hyperplasia and cystic colitis were described in association with neoplastic and inflammatory diseases of the large intestine (Clark 1970; Robinson et al. 1973; Dyson 1975; Kealy 1976b; Kaplan et al. 1984). Early ulcerative lesions in Crohn's disease were reported to affect selectively the epithelium overlying the lymphoid follicles in the large intestine (Morson 1972; Rickert and Carter 1980). The functional significance of the mucosa associated lymphoid follicles in the large intestine and their role in the pathogenesis of different diseases has

not yet been well defined.

Observations that B- and T-cells primed in the mucosa associated lymphoid follicles of the gastrointestinal tract (GI-tract) migrate to the lamina propria of other mucosal surfaces has lead to the concept of a common mucosal immune system (CMIS) involving the alimentary tract, the respiratory tract, the urogenital tract, the mammary gland, the salivary glands and the lacrimal glands (Bienenstock 1974; Bienenstock et al. 1978, 1979; McDermott and Bienenstock 1979; Bienenstock and Befus 1980; Befus and Bienenstock 1982). The follicle associated epithelium (FAE) of the subepithelial lymphoid follicles in these organs constitute adaptations for the transport of luminal antigen across the epithelial barrier (Bockman and Cooper 1973; Owen and Jones 1974a; Owen 1977). Special uptake and transport of macromolecules through follicle associated epithelium in the gastrointestinal tract has been described for tonsils, Peyer's patches, appendix, sacculus rotundus, cecal tonsils, colonic lymphoid patches and bursa of Fabricius (Bockman and Cooper 1973; Fournier et al. 1977; Owen 1977; Joel et al. 1978; Naukkarinen et al. 1978; Sachs et al. 1979; v. Rosen et al. 1981; Neutra et al. 1982; Beezhold et al. 1983; Bland and Britton 1984; Rosner and Keren 1984). These lymphoid organs share characteristic, morphological features:

1) close association between the epithelium and the underlying lymphoid tissue;
2) high numbers of intraepithelial cells;
3) specialized epithelial cells (M cells) in the follicle

associated epithelium (FAE).

M cells were first described in the epithelium overlying the small intestinal domes of the Peyer's patches of humans (Owen and Jones 1974a). Later they were also found in the follicle associated epithelium of the tonsils and the appendix (Owen and Nemanic 1978). In the large intestine, M cells have only been reported in the FAE of the colonic lymphoid patches in rats (Bland and Britton 1984).

The objectives of this study are:

1) to determine the distribution and to measure the amount of mucosa associated lymphoid follicles in the large intestine of calves;

2) to investigate their morphology and to establish the spatial relationship between lymphoid follicle and epithelium;

3) to describe the ultrastructural characteristics of the follicle associated epithelium.

The investigation of these objectives serves several purposes. Data for the distribution are urgently needed to find easily and consistently the sites where mucosa associated lymphoid tissue is present, and to facilitate collection of these tissues for diagnostic purposes. The description of the normal anatomy and histology of the aggregated lymphoid tissue (ALT) in the large intestine of calves will be used as control for comparative studies of colonic lesions in bovine virus diarrhea virus, Cryptosporidium sp., astrovirus and Breda virus infected animals. Finally structural analogies between mucosa associated lymphoid follicles in the large intestine and other sites in the gastrointestinal tract will be compared. Analogies would indicate

an immunological aspect of the colon apart from its mere reabsorptive function.

2. LITERATURE REVIEW

2.1. The Mucosa Associated Lymphoid System

Single lymphoid follicles or aggregates of lymphoid nodules are present in the mucous membranes of the alimentary tract and salivary glands, the conjunctiva, the respiratory tract and the urogenital tract of mammals and birds (Egberts et al. 1985). Many questions concerning the immunological role of this local mucosal immune system and its relation to the systemic immune system are unanswered. The current concept of the humoral component of the local immune system and its participation in the common mucosal immune system will be discussed using the gastrointestinal tract (GI-tract) as an example (Figures 1 and 2).

The mucosal surface of the gastrointestinal tract is continuously exposed to a multitude of different antigens. These can be classified as food antigens, endogenous flora, and pathogenic organisms (viruses, chlamydia, bacteria, fungi and parasites) and their products. The host is protected against these antigens by the following barriers, which help exclude most of the antigens and prevent thereby detrimental inflammatory reactions at the mucosal site (Mouwen et al. 1983; Egberts et al. 1984, 1985):

1) the mucus layer;
2) the epithelial layer with glycocalix, tight junctions, lysosomes and basal lamina;
3) the GALT consisting of mucosa associated lymphoid follicles, intraepithelial cells, diffuse lymphoid tissue in the lamina

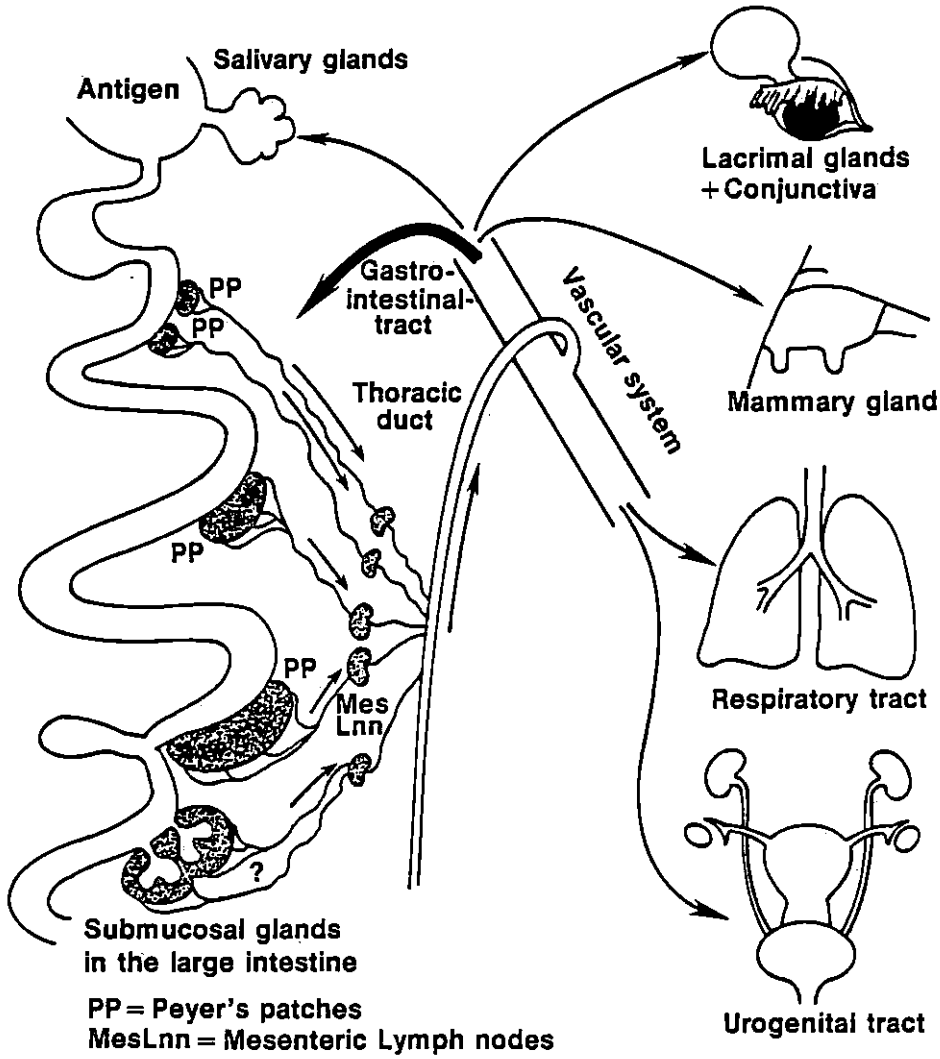

Figure 1. Antigen uptake in the gastrointestinal tract and immune response in the common mucosal immune system (modified from Owen 1977 and Woloschak and Tomasi 1983)

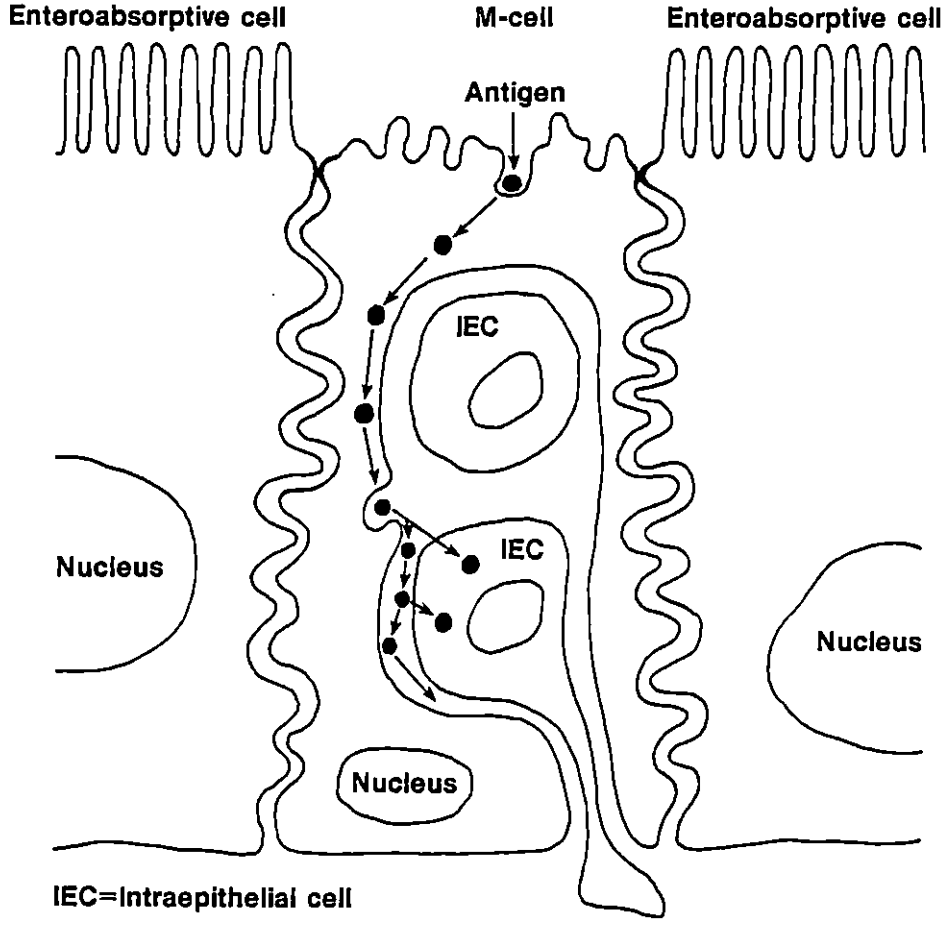

Figure 2. Antigen uptake and transport by the M cell (modified from Owen 1977)

propria and mesenteric lymph nodes.

To develop immunity and/or tolerance, antigen has to be presented to the immune system. Therefore uptake of antigenetic material has to be guarantied. To maintain its antigenicity, the antigen must not be digested. It has to retain a certain molecular weight (Tizard 1982) and structural uniqueness during the transfer through the epithelium.

The modus and capability of antigen uptake differs between newborns and adults.

In newborn animals large amounts of heterologous macromolecules can be absorbed without destruction for 24 to 48 hours after birth in those species, which have no transfer of antibodies through the placenta (Staley et al. 1972; Watson and Husband 1977; Banks 1982).

In adults two different routes for antigen uptake exist. The epithelial cells of the small intestine can phagocytose small, but immunologically significant amounts of macromolecular antigens (Walker 1981). Most of these antigens are digested within phagolysosomes in the enterocytes. A few, however, permeate the cell intact and are exocytosed at the base of the cell. They are presented to lymphocytes and macrophages in the epithelium or in the lamina propria in connection with Ia antigens, which were demonstrated at the basal and lateral side of enterocytes (Curman et al. 1979; Parr and McKenzie 1979; Mayrhofer et al. 1983).

A second way for uptake of macromolecules is through specialized cells (M cells) in the follicle associated epithelium. Walker (1981) hypothesizes that the chosen route depends on the concentration of the

antigen in the intestinal lumen. He suggests that antigen present in "physiological" or low concentration takes preferentially the way over M cells, while the general absorption through enteroabsorptive cells increases with increasing concentration of the antigen.

Preferential uptake and transport by follicle associated epithelium was shown for several macromolecular tracers for viruses, for chlamydia, for non invasive bacteria and for protozoa (Wolf and Bye 1984; Egberts et al. 1985; Marcial and Madara 1986). Tracer particles were released from the M cells into the intercellular spaces between M cells and intraepithelial cells and later found in IEC and in the germinal centers of lymphoid follicles (Owen 1977; v. Rosen et al. 1981).

The low number of lysosomes in M cells appears to favor the transit of nondestructed antigen which retains its antigenicity (Owen et al. 1981). Antigen presentation by M cells is, however, unlikely, because they do not express Ia determinants on their surface (Mayrhofer et al. 1983).

After leaving the M cell, antigen is transported partly free as particulate matter and partly by intraepithelial cells (IECs) to the germinal centers of the lymphoid follicles. T-lymphocytes, B-lymphocytes, macrophages, mucosal mast cells, plasma cells, and neutrophils are present as IECs in the intestinal epithelium. T-lymphocytes, which occasionally contain granules were shown to be the predominant cell type (Guy-Grand et al. 1978; Ermak and Owen 1985). It has been suggested, that some of these T-lymphocytes are precursors of the mucosal mast cells (Guy-Grand and Vassalli 1982; Ernst et al. 1985).

Mucosal mast cells have different staining properties than dermal mast cells (Wingren and Enerbaeck 1983) and their role in the immunological processes in the intestinal mucosa has not yet been established (Heatley 1983).

All cell types mentioned above have also been found in contact with the follicle associated epithelium (FAE). There is, however, controversy about their relative frequency. Roy et al. (1985) demonstrated with monoclonal antibodies, that T-lymphocytes are the most frequent cell type in the lymphoepithelium of rabbit Peyer's patches, sacculus rotundus, cecal patch and appendix. Immunohistochemical studies by Ermak and Owen (1985) showed that the follicle associated epithelium of mouse Peyer's patches contains predominantly B-lymphocytes. Another important intraepithelial cell in the follicle associated epithelium is the Ia positive macrophage (Owen 1982; Ermak and Owen 1985). LeFebre et al. (1979) coined the term intestinal macrophages or histiocytes for these cells and point out their role in both phagocytosis, transport and presentation of antigen in the Peyer's patches and in recirculation of antigen back into the intestinal lumen.

Antigens which reach the germinal centers of mucosa associated lymphoid nodules and are adequately presented, cause B-lymphocytes to differentiate and proliferate. The resulting immune response depends on kind, amount, duration of exposure and way of introduction of the antigen (Bourne and Newby 1981) as well as on the immune status of the host (Mayrhofer 1984). Antigen presented after being processed by macrophages is likely to be immunogenic, while soluble antigen reaching

suppressor T-cells directly is likely to be tolerogenic (Mayrhofer 1984).

Local, secretory immunity (IgA) without systemic antibody response (IgG) develops by the interaction of helper and suppressor T-cells (Richman et al. 1981). Two theories explaining the selective production of IgA antibodies by B-lymphocytes of the Peyer's patches are currently discussed:

1) Strober (1982) concludes that orally introduced antigen sensitizes T-switch cells in the Peyer's patches, which change the original population of IgM-producing lymphocytes to IgA-producing cells.
2) According to Gearhart et al. (1980), the chronic antigenic stimulation in the Peyer's patches may lead to multiple antigen-driven cell divisions with successive switches of immunoglobulin classes. This may result in an increased expression of 3' heavy chain DNA sites.

A systemic immune response (IgG) and allergic reactions (IgE) are in most cases prevented by the activation of antigen and idiotype specific suppressor T-cells and serum factors, which suppress IgM, IgG and IgE-producing B-lymphocytes (Thomas and Parrot 1974; Ngan and Kind 1978; Kagnoff 1978a,b; Benacerraf and Germain 1981; Richman et al. 1981; Kawanishi et al. 1982).

B-lymphocytes in the germinal centers of the gut associated lymphoid follicles proliferate and differentiate to IgA bearing and producing cells, but they do not immediately develop to antibody

secreting plasma cells (Woloschak and Tomasi 1983). Strober (1982) speculates that this is due to a lack of specific helper T-cells in the germinal centers.

The primed B-lymphocytes leave the Peyer's patches and migrate via afferent lymphatics to the mesenteric lymph nodes, where they undergo additional maturation. From the mesenteric lymph nodes, they enter via lymphatics the thoracic duct (Craig and Cebra 1971) or directly the vascular system (Benell and Husband 1981a,b). Experiments using tritiated thymidine and deoxycytidine as markers (Pabst and Trepel 1979) demonstrated that the large lymphocytes in the thoracic duct of pigs originate predominantly from the Peyer's patches and mesenteric lymph nodes and that they leave the vascular system to localize in the lamina propria of the small intestine and other organ systems (Craig and Cebra 1971).

The localization of the primed B-lymphocyte in the lamina propria of different organs shows a particular pattern, termed "homing." Most cells home selectively back to the lamina propria of their tissue of origin. This is, in the example of the Peyer's patches, the intestinal mucosa (Gowans and Knight 1964; Smith et al. 1980). A few, however, localize in the lamina propria of the respiratory tract (Rudzik et al. 1975; McDermott and Bienenstock 1979), the mammary gland (Goldblum et al. 1975; Roux et al. 1977; Weisz-Carrington et al. 1978, 1979), the urogenital tract (McDermott and Bienenstock 1979), the salivary glands (Weisz-Carrington et al. 1979) and the lacrimal glands (Weisz-Carrington 1979). This interconnection of different organs led to the concept of a

common mucosal immune system (Bienenstock 1974; Bienenstock et al. 1978, 1979; McDermott and Bienenstock 1979; Bienenstock and Befus 1980; Befus and Bienenstock 1982).

Husband and Gowans (1978) concluded that the localization of IgA precursors in the lamina propria is antigen independent (Halstead and Hall 1972; Parrot and Ferguson 1974), but that antigen influences the exact localization and the intensity of the immune response. The actual diapedesis of lymphocytes from the blood vessel to the lamina propria appears to have a multifactorial regulation (Bienenstock et al. 1983), involving lymphocyte characteristics, vascular specialization and mucosa derived factors (Table 1).

The primed B-lymphocytes develop in the lamina propria to IgA-secreting plasma cells. This occurs under the influence of helper T-cells (Lamm 1976; Woloschak and Tomasi 1983). Most plasma cells secrete dimeric IgA (Lamm 1976; McClelland 1976), which is selectively transferred into the intestinal lumen. IgA, which is relatively resistant against enzymatic degradation, is virus and toxin neutralizing and does not bind complement. These reactions result in immune exclusion, which implies decreased antigen contact and decreased antigen uptake (Befus and Bienenstock 1982), and is important in the prevention of food allergies (Bienenstock and Befus 1983).

In adult ruminants, IgA and IgG1 are secreted in nearly equal proportion into the intestinal lumen (Newby and Bourne 1976; Morgan et al. 1980). IgG1 originates from the serum and is also produced locally in the gut wall (Morgan et al. 1980). IgG1 has similar functional

Table 1. Potential causes for "homing" of lymphocytes primed in mucosa associated lymphoid follicles

LYMPHOCYTE CHARACTERISTICS
• organ derivation (Pierce and Cray 1982)
• protein or carbohydrate components of the plasma membrane of the lymphocyte (Butcher et al. 1980; De Sousa 1981)
• immunoglobulin on the surface (Lamm 1976; Cebra et al. 1980)
• secretory component (McWilliams et al. 1975)
• receptors for antigen on the surface
• receptors for hormones (Weisz-Carrington et al. 1978; McDermott and Bienenstock 1979; Bienenstock et al. 1983)
• receptors for chemotactic factors (Parrot 1981), e.g., metabolites of arachidonic acid (McConnel et al. 1980; De Sousa 1981)
• receptors for products of T helper cells or for the formation of histocompatibility complexes (T-IMF) (Curtis and Davies 1980)
CHARACTERISTICS OF THE VASCULAR SYSTEM OR THE MUCOSA
• perfusion (Ottoway and Parrot 1980)
• specialized blood vessels ("high endothelial venules") with endothelial cells, which have special surface determinants or secrete mediators (Cahill et al. 1977; McDermott and Bienenstock 1979; Butcher et al. 1980; Ottoway and Parrot 1980; Carey et al. 1981; Stevens et al. 1982)
• hormones (Roux et al. 1977; Weisz-Carrington et al. 1978; Bienenstock et al. 1979)
• iron (De Sousa 1981)

properties as IgA. It does, however, fix complement in the bovine species (Morgan et al. 1980).

2.2. Morphological Characteristics of Follicle Associated Epithelium (FAE) with Special Emphasis on M Cells

The epithelium overlying the domes above mucosa associated lymphoid follicles and lining the epithelial diverticula in lymphoid follicles is called follicle associated epithelium (FAE). It is composed of highly specialized epithelial cells (the M cells) and enteroabsorptive cells. The M cells are known to transport antigen and macromolecules after the intestinal closure (Bockman and Cooper 1973; Owen 1977; v. Rosen 1981). The function of enteroabsorptive cells within the FAE is currently incompletely understood. Goblet cells are rare or not present in the epithelium covering the mucosa associated lymphoid follicles.

2.2.1. Characteristics of enteroabsorptive cells in follicle associated epithelium

Enteroabsorptive cells on domes of Peyer's patches resemble morphologically those on adjacent villi. They are renewed in the same way as enterocytes with a time course similar to that determined for surrounding villi (Smith et al. 1980). In the rat, they differ, however, in their ability to absorb valine and in the expression of enzyme activities. The reduction in absorptive capacity for valine, which is characteristic for fully differentiated enterocytes, suggests, that either factors initiating differentiation in villus cores are missing or that the close proximity to lymphocytes inhibits the process

of cell differentiation (Smith and Syme 1982). Decreased lactase and glucosidase activities, but increased alkaline phosphatase activities were demonstrated in enterocytes within FAE by Smith in 1985. As similar results were also seen in T-cell deficient, athymic mice; it was concluded that T-cells do not appear to play a major role in these selective changes. Instead factors released during antigen sampling (e.g. by macrophages) may be the cause for the different maturation process (Smith 1985).

2.2.2. Characteristics of M cells in follicle associated epithelium

2.2.2.1. Location and origin The phenomenon of antigen uptake into mucosa associated lymphoid follicles has been known for a long time (Kumagai 1922, cited in Wolf and Bye 1984) and morphologically different cells with special absorptive capacity had already been described in the 1960s (Schmedtje 1965, 1966, Shimizu and Andrew 1967, Bockman and Cooper 1973). A detailed description and functional interpretation of these specialized cells was, however, first given by Owen and Jones (1974a) for the FAE of Peyer's patches in humans and rats. The name M cell was chosen because of characteristic microfolds observed on the surface of these cells in humans. Currently the initial "M" stands for the membranous appearence of M cells due to invaginating mononuclear cells.

Since the original description morphologically and functionally similar cells were also identified in:

- tonsils of rabbits, mice, dogs, hamsters, monkeys and humans (Owen and Nemanic 1978);

- Peyer's patches of mice (Bockman and Cooper 1973; Bockman and Stevens 1977; Owen 1977; Owen and Nemanic 1978; Smith and Peacock 1980; Smith et al. 1980; v. Rosen et al. 1981; Wolf et al. 1981, 1983; Bhalla and Owen 1982; Owen and Bhalla 1983), calves (Landsverk 1979, 1981a,b; Torres-Medina 1981, 1984; Woode et al. 1984; Liebler 1985), pigs (Chu et al. 1979, 1982; Torres-Medina 1981), rats (Owen and Nemanic 1978; Owen et al. 1981; v. Rosen et al. 1981; Smith and Syme 1982; Owen and Bhalla 1983), hamsters (Owen and Nemanic 1978), rabbits (Owen et al. 1982; Inman and Cantey 1983), dogs (Owen and Nemanic 1978), monkeys (Owen and Jones 1974b; Owen and Nemanic 1978), humans (Owen and Jones 1974a,b; Owen and Nemanic 1978), chickens (Befus et al. 1980; Burns 1982);
- isolated lymphoid follicles in the intestine of guinea pigs (Rosner and Keren 1984);
- cecal tonsils of mice (Owen and Nemanic 1978; Bhalla and Owen 1982) and rats (Owen and Nemanic 1978);
- appendix of humans (Bockman and Cooper 1975; Owen and Nemanic 1978) and rabbits (Schmedtje 1965, 1966; Bockman and Cooper 1973; Olah and Everett 1975; Bockman and Stevens 1977; Owen and Nemanic 1978; Bockman and Boydston 1982);
- colonic lymphoid patches of rats (Bland and Britton 1984);
- bursa of Fabricius of chickens (Bockman and Cooper 1973; Bockman and Stevens 1977; Naukkarinen et al. 1978);
- bronchus associated lymphoid tissue (BALT) of rabbits and rats

(Bienenstock and Johnston 1976; Fournier et al. 1977; Racz et al. 1977).

In a recent review, Egberts et al. (1985) postulate that "M cells are present in the lamina epithelialis in every region where it overlies accumulations of lymphoid tissue in the intestine." The finding of these specialized cells in the BALT may even extend this statement (Bienenstock and Johnston 1976; Fournier et al. 1977; Racz et al. 1977).

The relative frequency of enterocytes and M cells in FAE varies between animal species, locations of the lymphoid follicles, and levels on the dome surface.

Most animal species have only a few M cells interspersed in the dome epithelium (Bockman and Cooper 1973; Owen and Jones 1974a,b; Bockman and Cooper 1975; Owen 1977; Owen and Nemanic 1978; Chu et al. 1979, 1982; Befus et al. 1980; Smith and Peacock 1980; v. Rosen et al. 1981; Bhalla and Owen 1982; Bockman and Boydston 1982; Burns 1982; Owen and Bhalla 1983; Rosner and Keren 1984). The frequency of M cells in Peyer's patch FAE of monkeys is higher (Owen and Nemanic 1978) and ileal domes of newborn calves are predominantly (Landsverk 1981a; Liebler 1985) or exclusively covered by M cells (Torres-Medina 1981, 1984).

In young calves, the cell composition shows quantitative differences between jejunal and ileal domes. In the upper jejunum the enteroabsorptive cells are predominant and in the ileum the M cells are predominant (Liebler 1985).

Bye et al. (1984) find M cells on all levels of the dome surface of mice. In contrast, investigations by Smith and Peacock (1980) showed

that there are no M cells in the follicle associated crypts and fewer M cells at the base than at the tip of the dome.

Currently three different opinions about the origin of M cells exist:

1) Smith et al. (1980) conclude that they originate from fully differentiated enterocytes.
2) According to Bye et al. (1984), M cells may originate from immature crypt cells or differentiated enterocytes. The presence of immature and mature M cells on all levels of the dome suggests that M cells are formed on all regions of the dome (Bye et al. 1983).
3) Autoradiographic studies by Bhalla and Owen (1982) indicate that M cells arise from cuboidal cells which have lost the potential for further cell division and are partly differentiated.

Regardless of these different hypotheses about the origin of the M cell, there is general agreement (Smith et al. 1980; Bhalla and Owen 1982; Bye et al. 1983, 1984), that M cells develop due to the close association with monocytic cells or their products. A recent publication by Sicinski et al. (1986), however, denies this on the basis of morphometric evaluations. These demonstrate that mice depleted of IECs have the same number of M cells as control mice, no intermediate forms between M cells and enterocytes exist (contradiction to Bye et al. (1984)) and no correlation between the length of microvilli and the number of IECs in contact with M cells exists.

2.2.2.2. Morphological features M cells have been described in 1 um thin sections as pale cells, which have an indistinct brush border and are indented by intrusive cells (Owen 1977). A conclusive identification of these cells requires, however, electron microscopy (Wolf and Bye 1984).

Main characteristics distinguishing M cells from enterocytes are the morphology and the biochemical features of their apical membrane, which are of major importance for functional properties.

The glycocalix of M cells in most animal species, with the exception of the calf (Landsverk 1981a), is weakly developed (Owen and Jones 1974a; Owen 1977; v. Rosen et al. 1981; Inman and Cantey 1983; Egberts et al. 1984). Distribution of anionic sites (found by binding of cationic ferritin) and lectin-binding sites are equal on enterocytes and M cells (Neutra et al. 1982; Owen and Bhalla 1983; Madara et al. 1984). The latter indicates, that M cells and enterocytes share common glycoconjugates on the surface. Observations on freeze fracture replicas revealed, in mature and immature M cells of mouse Peyer's patches, lower numbers of intramembrane particles, which corresponds with reduced protein contents of the cell membrane, and an abundance of cholesterol (Bye et al. 1983; Madara et al. 1984). No cholesterol was present in the membranes of the intracellular vesicles, indicating that the uptake of material from the lumen occurs on cholesterol-free stretches of the membrane (Madara et al. 1984).

Histochemical studies on the enzyme activity of M cells showed reduced activity for alkaline phosphatase (Schmedtje 1965; Landsverk

1981a; Owen and Bhalla 1982, 1983), ATPase and aminopeptidase (Landsverk 1981a) and increased activity for esterase (Schmedtje 1965; Owen and Bhalla 1982, 1983).

The morphological appearance of the apical membrane protrusions varies between animal species. Short microvilli with irregular numbers, diameter, and length were described in mice (Bockman and Cooper 1973; Owen 1977; Abe and Ito 1978; Smith and Peacock 1980; Bhalla and Owen 1982; Owen and Bhalla 1983; Bye et al. 1984; Madara et al. 1984), rats (Owen and Bhalla 1983; Madara et al. 1984), guinea pigs (Madara et al. 1984; Rosner and Keren 1984), and calves (Torres-Medina 1981). Long microvilli have been reported in rabbits (Inman and Cantey 1983) and piglets (Sachs et al. 1979; Chu et al. 1979, 1982), and microfolds in humans (Owen and Jones 1974a,b). According to Landsverk (1981a) and Liebler (1985), both microvilli and microfolds are present in bovine ileal M cells.

Differences in microvillus development from cell to cell in the FAE of mouse Peyer's patches were interpreted as variations dependent on M cell maturation (Bye et al. 1984).

There are few or no rootlets of actin filaments in the microvilli of M cells. This reduces the potential for active contraction. The terminal web is poorly defined (Owen and Jones 1974a; Owen 1977; v. Rosen et al. 1981; Bhalla and Owen 1982).

M cells form apical junctional complexes with adjacent M cells and enterocytes. A statistical evaluation found increased numbers of longer tight junctions on domes of mouse Peyer's patches. Their numbers were

correlated to M cell numbers, the exact cell types participating were, however, not identified (Madara et al. 1984).

The cytoplasm of M cells is electron lucent. The area of the terminal web is devoid of organelles, but contains numerous vesicles and tubules (Bockman and Cooper 1973, 1975; Owen and Jones 1974a,b; Owen 1977; Befus et al. 1980; Landsverk 1981a; v. Rosen et al. 1981; Rosner and Keren 1984). M cells and enterocytes have similar numbers and types of cellular organelles (Wolf and Bye 1984). The number of lysosomes is, however, decreased in M cells (Landsverk 1981a; Owen et al. 1981). The nucleus of the M cell is basally located (Owen and Jones 1974a; v. Rosen et al. 1981).

In the colon, M cells have only been found in the epithelium overlying the colonic lymphoid patches in the rat. Bland and Britton (1984) identified two morphologically distinct types of M cells based on their special absorptive capacity for India ink. These M cells have a normally developed glycocalix, brush border and terminal web (Bland and Britton 1984). Further ultrastructural characteristics of M cells in large intestinal lymphoid aggregates will be discussed in this literature review part 2.3.2.

Another important feature of M cells to which the term membranous cell refers, is their close association with intraepithelial cells (IECs). Often several of these invaginate one M cell and occupy a large cytoplasmic vacuole (Schmedtje 1965). Only a thin apical bridge of attenuated cytoplasm separates them from the intestinal lumen (Bockman and Cooper 1973; Chu et al. 1979; v. Rosen et al. 1981). This may cause

a reticulate or pseudostratified appearence of the epithelium. Most of the IECs are lymphocytes (Bockman and Cooper 1973, 1975; Owen and Jones 1974a; Owen 1977; Landsverk 1981a; v. Rosen et al. 1981), lymphoblasts (Owen and Jones 1974a; Bhalla and Owen 1982), and macrophages (Abe and Ito 1978; Bockman and Boydston 1982; Owen 1982; Owen et al. 1982, 1983). Occasionally plasma cells (Abe and Ito 1978; Bhalla and Owen 1982) and rarely neutrophils (Inman and Cantey 1983) are present.

IECs are distributed in the FAE of mouse Peyer's patches in a non random fashion, because they cluster in M cells (Rowinski et al. 1985). In the dome epithelium overlying the rabbit appendix variations in number and localization of IECs dependent on their site on the dome surface were described (Schmedtje 1980). The base of the dome is free of IECs. Above this zone a few randomly arranged, basally positioned IECs were seen, in the upper part of the FAE large clusters of IECs were found in M cells and the tip was largely free of IECs.

M cells like other epithelial cells rest upon a basal lamina, which can be discontinuous (Befus et al. 1980) or undetectable (Bockman and Boydston 1982) due to transition of IECs.

These described morphological features, especially the specialized apical membrane, the reduced number of lysosomes, and the close contact to IECs, are the basis for a common functional characteristic of mature M cells. They have the ability of increased or exclusive uptake and transport of macromolecules and microorganisms.

The following macromolecules and agents have been reported to be transported by M cells:

- native ferritin (Bockman and Cooper 1973; Fournier et al. 1977; Beezhold et al. 1983; Rosner and Keren 1984);
- cationized ferritin (Neutra et al. 1982);
- carbon particles (Bockman and Cooper 1973; Joel et al. 1978; Naukkarinen et al. 1978; Sachs et al. 1979; Beezhold et al. 1983; Bland and Britton 1984; Rosner and Keren 1984);
- latex particles (LeFebre et al. 1978; Beezhold et al. 1983);
- horse radish peroxidase (Owen 1977; v. Rosen et al. 1981; Beezhold et al. 1983);
- ricin (Neutra et al. 1982);
- wheat germ agglutinin (Neutra et al. 1982);
- cholera toxin (Shaklamov et al. 1981);
- reovirus type 1 (Wolf et al. 1981, 1983);
- reovirus type 3 (Wolf et al. 1981);
- poliovirus (Bodian 1955);
- rotavirus (Landsverk 1981b; Torres-Medina 1984);
- Breda virus (Pohlenz et al. 1984a);
- astrovirus (Woode et al. 1984);
- TGE virus (Chu et al. 1982);
- adenovirus-like particles (Chu et al. 1982);
- chlamydia (Landsverk 1981b);
- Vibrio cholerae (Owen et al. 1982, 1983);
- Mycobacterium (Myrvik et al. 1979);

- Cryptosporidium sp. (Marcial and Madara 1986).

Earlier adhesion of Escherichia coli (RDC1) to M cells than to enteroabsorptive cells (Inman and Cantey 1983) and primary colonization of Peyer's patches by Salmonella spp. (Carter and Collins 1974; Hohmann et al. 1978) have been reported.

2.3. Lymphoid Patches and Discrete Lymphoglandular Complexes in the Large Intestine

2.3.1. Distribution and amount

Nodular mucosa associated lymphoid tissue occurs as solitary lymphoid follicles or as patches formed by accumulations of solitary lymphoid follicles in the large intestine. They have been described as normal anatomic structures in:

- humans (Orth 1901; Kealy 1976b; Watanabe et al. 1983);
- primates (Scott 1979);
- cattle (Carlens 1928; Rooney 1956; Grau and Walter 1958; Hebel 1960; Nickel et al. 1982);
- sheep (Carlens 1928; Grau and Walter 1958; Hebel 1960);
- pigs (Carlens 1928; Biswal et al. 1953; Grau and Walter 1958; Hebel 1960; Ferguson et al. 1980);
- horses (Carlens 1928);
- dogs (Hebel 1960; Atkins and Schofield 1972);
- cats (Hebel 1960);
- rats (Bland and Britton 1984);
- Australian echidna (Schofield and Cahill 1969).

In cattle, distribution and size of lymphoid aggregates in the large intestine have been described by Carlens 1928, Rooney 1956, Hebel 1960 and Nickel et al. 1982 as follows:

One patch of lymphoid follicles extends as a continuation of the ileal Peyer's patch at the ostium ileale between cecum and colon. It has an irregular outline and is surrounded by smaller patches. A second accumulation of lymphoid follicles occurs at the junction between the proximal loop and the coiled loop of the ascending colon. This is, in newborn calves, about 15 - 20 cm and, in adults, 70 - 130 cm behind the ileocecal entrance. In calves, this 5 - 15 cm long circular patch is followed by a 30 - 50 cm long segment of colon containing solitary lymphoid follicles. In other sections of the large intestine lymphoid follicles are rarely found. Both patches involute in older animals and the remnants may be difficult to identify (Carlens 1928).

Table 2 lists comparative data for distribution, amount and characteristics of lymphoid follicles in the large intestine of several other animal species.

2.3.2. Morphological characteristics

In cattle, the patch of lymphoid follicles outside the ileocecal entrance has (according to Carlens (1928)) the characteristics of Peyer's patches in the upper small intestine, whereas Rooney (1956) finds numerous wide gland openings. The second aggregation at the beginning of the coiled loop of the ascending colon is characterized by irregularly thickened, broadened and elevated intestinal folds (Rooney 1956). These are caused by the underlying lymphoid nodules. Ridges

Table 2. Comparative data from literature for distribution, amount and characteristics of mucosa associated lymphoid nodules (LN) in the large intestine of sheep, pig, horse, dog, and the Australian echidna

	CECUM	ILEOCECAL ENTRANCE (ICE)	COLON	RECTUM
SHEEP (Carlens 1928)	---	patch of LN at ICE continuous with ileal PP[a]; extends into colon; small or 10 - 20 cm long	small patches between ICE and PC patches[b]; H: like ileal PP 1 - 2 large (21x2.6 cm^2) patches in asc. colon 60 - 80 cm (30 - 55 cm in lambs) distal to ICE; H: like ileal PP solitary LN for 130 - 180 cm distal of PC patches; H: LIC	solitary LN begin 50 cm proximal to anal ring; circular patches in zona columnaris; H: ---
PIG (Carlens 1928; Biswal et al. 1953)	solitary LN H[c]: LIC[d] and LN in lamina propria	patches of LN at ICE continuous with ileal PP; irregular shape; solitary LN around it	diffusely solitary LN; more numerous in distal 3/5; H: LIC and LN in lamina propria and submucosa	diffusely solitary LN; H: LIC and LN in lamina propria and submucosa
HORSE (Carlens 1928)	diffusely solitary LN; increased # in apex; H: LIC and LN in lamina propria	---	solitary LN diffusely in colon; H: LIC and LN in lamina propria	solitary LN in ternimal rectum; increased # at anal ring; H: ---
DOG (Hebel 1960; Atkins and Schofield 1972)	diffusely solitary LN; three per cm2 H: LIC	---	solitary LN in colon adjacent to ICE; less than three per cm2; H: LIC	solitary LN; H: ---
AUSTRALIAN ECHIDNA (Schofield and Cahill 1969)	---	---	diffuse, discrete patches (ca. 1cm in diameter) containing 20 - 30 pits each; H: LIC	---

[a]Peyer's patches.

[b]Patches of lymphoid nodules in proximal colon.

[c]Histology.

[d]Lymphatic intestinal crypt.

encircle crater-like holes, which are often filled with soft mucus plugs. The holes are considered to be gland openings (Carlens 1928).

Histologically, at least two distinct types of mucosa associated lymphoid follicles can be differentiated in the bovine large intestine (Carlens 1928):

1) lymphoid nodules, which are mainly located in the lamina propria and reach the intestinal surface between crypts, and
2) extensions of intestinal epithelium through the muscularis mucosae into the submucosal tissue, where they are surrounded by lymphoid follicles. These structures are called lymphatic intestinal crypts (Carlens 1928), lymphoglandular complexes (Bautzmann 1948) or extraduodenal submucosal glands (Grau and Walter 1958).

Carlens (1928) described lymphatic intestinal crypts as specific formations of the colonic lymphoid patch, while Rooney (1956) found them in the patch at the ileocecal entrance as well. Other authors (Grau and Walter 1958; Hebel 1960) describe extraduodenal submucosal glands in the whole intestine, most frequently at the beginning of the jejunum, in the ileum just before the ileocecal entrance, at the beginning of the colon and in the rectum. Elias (1947) considered them to be a typical feature of the ileum.

The submucosal glands were morphologically further subdivided into "bushel" (bush) glands, which occur mainly in the pig, and "shrub" glands, which are typical for ruminants (Grau and Walter 1958; Hebel 1960). In "bushel" glands, a whole bundle of epithelial diverticula

extends parallelly to each other, without branching from the epithelial surface into the submucosa, while in "shrub" glands, one diverticulum extends into the submucosa, where it branches.

The epithelium lining the gland-like diverticula is described as basically indistinguishable from the adjacent mucosa (Rooney 1956, Hebel 1960). The number of goblet cells is, however, reduced (Hebel 1960). In the deepest parts of the diverticula, atrophic changes are frequent. These give the lining epithelial cells an endothelial or stratified mucous epithelium-like character (Rooney 1956).

Except in rats (Bland and Britton 1984), results of ultrastructural studies of the follicle associated epithelium of mucosa associated lymphoid follicles in the large intestine have not been reported.

The colonic lymphoid patches in the rat consist of discrete lymphoid follicles in the lamina propria and submucosa. They are covered at the intestinal surface by a specialized lymphoepithelium. This is characterized by specific epithelial cells, which are called M cells in analogy to the cells over Peyer's patches. Goblet cells are absent in these areas.

Bland and Britton (1984) describe two types of M cells:

1) Type one has electron dense cytoplasm, large numbers of apical vesicles and lysosomes, and prolonged extensions of the apical cytoplasm forming thin partitions between the gut lumen and underlying intercellular spaces.
2) Type two has a less electron dense cytoplasma, distorted mitochondria and little endoplasmic reticulum.

Both have completely developed microvilli, glycocalix and terminal web. Functionally they have been shown to adsorb ferritin-ink label preferentially onto their surface.

3. MATERIAL AND METHODS

3.1. Experimental Animals

Fifteen calves from ten days to three months of age were used to determine the distribution, amount and morphology of aggregated lymphoid tissue (ALT) in the large intestine (Table 3). Seven of these calves were selected from necropsy material (group A), five were conventionally kept, healthy calves (group B) and three were germfree raised animals (group C), which had served in an experiment with Moraxella bovis. They had been exposed to this microorganism for 2, 10 or 45 hours by ocular inoculation.

3.2. Tissue Sampling and Fixation

Different methods of tissue sampling and fixation were used in animals of group A, B and C, depending on the intended method of further investigation (macroscopic, microscopic or ultrastructural).

In group A, which was only used for macroscopic determination of the distribution and amount of ALT, the intestine was prepared by a method originally described by Carlens (1928). Cecum and colon were removed and opened along the mesenteric attachment site. The mucosal surface was rinsed gently with cold water for cleaning purposes. Then the intestines were washed in cold, running water for 12 hours, fixed in 2% acetic acid for 12 - 24 hours, stained in 0.5% methylene blue for 3 - 5 minutes and rinsed for 2 - 3 hours to remove excess dye. Remnants of mesentery and fat were trimmed off.

Tissues from animals of group B were used for macroscopic

Table 3. Experimental animals and methods of tissue fixation and examination

	calf #	age in days(d)/ weeks(w)	sex m	sex f	breed	clinical diagnosis	ILF[a] FA[g]	ILF[a] GA[h]	AAP[b]	LM[c]	GMA[d]	TEM[e]	SEM[f]
group A	1	10 d		+	Holstein	hem. diathesis			+				
	2	14 d		+	Holstein	----			+				
	3	14 d	+		Holstein	hem. diathesis			+				
	4	14 d	+		Guernsey	rotav. infect.			+				
	5	18 d	+		Guernsey	rotav. infect.			+				
	6	6 w			Simmental X Hereford	rabies neg.			+				
	7	8 w		+	Holstein	peritonitis			+				
group B	1	12 w	+		Holstein	healthy	+			+			
	2	12 w	+		Holstein	healthy	+			+			
	3	12 w	+		Holstein	healthy	+			+			
	4	12 w	+		Holstein	healthy	+			+			
	5	12 w	+		Holstein	healthy	+			+			
group C	1	3 d	+		Pinzgauer	germfree	+	+			+	+	+
	2	6 d		+	Angus	germfree	+	+			+	+	+
	3	7 d		+	Angus	germfree	+	+			+	+	+

[a] Intraluminal fixation.

[b] Acetic acid preparation.

[c] Light microscopy.

[d] Glycomethacrylate embedding and serial sections.

[e] Transmission electron microscopy.

[f] Scanning electron microscopy.

[g] Formalin.

[h] Glutaraldehyde.

determination of distribution and amount of ALT and for histological examination. For this purpose, the large intestine was infused with 10% neutral buffered formalin after the distal ileum and distal rectum had been ligated. During the infusion the gut was carefully detached from the mesentery and placed in formalin for storage.

Tissues from animals in group C were used for serial sectioning and ultrastructural investigations. Therefore, a special method for the tissue collection was chosen. Animals were intravenously anesthesized in the isolator with 30 mg/kg of sodium pentobarbital[1]. After removal from the isolation unit the abdominal cavity was opened in the right flank and the cecum was exteriorized for orientation. Then the ileum was ligated about 10 cm before the ileocecal entrance (ICE) and the proximal colon was ligated 40 cm behind the ICE, shortly after entering the spiral loop of the ascending colon (Figure 3). Cold, 3% glutaraldehyde in 0.1M cacodylate buffer was injected in the cecum until the closed segment consisting of distal ileum, cecum and the beginning of the proximal colon was widely dilated. After removal this segment was placed for at least one hour in an identical fixative. The remaining colon was intraluminally fixed with 10% neutral buffered formalin. The distal rectum and anus were opened longitudinally, cut in half and stapled on acetate sheets. One of them was fixed in formalin, while the other was placed in glutaraldehyde (Figure 3).

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[1] Nembutal^{R} Sodium Solution, Abbott Laboratories, IL.

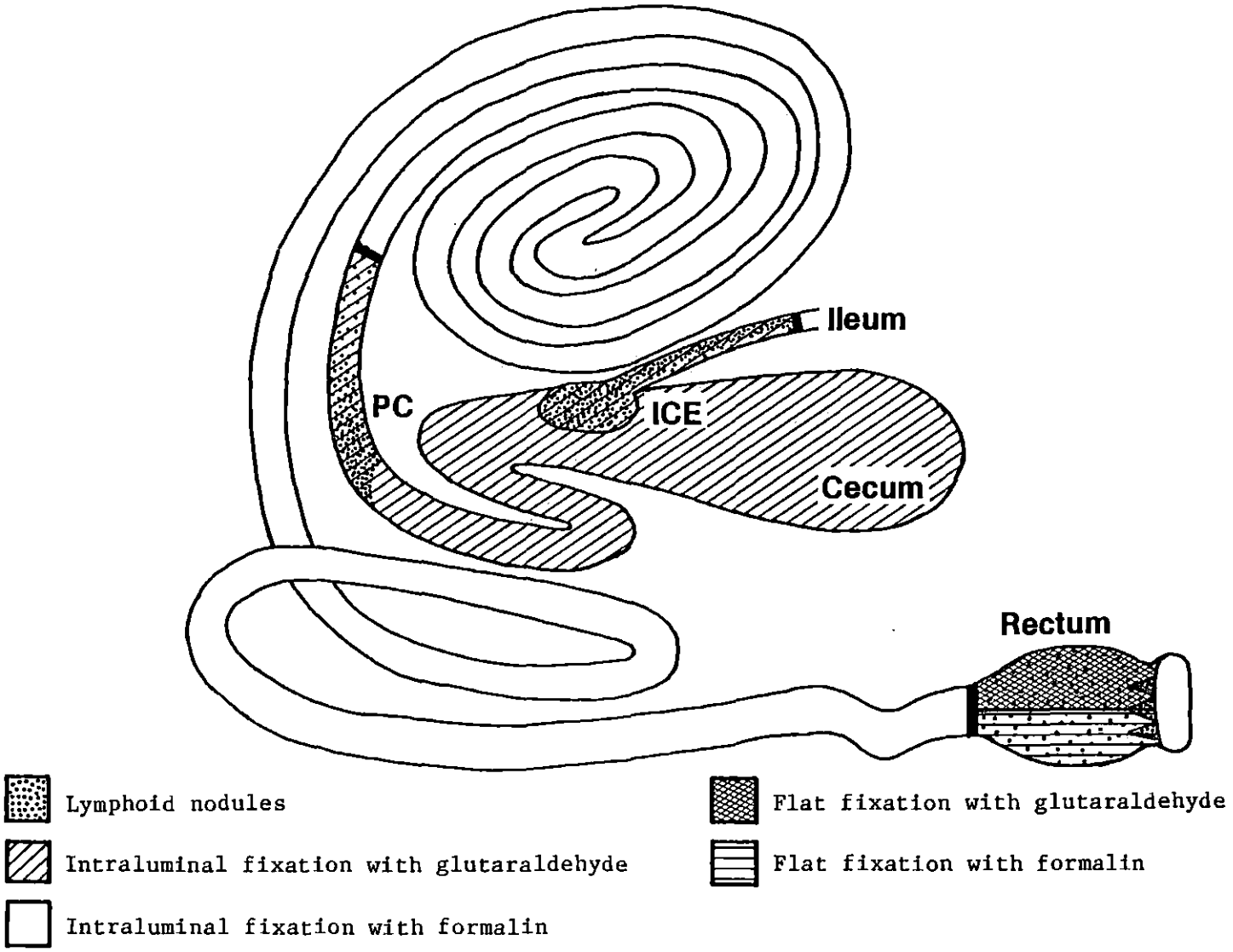

Figure 3. Tissue fixation at necropsy in gnotobiotic calves (group C)

3.3. Techniques for Tissue Preparation and Examination

3.3.1. Macroscopic investigations

Tissues from animals in groups A and B were used for macroscopic investigations applying different methods.

The intestinal walls fixed in acetic acid (group A) were spread flat on a slide-viewing-box and observed in translucent light. Thin transparent foil was spread over the tissue and the outline of the intestinal wall and the mucosa associated lymphoid nodules were traced. The tracings were mounted on paper. These originals were reduced to 15% of their size by photomechanical transfer (PMTs)[2].

The intraluminally fixed large intestine (group B) was viewed in translucent light[3].

The following parameters were measured and recorded in both groups:

- length of large intestine in cm;
- area of large intestine in cm^2;
- area of aggregated lymphoid tissue at the ileocecal entrance in cm^2;
- circumference of the large intestine at the ileocecal entrance in cm;
- presence of solitary lymphoid follicles between the ileocecal entrance and the aggregated lymphoid tissue in the proximal colon;

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[2]Graphic Arts Basic Printing Methods, No. GA-11-1, Kodak, NY.

[3]Fiber-Lite^R, Model 180, Dolan-Jenner-Industries, NY.

- distance between the ileocecal entrance and the aggregated lymphoid tissue in the proximal colon in cm;
- length of aggregated lymphoid tissue (patch) in the proximal colon in cm;
- area of aggregated lymphoid tissue in the proximal colon in cm^2;
- circumference of the large intestine at the beginning of aggregated lymphoid tissue in the proximal colon in cm;
- circumference of the large intestine at the end of the aggregated lymphoid tissue in the proximal colon in cm;
- extension of solitary follicles along the colon in cm;
- area of aggregated lymphoid tissue in the terminal rectum in cm^2;
- circumference of ampulla recti in cm.

In group A, the area measurments were determined from the PMT's with an Image Analyzer[4] following the instructions of the operator manual[5], while in group B the areas were approximated by multiplying average length with average width of the patch at the ileocecal entrance or with average circumference of the patch in the proximal colon.

3.3.2. Light microscopy

Tissues from calves of group A, B and C were prepared for histological examination.

From group A at least four locations along the large intestine,

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[4] SEM-IPS, Zeiss, West Germany.

[5] SEM-IPS Manual Vol.2, 1984, Kontron Electronics, West Germany.

where dark blue nodules were present, were prepared to see whether they were indeed lymphoid follicles. The tissues were postfixed in 10% neutral buffered formalin overnight.

From animals of group B, at least nine samples were collected at the ileocecal entrance and in the proximal colon as well as solitary follicles in the distal colon.

The tissues from animals of group A and B were dehydrated in alcohols of ascending concentration, cleared in xylene, infiltrated with and embedded in paraffin, cut in sections of 5 - 6 um thickness and stained with hematoxylin and eosin (H.E.).

From animals in group C, representative tissue squares (5 mm^2) containing lymphoid follicles were selected under the dissecting microscope at the ileocecal entrance and in the proximal colon for serial sectioning. The tissue was dehydrated in ethanol of increasing concentration, infiltrated with glycomethacrylate monomer in the refrigerator for 12 - 14 hours and embedded flat or on edge in glycomethacrylate polymer. The embedding moulds were sealed with paraffin to maintain anaerobic conditions and kept at room temperature. The blocks were serially cut in sections of 3 um thickness on a Sorvall^R "Porter-Blum" microtome[6] with Ralph knives (Bennett et al. 1976). The sections were floated on water, collected onto glass slides, dried for 15 minutes on a hot plate (60^oC), stained with 0.5% water soluble, freshly filtered toluidine blue for 6 minutes and cover-slipped.

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[6]Sorvall^R "Porter-Blum" Microtome, Type JB-4, Du Pont, CT.

3.3.3. Transmission and scanning electron microscopy

Only glutaraldehyde fixed tissue from animals of group C was used for electron microscopic investigations. Transmission and scanning electron microscopic samples containing lymphoid follicles were selected under a dissecting microscope from 2 locations at the ileocecal entrance and 6 locations in the proximal colon (Figure 4).

For scanning electron microscopy (SEM), squares of 1 cm^2 were cut out so that they contained lymphoid follicles in the center and tangentially sectioned lymphoid follicles at the cut edges. The mucosal surface was rinsed with cold, 0.1M cacodylate buffer (pH 7.2) to remove as much mucus as possible. Tissues were treated with tannic acid (Sweney and Shapiro 1977), dehydrated in alcohols of increasing concentration, transferred into freon, and critical point dried with a critical point dryer[7]. The samples were mounted on aluminum stubs with High Purity Silver Paste[8] and sputtercoated[9] with a 50 A thick layer of gold-paladium. They were viewed in a Cambridge Stereoscan 200 microscope[10] with a working distance of 6 - 10 mm and an accelerating voltage varying from 20 to 25 KV.

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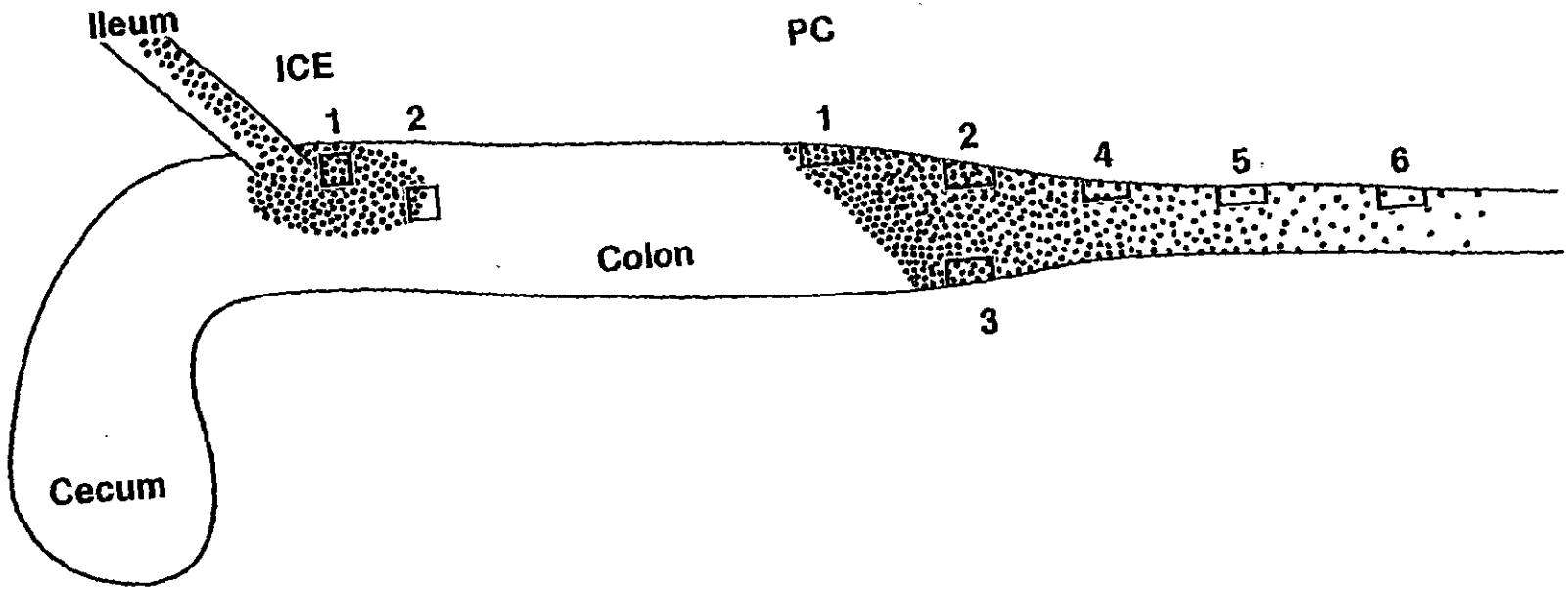
[7] DCP1, Denton Vac., Inc., NJ.

[8] SPI, Structure Probe, Inc., PA.

[9] Attachment to Edwards High Vacuum Evaporator, Type 306, BOC Ltd., England.

[10] Cambridge Stereoscan 200 Microscope, Cambridge, England.



Lymphoid nodules

Figure 4. Tissue collection for transmission (TEM) and scanning electron microscopy (SEM) in gnotobiotic calves (group C)

The material which had been selected for transmission electron microscopy (TEM) was cut in 0.5 mm thick slices under the dissecting microscope and only slices containing lymphoid follicles were further processed. After postfixation in 1% OsO_4 and dehydration in acetone, the tissue was embedded in epoxy resin, cut in 1 um thick sections with a ultramicrotome[11] and stained with 1% water soluble toluidine blue in sodium borate. Thin sections of selected areas were cut at about 600 A using a diamond knife and collected on 3 mm diameter copper grids (mesh size 200 um). Tissues were contrasted with uranyl acetate and lead citrate and viewed in a HS-9 electron microscope[12].

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[11]Ultramicrotome, Type 4802 A, LKB, Sweden.

[12]HS-9 Transmission Electron Microscope, Hitachi, Japan.

4. RESULTS

4.1. Results of Macroscopic Investigations

4.1.1. Distribution of mucosa associated lymphoid nodules in the large intestine

The aggregated lymphoid tissue (ALT) in the large intestine of calves consists of three accumulations of lymphoid nodules (patches) and of diffusely distributed, solitary lymphoid nodules.

Patches were present in all calves at the ileocecal entrance (IC patch), in the proximal colon (PC patch) and in the terminal rectum (RC patch).

The patch at the ileocecal entrance (Figures 5 and 6) was continuous with the ileal Peyer's patch and extended from the ileocecal entrance into the colon. It had an irregular ovoid shape. Solitary lymphoid nodules were scattered around it.

The patch in the proximal colon (Figure 7) was located at the transition between the proximal loop and the coiled loop of the ascending colon. The location was easily detectable, because the circumference of the large intestine decreased markedly at this point (Table 4) causing a funnel-shaped appearance of the distended loops (Figure 7). The distance from the ileocecal entrance to the beginning of the PC patch varied from 12.5 to 42 cm. Densely packed lymphoid follicles began at the antimesenteric site of the colon and extended circularly after 1.8 to 5.0 cm. The length of the PC patch varied from 4 to 30 cm. In two animals, the patch ended abruptly, while in 13 the

Figure 5. Patch of mucosa associated lymphoid nodules at the ileocecal entrance and scattered solitary lymphoid nodules in the cecum

calf A7; acetic acid fixation; methylene blue stain; translucent light

Figure 6. Patch of mucosa associated lymphoid nodules at the ileocecal entrance extending into the colon

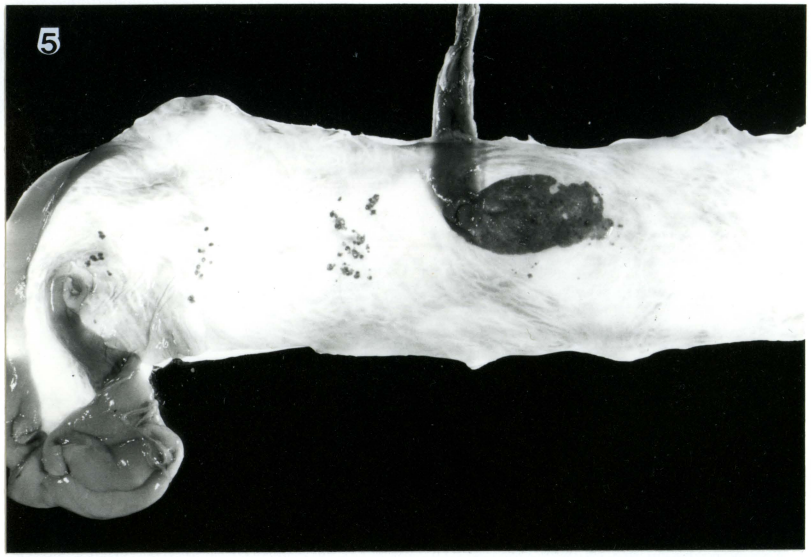
calf B3; intraluminal fixation; translucent light

Figure 7. Patch of mucosa associated lymphoid nodules in the proximal colon; funnel-like narrowing of colon

calf B1; intraluminal fixation; translucent light

Figure 8. Scattered lymphoid nodules distal to the patch in the proximal colon

calf B3; intraluminal fixation; translucent light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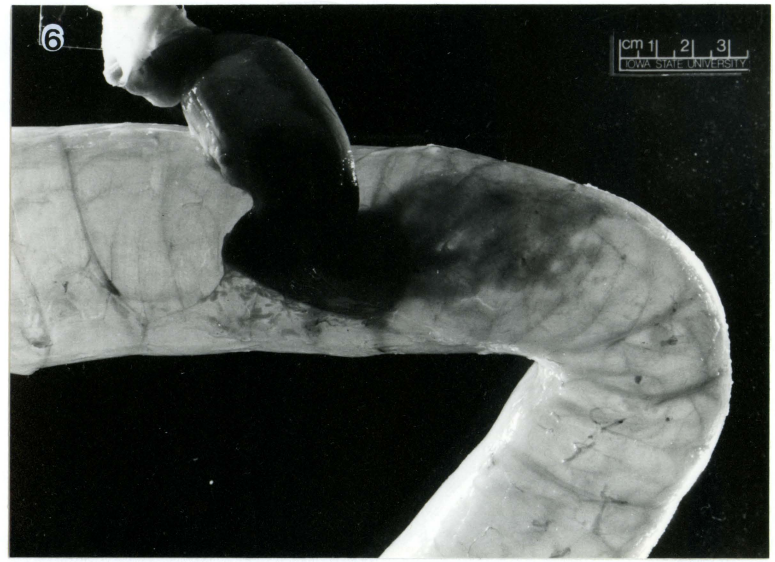
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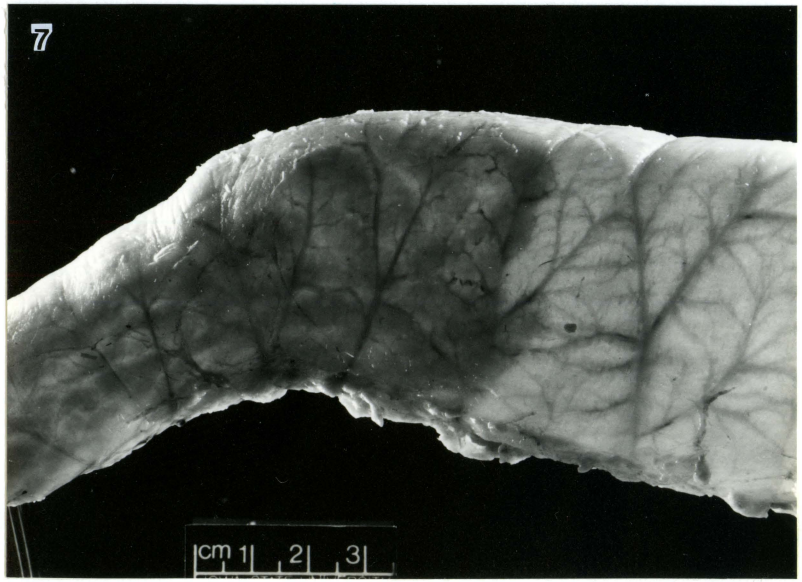
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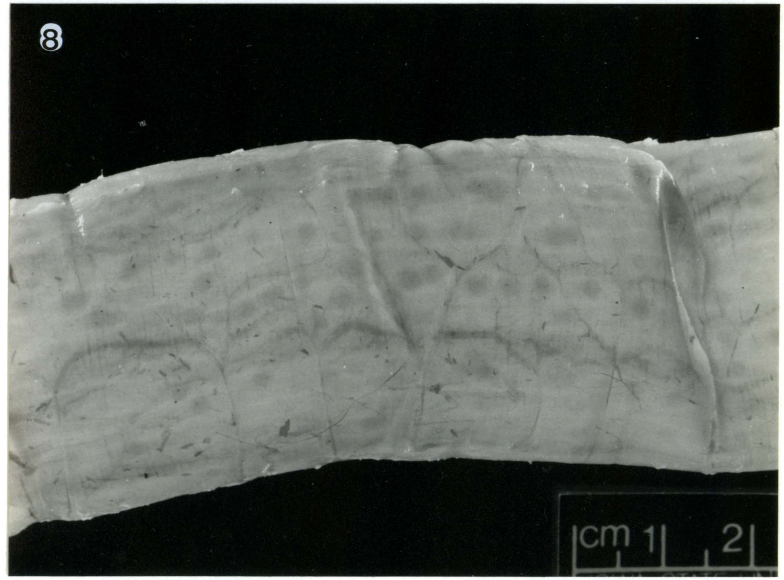

8
cm 1 2

density of lymphoid follicles decreased slowly and a continuous transition to solitary follicles occurred (Figure 8).

At the end of the rectum, in the plicate zone, small triangular patches were evenly distributed around the whole circumference of the colon. The base of the triangles bordered on the cutaneous zone of the anal canal and the tips pointed cranially.

The frequency and distribution of solitary lymphoid nodules showed variations between individual animals. Three of the 15 calves had a few, scattered lymphoid nodules in the body of the cecum. Four of the 15 calves had solitary lymphoid nodules and small patches between the IC patch and the patch in the proximal colon. In 13 calves, solitary lymphoid nodules were present following the patch in the proximal colon. The extension of these lymphoid nodules along the large intestine ranged from 6 to 201 cm. All examined animals (Table 5) had scattered lymphoid nodules in the ampulla recti.

Data for the distribution and extension of mucosa associated lymphoid follicles and reference data for the length and circumference of the large intestine are listed in Tables 5 and 4. Tracings of the large intestinal outline and mucosa associated lymphoid follicles present are shown in Figure 9.

Table 4. Length and circumference of the large intestine (l.i.) of calves in groups A, B and C

	calf #	age in days(d)/ weeks(w)	length of l.i. in cm	circumference at ICE[a] in cm	circumference at beginning of PC patch[b] in cm	circumference at end of PC patch in cm	ampulla recti in cm
group A	1	10 d	227	8.7	5.8	4.6	12
	2	14 d	195	7.2	6.4	5.3	12
	3	14 d	283	6.0	5.5	3.7	--[c]
	4	14 d	334	8.7	6.5	5.2	14
	5	18 d	230	8.8	4.7	3.0	--
	6	6 w	470	11.0	9.5	5.7	16
	7	8 w	312	10.2	8.1	5.1	12
group B	1	12 w	297	24	17	10	--
	2	12 w	301	13	10	9	--
	3	12 w	308	25	14	10	--
	4	12 w	420	20	19	12	--
	5	12 w	433	15	10	9	--
group C	1	3 d	195	10.0	7.0	4.7	--
	2	6 d	221	13.0	7.5	5.2	--
	3	7 d	208	12.5	7.0	5.5	--

[a]Ileocecal entrance.

[b]Proximal colon patch.

[c]Rectum was not evaluated.

Table 5. Localization of mucosa associated lymphoid nodules in the large intestine (l.i.) of calves in groups A, B and C

	calf #	age in days(d)/ weeks(w)	IC patch[a] length in cm	IC patch[a] width in cm	distance ICE[b] to PC patch[d] in cm	length of PC patch in cm	length of l.i. with SLN[c] distal of PC patch in cm	SLN in cecum	SLN between IC patch and PC patch
group A	1	10 d	2.3	0.9	33	8	8	-	+
	2	14 d	2.3	1.2	23	11	85	-	+
	3	14 d	2.7	2.3	--[e]	13	19	-	-
	4	14 d	3.5	2.8	31	12	6	+	+
	5	18 d	4.4	2.0	34	9	16	-	+
	6	6 w	4.0	3.5	40	26	196	-	-
	7	8 w	7.0	3.4	35	11	26	-	-
group B	1	12 w	6.0	5.0	33	17	201	-	-
	2	12 w	5.5	5.0	33	22	18	-	-
	3	12 w	5.5	3.5	37	15	168	+	-
	4	12 w	8.0	4.0	42	14	16	+	-
	5	12 w	6.0	4.5	25	30	18	-	-
group C	1	3 d	3.0	2.4	17	4	0	-	-
	2	6 d	2.7	1.7	17.2	15	106	-	-
	3	7 d	2.2	2.0	12.5	14	0	-	-

[a]Patch of lymphoid nodules at ileocecal entrance.

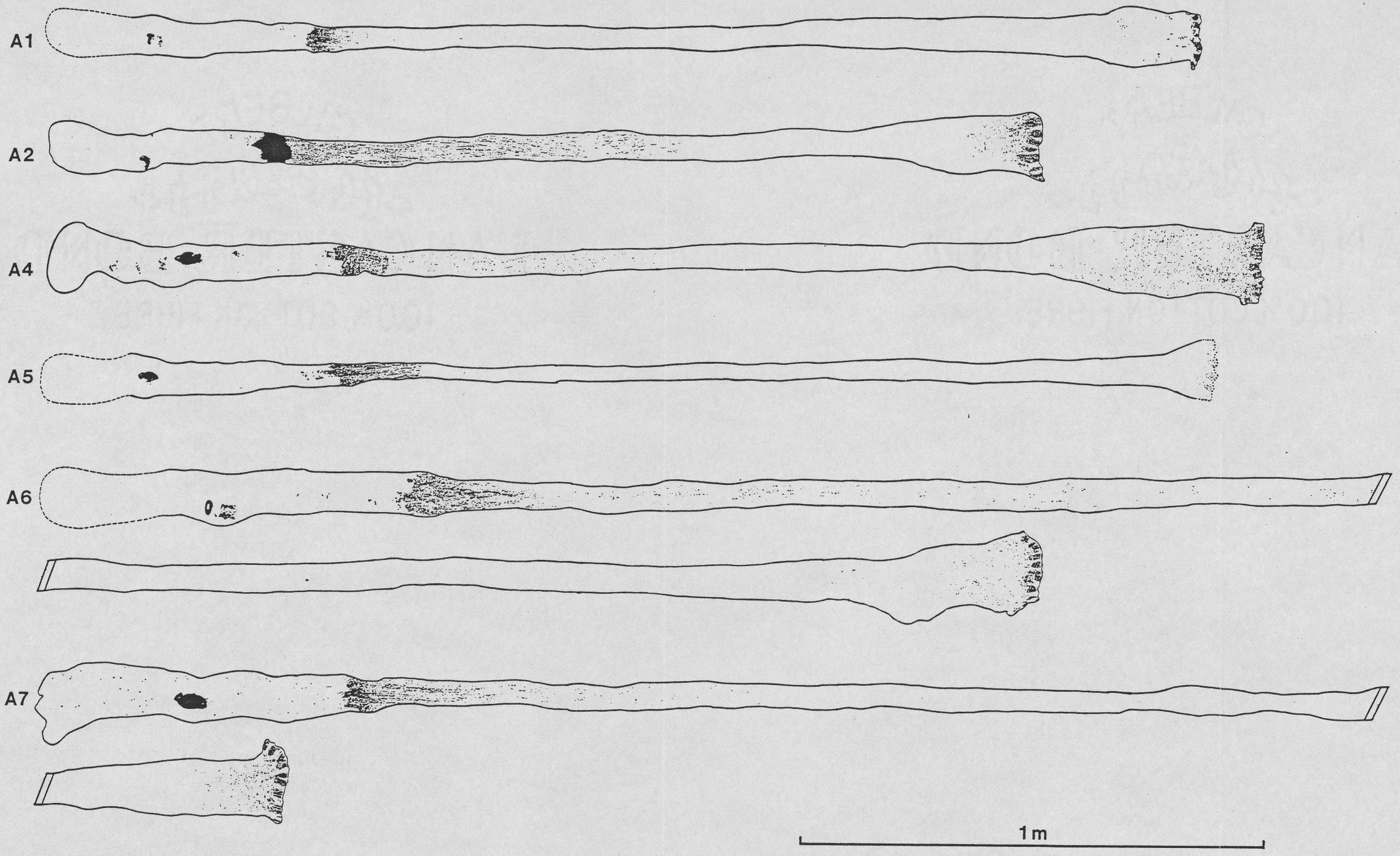
[b]Ileocecal entrance.

[c]Solitary lymphoid nodules.

[d]Patch of lymphoid nodules in proximal colon.

[e]Was not measured.

Figure 9. Tracings of large intestinal outline and mucosa associated lymphoid nodules in animals of group A (reduction: 89%)


A1
A2
A4
A5
A6
A7
1m

4.1.2. Area of mucosa associated lymphoid nodules in the large intestine

The area of aggregated lymphoid tissue (ALT) was measured by planimetry in animals of group A, while it was approximated in animals of group B.

The absolutely measured areas varied greatly between individual animals (Table 6). The area of the large intestine was on the average 2346 cm^2 in animals of group A and 3747 cm^2 in animals of group B. The average of the total area of ALT was 173.5 cm^2 in animals of group A and 252.8 cm^2 in animals of group B.

The data for the total area of ALT expressed as percentage of the area of the large intestine showed less deviation between individual animals. The average percentage of the area of ALT in the large intestine was 7.8% in group A animals (Figure 10a). The patch at the ileocecal entrance occupied 0.6%, the patch in the proximal colon and the adjacent solitary follicles 4.8% and the mucosa associated lymphoid follicles in the rectum 2.4% of the area of the large intestine (Figure 10b).

In animals of group B, only the ALT at the ileocecal entrance and in the proximal colon were evaluated. The average percentage of ALT in these two locations was 7.1% of the area of the large intestine (Figure 11a), which compares with an average of 5.4% (0.6% plus 4.8%) in animals of group A. The patch at the ileocecal entrance contributed 0.9% and the patch in the proximal colon 6.2% to this percentage (Figure 11b).

The data for individual animals are shown in Figures 12a and 12b.

Table 6. Area of large intestinal wall (l.i.) and area of mucosa associated lymphoid nodules at the ileocecal entrance, in the proximal colon and in the rectum in calves of groups A and B

	calf #	age in days(d)/ weeks(w)	area of l.i. in cm^2	IC patch[a] in cm^2	area of PC patch[b] in cm^2	RC patch[c] in cm^2	total area of ALT[d] in cm^2
group A	1	10 d	1876	5.87	43.32	34.93	84.12
	2	14 d	1628	4.77	148.93	75.11	228.81
	4	14 d	2200	22.27	79.67	85.25	187.19
	5	18 d	1490	9.91	97.79	--[e]	107.7
	6	6 w	4221	17.25	151.40	68.52	237.67
	7	8 w	2660	24.84	110.00	60.82	195.66
group B	1	12 w	4009	30.00	229.5	--	259.5
	2	12 w	2709	27.5	209	--	236.5
	3	12 w	3080	19.25	180	--	199.25
	4	12 w	5040	32	217	--	249
	5	12 w	3897	27	292.5	--	319.5

[a]Patch of lymphoid nodules at ileocecal entrance.

[b]Patch of lymphoid nodules in proximal colon.

[c]Patches of lymphoid nodules in rectum.

[d]Aggregated lymphoid tissue.

[e]Rectum was not evaluated.

Figure 10. Average percentage of aggregated lymphoid tissue (ALT) in the large intestine of calves in group A

a. % total ALT in the large intestine

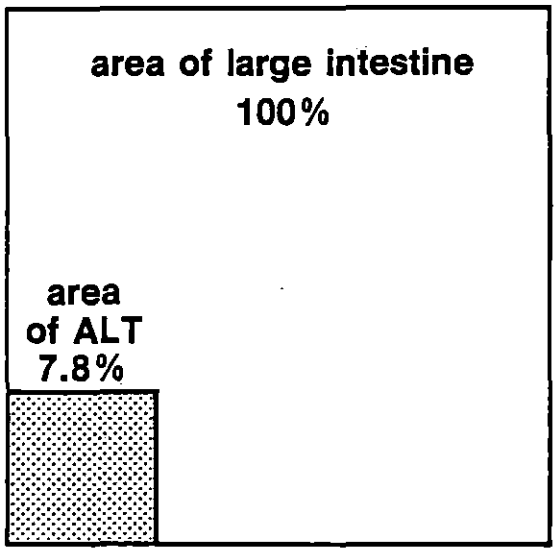
b. % ALT at the ileocecal entrance (ICE), in the proximal colon (PC) and in the rectum (RC)

Figure 11. Average percentage of aggregated lymphoid tissue (ALT) in the large intestine of calves in group B

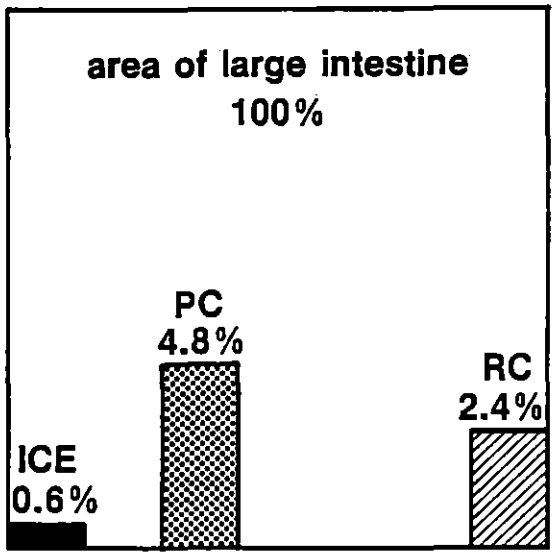
a. % total ALT in the large intestine

b. % ALT at the ileocecal entrance (ICE) and in the proximal colon (PC)

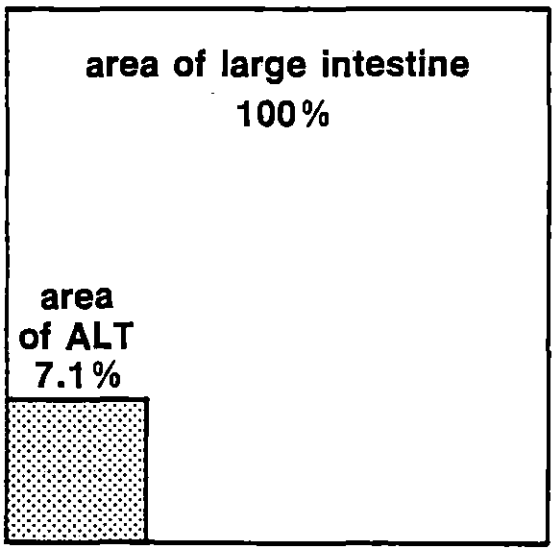
10a.



10b.



11a.



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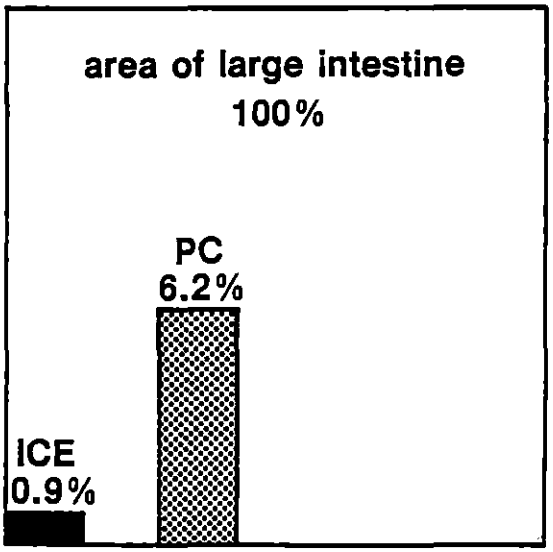


ALT at the ileocecal entrance (ICE)

ALT in the proximal colon (PC)

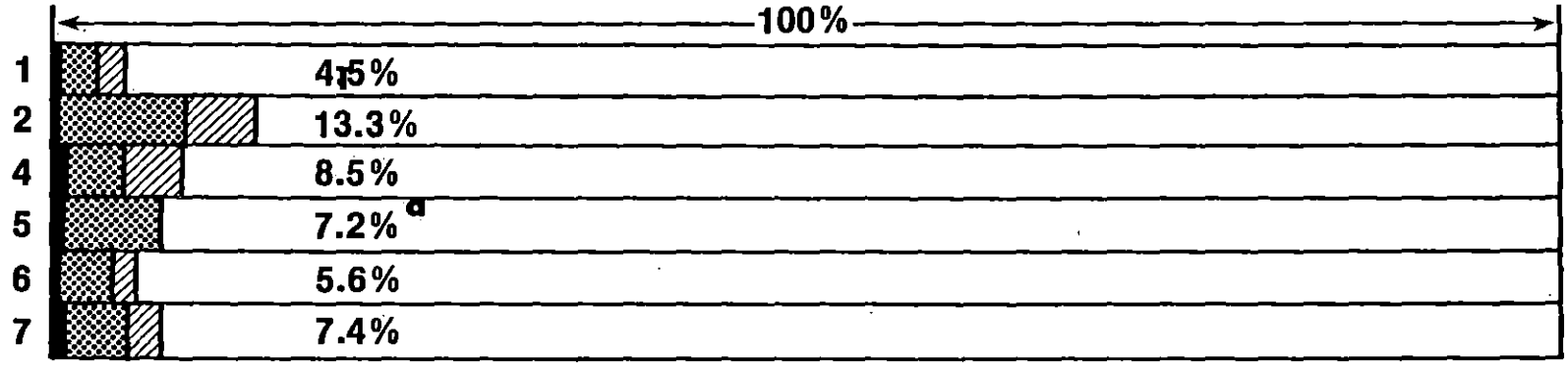
ALT in the rectum (RC)

Figure 12. Percentage of area of aggregated lymphoid tissue at the ileocecal entrance, in the proximal colon and in the rectum in individual calves; % figures indicate the total percentage of aggregated lymphoid tissue

a. calves selected from necropsy material (group A)

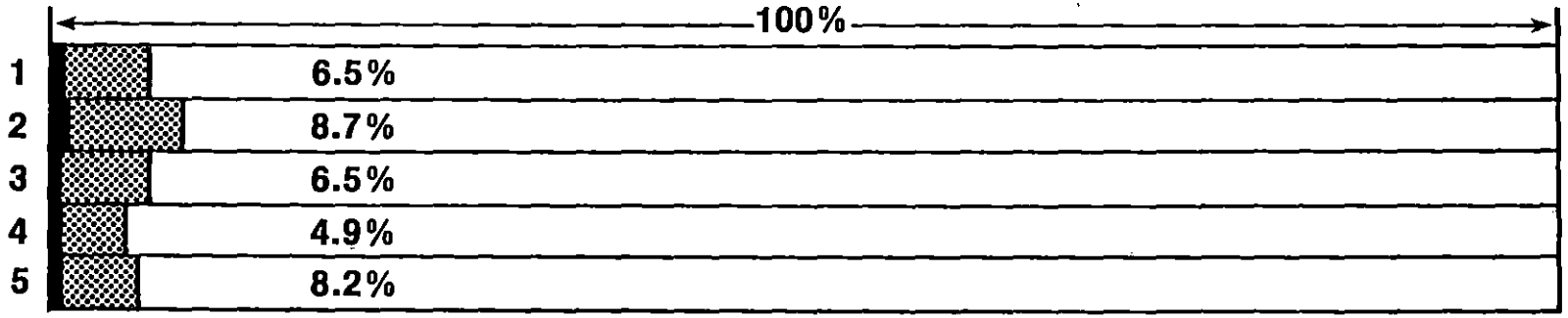
b. conventionally kept, healthy calves (group B) (ALT in the rectum was not evaluated)

12a.



[a]rectum was not evaluated

12b.



ALT at the ileocecal entrance (ICE)

ALT in the rectum (RC)

ALT in the proximal colon (PC)

4.2. Results of Microscopic Investigations

4.2.1. Light microscopy in calves selected from necropsy material (group A) and conventionally kept, healthy calves (group B)

4.2.1.1. Group A The acetic acid fixation did not provide good tissue preservation and the epithelium was lost, but the mucosa associated lymphoid nodules were still recognizable in the sections. All samples taken from the patch at the ileocecal entrance, from the beginning and the end of the patch in the proximal colon and from the terminal rectum contained lymphoid nodules. No lymphoid nodules were present in two sections, where solitary lymphoid nodules from the cecum and from the end of the patch in the proximal colon had been selected. Because these lymphoid nodules were very small, they may have been phased away at the beginning of the sectioning procedure before tissue was collected on the glass slide. Therefore, it was concluded that the macroscopically identified dark blue dots were in fact mucosa associated lymphoid nodules.

4.2.1.2. Group B Lymphoid nodules in the large intestine occured as two morphologically different units:

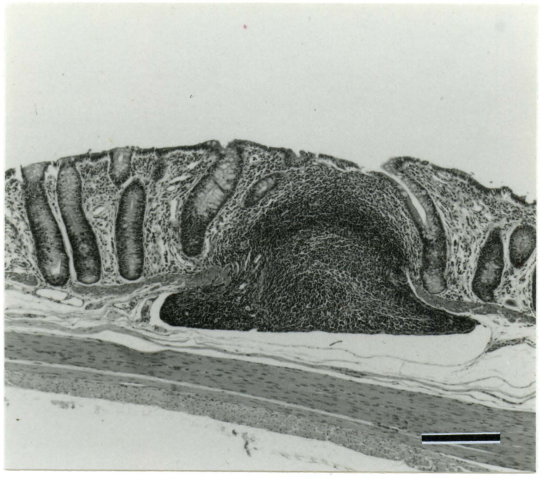
1) Type 1 (under the dissecting microscope and in the scanning electron microscope later identified as star-like structures) (Figure 13). The lymphoid follicles were partly located in the submucosa and partly in the lamina propria. They interrupted the muscularis mucosae. They had one germinal center, which was covered at the luminal side by a cap of small lymphocytes.

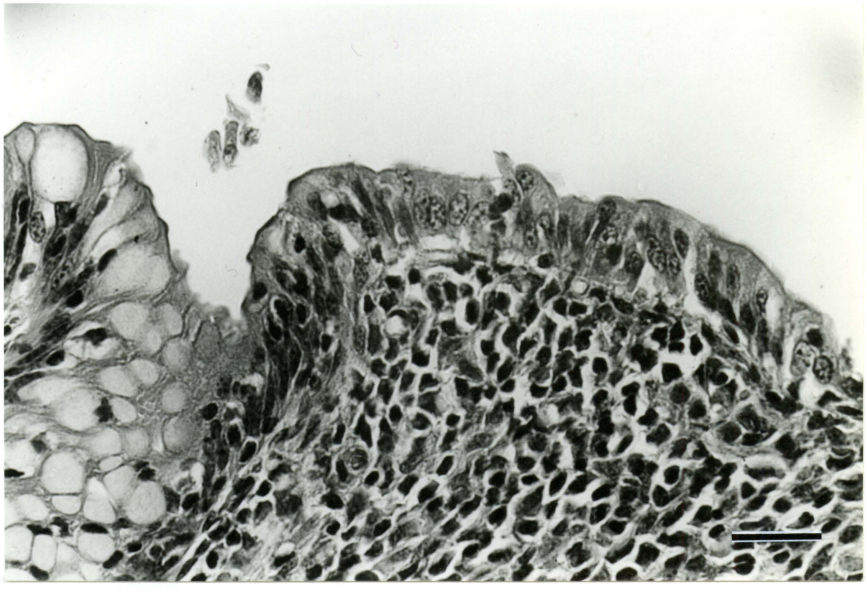
Figure 13. Tangential section through center of star-like structure; lymphoid follicle with germinal center in tunica submucosa and lamina propria; corona of small lymphocytes; lamina propria filled with lymphocytes and covered with morphologically distinct epithelium (FAE)

calf B1; PC patch; H.E.; bar = 250 um

Figure 14. FAE characterized by distinct epithelial cells, numerous IECs and lack of goblet cells

calf B1; PC patch; H.E.; bar = 25 um





The lamina propria of large intestinal ridges between two crypts was filled with lymphocytes. The width of this ridge varied. It was covered by epithelium, which lacked goblet cells and contained numerous intraepithelial cells (Figure 14).

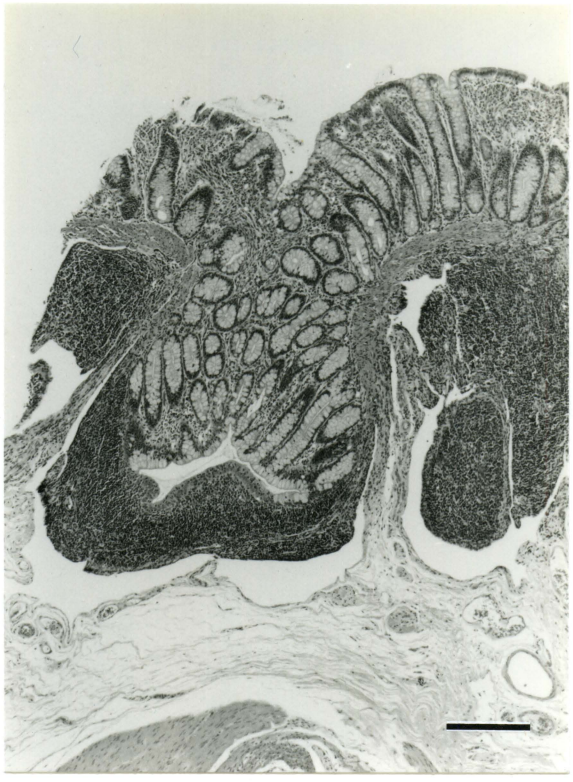
2) Type 2 or lymphoglandular complexes in the submucosa (under the dissecting microscope and in the scanning electron microscope later identified as pits on the surface) (Figure 15). The lymphoid nodules were almost completely located under the muscularis mucosae, which was interrupted by epithelial diverticula extending from the intestinal lumen into the submucosa. Usually one large diverticulum extended from the surface into the lymphoid nodule, where it branched into smaller diverticula. In one animal, lymphoglandular complexes were seen, where the diverticulum split first into 2 or 3 smaller diverticula and these entered the lymphoid nodules and branched. The entering diverticulum had a slightly larger diameter than the crypt openings. In two animals the diameter was so wide that an open connection between the intestinal lumen and the center of the lymphoid follicle was seen. In the center of the lymphoid nodule folds or dome-like protrusions covered by morphologically distinct epithelium were present (Figure 16). This follicle associated epithelium was devoid of goblet cells and contained numerous intraepithelial cells. Depending on the plain of the tangential section, lymphoid nodules isolated in the submucosa without connection to the

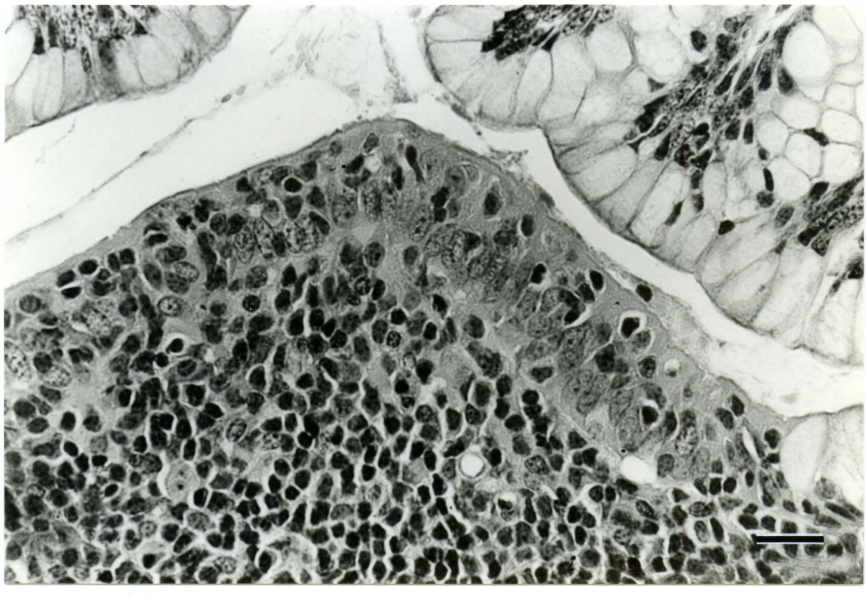
Figure 15. Tangential section through center of lymphoglandular complex; lymphoid nodule in submucosa; epithelial diverticulum extending from intestinal lumen into the lymphoid nodule; dome-like protrusions in center of lymphoid nodule covered with morphologically distinct epithelium (FAE)

calf B1; PC patch; H.E.; bar = 250 um

Figure 16. FAE lining dome-like protrusion in lymphoglandular complex characterized by distinct epithelial cells, numerous IECs and lack of goblet cells;

calf B1; PC patch; H.E.; bar = 25 um





surface occurred, with variable numbers of diverticula, and with varying stretches of FAE covered folds The folds and protrusions were separated by a corona of densely packed small lymphocytes from the germinal centers. In general several germinal centers were associated with one lymphoglandular complex. In one animal the germinal centers were occasionally replaced by thin crescents of lymphocytes and several cystic dilated, mucus-filled diverticula were present.

Lymphoid nodules in the submucosa without apparent connection to the intestinal surface and without diverticula were frequent. They were interpreted to be tangential cuts through star-like structures or lymphoglandular complexes.

The two types of lymphoid nodules described had a distinct distribution along the large intestine.

In the patch at the ileocecal entrance, only lymphoglandular complexes were seen with the exception of one animal, which also had star-like structures. The patch in the proximal colon consisted mainly of lymphoglandular complexes, but in four of the five calves also a few star-like structures were present. The proportion changed toward the end of the patch in the proximal colon. The number of lymphoglandular complexes declined and the relative number of star-like structures increased. After an average of 21 cm from the beginning of the PC patch, only solitary lymphoid nodules formed by star-like structures were present.

In the patches at the ileocecal entrance and in the proximal colon,

large amounts of parafollicular lymphoid tissue occurred, while it was missing around the solitary lymphoid nodules.

4.2.2. Three-dimensional reconstruction of the lymphoepithelial relationship in star-like structures and lymphoglandular complexes from serial sections

The morphologically different types of lymphoid nodules, which were seen in histological sections of animals in group B, star-like structures and lymphoglandular complexes, were selected in animals of group C under the dissecting microscope to confirm the spatial arrangement between epithelium and lymphoid tissue by serial sections.

(1) star-like structures (Figures 17 and 18)

The lymphoid follicle was mainly located in the lamina propria. The muscularis mucosae was, however, disrupted and a part of the lymphoid follicle penetrated into the submucosa. The lamina propria above the lymphoid follicle was infiltrated by lymphocytes and covered by follicle associated epithelium. This morphologically distinct ridge looked like a round, blunt elevation and will be referred to as the center of the star-like structure in the following. It ended on a slightly lower level than the large intestinal ridges and was surrounded by wide crypts, which were named furrows. The center of the star like structure was connected to the adjacent ridges by radiating crests. The furrows ended at the muscularis mucosae. The base of the furrows was lined by enterocytes and goblet cells. The upper half of the furrows had morphologically distinct follicle associated epithelium on the side

Figure 17. Three-dimensional reconstruction of star-like structure by superimposing selected serial sections cut parallel to the surface from lumen to submucosa; center of star-like structure containing lymphoid tissue is surrounded by furrows; furrows are partly lined with FAE

Lymphoid follicle

Morphologically distinct follicle associated epithelium (FAE)

Epithelium composed of enteroabsorptive cells and goblet cells

Lamina muscularis mucosae

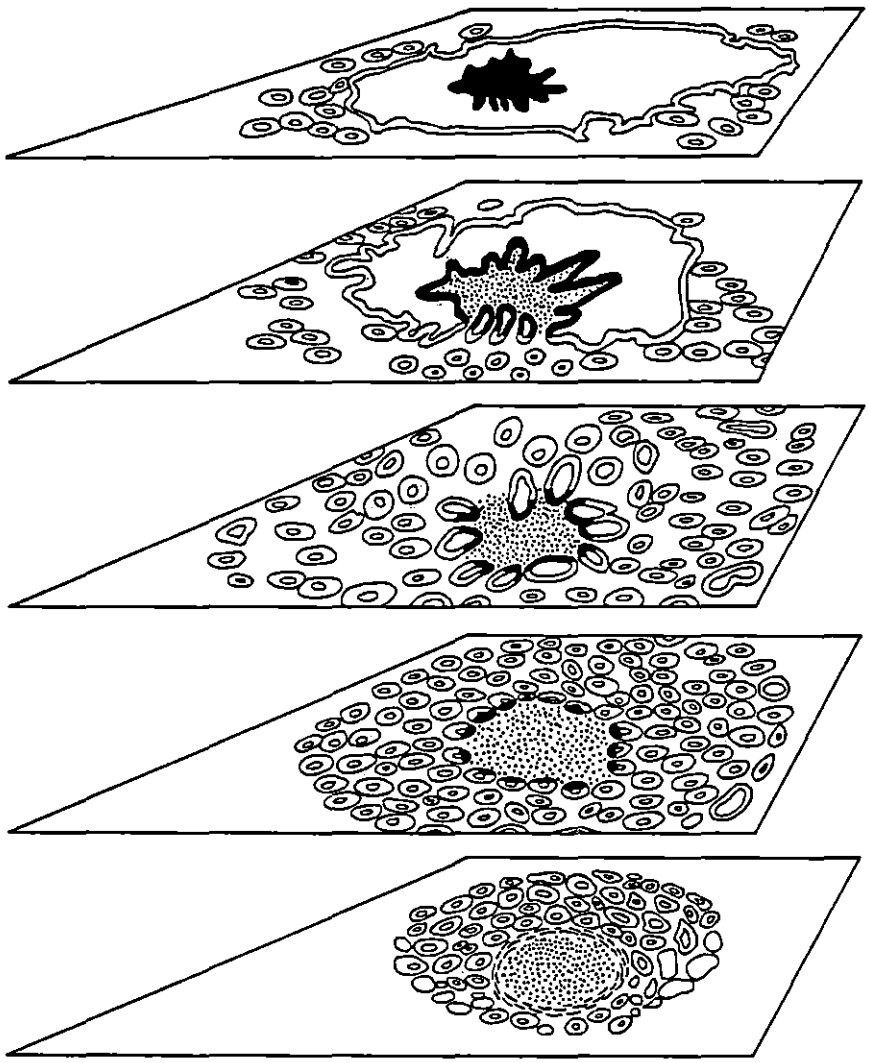


Figure 18. Schematic drawing of star-like structure

a. horizontal plain close to the luminal surface

b. vertical plain cut as indicated in 18a.

1 Lymphoid follicle

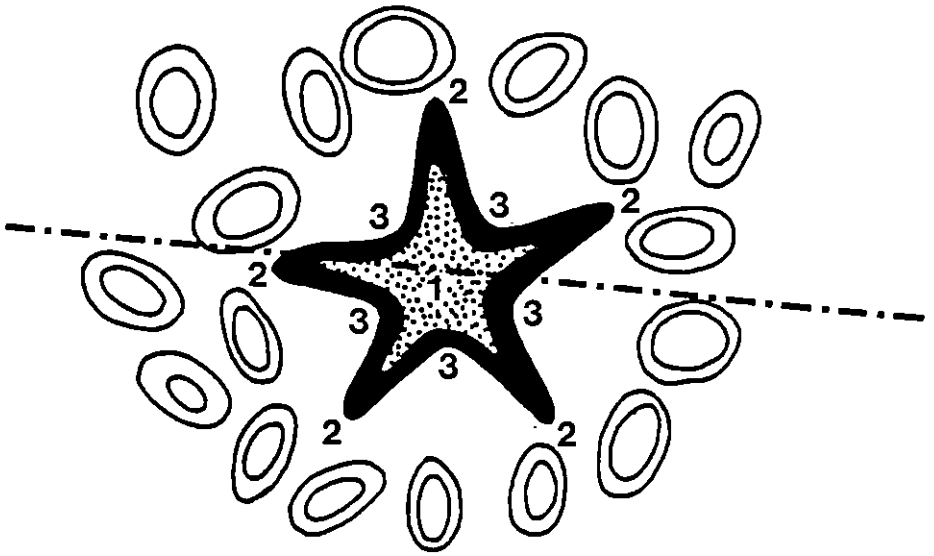
2 Crest

3 Furrow

Morphologically distinct follicle associated epithelium (FAE)

Epithelium composed of enteroabsorptive cells and goblet cells

Lamina muscularis mucosae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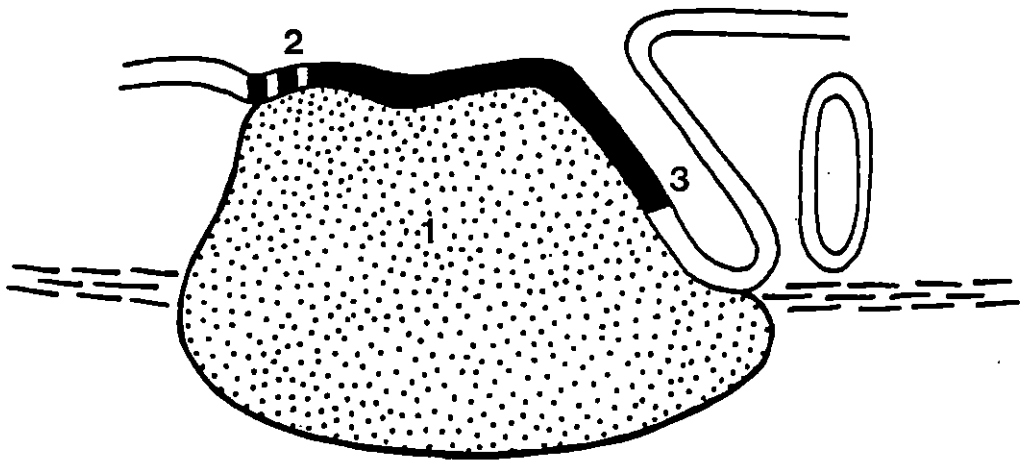
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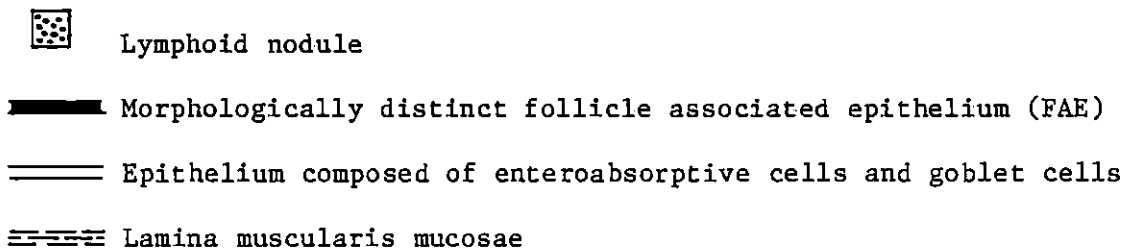
Figure 19. Three-dimensional reconstruction of lymphoglandular complex by superimposing selected serial sections cut parallel to the surface from lumen to submucosa; lymphoid nodule in submucosa; epithelial diverticulum branches in the lymphoid nodule; dome-like transversal fold in the center of the lymphoid nodule covered by FAE



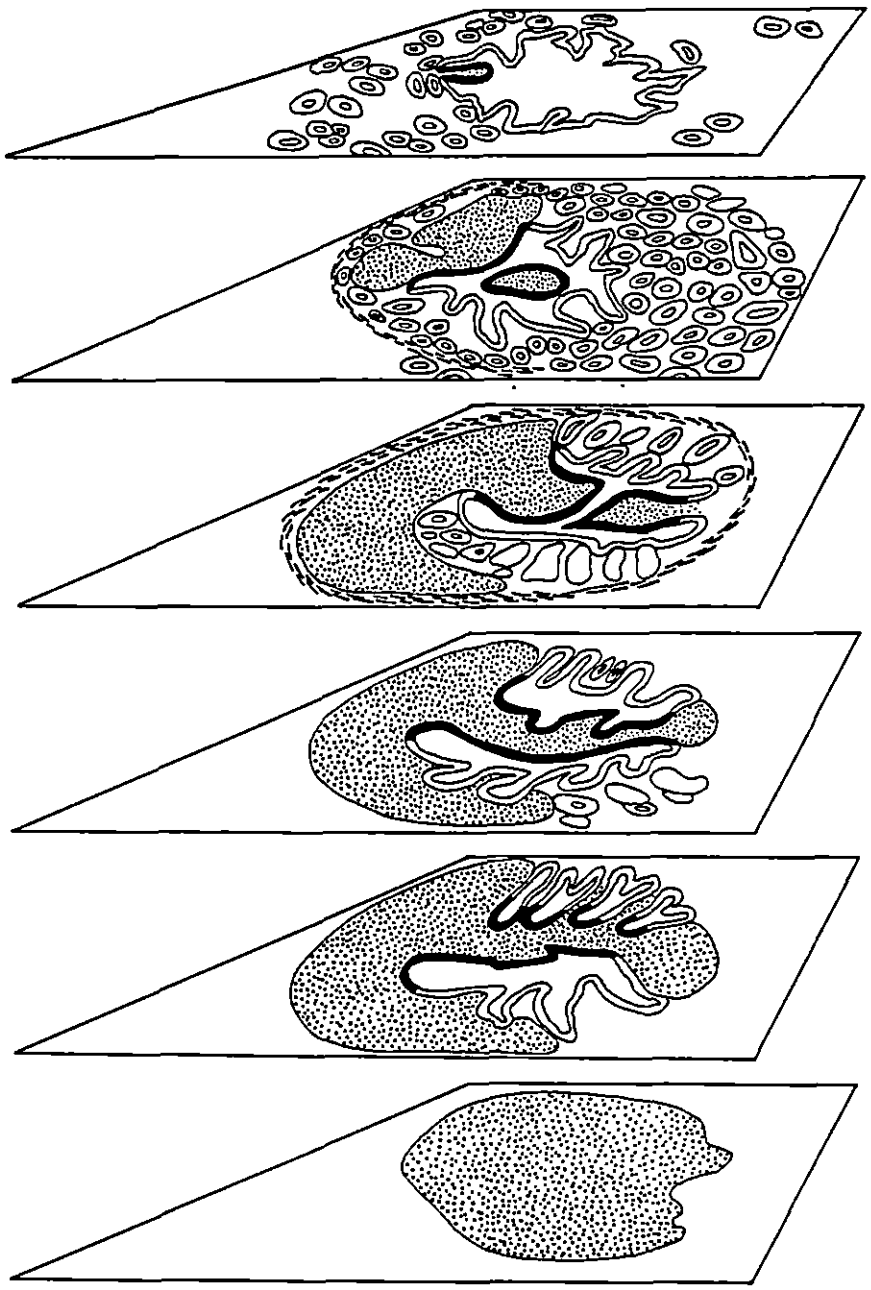


Figure 20. Schematic drawing of lymphoglandular complex

a. horizontal plain through diverticulum close to the luminal surface; FAE on apical end of the fold is present in the center of the diverticulum

b. vertical plain through center of lymphoglandular complex as indicated in 20a.

c. vertical plain tangentially through the lymphoglandular complex as indicated in 20b.

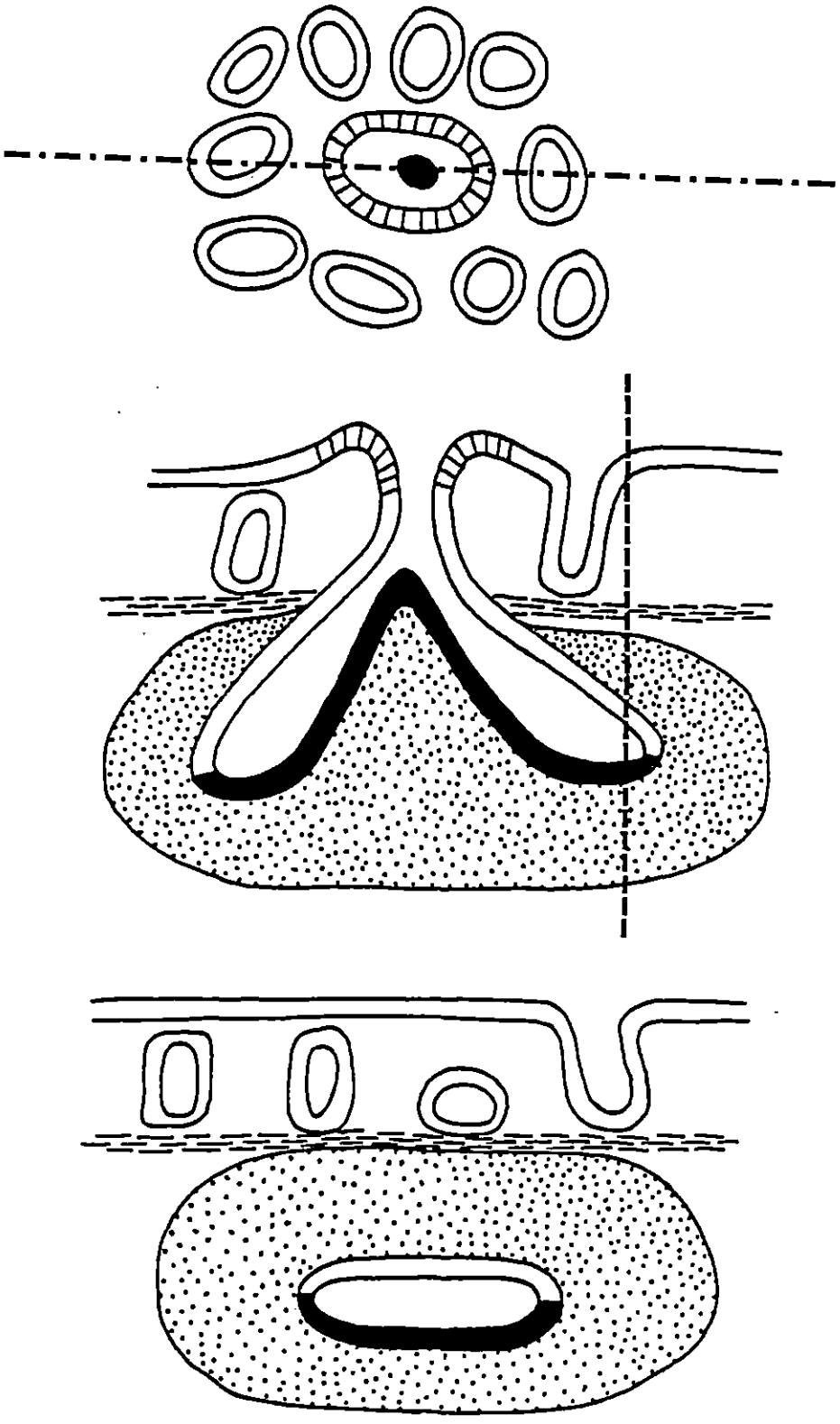
Lymphoid nodule

Morphologically distinct follicle associated epithelium (FAE)

Epithelium composed of enteroabsorptive cells and goblet cells

Entrance in lymphoglandular complex ("pit")

Lamina muscularis mucosae



adjacent to the lymphoid follicle. This FAE continued at the center of the star like-structure. It was characterized by pale cells without distinct brush border, numerous intraepithelial cells and the lack of goblet cells. A few scattered enterocytes were present.

(2) lymphoglandular complexes (LGC) (Figures 19 and 20)

The lymphoid nodule was completely located underneath the muscularis mucosae. One diverticulum with variable diameter extended through the muscularis mucosae. It entered the lymphoid nodule and branched in numerous smaller diverticula. In the center of the lymphoid nodule an assymmetrically shaped, transverse fold of lymphoid tissue protruded from the bottom. Its apical end protruded above the level of the muscularis mucosae in some sections. Only this fold was covered by a FAE as described for the star-like structures.

4.2.3. Scanning electron microscopy

On the surface of the selected intestinal mucosa (Figure 4), star-like structures and pit openings were seen (Figure 21), which were not present in areas where no ALT had been identified. Tangential sections viewed at the cut edge confirmed the results of serial sections and revealed, that the star-like structures corresponded with lymphoid follicles in the lamina propria and in the submucosa, and the pit openings with lymphoglandular complexes in the submucosa (Figure 22).

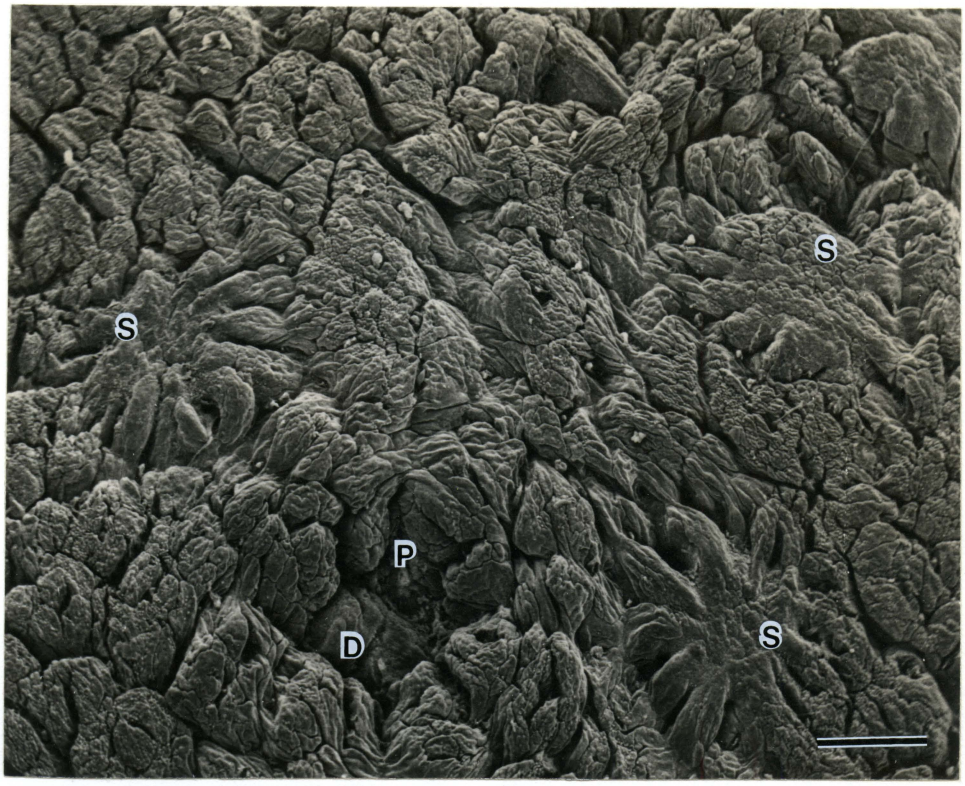
The star-like structures seen on the surface varied from 200 to 700 um in diameter. They were surrounded by 4 to 12 furrows.

Figure 21. Three symmetrical star-like structures (S) and one pit-like opening (P) with a dome-like protrusion (D) in the depth

calf C2; PC patch; SEM; bar = 250 um

Figure 22. Tangential section through lymphoglandular complex (L) and star-like structure (S); branching diverticula are present in the lymphoglandular complex; association between star-like structure seen on surface and underlying lymphoid follicle is evident

calf C3; PC patch; SEM; bar = 125 um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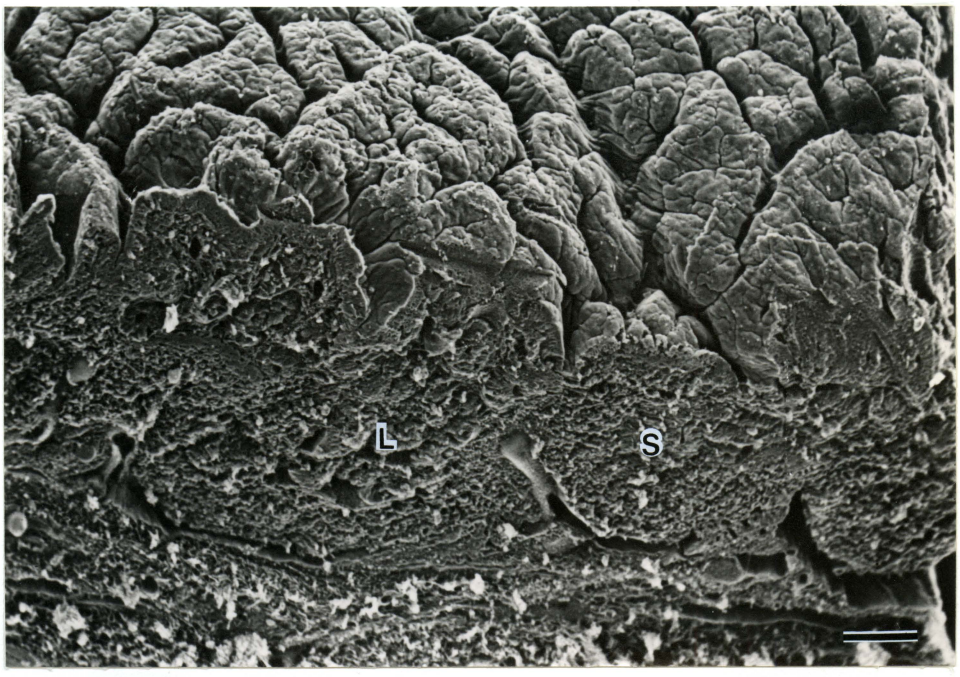
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Symmetrical and distorted forms were seen, probably due to different intraluminal pressure upon fixation (Figures 23 and 24).

The epithelium in the center consisted of cells with variable size, shape and surface structure. Small groups of cells were protruding causing a contorted appearance of the surface. The cells were bulging, thus delineating the cell borders by crevices (Figure 25). Large polygonal cells with dense, uniformly long microvilli of small diameter were interpreted to be enteroabsorptive cells. Smaller cells with less dense, thicker microvilli of irregular length were most frequent (Figure 26). They resemble M-cells in the upper small intestine of calves. Some cells had lost all microvilli. A few small, round cells with uniformly long, thick microvilli, which were longer than the ones on surrounding cells, were scattered (Figure 27). They were in connection with transmission electron microscopic observations (Figure 28) identified as tufted cells. No goblet cells were present in the FAE of the star-like structures.

Along the crests towards the periphery a gradual transition to enteroabsorptive cells and goblet cells occurred (Figure 29).

The furrows were lined by enterocytes and goblet cells with the exception of a gutter-like area adjacent to the lymphoid nodule (Figure 29). In this fold, medium-sized, round cells, which had very long thin microvilli in the center, were mixed with enterocytes (Figure 30).

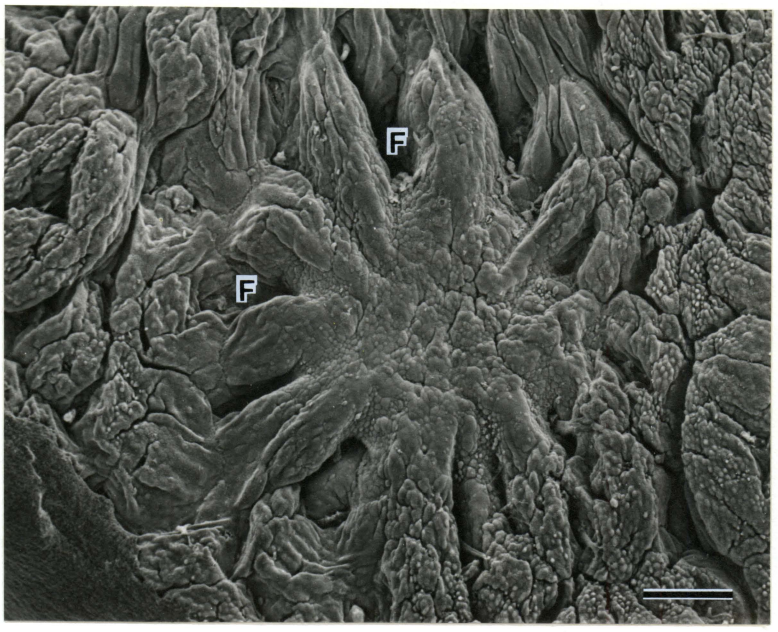
In most lymphoglandular complexes only the opening was seen on the surface. The epithelial lining of the opening consisted of enteroabsorptive cells and goblet cells. The view into these pits was in

Figure 23. Symmetrically shaped star-like structure; center surrounded by 12 furrows (F)

calf C2; PC patch; SEM; bar = 100 um

Figure 24. Distorted star-like structure

calf C3; PC patch; SEM; bar = 100 um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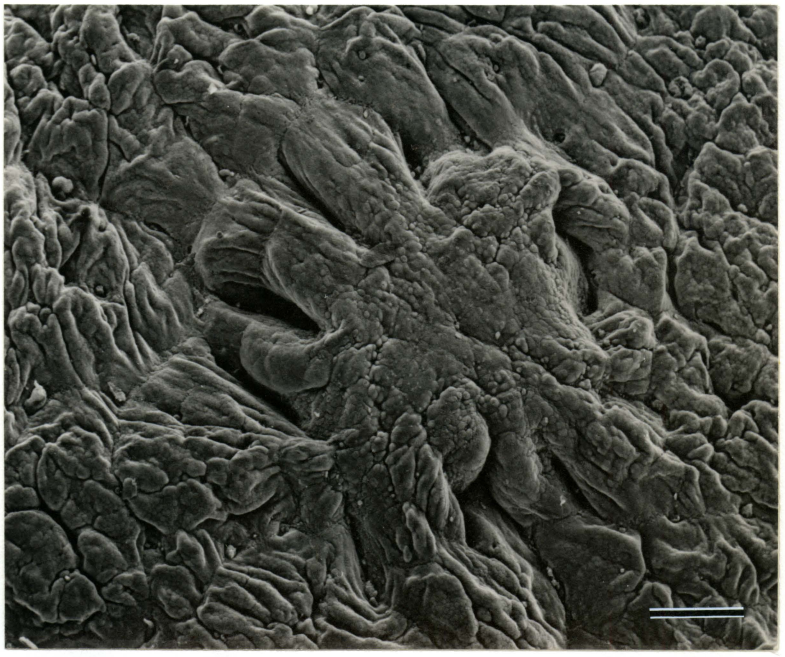
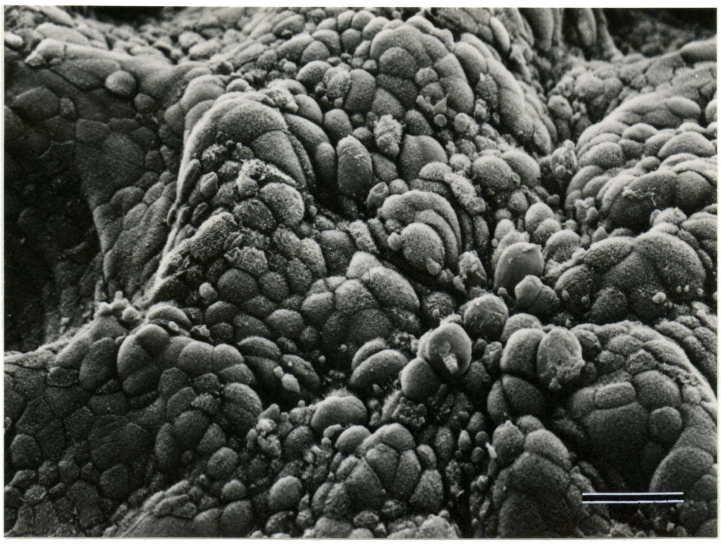


Figure 25. Center of star-like structure; FAE characterized by bulging cells of various shape and size

calf C3; IC patch; SEM; bar = 25 um

Figure 26. FAE in center of star-like structure; characterized by small cells with irregular microvilli, compatible with M cells

calf C2; PC patch; SEM; bar = 2.5 um



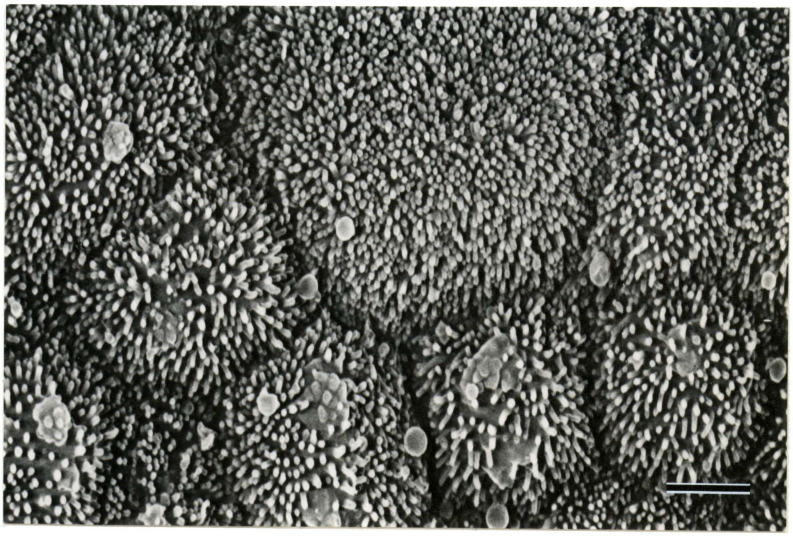
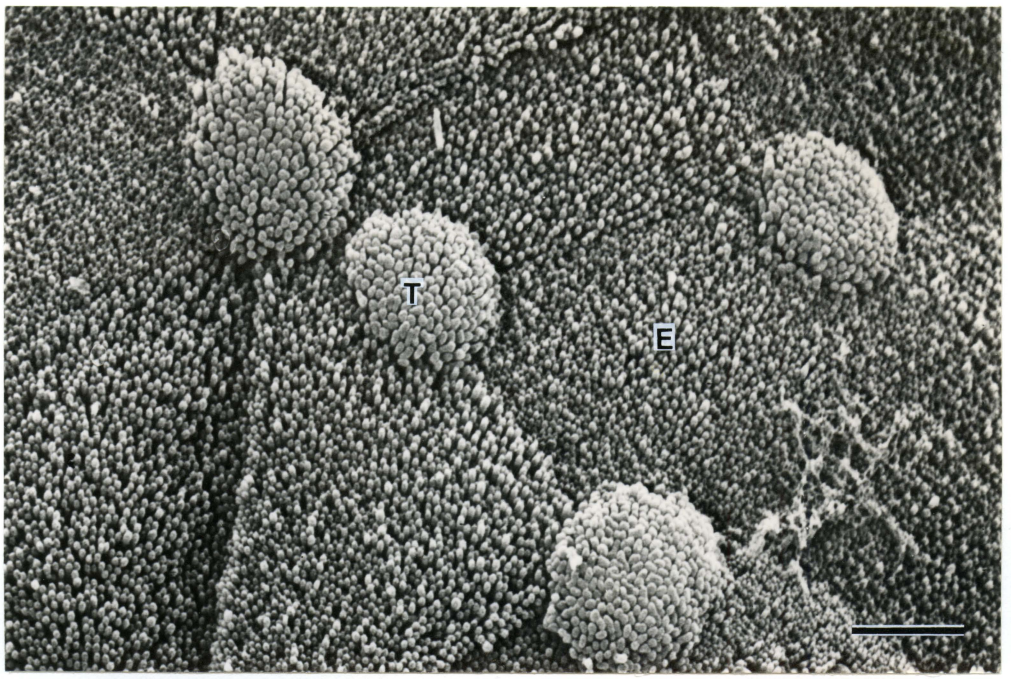


Figure 27. Periphery of star-like structure; enterocytes (E) and small round cells (T) with thick, densely packed, longer microvilli

calf C2; PC patch; SEM; bar = 2.5 um

Figure 28. Pear-shaped tufted cell (T) characterized by longer, thicker microvilli than in enteroabsorptive cells, well developed, straight microfilaments reaching from the tip of the microvilli to the basally located nucleus; scattered, small, electron dense granules

calf C2; PC patch; TEM; bar = 5 um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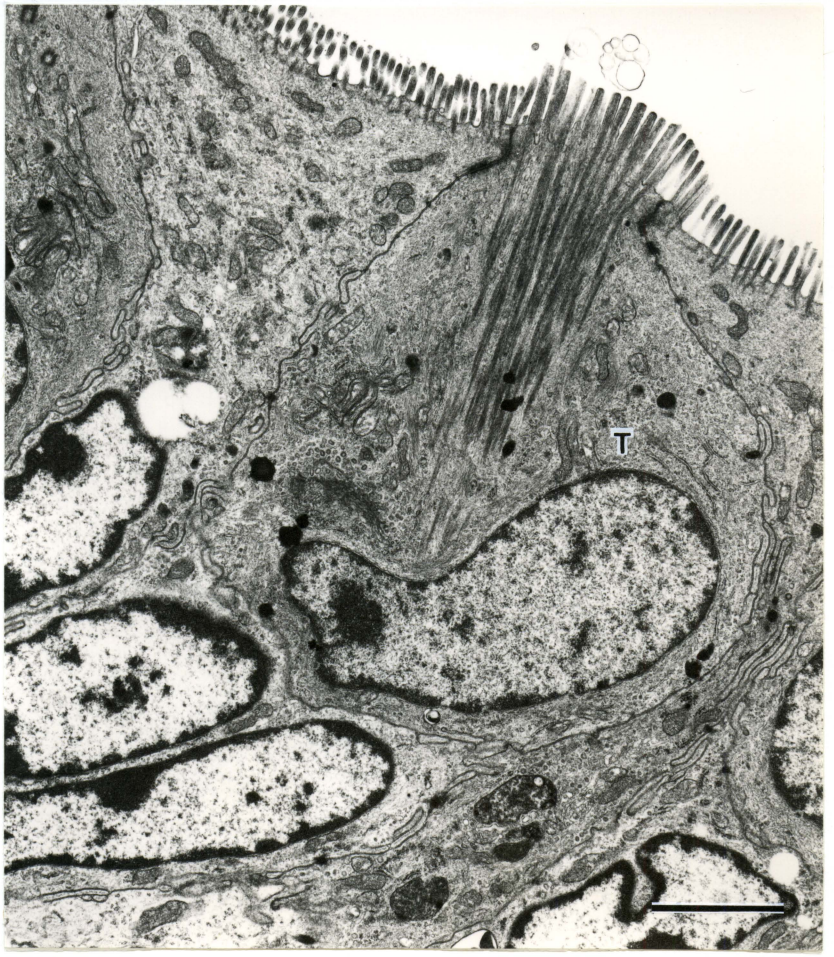
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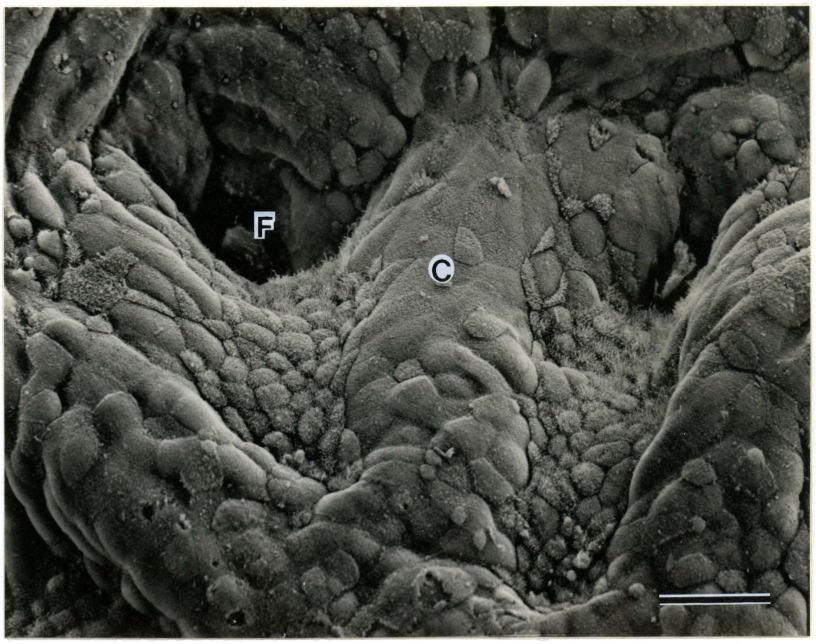
Figure 29. Crests (C) and furrows (F) of star-like structure; along the crests towards the periphery there is transition from FAE to enteroabsorptive cells; in the furrows are areas of morphologically distinct cells

calf C2; PC patch; SEM; bar = 25 um

Figure 30. FAE in furrow characterized by small, round cells with very long microvilli and a few enteroabsorptive cells

calf C2; PC patch; SEM; bar = 2.5 um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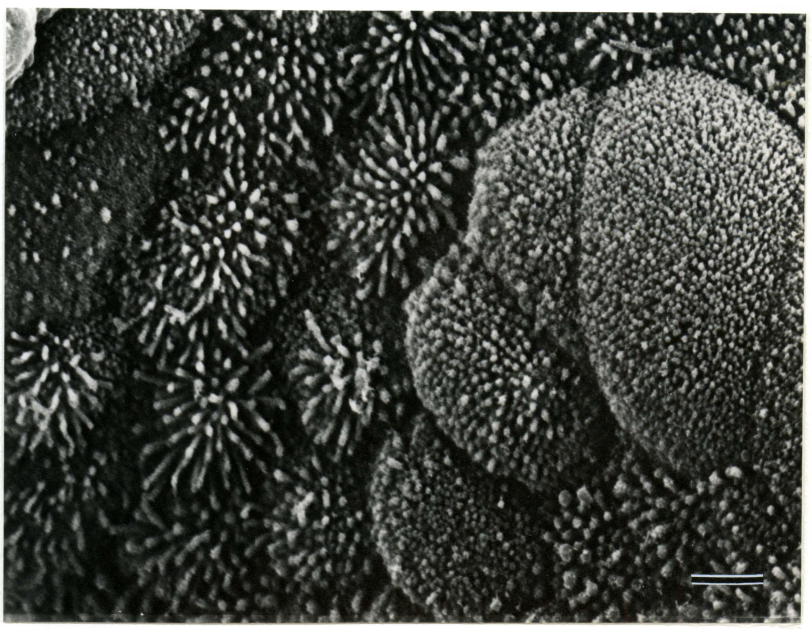


most instances obstructed by mucus plugs. A few openings, however, were wide enough to see the center in the depth, where dome-like protrusions were present (Figure 31). Other lymphoglandular complexes cut in tangential section confirmed the SEM observations and the results of the serial sections, that the lymphoglandular complexes contained a dome-like protrusion or fold covered with morphologically distinct follicle associated epithelium (FAE). In addition to enteroabsorptive cells, patches of M-like cells as described on the star-like structures were present in this FAE (Figure 32).

Star-like structures and lymphoglandular complexes were found in close association and star-like structures were seen at the rim of lymphoglandular complexes (Figure 31).

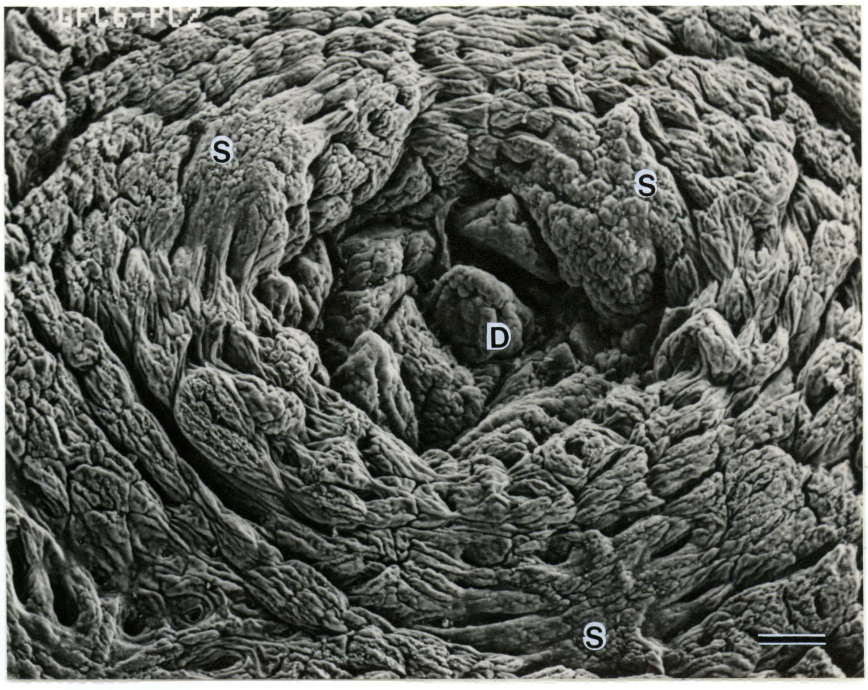
As already described for the histological sections of animals in group B, star-like structures and lymphoglandular complexes have a distinct distribution along the large intestine (4.2.1.). This distribution was, however, different in the young (3, 6, and 7 days), germfree calves of group C and in the 12-week-old, conventionally raised calves of group B. In calves of group C, mainly star-like structures were present at the patch at the ileocecal entrance. In the patch of the proximal colon both types (star-like structures and lymphoglandular complexes) were present, but the frequency of the star-like structures was higher. The solitary follicles distally of the patch in the proximal colon were alike in animals of group B star-like structures.

Figure 31. Lymphoglandular complex with wide opening; dome-like protrusion (D) in the center; three star-like structures (S) at the rim

calf C2; PC patch; SEM; bar = 125 um

Figure 32. Dome-like protrusion in depth of lymphoglandular complex (see fig. 31); patch of small, round, bulging cells (arrowheads) compatible with M cells on dome surface

calf C2; PC patch; SEM; bar = 25 um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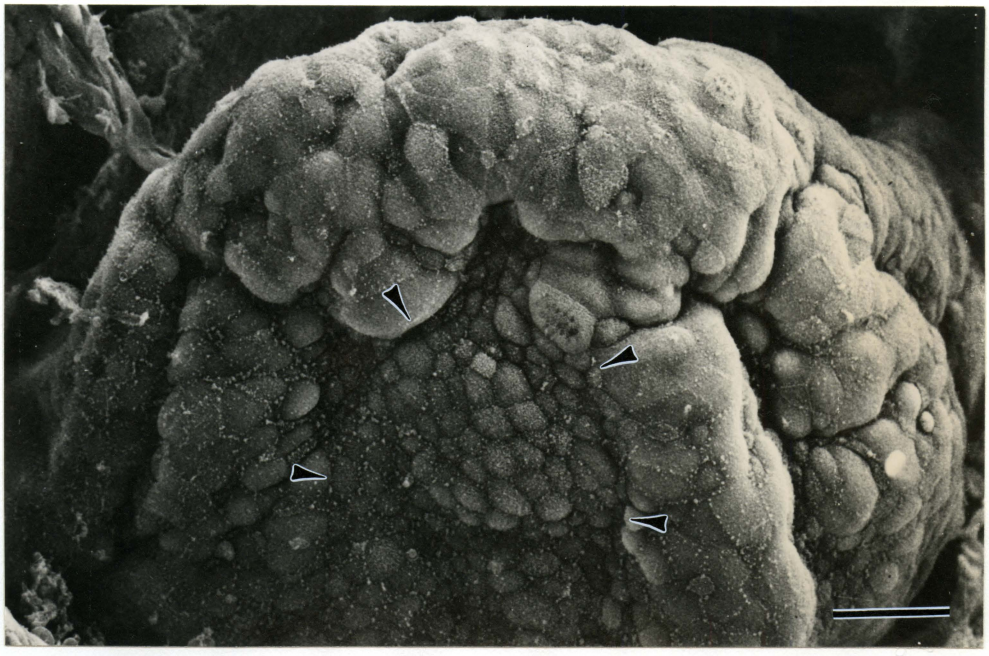


4.2.4. Transmission electron microscopy

Transmission electron microscopic observations revealed several different cell types within the follicle associated epithelium lining the surface and adjacent furrows of star-like structures and parts of the diverticula of the lymphoglandular complexes. The different cell types are described first separately. Their localization and frequency in these morphologically distinct structures are explained secondly.

4.2.4.1. Cell types In addition to the main population of mature and immature enterocytes and goblet cells, there were four cell types present, commonly not reported in the large intestinal epithelium.

(1) cells compatible with M cells as seen in the small intestine

These cells were distinctly different from adjacent enterocytes by their electron lucent cytoplasm. They varied in size and in the shape of their apical surface. Most of them bulged into the lumen (Figure 33), some apical surfaces were of neck-like appearence (Figure 34). In a few the surface was indented (Figure 35). The luminal projections of the plasma membrane changed without regularity. The following variations were found:

- no projections at all (Figure 36);
- few, thick, short microvilli, irregularly distributed over the apical cell surface;
- few, short, branching, bridging or fused microvilli;
- many, thick, short microvilli, evenly distributed over the apical cell surface (Figure 37);

Figure 33. FAE of dome-like protrusion in lymphoglandular complex; M-like cells with pale cytoplasm, bulging apical surface, few, short, microvilli, no distinct rootlets and numerous apical vesicles

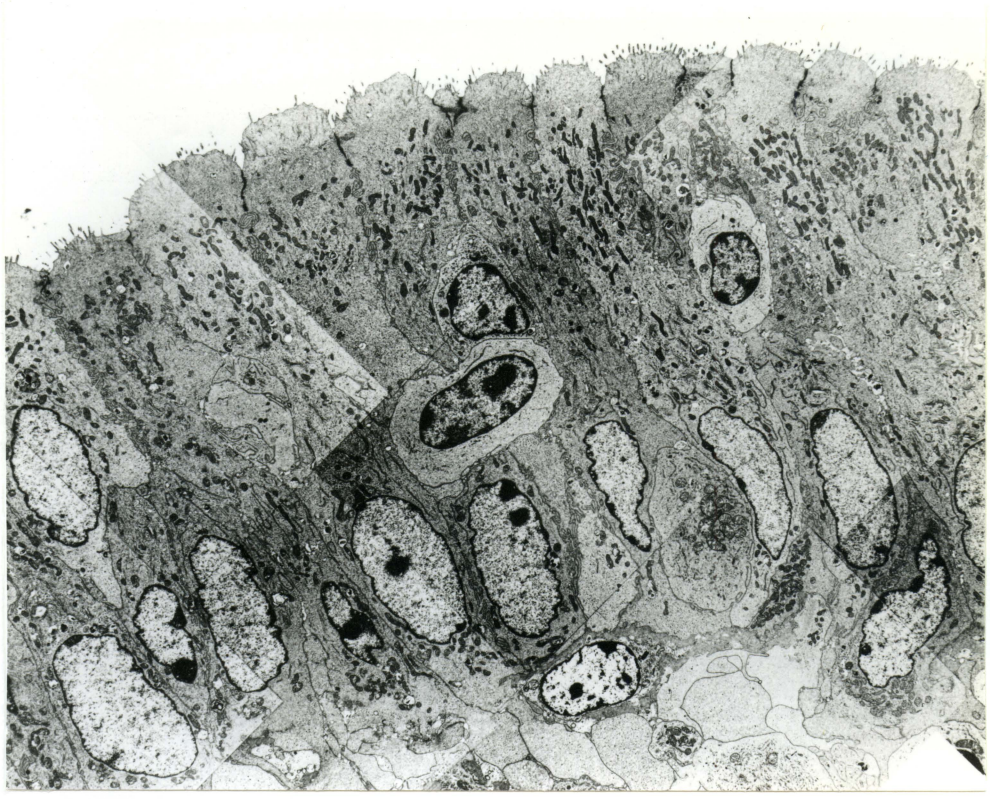
calf C2; PC patch; TEM; montage; low magnification

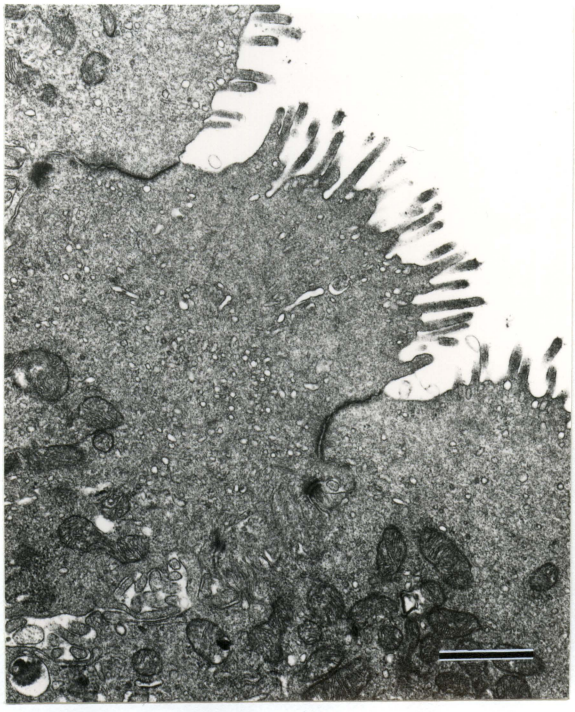
Figure 34. FAE of dome-like protrusion in lymphoglandular complex; M cells with neck-like protruding apical surfaces, few, irregularly long microvilli without microfilaments and rootlets; tubular invaginations between microvilli; numerous vesicles in apical cytoplasma

calf C2; PC patch; TEM; bar = 1 um

Figure 35. FAE on surface of star-like structure; M-like cell (M) is indented; adjacent cells were described as intermediate cells (I)

calf C2; PC patch; TEM; bar = 2 um





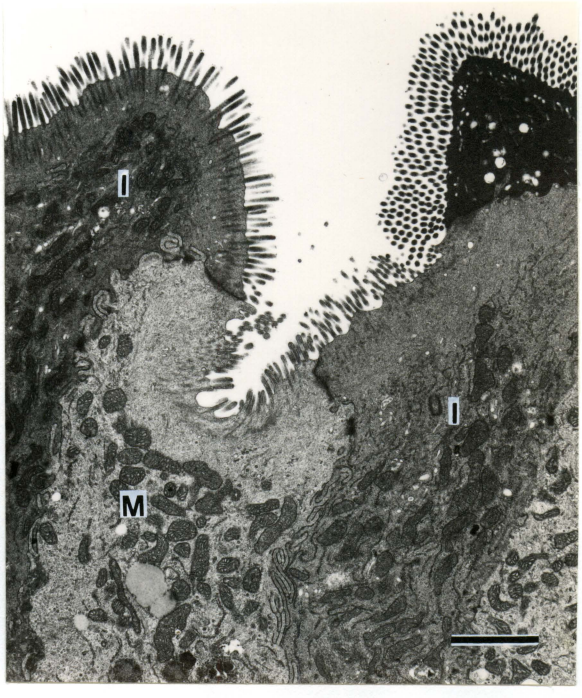

I
I
M

Figure 36. FAE in center of star-like structure; two M-like cells without surface projections and one M-like cell with a few, stubby microvilli; numerous vesicles in the apical cytoplasm; interdigitating lateral cell membranes

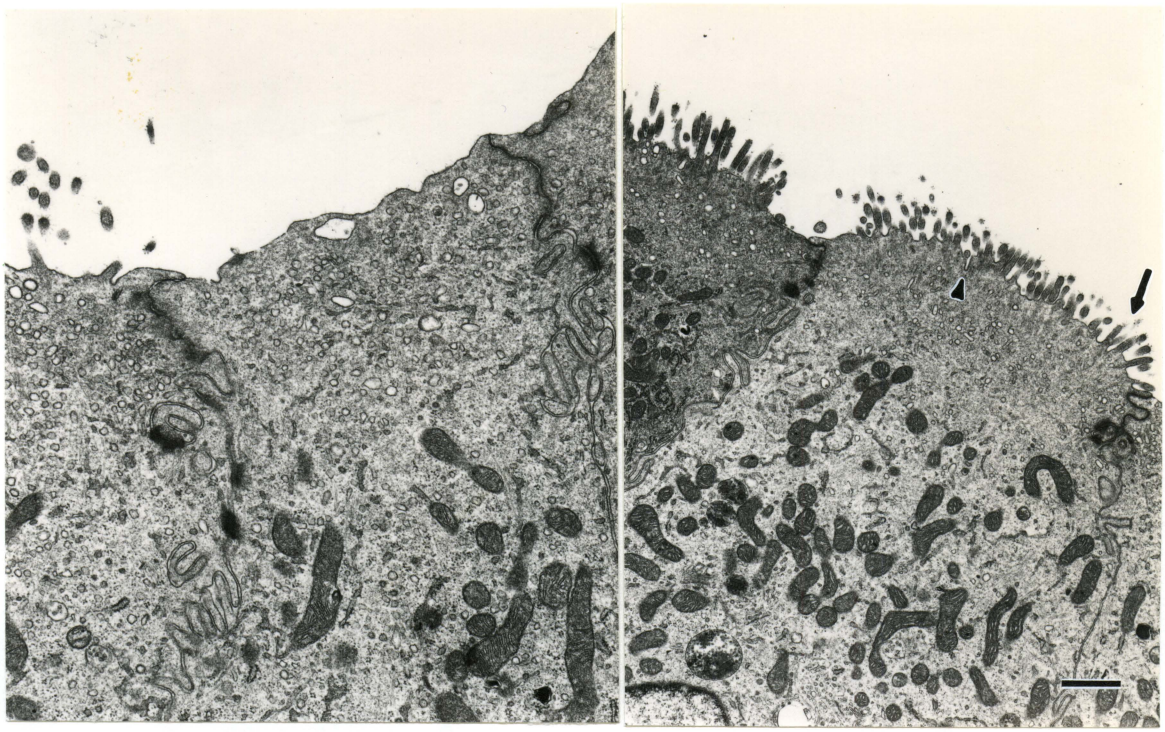
calf C3; IC patch; TEM; bar = 1 um

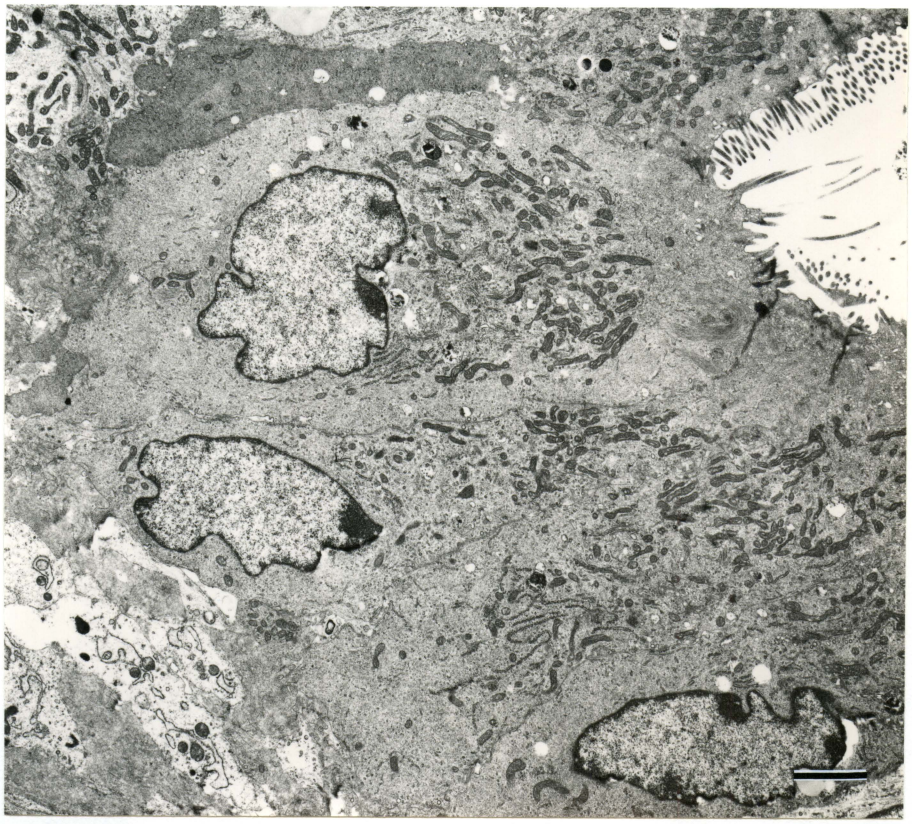
Figure 37. FAE in furrow of star-like structure; M-like cells with many thick, short microvilli evenly distributed over the surface; branching microvilli (arrow); no distinct microfilaments and rootlets; tubular invaginations of the apical plasma membrane between the microvilli (arrow); numerous vesicles in apical cytoplasm

calf C3; IC patch; TEM; bar = 1 um

Figure 38. FAE in furrow of star-like structure; M-like cell with bundles of very long microvilli and a few short, stubby microvilli; no microfilaments and rootlets are present; pale cytoplasm; numerous vesicles in apical cytoplasm

calf C2; PC patch; TEM; bar = 2 um





- a few, long microvilli arranged in bundles;
- short, thick microvilli and a few very long, thin microvilli (Figure 38).

In some cells the cytoplasm had herniated and formed one or several blebs on the surface (Figures 39 and 40). The microvilli contained few or no microfilaments.

The M-like cells were connected by tight junctions to adjacent cells. The lateral plasma membranes between M-like cells and between M-like cells and enterocytes were interdigitating. Numerous desmosomes were scattered along the lateral plasma membranes, more prominently at the cell periphery.

The association between the basal plasma membrane and the basal lamina was often obscured by interposed intraepithelial cells. A few M-like cells had cytoplasmic foot processes, which penetrated the basal lamina.

The apical plasma membrane had vesicular and tubular invaginations. Some of them were coated pits. Most cells had abundant vesicles and no organelles in the apical cytoplasm. The number of vesicles varied between cells. Some M-like cells were filled with large vacuoles interpreted to be fat. Numerous mitochondria were accumulated above the nucleus, which was in general in a basal position. Few lysosomes were seen. Intraepithelial cells indented the cytoplasm and deformed the cells.

(2) intermediate cells (Figures 41 and 42)

These cells carried some characteristics of enterocytes and some of

Figure 39. FAE in center of star-like structure; bulging cell characterized by sparse, short microvilli and several herniations of the apical plasma membrane ("blebs")

calf C3; IC patch; SEM; bar = 2.5 um

Figure 40. FAE in center of star-like structure; M-like cells with large "blebs" of clear cytoplasm extending from the apical surface into the lumen; sparse, short microvilli with indistinct microfilaments; microfilaments are maintained under the cytoplasmic "blebs"

calf C3; PC patch; TEM; bar = 1 um

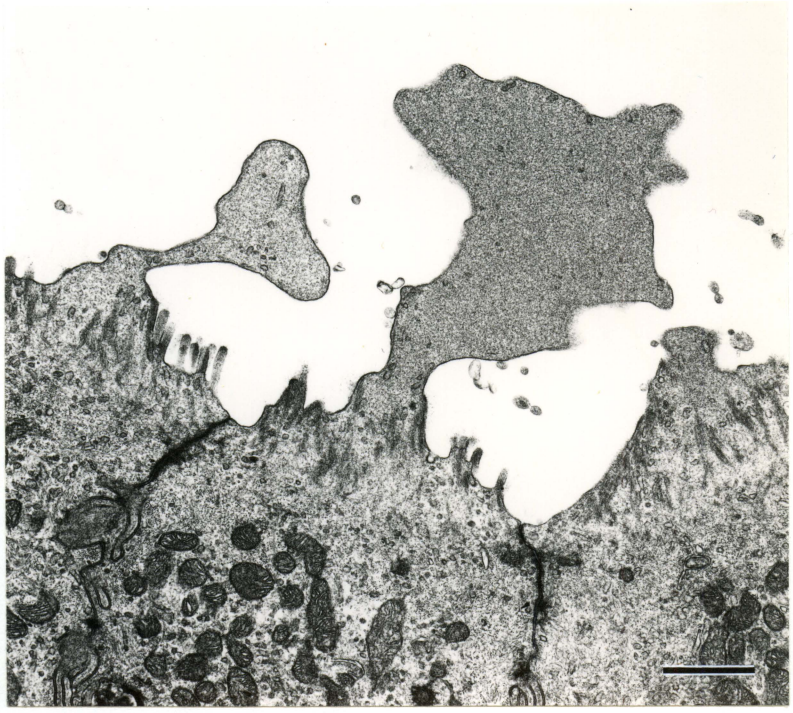
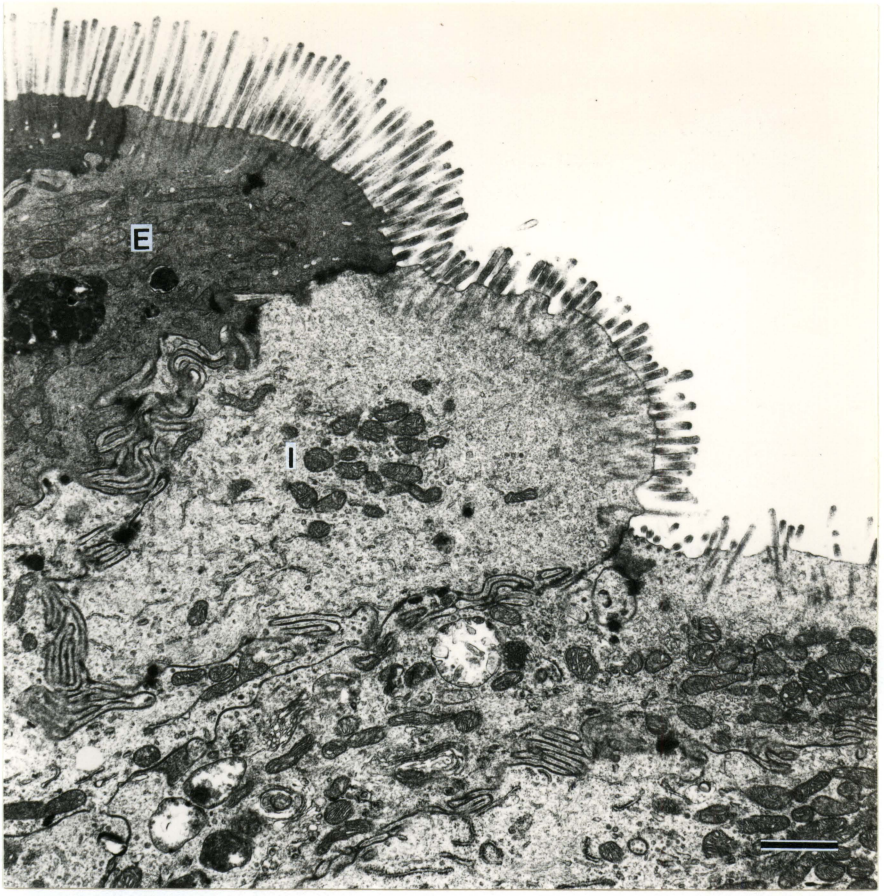


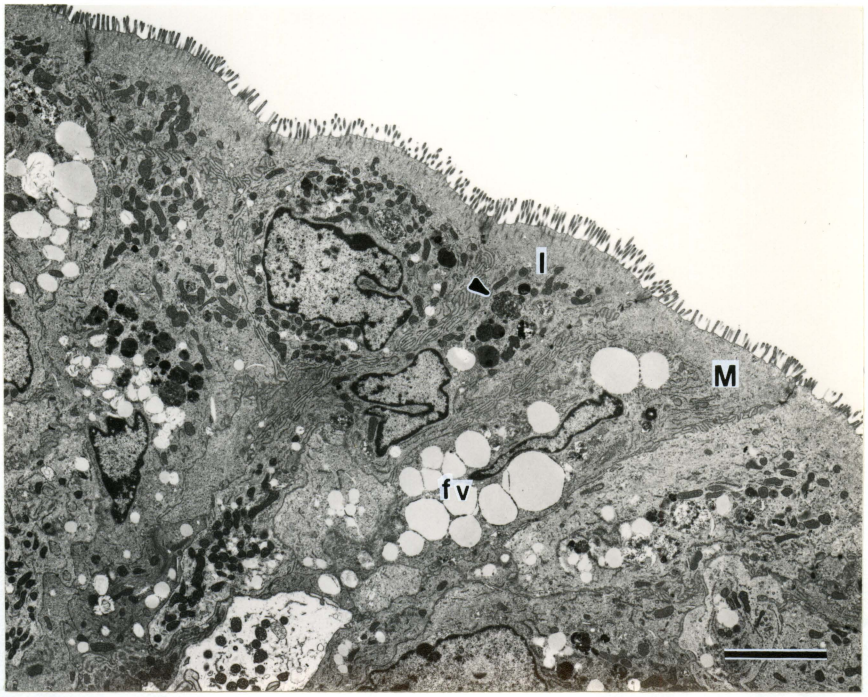
Figure 41. FAE in center of star-like structure; with intermediate cell (I) and enteroabsorptive cell (E); intermediate cell characterized by pale cytoplasm, densely packed, irregularly long microvilli with rootlets, uneven apical plasma membrane and few vesicles in the apical cytoplasm; interdigitating lateral plasma membranes

calf C3; PC patch; TEM; bar = 1 um

Figure 42. FAE in furrow of star-like structure with intermediate cells (I) and M-like cell (M); clear vacuoles (fv) interpreted to be fat in both cell types, large phagolysosomes (arrowhead) in intermediate cells only; notice the lobulated nuclei of the intermediate cells

calf C3; PC patch; TEM; bar = 5 um


E
I


I
M
fv

M cells. Their microvilli were nearly as long and dense as microvilli on enterocytes, and had microfilaments and rootlets. They had, however, pale cytoplasm, their apical plasma membrane was uneven and no distinct terminal web was present. The apical cytoplasm was devoid of cellular organelles like in M cells, but had few vesicles and contained interwoven microfilaments. Some cells had no microvilli, but rootlets in the apical cytoplasm were still present. Large membrane bound phagolysosomes were frequent (Figure 42). Like in M cells, numerous mitochondria were present above the nucleus, which was lobulated.

(3) tufted cells (Figure 28)

These pear-shaped cells reached the intestinal lumen with a narrow apex. They had very dense, thick microvilli longer than those on enterocytes. These microvilli contained long, straight microfilaments reaching the supranuclear region. Caveolae and tubules were present in the apical cytoplasm. The nucleus was often kidney-shaped. A few small, electron dense granules were seen throughout the cell.

(4) enterochromaffin cell

A few of these cells were interspersed in the diverticular epithelium. They were triangular shaped and had numerous electron dense granules between the nucleus and the basement membrane.

4.2.4.2. Distribution and frequency

(1) star-like structures

The epithelium of the furrows adjacent to the lymphoid follicles was characterized by M cells. Occasionally cells with a few very long, thin microvilli were found. At the base of the

furrows, there was a sudden transition to enterocytes and goblet cells.

The center of the star-like structures was lined by intermediate cells and M cells in about equal numbers. Many of these cells had one or more blebs of clear cytoplasm on the surface. A few mature enteroabsorptive cells were scattered, but no goblet cells were present. On the crests, M cells, intermediate cells, enterocytes, and goblet cells were mixed and a slow transition to a population of enterocytes and goblet cells was seen towards the periphery.

(2) lymphoglandular complexes

The surface openings of the diverticula were lined by mature enterocytes and goblet cells. The deeper parts of the diverticula consisted of immature enterocytes and goblet cells. A few enteroendocrine cells were present. The epithelial lining of the dome-like protrusions and folds was characterized by M cells and few intermediate cells (Figure 43). Goblet cells were absent. Tufted cells were interspersed between M cells and enterocytes. The presence of tufted cells appeared to be a special feature of the lymphoglandular complexes.

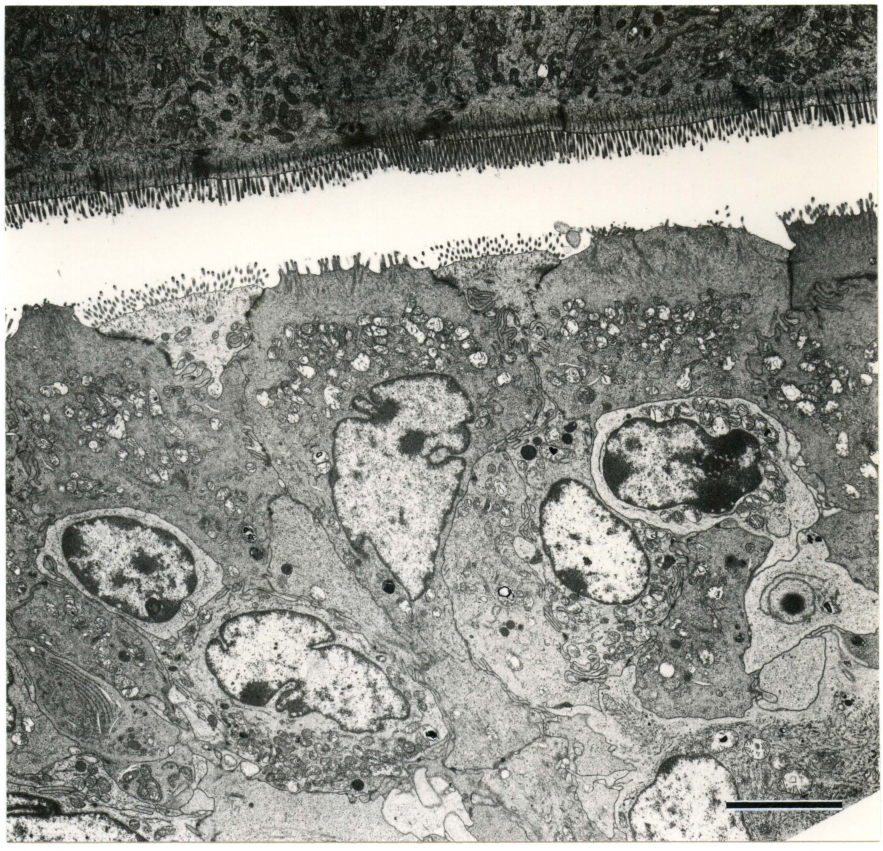
The follicle associated epithelium contained numerous intraepithelial cells. Some of them were small, round, with pale cytoplasm, few organelles and a large round nucleus. They were interpreted to be lymphoblasts. Macrophages and dendritic cells were common (Figure 44). They were large cells with a central nucleus and

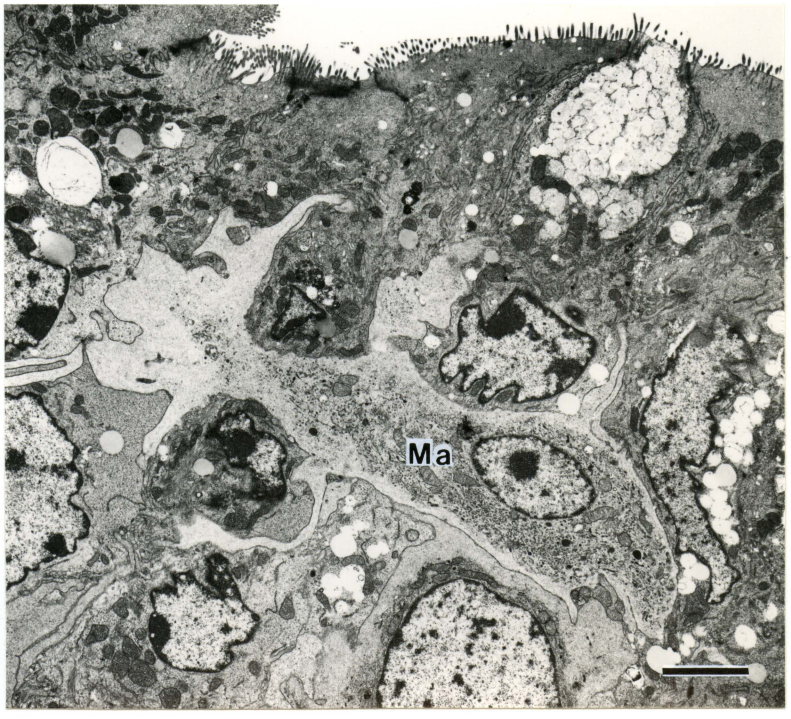
Figure 43. FAE on tip of dome-like protrusion in lymphoglandular complex; cells in FAE are paler compared to the enterabsorptive cells of the diverticulum; cells of FAE have few, shorter microvilli and no distinct terminal web; numerous IECs are invaginated into and sandwiched between M-like cells

calf C3; PC patch; TEM; bar = 5 um

Figure 44. Macrophage (Ma) in FAE on center of star-like structure in contact with several epithelial cells by long cytoplasmic protrusions; cellular organelles centered around the nucleus

calf C2; PC patch; TEM; bar = 5 um




Ma

pale clear cytoplasm which formed long protrusions between the epithelial cells. Cellular organelles, lysosomes, phagolysosomes and multivesiculated bodies were clustered around the nucleus. Several Golgi complexes were present. Intraepithelial cells occurred singly or in clusters. They were invaginated into or sandwiched between M cells and often in contact with several cells by deep indentations without any cellular connections.

5. DISCUSSION

Investigations to study in detail the distribution of mucosa associated lymphoid follicles and the surface characteristics of follicle associated epithelium in the small and large intestine were initiated, when Woode et al. (1984) described a bovine astrovirus, which selectively destroys M cells in domes above Peyer's patches in the ileum without causing obvious clinical signs of disease. Preliminary results of a reevaluation of samples from colon of gnotobiotic calves experimentally infected with astrovirus II suggest, that lesions are present in the follicle associated epithelium of the star-like structures of the IC and PC patch (J. F. Pohlenz, Dept. Vet. Pathol., ISU, personal communication). A selective destruction of M cells, which are cells in the FAE highly specialized for antigen transport, may interact with the induction of immunity or tolerance against food antigens or endogenous intestinal bacteria. Such subtle lesions in the FAE above the mucosa associated lymphoid nodules may cause or contribute to local hypersensitivity reactions in the intestinal mucosa or result in systemic food allergies.

Other viral and bacterial infections may also originate from these unique sites of the mucosal immune system and result in specific lesions as seen in diverticular disease of humans (Kealy 1976b) or bovine virus diarrhea (Ramsey 1956; Kent and Moon 1973; Barker and Dreumel 1985) and cryptosporidiosis (Pohlenz et al. 1984b) in the bovine species. Therefore the examination of large intestinal wall containing mucosa

associated lymphoid nodules is important for diagnostic purposes.

In the calves examined, patch-like accumulations were consistently found at the ileocecal entrance, in the proximal colon 12.5 to 42 cm (depending on the length of the large intestine) distal to the ileocecal entrance, and in the rectum, while the frequency and distribution of solitary lymphoid follicles was rather variable. These results confirmed previous studies (Carlens 1928; Rooney 1956; Hebel 1960). Therefore the patches would be preferential locations for tissue collection.

The distribution of aggregated lymphoid tissue along the large intestine is similar in all ruminants examined (sheep and cattle) (Carlens 1928). It is, however, markedly different in other animal species. Solitary follicles only were described in humans, pigs and horses, while Bland and Britton (1984) found patches only in the colon of rats. This may be due to differences in the embryological development of the spiral convolute of the bovine large intestine (Asari et al. 1985).

It was evident in the dilated loops of animals in groups A and B, that the colonic diameter was reduced at the transition between the proximal and the spiral loop of the ascending colon, thus giving the large intestine in this area a funnel-shaped appearance. By this the flow of ingesta will be altered and thereby the duration of contact between the antigens and the lymphoid nodules of the patch in the proximal colon will be changed. Detailed studies of intestinal motility in this area are needed to determine the flow of ingesta.

The percentage of intestinal area occupied by mucosa associated lymphoid follicles was relatively consistent in all calves examined regardless of age, health status, breed or sex. The average amount of aggregated lymphoid tissue in the large intestine was 7.8%. This compares to an average of 8.6% in the small intestine (Liebler 1985).

The variability of the absolute amount of ALT and especially of the number of solitary follicles, raises the question, whether the development of lymphoid nodules is embryologically predetermined or develops post partum. The segment of large intestine adjacent to the patch in the proximal colon, in which solitary lymphoid follicles were found, varied from 0 to 106 cm between two germfree animals of the same breed (Angus) and age (Table 5). This might suggest that the presence of lymphoid nodules may not be age, breed, or infection dependent but rather predetermined in the individual animal.

An influence by intrauterine viral infections can, however, not be excluded, because none of the dams was surveyed during the pregnancy for viral infections or seroconversion and none of the calves were tested post partum for antibodies against bovine virus diarrhea virus and other infections. In cattle which develop bovine virus diarrhea in their later life, lesions associated with small intestinal Peyer's patches and with the ALT in the proximal colon are significant. The lymphoglandular complexes in the proximal colon are cystic, dilated and filled with mucoid material. The lymphoid follicles are atrophic and the FAE is flattened (Barker and Dreumel 1985). It has not been determined whether decreased or impeded development of the mucosa associated lymphoid

nodules can be caused by infection with a non cytopathogenic bovine virus diarrhea virus during prenatal life.

Destruction of lymphoid follicles in the bursa of Fabricius of the chicken was induced by experimental application of testosterone (Lupetti et al. 1984). When in this process the FAE was also destroyed, no redevelopment of lymphoid follicles was observed. A similar effect might by caused in calves by hormonal growth promotors, mycotoxins or viral infections (e.g. bovine virrus diarrhea) resulting in a congenitally reduced amount of mucosa associated lymphoid tissue.

Comparison of histological sections of animals in groups B and C suggested that the size of each lymphoid follicle and the development of germinal centers may be influenced by contact with antigen. The question, whether this secondary development can compensate for a congenitally lower amount of ALT, is open and needs further investigation.

The amount of ALT may be responsible for differences between individuals in resistance to diseases. A reduced number of mucosa associated lymphoid nodules may cause increased susceptibility to disease or predisposition to allergic reactions (Ngan and Kind 1978; Cebra et al. 1980; Richman et al. 1981). On the other hand, when the FAE of the ALT is considered as a channel for infection (Wolf et al. 1981, 1983), then a reduced number might be associated with decreased incidence of infection (Owen 1983).

The morphology of lymphoid nodules varies within the animal species, between animal species, and along the intestinal tract.

In the bovine species both, lymphoid nodules in the lamina propria, named star-like structures because of their SEM appearance, and lymphoglandular complexes, are present in the large intestine. The relative frequency of these two types varied between animals in groups B and C. More star-like structures were found in the young, germfree animals. Whether this is due to age, development of the mucosal immune system of the newborn in comparison to the weaned animal, nutritional influences, or antigen contact, is unknown. No intermediate forms were seen, and even in the older calves, the solitary follicles adjacent to the patch in the proximal colon were only formed by star-like structures.

In other animal species in general, either lymphoid nodules in the lamina propria or lymphoglandular complexes have been described exclusively. In pigs lymphoglandular complexes were found only. This was confirmed by extensive histological examination of randomly collected lymphoid nodules from the large intestine of 5 to 13 week-old pigs (D. C. Morfitt, Dept. Vet. Pathol., ISU, personal commununication). In contrast, in the rat the colonic lymphoid patches consist of lymphoid follicles in the lamina propria and submucosa without epithelial diverticula (Bland and Britton 1984).

The mucosa associated lymphoid nodules along the alimentary tract, tonsils, Peyer's patches, appendix, sacculus rotundus, cecal tonsil, colonic lymphoid patches and bursa of Fabricius have different spatial arrangement between the lymphoid tissue and the epithelium. The star-like structures in the large intestine of calves resemble the lymphoid

follicles present in the Peyer's patches and solitary follicles in the small intestine (Grau and Walter 1967; Landsverk 1984) and in the sacculus rotundus (Snipes 1978). They consist of one lymphoid follicle with germinal center, corona and dome, covered by follicle associated epithelium. The lymphoglandular complexes are rather comparable to the oral tonsils (Olah 1978).

Depending on animal species and localization, typical branching patterns of the epithelial diverticula exist (Grau and Walter 1958; Kassay and Sandor 1962). The large intestinal lymphoglandular complexes in the bovine, which are characterized by one diverticulum branching underneath the muscularis mucosae (Grau and Walter 1958), resemble the monocryptic, pharyngeal tonsil of the rabbit (Olah and Everett 1975; Olah 1978). In oral tonsils, however, no folds covered by FAE in the depth have been described.

The distribution of these morphologically different types of lymphoid nodules in the alimentary tract raises the question whether different functions are associated with star-like structures or lymphoglandular complexes or whether the presence of similar morphological structures, for instance lymphoglandular complexes in oral tonsils and in colonic lymphoid patches, indicates a similar functional role. Reports of colonic diseases describe a predominant involvement of lymphoglandular complexes. Clark (1970) suggests, that lymphoglandular complexes weaken the muscularis mucosae and promote the herniation of epithelium in ulcerative colitis of humans. Scott (1982) speculates that by entering the lymphoglandular complexes, invasive agents and

especially parasites can avoid the barrier of the lamina muscularis mucosae.

Both star-like structures and lymphoglandular complexes contain areas of specialized follicle associated epithelium. This FAE is in general associated with dome-like projections, which are exposed to the intestinal surface in star-like structures or hidden from the surface in lymphoglandular complexes. This may allow for contact with different types of antigens and different duration of antigen exposure. Further studies of the physiology and pathophysiology are needed to investigate the function of these units and their potential differences.

The follicle associated epithelium on the star-like structures and in the lymphoglandular complexes contains cells compatible with M cells in the domes of small intestinal Peyer's patches. They share the following characteristics with the M cells, which were originally described by Owen and Jones (1974a) and recently reviewed by Wolf and Bye (1984) and Egberts et al. (1985):

- electron lucent cytoplasm;
- short, thick microvilli without or with only a few microfilaments;
- numerous apical vesicles;
- association with many intraepithelial cells.

The presence of M cells in these unique colonic units, has not yet been described for the bovine species and has only been found in pigs (D. C. Morfitt, Dept. Vet. Pathol., ISU, personal communication). In rats, the cells of the FAE had apical surface characteristics similar to

other enterocytes, they had, however, special uptake capacity for India ink just as M cells do. Therefore they were termed M-cells (Bland and Britton 1984).

In the bovine species also cells with ultrastructural characteristics of both M cells and enterocytes were found. These were named intermediate cells. One can hypothesize,

(1) whether they are a morphologically and functionally different cell type or

(2) whether they constitute intermediate developmental stages between M cells and enterocytes.

Their number is increased on the center of the star-like structures in comparison to the furrows and to the folds and domes of the lymphoglandular complexes. When the current concepts of M cell origin and renewal are considered (Smith et al. 1980; Bhalla and Owen 1982; Bye et al. 1983, 1984), then the first hypothesis is rather likely. A different functional role is suggested by the numerous, large phagolysosomes present in most intermediate cells. Some of these resemble remnants of dead cells. Thus intermediate cells may act as a route for transport of aged cells into the intestinal lumen.

In furrows of the star-like structures, small areas of cells with very long microvilli appearing as cilia were seen in the scanning electron microscope. Transmission electron microscopic investigations revealed cells with the characteristics of M cells in these areas. On a few of these M-like cells, however, single long microvilli without microfilaments were found. Therefore we assumed that most of the long

microvilli may have been lost during the thin sectioning procedure. It is yet undetermined, whether these cells with their extremely long microvilli serve special functions in the mucosal immune systeme. Similar cells were also described as fungiform cells at the entrance of the tonsils (Olah and Everett 1975).

In the diverticula and on the folds of lymphoglandular complexes, tufted cells were found. In the bovine species, this cell type has been reported to be scattered at the mucosal surfaces of the repiratory tract (Allan 1978) and the glandular stomach (Weyrauch 1979) and in the excretory ducts of liver and pancreas, in the conjunctiva and in the organ of Jacobson (Weyrauch 1979). These cells have not been described in the bovine colon, but are considered an occasional finding in the murine colon (Silva 1966). No definite function of the tufted cells has been established yet. Their specialized microvilli lead to speculations about a chemo- or mechanoreceptive role (Nabeyama and Leblond 1974; Weyrauch 1979). Because of the presence of small electron dense granules, an endocrine function is also discussed (Weyrauch 1979). In the lymphoglandular complexes they may contribute to the coordination of the motility of the surrounding smooth muscle, and therefore regulate the flow of ingesta through the epithelial diverticula.

The results of the histological and ultrastructural examinations revealed morphological similarities between the lymphoid nodules in the colon of calves and lymphoid nodules in other parts of the alimentary tract with established immunological functions.

Further investigations are needed to establish functional

similarities. They should include studies of absorptive capacity for and transport of macromolecular tracer materials. The results might help to confirm that the morphologically M-like cells function also like M cells. They would also reveal, whether the intermediate cells have similar functional capacities as M cells.

6. SUMMARY

The localization, distribution and area of aggregated lymphoid tissue in the large intestine were determined in fifteen 3 day to 12 week old calves of different breeds. Seven of these originated from necropsy material (group A), five were healthy, conventionally kept animals (group B) and three were germ-free raised calves (group C).

Patches of lymphoid nodules were present in all calves at the ileocecal entrance (ICE), 12.5 to 42 cm distal of the ileocecal entrance in the ascending colon (PC patch) and in the terminal rectum. The PC patch varied from 4 to 30 cm in length. Solitary follicles were found in the cecum of three calves, between ICE and PC patch in four calves, adjacent to the PC patch extending up to 201 cm along the colon in a 12 week old calf and in the terminal rectum.

The mucosa associated lymphoid follicles occupied, on the average, a total of 7.8% of the large intestinal wall in animals of group A, with 0.6% of it at the ICE, 4.8% in the proximal colon and 2.4% in the rectum.

Light and scanning electron microscopy revealed that the lymphoid nodules in the large intestine occur as two morphologically different units:

(1) star-like structures;

(2) lymphoglandular complexes (seen as pits on the surface).

The spatial arrangement between the epithelium and lymphoid tissue of these units was evaluated in 3 um thick serial sections. The star-like structures were characterized by lymphoid follicles in the lamina

propria and the tunica submucosa and lymphocytes in the lamina propria above the lymphoid follicle covered by a distinct follicle associated epithelium, which contained no goblet cells, but numerous intraepithelial cells. They were surrounded by wide crypts, named furrows, which were on the upper half also lined by FAE. In the lymphoglandular complexes, the lymphoid nodule, often consisting of several germinal centers, was almost completely located in the tunica submucosa. One epithelial diverticulum entered through the muscularis mucosae and branched inside the lymphoid nodule. In the center, a fold or dome-like protrusion covered with distinct FAE as described for the star-like structures was present.

Ultrastructural (SEM and TEM) investigations in three, 3 to 7 day old, germ-free calves demonstrated, that the FAE was composed of different cell types. Most frequently cells compatible with M cells in the small intestine of calves, named M-like cells, and cells with morphological characteristics of both M cells as well as enteroabsorptive cells, named intermediate cells, were found. Enteroabsorptive cells and a few tufted cells were scattered. The FAE of the star-like structures and of the LGC differed only in the relative frequency of the cell types. In the furrows of the star-like structures, cells with M cell characteristics, but additionally a few very long microvilli without microfilaments were present.

Further studies are needed to compare the functions of the different cell types found in the FAE of lymphoid nodules in the large

intestine with M cells found in the FAE of lymphoid nodules in the small intestine.

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