# DEVELOPMENT OF THE INTESTINAL TRACT LYMPHATIC SYSTEM IN GOATS



BY

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# DECLARATION

I, REMMY JOHN ASSEY, do hereby declare to the Senate of Sokoine University of Agriculture that, this dissertation has not been submitted for a degree award to any other University and it is my original work.

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## ABSTRACT

Gross and microscopic studies of the Gut-Associated Lymphoid Tissue (GALT) and Mesenteric Lymph Nodes (MLN) were performed on ten newborn kids, fifteen 3 months old kids and fifteen 8 - 12 months old goats. Morphological changes of the intestinal tract lymphatic system with age, the relationship of morphological changes between and within intestinal lymphoid organs in particular the Peyer's patches and the MLN during growth, possible role of the Peyer's patches in the immune system of the caprine and lymph flow from the intestine were studied.

Kids were born with an average of 35 histologically mature Peyer's patches in the jejunum (Jejunal Peyer's patches-JPP) which ranged in size from five follicles to 4.5 cm. long patches. A long Peyer's patch whose width was not uniform was also found in newborn kids. It was broader caudally and it extended from the jejunum to the ileocecocolic junction (Jejunal-Ileal Peyer's Patch-JIPP). The average length of the JIPP was 0.83 m. Two round Peyer's patches were found in the colon (Colon Peyer's Patches - CPP ). The Peyer's patches were present on 17% of the intestine length.

The shape of the follicles and the interfollicular area breadth were different in the JPP, JIPP and CPP. Interepithelial lymphocytes (IEL) number on domes decreased from cranial end to caudal end of the intestine. The trend was not observed on the villi epithelium. Intestinal tract villi epithelial cells in kids

which had not suckled were columnar and had their nuclei in the apices.

In average newborn kids had 5 (1 - 10) jejunal, 1 (1 - 3) ileocecocolic and 3 (2 - 6) colic lymph nodes. Their total weight was 0.11% of post-slaughter animal body weight corrected for stomach compartments weight. The MLN were immature. Within twelve hours after birth and colostrum ingestion, secondary lymphoid nodules appeared on the MLN.

At three months age, kids had about 38 JPP with size ranging from five follicles to patches 5.4 cm. long. The width of the JIPP was also not uniform, and it was 1.4 m. long. There were 2 CPP. Peyer's patches were present on about 18% of the intestinal length. There were 3 (1 - 9) jejunal, 1 (1 - 2) ileocecocolic and 3 (2 - 5 ) colic lymph nodes. Their total weight was 0.29% of the post-slaughter animal body weight corrected for stomach compartments weight. The MLN were histologically mature.

Goats of 8 - 12 months age had about 33 JPP and 2 CPP. In a single observation, three small patches were found in the cecum. JIPP had grossly atrophied and measured about 1.5 m. in length. Peyer's patches were present on about 13% of the intestine length. There were 5 (1 - 12) jejunal, 2 (1 - 4) ileocecocolic and 4 (2 - 5 ) colic lymph nodes. Their total weight was 0.16% of the post-slaughter animal body weight corrected for stomach compartments weight. The MLN were histologically mature. There was a general trend of a decrease of IEL number

on the domes and villi from cranial end to caudal end of the intestinal tract.

Domes epithelia were columnar and had a mean of 1.2 ± 0.3, 1.8 ± 0.5 and 0.2 ± 0.1 goblet cells per dome in the newborn, 3 months and 8 - 12 months old goats, respectively. IEL number on dome and villi showed a general increase with age.

There was no significant difference ($P > 0.05$) between the means of the JPP number of the three age groups. There was also no significant correlation neither between length of Peyer's patches and intestine length nor between MLN and body weights. There was no significant difference ( $P > 0.05$) between the proportion of intestine on which the Peyer's patches were present in newborn and 3 months old kids, while there was significant difference ( $P < 0.05$) between these two groups and the 8 - 12 months old group. There was significant difference ($P < 0.05$) between the means of the MLN weight of the three age groups and there was also significant difference ($P < 0.05$ ) between the means of MLN-body weight proportion of the three age groups.

The JPP, JIPP and CPP had a common character of being histologically mature at birth, but differed in their follicular histology and life history. The JPP follicles were short, broad and pear-shaped, had large interfollicular areas and did not show signs of atrophy with age. The JIPP follicles were cylindrical or sac-shaped and atrophied with age like the thymus and avian Bursa of Fabricius. The atrophy of the JIPP started

at the mid portion of the patch. Similarity between the caprine JIPP and the avian Bursa of Fabricius with regard to their prenatal maturation, their proximity to the gut and their postnatal involution is compatible with the assumption that the caprine JIPP is the equivalent of the avian Bursa Fabricii.

A bean-shaped jejunal lymph node caudally located on the group chain was found in three out of seven animals. The node with the single ileocecocolic node drained the JIPP. Efferent from the bean-shaped jejunal node joined efferent of the ileocecocolic lymph node and the vessel formed joined the efferent of the colic lymph nodes to form the colic trunk. In three animals, efferents of one or two colic lymph nodes drained into the ileocecocolic lymph node. The colic trunk confluenced with the jejunal trunk to form the intestinal trunk which emptied into the cisterna chyli. Goats did not have visceral trunk.

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# CHAPTER 1

# INTRODUCTION

## 1.1 The lymphatic system in general

The lymphatic system is an organized integrated defense mechanism for the protection of the animal's body against pathogenic organisms and other injurious substances. The organs making up the effector arm of the system are the primary lymphoid organs- thymus, bone marrow, avian Bursa of Fabricius or a mammalian equivalent and secondary lymphoid organs - spleen, lymph nodes and the subepithelial lymphoid accumulations of the respiratory and gastrointestinal tracts.

The lymphoid organs may be invariably localized, e.g. spleen, tonsils, thymus and lymph nodes or variably located in the body system e.g. the solitary and aggregated lymphoid follicles. Although the lymphoid organs are anatomically discrete, they are strategically placed accumulations of migratory lymphocyte pool.

## 1.2 Lymphoid tissue of the gut

The intestinal tract with its enormous mucosal surface forms an important host - environment interaction site, hence a potential antigen portal of entry into the animal body. The gut associated lymphoid tissue (GALT) and the chain of lymph nodes draining the intestinal tract forms the principal cellular or active immunity defence mechanism for the tract acting in synergy with innate resistance features.

The GALT forms one of the major subdivisions of the immune system and contains several lymphoid populations that can be distinguished on morphologic and anatomic considerations. The lamina propria of the intestinal tract is populated with lymphocytes and plasma cells while between the epithelial cells there are lymphocytes (intraepithelial lymphocytes). In addition, scattered solitary lymphoid follicles (or nodules), small aggregates of follicles as well as organized lymphoid nodules (patches of aggregated nodule, folliculi lymphatici aggregati ) - the Peyer's patches line the intestinal tract.

In the subepithelial lymphatic tissue of the digestive tract there are three times as many lymphocytes as in the circulating blood and, taken as a whole, the subepithelial lymphatic tissue of the digestive tract amounts to 6½ times that of all the other lymphatic tissues combined (Grau, 1979). 25% of the intestinal mucosa is lymphoid tissue (Kagnoff, 1981).

## 1.3 Functional role of the GALT

Within the GALT, the patches of aggregated nodules (PP) have been found in avian species (Hood et al.,1978; Burns, 1982) and in the laboratory and domestic mammals (Titkemeyer and Calhoun, 1955; Saar and Getty, 1975; Schummer et al., 1979).

In the avian species, the Bursa of Fabricius, a gut-epithelium derived from primary lymphoid organ, has been shown to be essential in the differentiation and maturation of

immunocompetent lymphoid cells destined to make humoral antibodies (Cooper et al., 1966; Ivanyi et al., 1972; Fitzsimmons et al., 1973; Baba et al., 1981).

Although significant component of the circulating lymphocytes population in mammals as in the birds displays surface immunoglobulins, the organ which functions as the Bursa of Fabricius analoguosly in mammals, has not yet been confirmed. In vitro experiments in mice involving fetal liver (Owen et al., 1974 ), bone marrow and fetal liver cell cultures (Yoffey and Courtice 1970; Roitt, 1977) indicate that the hemopoietic tissue itself provides the appropriate microenvironment for maturation of B-Lymphocytes. However, the PP and other parts of GALT have been proposed to be the Bursa of Fabricius (BF) equivalents because of their having some common features. Consequently, the search for the bursa homologue has centered on the GALT as the proposition is not generally accepted (Faulk et al., 1971; Friedberg and Weissman, 1974).

Archer et al. (1963), comparing the structural morphology of the mature appendix of the rabbit and bursa of the chicken as well as their lymphoid development and their intimate association with the epithelium, hypothesized that the rabbit appendix may be a central (primary )lymphoid tissue analogous to the BF of the chicken. The hypothesis was further supported by Sutherland et al. (1964), who showed that appendectomy in rabbit depressed antibody production just as bursectomy did on chicken. Fitchelius (1967) using phylogenetic data and in vivo DNA synthesis by lymphocytes within

the gut epithelium suggested that the gut epithelium is a primary lymphoid organ with same influence on lymphocytes and lymphoid tissue as the BF in birds. X-irradiation and removal of the sacculus roturdus, appendix and PP in rabbits reduce significantly levels of circulating immunoglobulins, cause selective failure of antibody producing capacity to a range of antigens and lower the total number of circulating lymphocytes (Cooper et al., 1966, 1968). From these observations, the authors concluded that the PP type of lymphoid tissue in the rabbit is a homologue of the BF of chicken. Through similar experiments in the rabbit followed by reconstitution, Perey et al. (1970) observed the GALT to function as a homologue of the BF.

However, proximity to the contents of the intestinal lumen in their normal flow has been shown to be essential to the lymphopoietic function of the BF (Thompson and Cooper, 1971) and the GALT (Perey and Good, 1968).

Mammalian lymphoid follicles of GALT in analogue to the avian BF have been shown to be sites in which lymphoid differentiation along plasma cell lines is induced (Cooper and Lawton, 1972). Other studies suggested the domes of the PP to serve as a bursa equivalent(Waksman, 1973; Waksman et al. 1973). Micropinocytotic capability studies (Bockman and Cooper, 1973) demonstrated that epithelial cells closely associated with lymphoid follicles in the chicken BF, rabbit appendix and mouse PP are capable of transporting ferritin and indian ink traces from the lumen.

More investigations on the functional role of the PP in pigs (Chapman et al., 1979; Muller-Schoop and Good, 1975), sheep (Reynolds et al., 1981; Reynolds and Morris, 1983a; Miyasaka et al., 1984a, 1984b; Reynolds and Morris, 1984) and in calves (Landsverk, 1984) have showed some developmental, morphologic and functional similarities of the PP with the avain BF. Still evidence has been presented for the existence of two distinct types of PP in sheep (Reynolds and Morris, 1983a; Reynolds et al., 1985). Whereas the jejunal PP persists throughout the life of sheep, the ileal PP shows prenatal maturation and involution after puberty (Reynolds, 1980; Reynolds and Morris, 1983a; Reynolds et al ., 1985). The ileal PP bears some characteristic of the primary lymphoid organ, and it has been proposed to be the 'bursa equivalent' (Reynolds and Morris, 1983a; Miyasaka et al., 1984a, 1984b ).

On the contrary, other workers have showed the PP in mice (Evans et al., 1967; Joel et al., 1971; Raff and Owen, 1971; Friedberg and Weissman, 1974), rabbits (Faulk et al., 1971) and in the bovine fetus (Schultz et al., 1973) not to behave as a primary lymphoid organ. Two of the workers, (Joel et al., 1971 and Raff and Owen, 1971) showed that in the mice the lymphoid differentiation in the PP is in part at least, thymus dependent.

Their findings therefore supported a view that the PP are not the analogues of the BF.

There are, therefore, quite divergent opinions as to whether the mammalian PP or the GALT is a primary lymphoid organ, i.e. the 'bursa equivalent' or not. However, the division of lymphoid organs into central (primary) or peripheral (secondary) is generally based on a number of non immunological criteria amongst which are morphology, stages of development at birth and the pattern of cellular repopulations (Faulk et al., 1971).

Much of the research on the PP and other GALT structures has been done on mice, rabbits, pigs, sheep and few on cattle. The caprine is becoming of economic importance in meat and milk production as well as an animal for biomedical studies in the world today. Knowledge of the GALT with its age associated changes in the caprine per se and as a representative of the ruminants is of importance in enlightening on its role in the immune system. In this investigation, morphologic and histological changes of the GALT with emphasis on the PP of the small and large intestine in three age groups of conventionally reared goats is to be studied. The groups shall consist of neonates, three months and eight to twelve months olds. Using the morphological changes during postnatal growth and the stage of development at birth, the PP may therefore be categorized.

Few investigations have been done on the mesenteric lymph nodes of the caprine (Ozguden, 1967; Von Forstner, 1973). Lymphoid tissue has been shown to change with age in animals, growing actively from birth to puberty or maturity, after which a levelling or decrease of organ weight is apparent (Yoffey and

Courtice, 1970; Lubis et al., 1982). It has also been shown in the dog (Resnick, 1967) that the lymph nodes are necessary for tissue repair more so in young animals than in the mature and consequently young animals have and need a lymphatic system that is more active than in the mature animals.

The study of the morphological and histological changes in the caprine mesenteric lymph nodes will therefore, apart from showing their changes with postnatal growth, be of necessity in assessing their role in the gut immunity and to the whole body immune system in general. Gross and histological investigations will be carried out on the jejunal, ileocolic and colic lymph nodes in the same three groups of goats for the GALT study. Comparison shall be made between the GALT and the mesenteric lymph nodes on their age associated changes. Using goats with a fully developed ileal Peyer's patch, the drainage areas of the mesenteric lymph nodes will be demonstrated by the subserosal dye-injection method.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 General introductory remarks

The lymphatic system consists of two main elements. First, the extensive network of vessels (vascular component) containing lymph, and secondly, the scattered masses of lymphoid tissue (cellular component) with which these vessels are associated. The lymphoid tissue is made up of two types of cells, fixed or reticular cells and the mobile elements which are represented by lymphocytes, plasma cells and macrophages. The lymphoid organs may be classified into central (primary) lymphoid organs, which include the thymus, bone marrow, bursa of Fabricius of birds or the bursa equivalent of mammals and peripheral (secondary) lymphoid organs, namely the lymph nodes, spleen, tonsils and the gut and respiratory tract associated lymphoid tissue. The lymphoid organs may be specifically localized, e.g. spleen, tonsils and the thymus or widely spread in the body systems, e.g. lymph nodes, solitary and aggregated lymphoid follicles.

The primary and secondary lymphoid organs together with their interconnecting blood vessels and lymphatics are collectively known as the 'lymphon'. The 'lymphomyeloid complex' includes the myeloid and connective tissue in which the lymphocytes establish a close relation during their migration. Lymphatics generally imply lymph conveying vessels.

Phylogenetically, the immune reaction is developed in the vertebrates only. Primitive lymph nodes are seen in the amphibians (Yoffey and Courtice, 1970 ), and it is in birds where the first true lymph nodes are encountered (Rusznyak et al., 1960). The first vertebrate to develop a mesenteric lymph node is the crocodile (Schilling, 1928). The lymphatic system reaches its highest complexity of structure and function in mammals, especially in the Placentalia.

Little information was available on the lymphatic system in the caprine. More research has been done on the mice, rabbit, pig, cattle and sheep. References shall be made mainly to the sheep where necessary.

## 2.2 Ontogenesis and histogenesis of lymph nodes and the gut-associated lymphoid tissue

### 2.2.1 Lymph nodes

Lymph vessels and lymph node sinuses are lined by endothelium. Two theories are in existence describing their origin (Rusznyak et al., 1960; Yoffey and Courtice, 1970).

a. The original theory (also known as the centripetal theory). This holds that the endothelium of the lymphatic vessels is of mesenchymal origin and by means of fusion of the peripheral clefts towards the large venous trunks, links are established with the large veins.

b. The angioblastic theory (also known as the centrifugal theory). The lymphatic endothelium has its source from the embryonic venous system and the lymphatic vessels grow from their junction with the great veins towards the periphery by means of budding and ramification.

Collected evidence seems to favour the centripetal theory (Rusznyak et al., 1960).

In the developing embryo, the lymphatics are first observed as primary lymph sacs lying close to the primitive main venous trunks. The embryonic lymph sacs are formed in a cranio - caudal sequence by coalescence of developing lymphatic channels. This results in the formation of six lymph sacs in the embryo, two paired jugular and sciatic lymph sacs and two unpaired retroperitoneal and cisterna chyli. These lymph sacs disappear in the fetus except the cisterna chyli.

Adjacent or along these primitive lymphatic channels, at genetically predetermined loci, strands of mesenchymal tissue develops and become infiltrated by these vessels together with blood capillaries to form the lymph node anlage. Alternatively, the lymph node anlage may arise from lymphatic plexuses by the proliferation of mesenchymal elements into the meshes of the vascular plexuses.

As the network of lymphatic channels within the lymph node anlage forms, the mesenchymal tissue remaining between these channels forms connective tissue bridges or trabeculae, which join in the periphery a connective tissue capsule forming around the lymph node from surrounding mesenchyme. The confluence of adjacent lymphatics forms the marginal plexus, which later becomes the marginal sinus of the lymph node. The primitive cortex of the developing lymph node arises as a result of differential growth of the mesenchymal components along the more convex region of the developing node with narrowing of the lymphatic sinuses and the preferential accumulation of lymphocytes in this area (Hostetler and Ackerman, 1966).

In the antigen-free intrauterine environment, the development of the lymph nodes occurs without much differentiation between cortex and medulla (Silverstein, 1964).

### 2.2.2 Gut-associated lymphoid tissue (GALT)

In the domestic mammals, the ontogeny of the Peyer's patches (PP) has been studied in cattle (Doughri et al., 1972), pig (Chapman et al., 1974) and in sheep (Reynolds, 1976; Reynolds and Morris, 1983a).

The first appearance of the PP in the bovine fetal gut is in the mucosal lamina propria in close association with the crypts of Lieberkuhn. The anlage consists of cells of the lymphoid series appearing as nodules initially adjacent to lymphatic channels.

The developing nodule enlarges both toward the mucosa and toward the serosa, extending to varying depths into the submucosa. The former direction is the primary one. The epithelium directly overlying the lymphoid nodule is penetrated by some lymphocytes.

In the fetal lamb, the PP can be histologically detected as early as 60 - 70 days post conception. By 100 days, the major histological structures of the PP, i.e. dome, follicle and interfollicular regions can be identified. The dome is composed of reticular fibers interspersed with few lymphocytes and macrophages and its epithelial covering cells are more cuboidal than the epithelial cells overlying adjacent villi. The follicles show intense lymphopoiesis and are separated by connective tissue on both sides and at the base. In both bovine and ovine fetus, the jejunal PP develops and matures before the ileal PP (Doughri et al.,1972; Reynolds and Morris, 1983a).

## 2.3 Ontogeny of the lymphoid cells

The mode of origin, differentiation and maturation pathways of lymphocytes in the fetal caprine is not known. However, in other animals the modes of origin of various blood cells including the lymphocytes are held by two sharply contrasted views - the monophylectic and the polyphylectic theories. Evidence seems to favour the monophylectic view (Yoffey and Courtice, 1970; Arey, 1974).

In the rabbit, like in the birds, multipotential hemopoietic stem cell capable of populating both the lymphoid and myeloid tissue is of yolk sac origin (Moore and Metcalf, 1970). Through processes of unknown nature, pluripotent stem cells originating from yolk sac are transported to the liver, then to the thymus and bone marrow by the blood stream in the early stages of embryogenesis (Tuboly et al., 1984). Within the thymus microenvironment, primitive lymphoid cells are primed to differentiate to T-lymphocytes responsible for cell-mediated immunity. Similarly, within the bursa microenvironment in the avian species or in its equivalent in mammals, primitive lymphoid cells are differentiated to immunocompetent cells destined to make humoral antibodies. The primary lymphoid cells are then disseminated to the peripheral lymphoid organs.

There is no evidence of in situ formation of lymphocytes in the lymph nodes (Silverstein, 1964; Metcalf and Brumby, 1966; Tuboly et al., 1984). However, in the rabbit, in situ transformation supplemented by lymphocyte immigrants is evidenced to occur (Hostetler and Ackerman, 1966).

Three concepts are advanced for the origin of the GALT lymphocyte population (Doughri et al., 1972).

a. Lymphoid elements arise from transformation of epithelial cells of the primordial gut.

b. Lymphoid elements migrate from the vascular system into a mesenchymal anlage of the patch.

c. The GALT develops from interaction of budding primordial gut epithelium with adjacent mucosal lamina propria.

In the bovine fetus, there is no evidence of epithelial cells budding or transformation of epithelial cells into lymphoid cells in the PP. The lymphoid cells originate from undifferentiated mesenchymal cells of lamina propria (in situ transformation) and are supplemented by some lymphoid cells originating from the circulation (Doughri et al., 1972). The rabbit appendix lymphoid tissue is formed in a similar manner (Hostetler and Ackerman, 1966).

The origin of the lymphocytes has not yet been determined in the fetal lamb. However, fetal thymectomy produces a reduction in the number of circulating lymphocyte as well as a reduction in the lymphocyte population in lymph nodes, spleen and cortical and interfollicular areas of Peyer's patches indicating that a large proportion of the cells are of thymic origin (Cole and Morris, 1971; Reynolds, 1976; Pearson et al., 1976).

## 2.4 The fetal lymphoid apparatus

The thymus is the first lymphoid organ to form in the rabbit, dog, bovine and ovine (Hostetler and Ackerman, 1966; Bryant and Shifrine, 1972; Schultz et al., 1973 and Cole and Morris, 1973; respectively ). The spleen is the second lymphoid organ to be visible grossly in the bovine (Schultz et al., 1973) while in the sheep it is grossly visible almost at the same period with the lymph nodes (Cole and Morris, 1973).

The PP are grossly detected as small discrete focal aggregations in the terminal part of the jejunum 120 days post conception in the fetal lamb (Cole and Morris, 1973). With increasing age the fetal PP follicle size increases and before birth the fetal lamb has 25-40 PP in the jejunum and one single PP in the terminal ileum which are histologically mature at birth (Reynolds and Morris, 1983a).

The mesenteric lymph nodes are identifiable grossly in the fetal lamb at around 50 to 60 days gestation (Reynolds, 1976: Cole and Morris, 1973). As gestation continues, the lymph node tissue is slowly populated by lymphocytes and together with the proliferation of the primitive connective tissue stroma the lymph nodes increase in size. The cortex and medulla of the mesenteric lymph nodes are not clearly differentiated until after birth and germinal centers do not form unless antigen is deliberately injected into the gut (Reynolds, 1976). Fetal lamb lymph nodes have in some cases been shown to be slightly differentiated into cortex and medulla before birth (Cole and Morris, 1973) with the cortex being thin and sparsely populated by lymphocytes and some primary follicles are present (Tuboly et al., 1984). Under normal course of events, germinal centers do not develop in the fetal lymph nodes and cells identifiable as having immunological function are not seen (Silverstein, 1964).

Lymphocytes are present in the peripheral blood early in fetal life, 40 days post conception in fetal lamb (Cole and Morris, 1973). As the lymphoid organs increase in size during fetal life in the lamb, the number of circulating lymphocytes increases throughout gestation up to 20 - 30 fold near birth. The adult pattern of lymphocyte recirculation is established quite early in fetal lamb (Cahill and Trnka, 1980).

## 2.5 Development of the immunologic competence

The caprine fetus grows in an environment free of antigens and maternal antibodies due to the nature of the placenta. The observed prenatal maturation of the PP and the population of the lymph nodes with lymphocytes in the fetal lamb occurs in absence of any antigenic stimulus. Nevertheless, immunologic competence is developed early in the fetal lamb before the lymphoid system has reached its full anatomic development (Tuboly *et al.*, 1984). The fetus does not, however, acquire an ability to react against all antigens at the same time, but in a sequential fashion and the production of antibodies against some antigens does not occur until after birth (Cole and Morris, 1973; Fahey and Morris, 1978; Tuboly *et al.*, 1984). The magnitude of the responses increases with age, parallel with the lymphoid size and maturity increase, and the specific immune responses exerted are fully developed and adult in character (Fahey and Morris, 1978). Immunologic competence is also evident in the fetus in man (Silverstein and Lukes, 1962), chicken (Lydyard *et al.*, 1967), pig

(Binns and Symons, 1974 ; Chapman et al., 1974; Kovaru et al., 1980) and in the bovine (Schultz et al., 1973; Renshaw et al., 1977; Osburn et al., 1982). Magnitude of immune response increase with fetal age also exists in the bovine (Schultz et al., 1971; Renshaw et al., 1977) and in the pig (Binns and Symons, 1974; Kovaru et al., 1980).

## 2.6 Post natal age-associated changes in the lymphoid tissue

In the ruminants, significant transmission of passively acquired immunity occurs in a brief interval after birth by way of the colostrum and neonatal gut. In the rats and mice, the transmission continues almost throughout lactation (Brambell, 1970). The maternal immunoglobulins reach maximum concentration in the neonatal ruminants by the second day after which they start to decrease. The production of autogenous globulins i.e. the onset of active immunity development by the young animal starts within a few days after birth.

The lympoid tissue is present in mammals throughout life, but its amount seems to undergo changes with age. It has been shown in the bovine that, with the exception of the thymus, weights of all lymphoid organs increase with age until puberty or maturity, after which a levelling of organ weight is apparent. In contrast, organ-bodyweight ratios tend to be highest in the young animals after which they decrease with increasing age (Lubis et al., 1982). Other calves show an increase in size and weight of the thymus up to 8 weeks of life

when involution starts (Venzke, 1975). The goat shows a marked involution of the thymus at 2 years age (Venzke, 1975), but considerable amounts of thymic tissue are present in the thorax even at 5 years (Schummer et al., 1981). The lamb thymus has the most rapid absolute rate of growth from birth to 50 days when it reaches its maximal size after which regression starts (Cole and Morris, 1973).

After birth, reactive changes associated with stimulation by the naturally occurring antigens take place in the lymphoid tissue of animals. This results in an increase in size of the lymphoid organs after birth and the development of germinal centers and secondary follicles.

Studies in germfree and conventional animals have shown that antigenic stimuli greatly influence the development, growth and proliferative activity of peripheral lymphoid organs (Gulliani et al:, 1974). The anatomical location of the mesenteric lymph nodes (MLN) like that of the PP places them closer to continuous antigenic stimulation than other lymph nodes. As a result, the MLN are the most reactive lymph nodes in the animal after birth, showing additional lymphoid follicles and germinal centers soon after birth. In addition, the medullary tissue become much more cellular and show mitotic figures as well as plasma cells (Cole and Morris, 1973). The MLN increase in size at a relatively greater rate than the peripheral lymph nodes and the total weight of the animal. For example, at birth the prescapular lymph nodes are

heavier than the MLN but after birth the MLN grow much more rapidly and at 3 months age they are about six times heavier than the prescapular nodes (Reynolds, 1980).

With increasing age in the dog, lymphocyte number increases in all regions of the MLN and primary and secondary follicles become larger. In addition, the medullary cords contain an increasing number of plasma cells, and the post-capillary venules together with the infiltration of the paracortex with lymphocytes increase markedly during the first 3 days after birth and more slowly thereafter (Bryant and Shifrine, 1972). In an extensive investigation on the lymph nodes in the cat, Sugimura (1962) observed several differences in lymph nodes associated with age. These include increase in the number of mature lymph nodes, thickening of the capsule, presence of cortical and medullary trabeculae, mature cortex arrangement, development of secondary nodules and narrowing of the sub-capsular sinus.

There is a general tendency for the PP to increase in size and number with increasing age until puberty when up to 238 patches can be found in man (Cornes, 1965). Similar trend has been observed in the pig up to one month age (Chu et al., 1979a). The PP in cattle show rapid growth in the first few weeks after birth (Heilmann and Steinbach, 1978) and is followed by a sustained but slower development until about the time of puberty and a subsequent decrease in their size during maturity and advanced

age (Lubis et al ., 1982). In the sheep, the total weight of PP tissue is greater than any other single lymphoid tissue at about 6 - 8 weeks after birth and from 12 weeks the ileocecal PP begin to involute and only a few follicles remain in this region of the intestine by the eighteenth month of age (Reynolds and Morris, 1983a). The PP in other regions of the intestine remain and continue to produce lymphocytes throughout the life of the animal. Antigenic stimuli has been shown not to be necessary for PP follicles growth during the intrauterine period up to about one month after birth. After this period, growth of the PP can only be sustained if they are part of an intestine that is functioning normally (Reynolds, 1976, 1980).

Few undifferentiated small lymphocytes are present in the lamina propria of the lambs intestine at birth, but later in life, the lamina propria becomes populated with an increasing number of lymphoid cells, which includes plasma cells (Reynolds, 1980). The intraepithelial lymphocytes (IEL) increase significantly after birth in piglets and lambs (Chu et al., 1979; Reynolds and Morris, 1983b). Antigenic stimuli from the intestinal lumen is necessary for the lamina propria and epithelial population with lymphocytes (Reynolds, 1980; Reynolds and Morris, 1983b) and as a result the lamina propria remains densely populated with lymphoid cells throughout the postnatal life of the animal (Reynolds, 1976).

Concomitant with the lymphoid organs size increase with age in the lamb, the number and concentration of lymphocytes in the thoracic duct and intestinal lymph increases. This is a reflection of the increase in size of PP and MLN and it appears to be associated with birth and with naturally occurring antigenic stimuli originating in the gut (Cole and Morris, 1973). The number of circulating small lymphocytes with surface immunoglobulins also increases with age (Cole and Morris, 1971; Reynolds, 1976) and the removal of the PP in utero or a few days after birth reduces significantly the output of such cells (Reynolds, 1980).

So far, four classes of immunoglobulins have been found in the lamb. These are $IgG_1$, $IgG_2$, IgM and IgA. These have been shown to change with age too, IgM being the first immunoglobulin to be produced in lambs deprived of colostrum. It is followed by $IgG_1$, then $IgG_2$ and lastly IgA (Cole and Morris, 1973).

## 2.7 Anatomy of the intestinal lymphoid organs in adult goats

### 2.7.1 The intestinal lymph nodes

A number of lymph nodes drain the intestinal tract of goats (See Von Forstner, 1973; Tanudimadja and Ghoshal, 1975 and Schummer et al., 1981). Amongst them are lympho nodi (Inn.) pancreoticoduodenale located on the ventral surface of the pancreas and on the right lobe of pancreas along the duodenum consisting of 3 nodes which are sometimes absent. The Inn. jejunales form the second group which consists of lymph nodes of various sizes numbering 2 - 40. They make up

the largest group of the intestinal lymph nodes and are located between the first centripetal and the last centrifugal coils of the spiral loop of the ascending colon. Others are Inn. colici and Inn. mesenterici caudales. Tanudimadja and Ghoshal (1975) reported on the presence of two Inn. cecales which seem to be equivalent to the 2 - 3 Inn. ileocolici found on the ileocolic artery near the junction of the ileum with the large intestine (Schummer et al., 1981). However, Von Forstner (1973) reported the absence of Inn. ileocecales in the goats and the presence of Inn. ileocecocolici instead.

Microscopically, the structure of the mesenteric nodes is similar to the general structure of other lymph nodes. However, due to the continuous bombardment with antigens via the gut, mesenteric lymph nodes always appear very reactive (Reynolds, 1980).

The structure consists of a capsule which sends trabeculae to the lymph node parenchyma. The parenchyma is divided into outer cortex with lymph nodules and inner medulla. Cortical parenchyma continues in columns known as medullary cords towards the hilus. Afferent lymph vessels enter the marginal sinus under the capsule thence into the intermediate (trabecular) sinus, traverse the cortex and dilate into the medullary sinus before leaving the lymph node at the hilus as efferent vessel.

The lymph node cortical and medullary parenchyma consists of reticular cells and fibers in which lymphocytes, macrophages and plasma cells are supported. The sinuses form a network of branching and anastomosing channels lined by endothelial cells and the lumina of the sinuses are traversed by a network of interconnected reticular cells attached to the sinus walls through numerous slender processes. Within the sinuses network there are lymphocytes, macrophages and plasma cells in the medullary sinuses.

Arteries and nerves enter the lymph node at the hilus where efferent lymph vessels and veins leave the organ. In the deeper cortex and corticomedullary region (Thymus dependent area) are cuboidal-lined post capillary venules (or high-endothelial venules) where the recirculating long lived lymphocytes migrate from blood into the lymphatic tissue. More details on the lymph node microanatomy can be found in any standard histology textbook and in Sugimura (1962); Hwang (1967); Yoffey and Courtice (1970) and Schummer et al. (1981). Ultrastructural morphology of the lymph nodes can also be found in Movat and Fernando (1965); Fujita et al. (1972) and in Gorgollon and Krsulovic (1973), the second referred work being on the mesenteic lymph nodes.

### 2.7.2 The gut-associated lymphoid tissue (GALT)

The GALT consists of the Peyer's patches (PP), the intraepithelial lymphocytes (IEL) and the lymphocytes of the lamina propria.

2.7.2.1 Peyer's patches (PP)

PP are circumscribed patches of organized lymphoid tissue containing more than five follicles (Doughri et al., 1972). They are found in the domestic animals and are located on the antimesenteric side of the intestine varying in number, size and shape with species, age and intestinal region (Titkemeyer and Calhoun, 1955; Cole and Morris, 1973; Saar and Getty, 1975; Chu et al., 1979a, 1979b; Schummer et al., 1979; Sackman, 1981; Burns, 1982; Lubis et al., 1982; Reynolds and Morris, 1983a).

The single PP occupying most of the ileum circumference in the terminal ileum of sheep measures up to 2.5 meters long whereas in the jejunum there are 25 - 40 separate PP measuring up to 10 cm. long and 1.5 cm. wide (Reynolds and Morris, 1983a). An average of 25 PP are found in goats (Titkemeyer and Calhoum, 1955).

Four morphologically distinct zones are identifiable in the rabbit PP, namely the dome, corona, follicle and interfollicular area (Waksman et al., 1973) (See figure 1). Similar morphological structures have been reported on the PP of sheep (Reynolds and Morris, 1983a), calves (Landsverk, 1984), mice (Sobhon, 1971) and in pigs (Chu et al., 1979b).

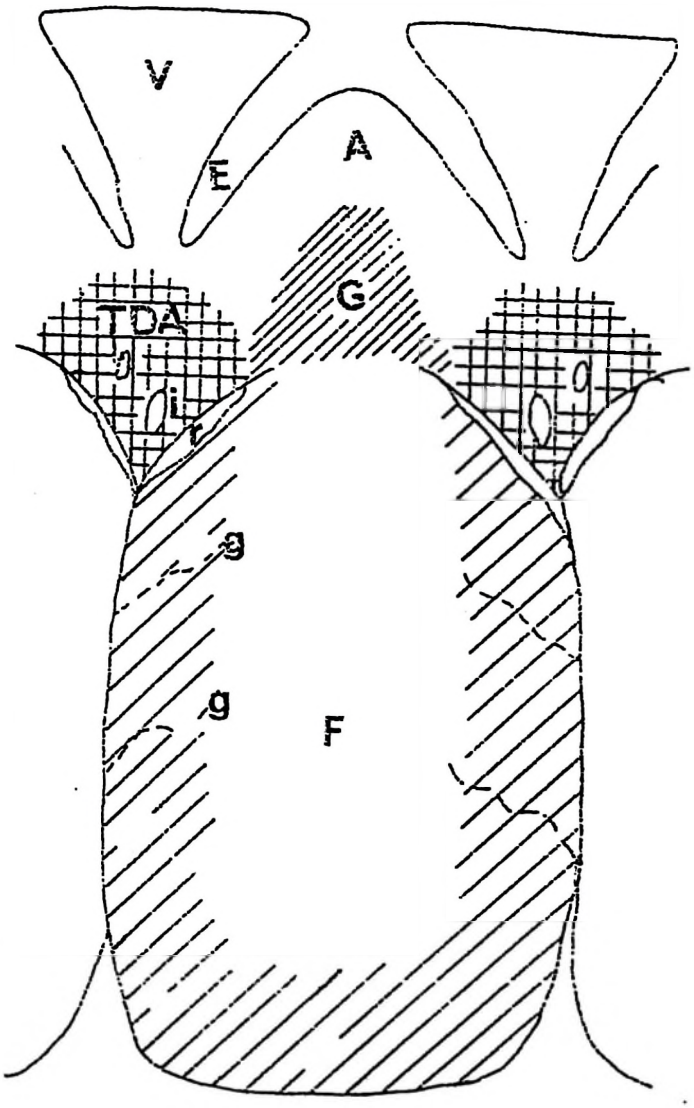


Fig. 1: Diagram to show the morphologic elements of a Peyer's patch follicle. A, dome; E, crypts extending from lumen; F, follicle germinal center ; G, corona; V, mushroom of adjacent villi, TDA, Thymus Dependent Area; g, capillaries; i, post-capillary venules; r, lymphatics.
(With minor modifications from Waksman et al., 1973)

The dome is composed of pleomorphic lymphocytes mixed with macrophages and it is covered by a specialized noncolumnar epithelium which lacks goblet cells in rabbits (Faulk et al., 1971; Waksman et al., 1973), calves (Landsverk, 1981) and in the chickens (Burns, 1982) but have few goblet cells in the pigs (Chu et al., 1979b). The dome epithelium provides an important site for contact between the intestinal contents and the lymphoid system. Interspersed in the dome epithelium in man, mice and chicken (Shimizu and Andrews, 1967; Owen and Jones, 1974; Owen, 1977; Smith and Peacock, 1980; Burns, 1982)or uniformly distributed over the dome epithelium in calves (Landsverk, 1981; Torres-Medina, 1981) are membraneous or microfold cells (M-cells). The M-cells contain multiple vesicles and the cells are said to transport lumenal material with subsequent uptake by lymphocytes providing a specific route for antigen uptake into the intestinal lymphoid system (Bockman and Cooper, 1973; Owen and Jones, 1974; Owen, 1977; Landsverk, 1981; Sackmann, 1981; Chu and Liu, 1984). The M-cells appear to represent an early stage in the life cycle of columnar cells and are not a separate cell type (Owen, 1977; Smith and Peacock, 1980). In addition, the presence of veiled cells resembling the antigen presenting cells of skin and lymph nodes have been demonstrated in the PP of rats, guinea pigs and pigs (Wilders et al., 1983). Epithelial cells not associated with lymphoid follicles do not express pinocytotic activity (Bockman and Cooper, 1973; Chu and Liu, 1984).

The corona is formed by an accumulation of small lymphocytes below the dome. Merging from below with the corona is the follicle which is precisely defined by connective tissue. The central core of the follicle, also known as the germinal center, is composed of small, medium and large lymphocytes, lymphoblasts, tangible body macrophages and reticular cells whereas the periphery is densely populated with lymphocytes and macrophages (Sobhon, 1971; Waksman et al., 1973). The macrophages are thought to 'eat away' the dying lymphocytes at the end of their life span (Sobhon, 1971).

There is evidence for the existence of histological difference between ileocecal PP and jejunal PP in sheep (Reynolds and Morris, 1983a; Larsen and Landsverk, 1985; Reynolds et al., 1985). Whereas the ileocecal PP follicles are long and tightly packed extending up to 3 mm. in the submucosa and the interfollicular area forms small triangular tissue, the jejunal PP follicles are pear shaped and the interfollicular tissue is wider. In the pig, the jejunal PP dome epithelium is more superficially located and is larger in total surface area in comparison to the ileal patches (Chu and Liu, 1984).

Functional studies have indicated that T-Lymphocytes are more frequent in the jejunal PP ( Larsen and Landsverk, 1985; Reynolds et al., 1985). B-lymphocytes localize in the follicle and they are more frequent in the ileocecal PP (Gerber, 1979; Kagnoff, 1981; Reynolds et al., 1981; Miyasaka et al., 1984b; Larsen and Landsverk, 1985 ). Similarly, IgM positive cells have been found to be localized more frequently in the ileocecal PP germinal centers than in the

jejunal PP (Larsen and Landsverk, 1985). Other studies have indicated that the PP are sources of IgA secreting plasma cells precursors that populate various mucosal tissues (Craig and Cebra, 1971) and the PP in sheep are said to be the major sites in which immunoglobulin-bearing small lymphocytes are formed (Gerber, 1979).

The interfollicular areas (also known as Thymus Dependent Area) consists of predominantly small lymphocytes coming from the blood stream by way of the post-capillary venules (Clark, 1963; Sobhon, 1971; Waksman et al., 1973; Landsverk, 1984). Other cells include macrophages and plasma cells, the latter being more frequent in interfollicular spaces than in the lymphoid follicles (Faulk et al., 1971). A high percentage of the lymphocytes in the interfollicular area are T-lymphocytes (Waksman, 1973; Kagnoff, 1981; Larsen and Landsverk, 1985).

Although the Peyer's patches contain both T- and B-dependent areas and germinal centers, the proportion of each type varies depending on age and antigenic experience (McConnell et al., 1981).

2.7.2.2 Intraepithelial lymphocytes (IEL)

Located within the epithelium are lymphocytes, and the size of the majority is larger than typical small lymphocytes (Shimizu and Andrews, 1967; Kagnoff, 1981). Ultrastructural studies have revealed that the epithelial lymphocytes are located between rather than within the intestinal epithelial cells (Chu et al., 1979a) and they are therefore termed interepithelial (Hirsh, 1980) rather

than intraepithelial as called by others (Marsh, 1975; Kagnoff, 1981). The IEL show a general pattern of decreasing in number from cranial end to caudal end of the intestine in the pig and at least part of the IEL leave the epithelium and enter the intestinal lumen (Chu et al., 1979a: 1979b). The possibility of some lymphocytes and plasma cells being extruded into the intestinal lumen has also been shown to exist in the domestic fowl due to discontinuities in the epithelium over the PP follicles (Burns, 1982).

Villi of isolated ileal segments show fewer IEL and significantly fewer lymphoid cells in the lamina propria, thus the gut contents exert an influence on the lymphoid cell population in the mucosa (Reynolds and Morris, 1983b).

IEL is a mixed population of small T-and B-lymphocytes (Hirsh, 1980) similar in composition to PP, spleen, blood and lymph (Ferguson and Parrott, 1972) but Guy-Grand et al. (1974) showed the majority of the IEL to be T-lymphocytes. The gut T-lymphocytes are the progeny of T-blasts arising in the PP as a result of antigenic stimulation, leaving the patches to reach the mesenteric lymph nodes, then the thoracic duct and finally disseminating from the blood to all the intestinal mucosa because of their peculiar gut-homing property (Guy-Grand et al., 1978). It is not known whether the IEL are pre-effector or mature effector T-cells although the majority are larger than typical small lymphocytes (Kagnoff, 1981).

## 2.8 Recirculation of lymphocytes

Lymphocytes recirculate through the body systems in the blood and lymph vessels. They leave the blood vessels in the specialised high-endothelial post-capilllary venules (HE-PCV) and re-enter the blood as the thoracic duct joins the venous blood. In vivo, there are two patterns of lymphocytes movements. First there is the continuous recirculation of lymphocytes between and through the lymphoid tissues, and second there is homing or migration of lymphocytes from one site to another (McConnell et al., 1981). Most of the recirculating lymphocytes in mice and normal sheep lymph are T-lymphocytes (Raff and Owen, 1971; Miyasaka et al., 1983). It is estimated that about 1 to 2% of the total pool of recirculating lymphocytes emerge into the blood from the thoracic duct each hour in the sheep. This output from the thoracic duct balances the number of lymphocytes that have left the blood through the HE-PCV in lymph nodes, the gut and in other tissues (McConnel et al., 1981).

The tissue distribution and migration pattern of the different types of lymphocytes can be easily followed by marking the cells with radioactive isotopes, dyes or chromosome markers (Yoffey and Courtice, 1970). Thereafter, such cells are returned to the donor or to syngenetic recipients and efferent lymphatics from a single lymph node, the thoracic duct or blood vessel can be cannulated and samples collected for analysis of marked cells.

### 2.8.1 Recirculation and kinetics of lymphocyte traffic through lymph nodes

Lymph flow in the lymph nodes is centripetal, flowing first through peripheral nodes then the central nodes. The central nodes, e.g. medial iliac lymph nodes have, therefore, a significant input of lymphocytes via the afferent lymph vessels whereas the peripheral nodes receive most of their lymphocytes from the blood. The mesenteric lymph nodes on the other hand are the only lymph nodes in the body that habitually receive large numbers of newly- formed lymphoid cells via the afferent lymph stream (Reynolds, 1976).

A resting lymph node in sheep receives about 0.014% of cardiac output producing a flow of about 25 mls. of blood per hour. This represents a lymphocyte throughput in the blood of about 110 million per hour and about 25% of the lymphocytes passing through the node in the blood actually enter the lymphoid compartment (Hay and Hobbs, 1977). The output of small lymphocytes per hour in the efferent lymph vessel of a node weighing about a gram in sheep is estimated to be 30 to 50 million (Hay and Hobbs, 1977; Trnka and Cahill, 1980). Of the small lymphocytes emerging from the node, a significant large percentage comes from the blood by passing through the specialised HEV and not more than 4% efferent lymph lymphocytes are actually produced in the node (Hall and Morris, 1965; Trnka and Cahill, 1980).

The recirculation of lymphocytes from blood to lymph occurs principally in the diffuse cortex and corticomedullary areas of lymph nodes through the specialised HE-PCV.

Only B- and T-lymphocytes are able to traverse the HE-PCV walls, the B-cells migrating to the follicles and the T-cells remaining in the diffuse cortex (Hood et al., 1978). In the normal animal, the transit time of T-lymphocytes through lymph nodes is considerably shorter than that of B-cells (Dorsch and Roser, 1975; Fossum et al., 1983). In man and mice, the HE-PCV cells synthesize a sulfated proteolipoglycan in greater concentration than in flat endothelium and mature lymphocytes migrate actively between the HE-PCV cells (McConnell et al., 1981). The proteolipoglycan role is hypothesized to control the selective extraction of lymphocytes from the blood but this remains to be clarified. In the rats, the molecule secreted by the HE-PCV endothelium is not a proteolipoglycan (Andrews et al., 1982) and a lymph fraction designated Adherence Enhancing Factor (AEF) has been shown to increase the binding of lymphocytes to the HE-PCV in vitro (Carey et al., 1981).

Emigration of lymphocytes from the mesenteric lymph nodes is predominantly via lymph in the sheep, but in pigs there is a direct emigration of lymphocytes into the blood draining both visceral and somatic lymph nodes (Bennell and Husband, 1981; Binns et al., 1985).

Following antigenic stimulation, there is an increase in the number of lymphoblasts in the efferent lymph. This is not associated with an altered transit time through a lymph node (Cahill et al.,1976)

but it is directly related to changes in the blood supply to the node (Hay and Hobbs, 1977) with a concomitant increase in both vascular permeability (Anderson et al., 1975)and lymphocyte input (Cahill et al., 1976 ). During the first few days of antigenic stimulation there is a large accumulation of cells in the nodes which is caused not only by the increase in lymphocytes input but also by the almost total shutdown in the outflow of cells into the efferent lymph (Hall and Morris, 1965).

The effect of antigen is therefore to increase the proportion of lymphocytes leaving the blood within the node and to increase the blood flow to the node resulting in a greater traffic of lymphoid cells into the efferent lymph. In contrast to the resting lymph node where not more than 4% of the efferent lymph lymphocytes are actually produced in the node, the large lymphocytes, transitional cells and plasma cell series which appear in the efferent lymph following antigenic stimulation are all produced in the node (Hall and Morris, 1965).

Shortly after the primary immune response, some memory B-and T-cells leave the lymph node via the efferent lymphatics and become distributed throughout the body. Lymphocytes in efferent lymphatics can be the carriers of the immunological memory (Hall and Morris, 1965). Once in the blood stream, these cells can enter any lymphoid organ through the HE-PCV. The end result is that after a primary immune response every lymphatic structure in the body becomes seeded with T-and B-cells specific for the antigen that first elicited the primary response (Hirsh, 1980).

2.8.2 Recirculation and kinetics of lymphocytes through the GALT

The GALT is largely responsible for the maintanance of mucosal immunity and the majority of cells leaving the Peyer's patches do so via the lymph and not directly via the blood (Chin and Cahill, 1984; Reynolds and Pabst, 1984). Virgin lymphocytes in the patches therefore, probably migrate to the upper interpithelial cell spaces or to the M-cells to recognize lumenal antigens. Alternatively, antigen is absorbed through pinocytic vacuoles of the M-cells or through the columnar epithelial cells to the lamina propria (Hirsh, 1980). Whichever way the antigenic material gains entrance to the lamina propria, sensitized lymphocytes then enter the lacteals to the mesenteric lymph nodes before entering the thoracic duct and finally the blood circulation. The fate of newly produced cells in the Peyer's patches follicles is unknown but they may die *in situ*, move directly to the blood or lymph or may migrate first to the gut mucosa (Meuwissen *et al.*, 1969).

Slight evidence suggests that intraepithelial lymphocytes do not leave the gut together with the epithelial cells, but re-enter the lamina propria (Fitchelius, 1968).

The environment where transformation of the lymphocytes from non-immune competent to competent cells occurs is not clearly known. In mice, it has been suggested that this occurs within the interepithelial cell spaces of the small intestine mucosa (Marsh, 1975).

The follicular areas of both Peyer's patches and lymph nodes are regarded as regions through which B-lymphocytes normally recirculate in the same way as deep cortical zone of lymph nodes are the traffic areas for the recirculation of T-lymphocytes (Howard et al:, 1972) but how the segregation occurs is not known. The presence of high-endothelial post-capillary venules in the interfollicular areas of Peyer's patches (Clark 1963; Gowans and Knight 1964; Sobhon, 1971; Kagnoff, 1981; Landsverk, 1984) indicates that recirculating lymphocytes homing to the GALT most probably do so through these areas. A high percentage of lymphocytes in the interfollicular areas of Peyer's patches are T-lymphocytes (Waksman, 1973; Kagnoff, 1981; Larsen and Landsverk, 1985) and therefore the interfollicular areas of Peyer's patches may probably represent the traffic areas of the T-cells (Waksman, 1973).

Lymphoid cells produced in the GALT can migrate to sites where there is no antigen,but the distribution can be modified by the presence of lumenal antigens (Stramignoni et al., 1969; Chapman et al., 1974). The mechanism by which antigens influence the distribution of lymphoid cells along the intestine has not been clearly established although a number of processes may be involved (Reynolds, 1980). One possibility is that antigens may alter the extravasation of immunoblasts into the lamina propria and secondly in post-natally isolated intestinal loops which show low lymphoid cell population for instance, blood flow is lower than normal and therefore few immunoblasts capable of migrating to the lamina propria.

Blood supply has been suggested as one of the most important factors for the development of gut lymphoid tissue (Stramignoni et al., 1969). Moreover, the lumen of isolated loop has few of the processes associated with normal digestion which may have an influence on the distribution of the lymphocytes.

### 2.8.3 Migration and homing of intestinal lymphoid cells

Lymphocytes do not diffuse through the tissues, rather their pattern of movement is non-random and directed. They have been shown to have preferential migratory pathways. In 1964, Gowans and Knight demonstrated that lymphoid-blast cells obtained from rat thoracic duct lymph show a marked preferential accumulation in the lamina propria of the small intestine and the mesenteric lymph nodes. Similar preferential migration of lymphoblasts from not only thoracic duct but also mesenteric lymph nodes, intestine and Peyer's patches to lymphoid tissue within or adjacent to the intestine have also been demonstrated by several authors (Griscelli et al., 1969; Howard et al., 1972; Parrott and Ferguson, 1974; Reynolds, 1976; Rose et al., 1976; Cahill et al., 1977; Guy-Grand et al., 1974, 1978; Bienenstock et al., 1980; Kagnoff, 1981; Chin and Hay, 1984). Large lymphoid cells and blast cells derived from intestinal lymph recirculate more rapidly than small lymphocytes (Reynolds, 1976 ).

Transfer of allogeneic lymphocytes from either bronchial-associated lymphoid tissue or Peyer's patches into X-irradiated recipients shows a prominent repopulation of gut and bronchial lamina propria as well as spleen with IgA containing cells suggesting the presence of a common mucosal immunologic system (Rudzik et al., 1975).

The dichotomy between peripheral and intestinal migration of lymphoblasts is not restricted to immunoglobulin-producing cells only, but also operates for T-lymphoblasts and does not extend to small lymphocytes (Parrott and Rose, 1978). However, Reynolds (1976), Cahill et al. (1977) and Hopkins and McConnell (1984) showed the dichotomy to extend to small lymphocytes. Cahill et al. (1977) proposed the pool of recirculating lymphocytes in sheep to consist of two major subdivisions, an intestinal pool and a nodal pool. The nodal pool traverses the PCV in all lymph nodes but not in the small intestine and the intestinal lymphocytes do not traverse the PCV in lymph nodes but recirculates via the small intestine from which they pass via afferent lymphatics to mesenteric lymph nodes and subsequently through the thoracic duct to the blood (Figure 2 ).

The molecular determinants governing lymphocyte migration and localisation are not yet understood. Nevertheless, it has been suggested that B-lymphocyte localisation in certain sex-hormone dependent target tissue e.g. mammary gland may be dependent on a receptor under sex-hormone control.

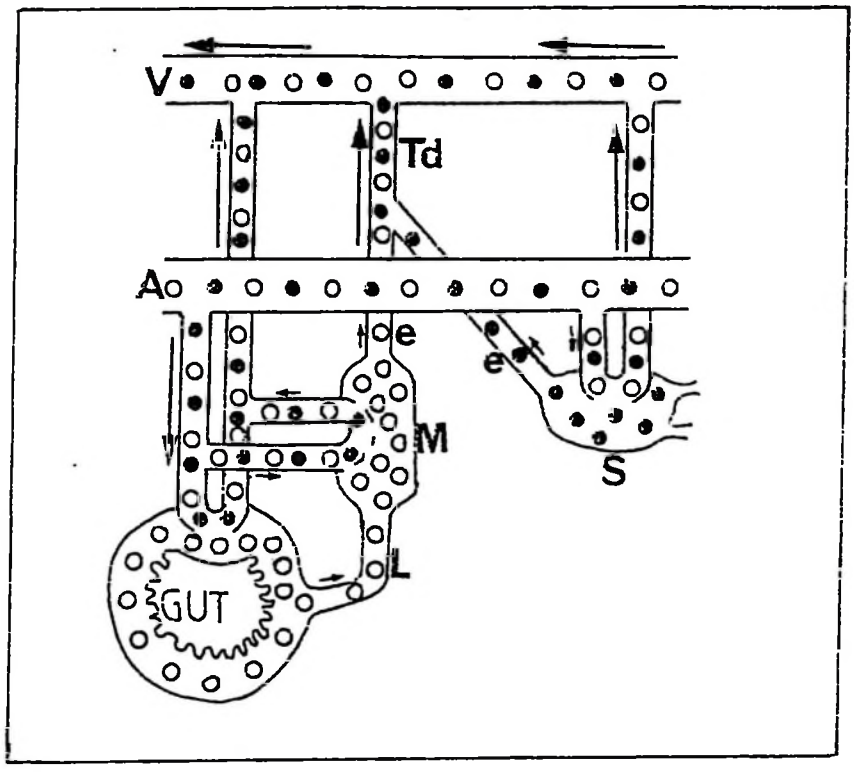


Fig. 2 : Diagram to show the migratory pathways of small lymphocytes derived from the GALT (O) and somatic lymph nodes (●). A, artery; L, Lacteal; M, mesenteric lymph node, S, somatic lymph node; Td, thoracic duct; V, vein; e, efferents.

( Modified from Hall, 1980 ).

The mesenteric lymph nodes and Peyer's patches have cells with a predilection to make IgA (Craig and Cebra, 1971; Rudzik et al., 1975; McWilliams et al., 1977; Bienenstock et al., 1980). Blast cells in the thoracic duct have originated almost exclusively in the gut-associated lymphoid tissue (Hopkins and McConnell, 1984). Moreover, both thoracic duct lymph and mesenteric lymph node contain lymph directly draining the gut and would be expected to contain cells primed against the variety of antigenic stimuli present in the gut. The secretory component produced by mucosal epithelial cells may be a candidate for allowing selective localisation of IgA-precursos (Bienenstock et al., 1980).

However, there is no evidence for the direct involvement of surface IgA, secretory component or the J-chain in homing of mesenteric lymph node and Peyer's patches lymphoblasts (McWilliams et al., 1975; Hopkins and McConnell, 1984).

Another speculation for the selective homing of B-and T-lymphocytes is that this is due to acquired specific receptors that occur during the process of differentiation either to antibody producing cells or to activated T-cells (Parrott and Ferguson, 1974). However, there is an important relationship in normal animals between the level of lymphoblasts accumulation within a particular region of the small intestine and the delivery of blood-borne cells to that region (Ottaway and Parrott, 1980).

Although the migration of lymphoblasts to the small intestine is basically determined by the tissue origin of the migrating cells, this requirement is less strict when the gut is infected or inflamed. Parasitized gut is indeed more permeable to peripheral blast cells, but yet there is real dichotomy in the migratory behavior of both peripheral and mesenteric lymphoblasts (Rose et al., 1976). The selective migration is not antigen dependent because injected lymphoblasts from mesenteric lymph node or thoracic duct lymph migrates in comparable numbers to normal intact gut and to grafted antigen free gut (Guy-Grand et al., 1974; Parrott and Ferguson, 1974). Thus specific immunological attraction may not be of major importance to the homing of lymphocytes to the gut. Antigenic stimulation within the Peyer's patches is necessary for the emergence of gut T-lymphoblast progenitors but their gut-homing property is entirely independent of the presence of antigen in the gut (Guy-Grand et al 1978; Parrott and Rose, 1978 ).

## 2.9 GALT functional role and category in the immune system

### 2.9.1 General function of the GALT

The intestinal immune system is important in host interaction with gut bacteria, viruses, parasites, drugs and chemicals. In addition, the gut lymphatic system also plays a specific role in the absorption of fat, fat-soluble materials and colostral proteins from the gut as well as picking up large molecules, particles and excess fluid and transport them to the central lymphatics and the venous system (Casley Smith, 1968;

Fahy et al., 1980 ). Through the migration of lymphocytes from one site to another, the various populations of lymphocytes in the intestine appear to be interrelated. Thus Peyer's patches contain a precusor population of lymphocytes that eventually populate the lamina propria and participate in the local mucosal immunity. Besides this, lymphocytes of gut origin can also disseminate to extraintestinal sites (Kagnoff, 1981) and may migrate into inflamed areas, hypertrophy and become large monoclear cells, epitheliod cells and even giant cells (Braunsteiner et al., 1958).

Peyer's patches are a major 'sampling site' for antigens encountered by the enteric route and the initiating site for IgA-mucosal immunity. Together with the other GALT they may also serve as the source of immunologically competent cells throughout the life of the animals (Cooper and Turner, 1969). The specialized type of epithelium over the Peyer's patches is suggested to have a transport function, possibly for lumenal antigenic material or for secretory immunoglobulin (Owen and Jones, 1974; Owen, 1977).

B-lymphocytes stimulated by antigen proliferate and mature into antibody forming cells. The terminal step in B-lymphocytes differentiation are plasma cells that synthesize and secrete immunoglobulins (antibodies). The latter are classifed according to the characteristic of the heavy-chain constant region to five classes, i.e. IgM, IgG, IgA, IgE and IgD. In all of the domestic animals except the ruminants, IgA is the major immunoglobulin in intestinal secretions (Hirsh, 1980). In calf Thiry-Vella loops,

IgM exceeds levels of IgA(Porter et al., 1972) suggesting that IgM may also play an important role in the local intestinal defence (Porter et al., 1972 ; Allen and Porter, 1977).

Immature T-lymphocytes when stimulated by antigen proliferate and/or mature to express a variety of effector functions. According to the nature of effector function mediated by the T-cells, the latter are further classified into helper, suppressor, cytotoxic and amplifier T-cells. Other cell types involved in immune effector mechanisms are macrophages, mast cells, eosinophils and basophils.

### 2.9.2 GALT as a primary (central) lymphoid organ

One of the functions of the avian bursa is that of a primary lymphoid organ responsible for the early differentiation of antibody producing cells. The majority of the bursal lymphocytes are derived from blood-borne progenitor cells which enter the bursa primordium during embryogenesis (Moore and Owen, 1966). In mammals, the search for an organ equivalent to avian bursa has focused for a number of years on the appendix (in rabbits) and Peyer's patches because they have many features in common with the bursa of Fabricius (See Cooper and Lawton (1972) and Perey et al., 1970).

True primary (central) lymphoid organs may be defined as those sites where lymphopoiesis occurs in the absence of antigenic stimulation (Schaffner et al., 1974). They are sites where precursos cells are started along specific

avenues of morphologic and functional specialisations. After receiving early instruction, some of these lymphoid cells leave the primary organ and migrate to peripheral (secondary) lymphoid tissues such as the spleen and lymph nodes.

Much experimental evidence has been collected in favour of the view that the GALT in mammals especially the Peyer's patches and appendix (in rabbits) are primary lymphoid organs analogous to the avain bursa of Fabricius.

a. From an evolutionary point of view of the immune system and the behavior of the avian bursa, it seems likely that mammals possess an analogous differentiating system in the GALT which has a similar relationship to antibody-producing cell lines like the bursa has (Burnet, 1968).

b. Using phylogenetic considerations, the high figures of lymphocytes in DNA synthesis within the gut epithelium, which is strikingly comparable to corresponding high figures within the thymus and bursa of Fabricius, and results from a study on the kinetics of labelled cells within the epithelium covering the lymphoid aggregates, Fitchelius (1966 , 1968) proposed that young lymphocytes are recruited from the blood and differentiate to cells capable of immunoglobulin synthesis after interaction with epithelial cells. The epithelium over the GALT may influence the lymphocytes in a way comparable to that which is suggested for thymus and bursa of Fabricius making the lymphocytes immunologically competent in some unknown

way. The author further proposed that mammals may be in the process of developing a special bursa equivalent to the epithelium covering the GALT, though the epithelium of the whole gut may function as a bursa of Fabricius.

c. Like thymus and bursa, GALT is a gut-associated lymphoepithelial tissue with follicular organization, derived from epithelial-mesenchymal interaction and maintaining a close lymphoepithelial relationship throughout the life of the animal (Perey and Good, 1968). Both the lymphoid follicles of the chick bursa and the rabbit appendix seem to develop from an outpouching of epithelial cells (Archer et al., 1963) and as they mature, they develop a clearly defined cortex and medulla assuming a structure similar to that of thymic lobules.

d. Ontogenetically, the GALT develops after the thymus, but before lymph nodes and spleen, and preceeds the appearance of germinal centers and immunoglobulin - producing cells elsewhere in the body (Papermaster and Good, 1962; Cornes, 1965; Schultz et al., 1973). In the rabbit, the thymus is the first organ to become lymphoid followed by spleen, gut and peripheral lymph nodes in that sequence (Archer et al., 1963). In the fetal lamb, however, the thymus, as in the rabbit, is the first identifiable lymphoid organ followed by spleen and lymph nodes which are discernible almost during the same period and subsequently the Peyer's patches (Cole and Morris, 1973). The occurrence of lymphoid follicles in typical thymus-independent regions of Peyer's patches in pigs well before

their appearance in thymus independent regions elsewhere and the occurrence of lymphoid cells in the patches prior to the development of immunoglonulin-producing cells elsewhere further supports the idea that the lymphoid follicles in Peyer's patches are mammalian bursa-equivalent sites (Chapman et al., 1974 ).

e. In lambs, Peyer's patches develop prenatally and like the thymus and the avian bursa they are histologically mature before birth (Reynolds, 1976; Reynolds et al., 1981; Reynolds and Morris, 1983a). Similar observations have been reported in various other species including man, dog and cattle (Cornes, 1965; Bryant and Shifrine, 1972; Doughri et al., 19729:

f. A primary lymphoid organ should be able to influence the differentiation of immunoglobulin-bearing lymphocytes without the help of an antigen. The GALT usually develops in the absence of antigenic stimulation as seen in germfree animals and in ruminant fetuses (Doughri et al., 1972; Reynold, 1976; Reynolds and Morris. 1983a). Injection of antigen into fetal sheep ileal segment do not cause premature growth of Peyer's patches and therefore lymphopoiesis in the follicles of the Peyer's patches of fetal lamb is not antigen dependent (Reynolds and Morris. 1984). The epitheliochorial type of placenta in sheep prevents the exposure of the developing fetus to the maternal immunoglobulins enabling it to grow in a naturally antigen-free environment (Brambell, 1970). Although there is evidence that lymphoid cells proliferate prenatally in the Peyer's patches of dogs and man, the nature of the

placenta in especially man (hemo-chorial type) exposes the fetus to maternal immunoglobulins and therefore not developing in an absolutely antigen-free environment.

During the first weeks after birth, in utero isolated Peyer's patches of lambs continues to show lymphopoiesis and growth which coincide with period when there is a rapid increase in the number of small lymphocytes with surface immunoglobulins entering the lymph (Reynolds, 1980; Reynolds and Morris, 1984). Antigen in the intestine is therefore not the cause of the lymphopoiesis seen in the Peyer's patches during the period when the population of immunoglobulin-bearing lymphocytes is rapidly expanding. Evidence has been presented showing that by one month after birth in lambs, there is a second phase of development of the patches in which they can only be sustained if they are part of an intestinal tract which is functioning normally (Reynolds, 1980; Reynolds et al., 1981).

The avain bursa (Thompson and Cooper, 1971) and rabbit appendix (Perey and Good, 1968) also show arrested lymphopoiesis in the follicles when they are isolated from the intestinal tract in early post-hatch or post-natal life. Both the early differentiation of lymphocytes and the subsequent contact between lymphoid cells and antigen absorbed from the gut occur within the Peyer's patches and avian bursa. It may therefore be that the primary lymphoid organ function of the avian bursa, Peyer's patches and appendix is limited to prenatal and a brief period after birth/hatching which is the period when the lymphopoiesis is clearly independent of antigens.

g. GALT of rabbits develop normally after neonatal thymectomy signifying a thymus-independent follicular lymphopoiesis in the appendix (Sutherland et al., 1970). However, in the avian species, Perey and Bienenstock (1973) documented the requirement for normal thymus - derived and bursa- derived cell interaction in the formation of lymphocytes with surface immunoglobulins. Nevertheless. the mechanism underlying this cooperation is not yet fully understood.

h. In the rabbits, the GALT are sites of intense cell proliferation and of the body lymphomyeloid tissues only the bone marrow is a more active source of newly formed cells. The Peyer's patches appear to be the primary location for cell replication and in the follicles many cells are in DNA synthesis (Meuwissen et al., 1969) hence supporting a view that the GALT in the rabbit expand the lymphocyte population by rapid proliferation and may play a role as a primary lymphoid organ.

i. The thymus and the avian bursa are maximally developed early in life and involute in later life (Cole and Morris, 1973; Schaffner et al., 1974) and the GALT shows a similar behavior (Cornes, 1965; Reynolds, 1976; Reynolds, 1980; Reynolds et al., 1981; Reynolds and Morris, 1983a). Peyer's patches reach maximum size in 6 - 8 weeks old lambs and then they gradually get smaller so that by 15 to 18 months they have completely involuted (Reynolds, 1976; Reynolds and Morris, 1983a). The fact that Peyer's patches involute in sheep places them in the same category as the primary lymphoid organs.

j. Epithelium overlying the avian bursa (Burns, 1982), Peyer's patches follicles in rabbits (Faulk et al., 1971 ; Waksman et al., 1973) and calves (Landsverk, 1981) is of the cuboidal type and lacks goblet cells. The follicular epithelial cells which are interspersed by specialised M-cells, express a high capacity to ingest material from the lumen of the intestine (Bockman and Cooper, 1973; Hess et al., 1973; Owen and Jones, 1974; Schaffner et al., 1974; Owen, 1977; Reynolds, 1980). Antigen taken in from the lumen may amplify the number of cells in the clone specific for that antigen or perhaps cause the cells to differentiate into the precursors of antibody producing cells (Reynolds, 1980).

The dome may also serve as a 'bursa equivalent' and the follicle as an 'implification site' for B-cells arising in the dome and stimulated to profiferate by endotoxins from intestinal bacteria (Waksman, 1973; Waksman et al., 1973). The presence of dome at birth before the occurrence of antigenic stimulation is consistent with its possible function as a bursa equivalent but in no way proves it (Waksman, 1973).

k. Mammalian GALT like the avian bursa contain cells fully capable of responding to antigens with antibody production and secretion, but under normal conditions it produces little antibody for secretion in situ (Cooper and Lawton, 1972). The Peyer's patches of mice do not respond to systemic antigenic stimulation by producing antibody in situ, but they are able to cooperate with bone marrow cells to produce antibody forming

cells of IgM, IgG and IgA classes. However, in vitro , the individual cells are capable of independent formation of IgM and IgG but not IgA (Knudson et al., 1975). After systemic or local immunisation, antibody - containing cells are absent in the follicular epithelial lymphoid cells (Bienenstock et al., 1980).

Rabbit appendix is the earliest site of immunoglobulin production (Thorbecke, 1960). The sequence of immunoglobulin synthesis in the bursa system is IgM to IgG (Schaffner et al., 1974) and a similar sequence exists in the rabbit and pig GALT (Perey et al., 1970; Chapman et al., 1974).

1. The avian bursa provides the proper environment and or stimuli for the development of immunoglobulin producing cells (Fitzsimmons et al., 1973 ). In ovo bursectomized chicks show significant inability to produce IgM and IgG, selective failure of antibody - producing capacity to a range of antigens, deficient antibody responsiveness and significant reduction of circulating immunoglobulins but no demonstrable impairment of allograft rejection or delayed allergy (Ivanyi et al., 1972; Cooper et al., 1966; Fitzsimmons et al., 1973).

The role played by the Peyer's patches and appendix on the development and differentiation of immunocompetent cells capable of producing immunoglobulins has been observed in mammals. Removal of either appendix or a major portion of the

GALT in neonatal rabbit has immunological consequences similar to those which follow bursectomy (Archer et al., 1964; Sutherland et al., 1964; Cooper et al., 1966, 1968; Stramignoni and Mollo, 1968; Perey et al., 1970). Extirpation, lethal irradiation and reconstitution experiments in rabbits show the GALT to supply the precursors of antibody-producing cells and the development of lymphoid cells capable of eliciting a primary humoral immune response can not take place in the absence of GALT (Cooper et al., 1968; Perey et al., 1970).

Peyer's patches make a similar contribution as the avian bursa to the development of B-lymphocytes in sheep by being the major site of B-lymphocytes development (Gerber, 1979; Reynolds, 1980; Reynolds et al., 1981). When a large proportion of the Peyer's patches is removed in lambs either in utero or during the first few days after birth, the postnatal increase in the output of small lymphocytes with surface immunoglobulins is dramatically reduced. The role played by the patches in producing the immunoglobulin-bearing small lymphocytes most likely is complete by the second month and possibly even by 2 to 3 weeks since at this age the percentage of these cells reaches the adult level (Reynolds, 1980).

In Bruton's congenital a-gamma-globulinemia of man (congenital deficiency in capacity for plasma cell differentiation and immunoglobulin synthesis ) the Peyer's patches are lacking or are very deficient (Good, 1955).

Amongst the Peyer's patches of the different regions of the gut, the ileal (ileo-cecal) patches (IPP) have been shown to consist of several follicles of immature B-cells expressing small amounts of surface immunoglobulins (Miyasaka et al., 1984b). Resection of a large proportion of the IPP before birth or during the first weeks of postnatal life results in a severe depletion of circulating B-cells in lambs (Gerber, 1979). More, the fetal IPP shows extensive lymphopoiesis in the obvious absence of maternal or exogenous antigen and it develops before the jejunal patches (Reynolds and Morris, 1983a, 1984). The IPP undergoes postnatal involution as the avian bursa (Reynolds, 1976; Reynolds and Morris, 1983a). The extent of emigration of newly formed B-cells from the IPP of young lambs is sufficient to replace the B-cell pool in the peripheral blood within 2 to 5 days (Reynolds and Pabst, 1984). The IPP is the major site of B-cell production (Gerber, 1979) and the great majority of IPP cells are B-cells (Reynolds et al., 1981; Miyasaka et al., 1984b) while T-cells constitute less than 2% of IPP cells (Miyasaka et al., 1983).

The difference between the histology and life history of the jejunal and ileal Peyer's patches raises a possibility that these two types of patches are also different functionally. While the jejunal patches may have other functions as a consequence of the recirculating lymphocytes through the associated large interfollicular tissue areas, the IPP behaves as a primary lymphoid organ and may therefore be the analogue of the avian bursa of Fabricius (Reynolds and Morris, 1983a; Landsverk, 1984; Miyasaka et al., 1984b; Larsen and Landsverk, 1985).

2.9.3 GALT as a secondary (peripheral) lymphoid organ

Based on some experimental findings, several authors have argued against the view of the mammalian GALT being analogous to the avian bursa.

a. It is reported that Peyer's patches produce progenitors of IgA (Craig and Cebra, 1971; Befus et al., 1978; Butcher et al., 1982; Husband and Gowans, 1978) and the proliferation in Peyer's patches together with their attainment of full size is in response to antigens absorbed from the intestinal lumen (Gulliani et al., 1974). Lymphopoiesis is arrested in GALT of rabbits following surgical in situ isolation of these tissues from the normal gut-lumenal flow without disruption of vascular integrity (Perey and Good, 1968; Stramignoni et al., 1969). In the bovine fetus, development of the lymphoid tissue of the GALT has been observed to be directly related to antigenic stimulation giving a probability that the GALT in this species is not a primary lymphoid organ (Schultz et al., 1973 ).

Nevertheless, experiments in birds show that analogous bursal implants grow in the peritoneal cavity but unlike thymus implants fail to continue their lymphopoietic activity in that site. This suggests the proximity to the contents of the intestinal lumen to be essential for the lymphopoietic function of the bursa

(Thomson and Cooper. 1971), which is comparable to the above observations in rabbits and in the bovine.

b. In contrast to the thymus in mammals and the thymus and bursa in birds, results in rats do not show well developed Peyer's patches in germfree animals and little or no follicular mitotic activity in the absence of extrinsic antigenic stimulation. Moreover, such stimulation induces the formation of prominent germinal centers, immunoblasts and plasma cells, characteristics which are not normally observed in the thymus or bursa. Thus the Peyer's patches classified on the ability to mount an immune response criterion, at least in the rats are secondary lymphoid organs (Cooper et al., 1968). The results, however do not exclude the possibility that these tissues may posses a dual role, serving both as antibody producers and as a bursal-analogue in controlling the development of the humoral antibody mechanisms in rats.

c. Like other peripheral lymphoid organs, Peyer's patches of mice contain functional T-and B-lymphocytes. The presence of T-lymphocytes in the developing Peyer's patches indicates that lymphoid differentiation in these organs is in part, at least, thymus dependent (Gowans and Knight, 1964; Evans et al., 1967; Joel et al., 1971; Raff and Owen, 1971). Some thymus derived cells migrate to the mammalian GALT but they occur in areas outside of the organized lymphoid follicles (Sutherland et al., 1970). Joel et al. (1971) estimated that at least

60 to 75% of the lymphocytes present in the Peyer's patches of newborn mice are derived from the thymus.

d. The pattern of repopulation of Peyer's patches in irradiated mice resemble that of lymph nodes, but not that of thymus (Evans et al., 1967) . In similar experiments using immunofluorescent markers, Friedberg and Weissman (1974) demonstrated prominent accumulations of T-lymphocytes in Peyer's patches of mice, and insufficient level of proliferation in the patches to account for the increase in even the splenic immunoglobulin-bearing cells. The authors concluded thus, it is unlikely at least in the neonatal mice that the Peyer's patches are primary lymphoid organs analogous to the avian bursa.

e. Culture of Peyer's patch cells from nonprimed rabbits can respond in vitro to sheep red blood cells. The response is independent of the absence of epithelial cells which cover the lymphoid aggregates and it is very similar to that of spleen cultures from normal rabbits. In contrast, thymic cultures can not mount a primary immune response in vitro. These findings place the Peyer's patches in the category of secondary lymphoid organs (Henry et al., 1970). However, these findings did not consider the possibility that some of the reacting Peyer's patch cells could be recirculating differentiated nonspecific B-cells, which may have homed to the patches through the post-capillary venules.

f. Immunoglobulin bearing B-lymphocytes can differentiate within the mammalian fetal liver and on this basis mammalian liver can be considered as a candidate for a mammalian 'bursa equivalent' (Owen et al., 1974). Postnatally, the bone marrow continuously generates and disseminates large numbers of lymphocytes which initially lack distinctive surface receptors, but undergo marked changes in surface properties and functional responsiveness during maturation, which takes place in spleen and lymph nodes (Osmond, 1980).

Peyer's patches probably have a number of functions and the ease with which evidence of any of these can be obtained may vary with the age and species of the animals. This probably contributes to the controversy about whether or not Peyer's patches are a bursa equivalent (Reynolds et al., 1981).

## 2.10 Lymph drainage of the intestinal tract

Lymph vessels pervade the entire body forming a second vascular system in addition to the blood vessels. The fluid lymph is of variable composition but generally that of the extremities has a certain resemblance to blood plasma although it contains more water and less protein. Cholesterol, fatty acids, colostral proteins and electrolytes are absorbed into the intestinal lymph from the gut, and as a result the composition of intestinal lymph is largely determined by the diet. Information on the formation and composition of lymph can be obtained from Yoffey and Courtice (1980) and Schummer et al. (1981).

The lymphatics of the intestinal tract as are of other regions of the body can be made grossly visible with the use of various materials and techniques (Taher, 1965; Ozguden, 1967; Yoffey and Courtice, 1970; Gooneratne, 1972; Tanudimadja and Ghoshal, 1973). From the 18th century, a variety of materials including water, gelatin, wax, oil, chinese ink, prussian blue, carmine and mercury had been used as injections into cadavers to demonstrate the lymphatics. Recently, a number of dyes such as trypan blue, Evan's blue, pontamine sky blue, patent blue violet or suspensions of particles e.g. indian ink have been used either by injecting into the lymphatics themselves or into the organ parenchyma. Another technique for demonstrating the lymphatics is the injection of radio-opaque material.

### 2.10.1 Lymph flow from the intestine

In the initial lymphatics such as the lacteals, small molecules of 2,000 - 5,000 molecular weight pass readily through the closed junctions but larger molecules such as plasma protein pass more slowly. Junctions where larger molecules can enter the lymphatics are more open when greater than normal pressure differences exists between the tissues and the lumen of the lymphatics. Moreover, at the initial lymphatics, many open junctions are found (Casley-Smith, 1968). These are caused by the poor support given to the cells by the poor basement membrane, and secondly, the flow of fluid due to the increased pressure differences tends to push the cells apart. Large particles and cells will also act as dilators causing

larger gaps between the cells for the passage of of larger molecules into the lymphatics.

Propulsion of the lymph in the vessels is brought about by vascular and extravascular forces. Lymph vessels have own contractibility caused by the wall muscle fibers. In addition, when a segment is filled with lymph, this alone is thought to initiate the lymph flow. Delivery of a limited fluid load increases the frequency of contraction and the rhythmic contractile activity of an afferent and efferent vessel is related to the lymphatic pressure measured from the vessels and not to other physiological parameters (Johnston, 1984). In the sheep, lymph vessels have been shown to regulate lymph flow through appropriate contractile activity and this activity can be modified with pharmacological agents (Chin and Cahill, 1984).

Vascular contractility force is supplemented by the utilisation of extravascular forces including physicochemical forces, the movement of muscles and organs, pulsation of arteries and veins and the effect of respiration (Casley-Smith, 1968; Schummer et al., 1981).

The lacteals from different villi join and a net like pattern of vessels is formed around the Peyer's patch follicles. The vessels then form the afferents of the mesenteric lymph nodes from which the efferents issue and form the colic and jejunal trunks. The two trunks confluence and form a larger intestinal trunk which empties into the cisterna chyli (Von Forstner, 1973). From the

cisterna lymph flows into the thoracic duct and the latter empties into the venous blood at the left venous angle.

Lymph movement in the thoracic duct is brought about by the intrathoracic suction force and the pull produced by the large veins near the heart (Schummer et al., 1981). In rats, much of the lymph from the abdominal viscera returns to the blood circulation at some points distal to the entry of the thoracic duct. The findings indicate the presence of lymphatic-venous anastomoses throughout the abdominal venous system in this species (Paldino and Hyman, 1964). Similar connections also seem to exist in the dog (Freeman, 1942).

In the sheep the main intestinal lymph ducts collect all the lymph from the intestine (Chin and Cahill,1984). Direct connections between the intestinal lymphatics and blood vessels in the abdominal and caudal thorax in normal sheep are very uncommon, but in thoracic duct occluded sheep vascular connections develop between the azygous vein and the thoracic duct (Heath, 1964).

2.10.2 Mesenteric lymph nodes drainage areas

The mesenteric lymph nodes afferent and efferent lymphatics are as follows (Tanudimadja and Ghoshal, 1975; Von Forstner, 1973; Schummer et al., 1981).

a. lympho nodi (Inn.) pancreoticoduodenales

receive afferents from the pancreas, initial segment of the jejunum, duodenum and distal part of ascending colon. Efferent vessels drain into the intestinal trunk.

b. Inn. ileocolici (Schummer et al., 1981) or Inn. cecales (Tanudimadja and Ghoshal, 1975) or Inn. ileocecocolici (Von Forstner , 1973) - drain the cecum, ileum and proximal part of colon and efferents may go to the colic lymph nodes or empty into the colic trunk.

c. Inn. jejunales-drain the jejunum, distal part of duodenum and proximal parts of ileum. Efferent vessels converge forming a single jejunal trunk which in turn opens in the intestinal trunk.

d. Inn. colici-receive afferent vessels from mainly the colon and the efferents converge forming the colic trunk which in turn joins the intestinal trunk.

The lumbar and intestinal trunks enter the cisterna chyli independently (Von Forstner, 1973).

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Gross and histological studies of the gut-associated lymphoid tissue and mesenteric lymph nodes

Three age groups of conventionally reared male goats (Capra hircus) of local breed were used in these studies. The groups were ten newborn kids (killed 1 - 12 hours after birth), fifteen 3 months old kids and fifteen 8 - 12 months old goats. Specimens from the 8 - 12 months old goats were collected at Vingunguti abattoir in Dar es Salaam while specimens from the other two groups were collected at Sokoine University of Agriculture, Morogoro.

### 3.1.1 Gross studies and specimens collection

a. Animals were aged using their dentition status according to Holst and Denney (1980) and Schummer et al.,(1979) (Appendix 1). Antemortem inspections were performed and those showing lesions or gross clinical signs of diseases were excluded.

b. Animals were then killed by exsanguination through the jugular veins after sedation with Xylazine. Those of 8 - 12 months age were slaughtered by cutting through the necks including the spinal cords at the atlanto-occipital joints. Immediately after death, animals of 3 months and 8 - 12 months age were weighed to the nearest 0.2 kg, and the newborn kids to the nearest gram .

c. Within five minutes after death, the abdominal cavities were opened through the linea alba. The pelvic symphyses were separated and the termination of the colons were marked. The distal ends of esophagus were ligated by double ligatures and resected by cutting between the ligatures. The gastrointestinal tracts were removed en masse from the cardia.

d. Thorough postmortem inspections of the thoracic and abbominal cavities organs were performed, and whenever lesions were observed such animals were excluded.

e. Double knots were placed at the pylori and resections performed by cutting between the ligatures. The livers, omenta and spleens were then dissected out from the stomach compartments. The stomach compartments have large varying capacities, and as there was no control over feeding restrictions on animals of the 8 - 12 months age, animals' liveweight attributed by different levels of stomach filling were reduced by weighing the four stomach compartments with their contents.

Their weights were then subtracted from the animals' body weight to obtain corrected body weights. To homogenise results, this was applied also on the other two age groups.

f. The intestine levels were marked by placing knots at the established bounderies (Habel, 1975; Schummer et al., 1979) (Appendix II ).

g. By observing through the serosae of the intestine, the duodenal, jejunal, jejuno-ileal (extended from jejunum to ileocecocolic junction ), cecal and colic Peyer's patches were located. About 2 cm. long intestine sections with Peyer's patches randomly selected in the duodenum and jejunum were ligated after squeezing out the contents. Bouin's fixative was injected into the ligated segments until mild distensions were evident. The cranial ends of Jejuno-Ileal Peyer's patches (JIPP) were located, ligated and injected with Bouin's fixative as above. This was repeated for the roughly located middle sections of JIPP, the caudal parts of JIPP, cecal and colic Peyer's patches.

h. The jejunal, ileocecocolic and colic lymph nodes were then separated, counted, weighed and recorded separately. Samples either whole or about 0.5 cm thick cross sections through the hili of the nodes were taken one from each group of lymph nodes and fixed in Bouin's fixative.

i. The mesenteries were then dissected out and intestine levels lengths were measured and recorded. About 1.5 cm. lengths of the Bouin's-injected ligated intestine segments were resected and fixed in Bouin's fixative.

The intestine specimens collection were completed within one hour after death.

j. Intestines were opened at their mesenteric attachments from the pylori to the ani sparing the intestine levels dermacations. They were then washed in running tap water until the washing fluid was free of mucus and ingesta. The intestine specimens were placed in 3% acetic acid for 24 hours to fix the nuclei (Cornes, 1965). To make the follicular contents more apparent, the specimens were thereafter washed in water twice then stained with 0.5% polychrome methylene blue (Color Index No. 52015 MERCK) for one minute. Excess stain was removed by rinsing the specimens in water and then allowed to stand in water for 30 minutes.

k. Peyer's patches individual lengths were measured and recorded for each level. In small patches, the number of follicles were counted with the help of a 10x magnifier. Only 5 or more follicles were counted as a Peyer's patch (Doughri et al., 1972). The total lengths of JIPP were obtained by adding 4.5 cm (which was the approximate length of the resected patch sections for histological studies) to the obtained JIPP lengths. For the other intestine levels patches, 1.5 cm. were added to the obtained lengths of the patches for the same reasons.

l. In some cases, stained intestine sections with Peyer's patches were resected and photographed.

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l. In some cases, stained intestine sections with Peyer's patches were resected and photographed.

m. All numerical results were analysed for means, standard deviations and standard errors of the means. Correlation analyses were done between corrected body weights and lymph node weights, lengths of the intestines and weights of lymph nodes, lengths of the intestines and total lengths of the Peyer's patches and between weights of the lymph nodes and the total lengths of the Peyer's patches. Significance tests for the correlation coefficients were performed according to Sokal and Rohlf (1973). The difference of the means of some parameters between the three age groups were tested for significance using the students t-test.

### 3.1.2 Preparation of samples for histological studies

Collected tissue specimens were fixed in Bouin's fixative for about 18 hours. Thereafter, specimens were washed in 50% ethanol until solutions were free of the yellow Bouin's color. They were then washed twice in 70% ethanol and then left in the same until processed.

Tissue specimens about 2 mm. thick were embedded in paraffin, sectioned at 5 microns and stained with Harris Haematoxylin- Eosin and Weigerts Iron Hematoxylin-Van Gieson using standard procedures (Gordon, 1982; Bradbury and Gordon, 1982; Stevens, 1982).

### 3.1.3 Histological studies

#### 3.1.3.1 Lymph nodes

The studies of microscopic structural variations of the lymph nodes were performed on the stained sections using a light microscope. Five sections of jejunal, ileocecocolic and colic lymph nodes from the newborn kids were used for the histological studies. Twelve, 8 and 10 sections of jejunal, ileocecocolic and colic lymph nodes, respectively from the 3 months old kids and 9, 14 and 11 sections of jejunal, ileocecocolic and colic lymph nodes, respectively from the 8 - 12 months old goats were used for similar studies.

The degree of differentiation of lymph nodes and classification were done according to Sugimura (1962).

#### 3.1.3.2 Quantitative measurements on gut-associated lymphoid tissue

From each age group, five stained section of each Peyer's patch were selected for the histological studies. On each section, the following parameters were determined.

a. Cortex-medulla differentiation of the follicles

b. Depth and breadth of follicles, interfollicular

zone breadth and dome size (follicle area protruding into the intestinal lumen) were measured using a calibrated graticule. Five randomly selected structures were measured and the means recorded.

c. On the five dome epithelia examined per section, goblet cells were counted and the means recorded.

d. The number of interepithelial lymphocytes per 100 absorptive epithelial cells and per 100 dome epithelial cells were counted with a x40 objective at five randomly selected domes and villi and the means recorded.

e. Using a calibrated graticule and 100x objective with oil immersion, ten randomly selected interepithelial lymphocytes on domes and on villi epithelia were measured and their means recorded.

f. Size of ten randomly selected large lymphocytes (lymphoblasts) and ten small lymphocytes in the follicle centres of randomly selected follicles were also measured at x100 objective with oil immersion and the means recorded.

All numerical data were analysed for means, standard deviations and standard errors of the means

## 3.2 Lymph drainage of the intestinal tract

The indirect injection technique was used to demonstrate the lymph flow from the intestinal tracts. Seven healthy male goats (Capra hircus) of local breed aged three months and weighing 5 - 8 kg. body weight were used in this study.

The injection medium (Evans blue) was prepared according to Tanudimadja and Ghoshal (1973). Natural hen egg-albumen was used.

Animals were starved for 2 days but given water ad libitum. They were weighed and anaesthesized with a combination of Xylazine and Ketamine Hydrochloride. Skins were prepared routinely and laparatomy performed along the linea alba.

Intestines were taken out of the abdominal cavities onto plastic sheets wetted with isotonic saline. About 0.2 mls. of the dye were injected subserosally on the antimesenteric surfaces from the pylori to the recti at an interval of about 15 cm. using 23 Gx 1 inch needles. For the demonstration of the lymph drainage of the Jejuno-ileal Peyer's patch, the interval was about 10 cm. and for the ceca the interval was about 5 cm. Colic Peyer's patches were also injected with the dye.

The intestines were returned into the abdominal cavities and the incisions were closed. One hour after last dye injections, animals were sacrificed by exsanguination through the jugular veins. They were then kept in a cool room at about 2 - 4$^{o}$C for 24 hours.

Walls of the right thoracic and abdominal cavities were opened and the lymph vessels from the intestine were traced. to where they joined the venous system by systematic dissections. The lymph flow patterns as outlined by the dye flow pattern were inserted on intestinal tract sketches. From the sketches, two most frequent flow patterns were prepared.

# CHAPTER 4

# RESULTS

## 4.1 Morphological changes of the gut-associated lymphoid tissue (GALT) with age

### 4.1.1 Gross morphological changes

Kids were born with an average of 35 Peyer's patches in the jejunum which had a mean length of 0.9 ± 0. 1 m.* Neither the duodenum nor the cecum had any patches. The colon had an average of two Peyer's patches which were round in shape. The single Peyer's patch which extended from jejunum to the ileocecocolic junction and hence termed Jejuno-ileal Peyer's patch (JIPP) measured about 0.8 m. in length. The JIPP were not uniformly wide, the caudal one third were wider than the cranial two thirds and always the caudal half covered more than 75% of the intestine circumference.

Individual Peyer's patches in the jejunum had sizes ranging from five follicles to 4.5 cm. long patches. Their shapes were variable, but mostly they were raised and flat, oval or elongate with round ends. The Peyer's patches extended on 17.0 ± 0.5 percent of the intestine length.

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* Data in Mean ± standard error of the mean.

At the age of 3 months, the average number of Peyer's patches in the jejunum was 38, and individual patches sizes ranged from five follicles to 5.4 cm. long patches (Fig. 3). The colon had two patches which were about 30 cm. apart (Fig. 4). No Peyer's patches were observed in the duodenum and there was one observation of Peyer's patches in the cecum which had a round shape. The total length of the jejunal Peyer's patches (JPP) had increased to a mean of 1.5 $\pm$ 0.1 m. The JIPP length had also increased to 1.4 $\pm$ 0.04 m. The JIPP breadth was as in newborn kids not uniform (Fig. 5). The caudal one third of the patch were broader than the cranial two thirds. Occassionally, in the caudal two thirds the Peyer's patch covered almost the entire intestine circumference. The Peyer's patches extended on 18.0 $\pm$ 0.6 percent of the intestine length.

In the goats of 8 - 12 months age, the average number of Peyer's patches in the jejunum was 33 with individual patches lengths ranging from five follicles to 8.1 cm. long patches. The patches shapes were as described above for the newborn and three months old kids. In only single observations were duodenal and cecal patches found. The JIPP had grossly atrophied follicles, but in one observation the cranial end of the JIPP had distinct grossly visible follicles which had undergone some degree of atrophy compared to those of three months old kids (Fig. 6). The area on which the JIPP extended was

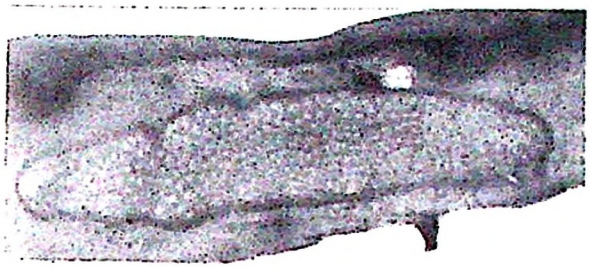

Fig. 3

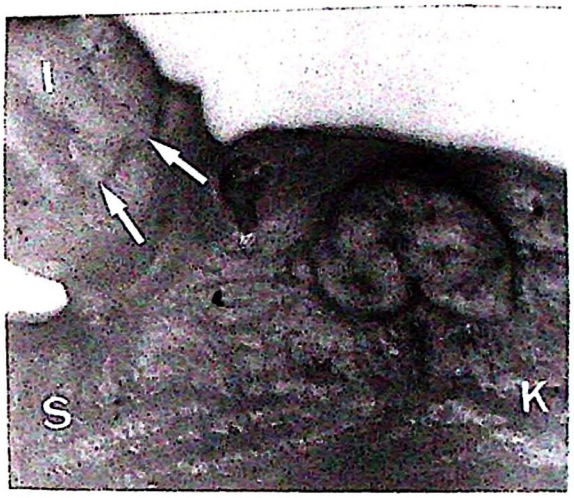


Fig. 4

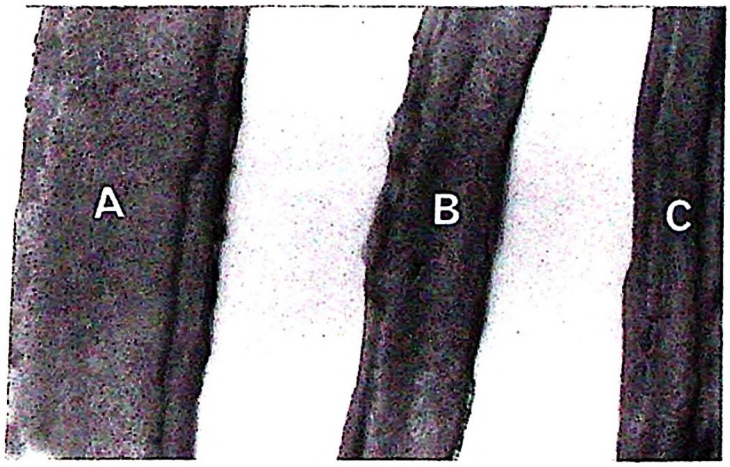


Fig. 5

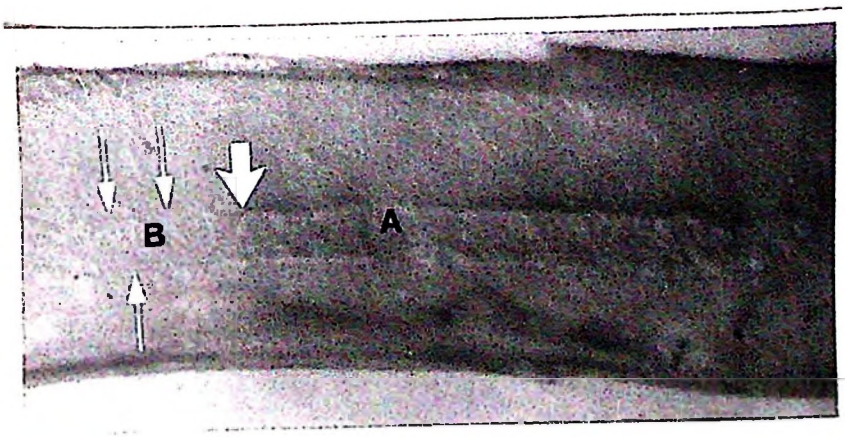


Fig. 6

Legends for Figures 3, 4, 5 and 6

Figure 3: JPP from a 3 month old kid. Domes of the follicles can be seen as whitish spots.

Figure 4: A colic Peyer's patch from a 3 months old kid. The patch is near the ileocecocolic junction. Note the termination of the JIPP (arrows). S, cecum; K, colon; I, ileum.

Figure 5: JIPP from a three months old kid. Caudal one-third (A) is wider than the middle (B) and cranial (C) portions.

Figure 6: Cranial end of JIPP from 8 - 12 months old goats. Note border (thick arrow) of grossly visible follicular part (A) and the atrophied part (B). The borders of the atrophied part are indicated by thin arrows.

marked by scattered individual follicles on the mucosal surface which were also detected on the serosal surface. An average of two Peyer's patches were observed in the colon. The mean percentage of intestine length on which Peyer's patches extended had decreased to 12.7 $\pm$ 0.5.

The mean total length of the intestine (Table 2, column 6) and Peyer's patches (column 21) increased at a relatively higher rate to three months age, but at a slower rate to the 8 - 12 months age. The mean proportion of intestine on which the Peyer's patches extended (Table 2, column 22) increased to the three months age, but had decreased to 8 - 12 months age (Fig. 7).

There was no significant difference ( $P > 0.05$) between the mean number of jejunal Peyer's patches (Table 2, column 10) of the three groups. There was significant difference ($P < 0.05$) between the means of the proportions of intestine covered by Peyer's patches (Table 2, column 22) of newborn and 8 - 12 months old goats, and between 3 months old kids and 8 - 12 months old goats. There was no significant difference ( $P > 0.05$) between the proportions of intestine covered by Peyer's patches of newborn and 3 months old kids.

Within the age groups, there was no significant correlation ($P > 0.05$) neither between corrected body weights (Table 1 column 3) and total mesenteric lymph nodes weight (column 10) nor between total intestine length (Table 2, column 6) and Peyer's patches length (Table 2, column 21). There was also no significant correlation

Table 1 : Means ($\bar{X}$) and standard errors of the means (SE) for body and mesenteric lymph nodes weight of newborn kids (I), 3 months old kids (II) and 8 - 12 months old goats (III)

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | X | 1.9 | 31.5 | 1.8 | 4.6 | 1.5 | 1.3 | 0.1 | 3.0 | 0.07 | 1.74 | 0.09 | 0.01 | 0.004 | 0.11 |
| | SE | 0.1 | 20.3 | 0.1 | 1.0 | 0.1 | 0.2 | 0.02 | 0.4 | 0.01 | 0.14 | 0.01 | 0.001 | 0.001 | 0.01 |
| II | $\bar{X}$ | 5.0 | 471.9 | 4.6 | 3.3 | 10.7 | 1.1 | 1.5 | 2.9 | 0.75 | 12.91 | 0.24 | 0.03 | 0.02 | 0.29 |
| | SE | 0.3 | 55.8 | 0.3 | 0.7 | 0.6 | 0.1 | 0.1 | 0.3 | 0.11 | 0.80 | 0.01 | 0.003 | 0.01 | 0.02 |
| III | $\bar{X}$ | 20.5 | 3520 | 17.0 | 5.1 | 21.4 | 1.6 | 2.8 | 3.5 | 3.30 | 27.41 | 0.13 | 0.02 | 0.02 | 0.16 |
| | SE | 0.7 | 230 | 0.6 | 0.7 | 0.8 | 0.2 | 0.4 | 0.2 | 0.31 | 0.99 | 0.01 | 0.002 | 0.002 | 0.01 |

Key to Table 1 :

1. Body weight (kg)
2. Weight of stomach compartments (gm)
3. Corrected body weights (kg)
4. Number of jejunal lymph nodes
5. Weight of jejunal lymph nodes (gm)
6. Number of ileocecocolic lymph nodes
7. Weight of ileocecocolic lymph nodes(gm)
8. Number of colic lymph nodes
9. Weight of colic lymph nodes(gm)
10. Total weight of 5, 7 and 9 (gm)
11. Ratio of (5) to (3) in percentage
12. Ratio of (7) to (3) in percentage
13. Ratio of (9) to (3) in percentage
14. Ratio of (10) to (3) in percentage

Table 2 : Means ($\bar{X}$) and standard errors of the means (SE) for Peyer's patches length and number, and length of the intestine of newborn kids (I), 3 months old kids (II) and 8 - 12 months old goats (III)

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | $\bar{X}$ | 0.29 | 5.51 | 0.30 | 0.09 | 1.14 | 7.33 | - | - | - | 34.9 | 0.9 | 16.6 | 1.0 | 0.8 | - | - | - | 1.9 | 0.03 | 2.3 | 1.2 | 17.0 |
| | SE | 0.01 | 0.13 | 0.03 | 0.01 | 0.04 | 0.14 | - | - | - | 1.5 | 0.1 | 0.8 | - | 0.03 | - | - | - | 0.1 | 0.003 | 0.2 | 0.04 | 0.5 |
| II | $\bar{X}$ | 0.46 | 8.95 | 0.56 | 0.12 | 1.75 | 11.84 | - | - | - | 37.1 | 1.5 | 17.0 | 1.0 | 1.4 | 1.0 | 0.02 | 14.3 | 2.0 | 0.05 | 2.8 | 2.1 | 18.0 |
| | SE | 0.02 | 0.31 | 0.03 | 0.01 | 0.09 | 0.37 | - | - | - | 2.1 | 0.1 | 0.8 | - | 0.04 | - | - | - | 0.1 | 0.003 | 0.1 | 0.4 | 0.6 |
| III | $\bar{X}$ | 0.82 | 13.63 | 0.71 | 0.24 | 4.25 | 19.63 | 1.00 | 0.02 | 1.9 | 33.1 | 1.7 | 12.5 | 1.0 | 1.5 | 3.0 | 0.01 | 4.1 | 1.7 | 0.07 | 1.8 | 2.5 | 12.7 |
| | SE | 0.04 | 0.61 | 0.04 | 0.01 | 0.22 | 0.77 | - | - | - | 1.8 | 0.1 | 0.7 | - | 0.11 | - | - | - | 0.2 | 0.01 | 0.1 | 0.1 | 0.5 |

Key to Table 2 :

1. Length of duodenum (m)
2. Lenth of jejunum (m)
3. Length of ileum (m)
4. Length of cecum (m)
5. Length of colon (m)
6. Total length of intestine (excluding rectum ) (m)
7. Number of Peyer's patches in the duodunum
8. Length of Peyer's patches in the duodenum (m)
9. % duodenum length covered by Peyer's patches
10. Number of Peyer's patches in the jejunum
11. Total length of Peyer's patches in jejunum (m)
12. % jejunum covered by Peyer's patches
13. Number of jejuno-ileal Peyer's patches
14. Length of jejuno-ileal Peyer's patches (m)
15. Number of Peyer's patches in cecum
16. Length of Peyer's patches in cecum (m)
17. % cecum covered by Peyer's patches
18. Number of Peyer's patches in colon
19. Length of Peyer's patches in colon (m)
20. % colon covered by Peyer's patches
21. Total length of Peyer's patches (m)
22. % length of intestine covered by Peyer's patches.

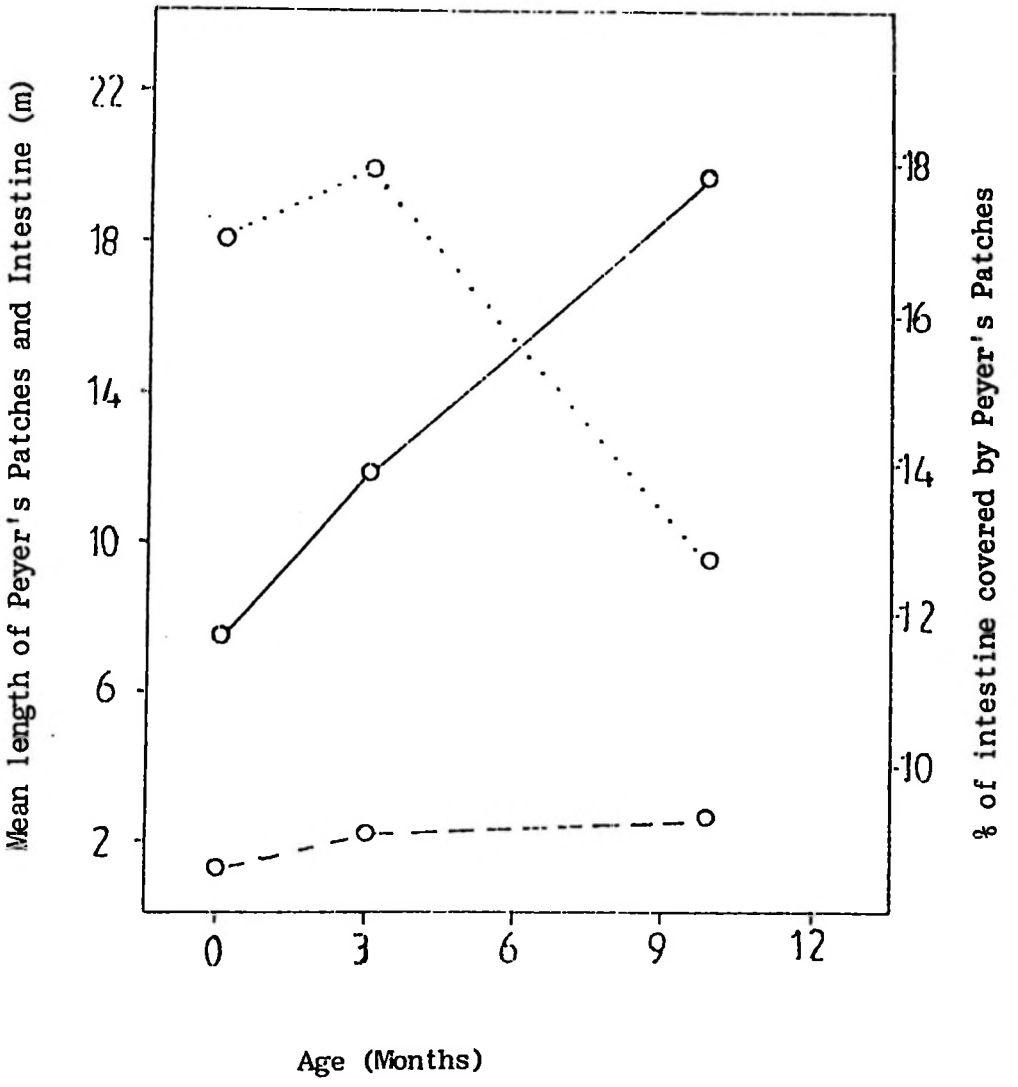


Fig. 7 : Postnatal changes in mean intestine length (————), mean Peyer's patches length, (-------) and mean % of intestine covered by Peyer's patches (.....)

between Peyer's patches lengths (Table 2,column 21) and mesenteric lymph nodes weight (Table 1, column 10).

### 4.1.2 Microscopic changes of GALT with age

92% of newborn kids Peyer's patches follicles were clearly differentiated into cortex and medulla. The follicles were fully developed and had typical Peyer's patches follicular morphology of dome protruding into the intestinal lumen, interfollicular area, corona and follicle center.

The follicles were separated from each other and from the interfollicular area by a connective tissue capsule. Their shapes were variable, but generally, the JPP follicles were short, pear-shaped while the JPP follicles were long cylindrical and tightly packed (Figs. 8 and 9 ). The colon follicles were short, round or oval - shaped.

In newborn kids which had suckled, the dome as well as the lamina propria of the villi were more populated with lymphocytes. In these kids, a large number of the villi epithelial cells had their cytoplasms filled with eosinophilic globules and their nuclei were deformed and displaced. The globules varied in size and position within the cells. The globules were not observed on the dome epithelium (Fig. 10). In the kids which had not suckled, the follicle centers and domes were less populated with lymphocytes compared to kids which had suckled (Figs. 11 and 9), the villi lamina propria had few

Fig. 8

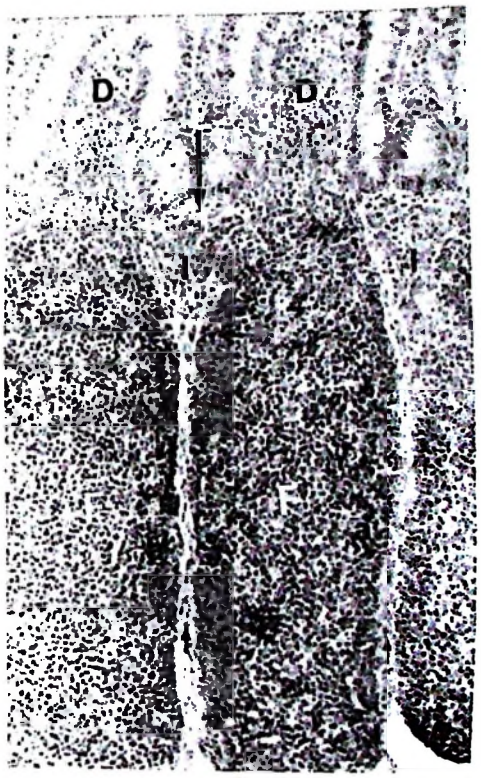


Fig. 9

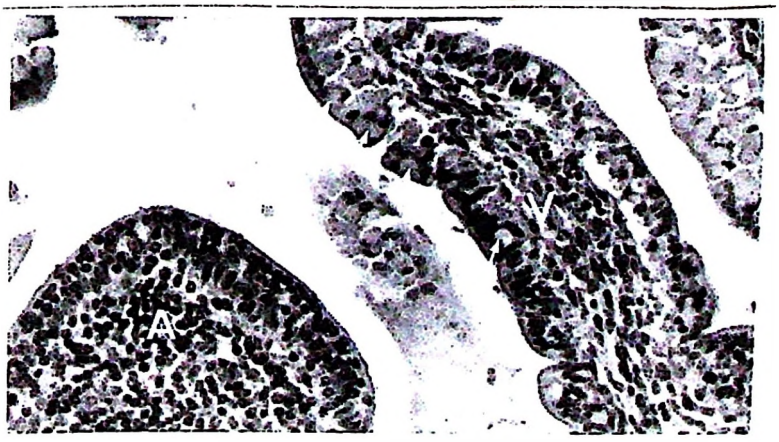


Fig. 10

Legends for Figures 8, 9 and 10

Figure 8 : JPP follicles of newborn kid. Follicles (F) are short and have wide interfollicular areas (I). (V.G. 50 X).

Figure 9: Mid JIPP follicles of a newborn kid which had suckled. The cylindrical follicles are separated from each other by connective tissue capsule (arrows). D, dome; F, follicle center; a, follicle cortex; I, interfollicular areas are small and triangular in shape; L, lamina muscularis mucosa. (H. & E. 125 X).

Figure 10: Dome (A) and villi (V) of JPP of a newborn kid which had suckled. Eosinophilic globules (arrows) are observed in villi epithelial cells but not in dome epithelium. Note high IEL on the dome epithelium. (H. & E. 250 X).

lymphocytes and the villi epithelial cells nuclei were located near the apices (Fig. 12). Occasionally, in kids which had suckled, material staining like the whey in the intestinal lumen could be found in the lacteals. However, in both cases, lymphocytes were more numerous in the dome epithelia than in the villi epithelia (Table 3, columns 7 and 11) and goblet cells were occasionally seen on the dome epithelia.

The follicles had outer cortical zone of mainly small lymphocytes mixed with few large lymphocytes (lymphoblasts), tingebody macrophages and reticular cells. On the lumenal side of the follicles, between the follicle center and the dome, the cortical layer form the corona (Fig. 11). True germinal centers were not observed, but in the kids which had suckled, the follicles showed increased population of lymphocytes in the medullary region of the follicles (compare Figs. 9 and 11 ).

The interfollicular areas were located at the follicles apex and were small triangular-shaped in the JIPP. They were bordered on either side by the adjacent follicles capsules and on the lumenal side by the lamina muscularis mucosa (Fig. 9). The JPP and colic Peyer's patches interfollicular areas were broader and on the serosal side they were bordered by the tunica muscularis (Fig. 8). Post-capillary high - endothelial venules were observed in the interfollicular areas (Fig. 13).

Table 3 is summary of the measured histological parameters of the Gut-Associated Lymphoid tissue (GALT) of newborn kids.

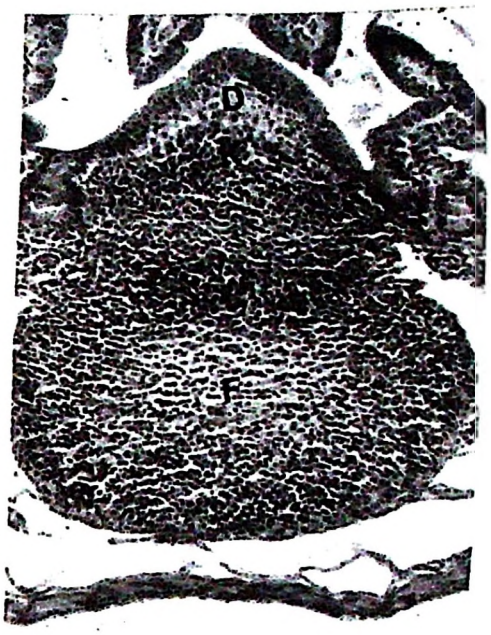


Fig. 11


Fig. 12

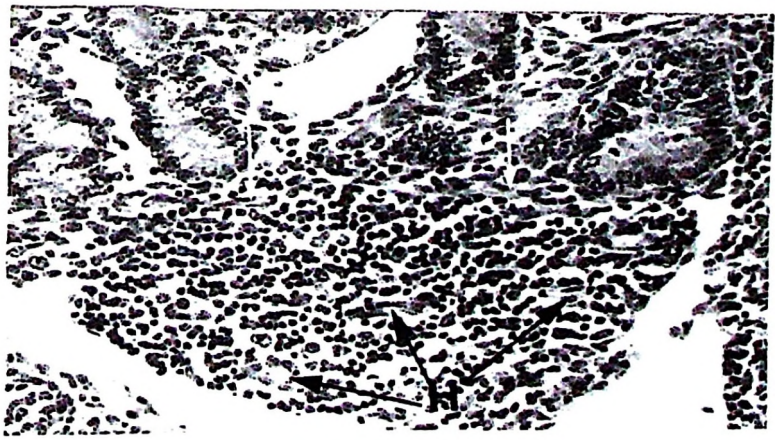

Fig. 13

Legend for Figures 11, 12 and 13

Figure 11 : JPP follicle of a newborn kid which had not suckled. Follicle center (F) and dome (D) have a low lymphoid cells population and the former has many reticular cells. K, Corona. Note lower dome IEL compared to Fig. 10. (H. & E. 125 X ).

Figure 12 : Villi epithelium of a newborn kid which had not suckled. Note apical position of the nuclei and low IEL. L, lacteals. (H. & E. 500 X).

Figure 13 : JPP interfollicular area of a newborn kid with high-endothelial venules (H). Lamina muscularis mucosa is indicated by short arrows. (H & E. 250 X).

Table 3 : Histological parameters of the GALT of ten newborn kids (Means ± Standard error of the means)

| Peyer's patches No. | Parameter | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 2 | 80 | 649±135 | 418±35 | 257± 45 | 234±32 | - | 47±16 | 7.0±0.2 | 3.5±0.1 | 4.9±0.1 | 7±2.0 | 4.1±0.1 |
| 3 | 80 | 733±118 | 286±11 | 124±15 | 184±29 | - | 40±11 | 7.0±0.4 | 3.9±0.1 | 4.9±0.1 | 7±1.0 | 4.5±0.2 |
| 4 | 100 | 734±102 | 282±13 | 154±20 | 155±17 | 2.0±0.5 | 18±4 | 7.5±0.4 | 4.0±0.04 | 4.6±0.2 | 11±3.0 | 4.7±0.1 |
| 5 | 100 | 649±72 | 343±67 | 162±26 | 185±19 | 2.0±0.8 | 17±2 | 7.3±0.1 | 4.1±0.1 | 4.6±0.2 | 10±3.0 | 4.6±0.2 |
| 6 | 100 | 288±21 | 270±30 | 166±24 | 97±4 | 2.0±0.5 | 18±2 | 7.0±0.1 | 3.5±0.05 | 4.3±0.4 | 4±0.5 | 4.5±0.1 |



Key to Table 3 :

Parameter No. : 1. Cortex-Medulla differentiation (%)
2. Depth of follicles (Microns)
3. Breadth of follicles (Microns)
4. Interfollicular areas breadth (Microns)
5. Dome size (Microns)
6. Goblets cells per dome epithelium
7. Number of IEL per 100 dome epithelial cells
8. Diameter of large lymphocytes (lymphoblasts) in the follicle center ( Microns)
9. Diameter of small lymphocytes in the follicles centre (Microns)
10. Diameter of IEL of dome epithelium (Microns)
11. No. of IEL per 100 filli epithelial cells
12. Villi IEL diameter (Microns)

Peyer's Patch Number:

2 - Jejunal Peyer's patch
3- Cranial Jejuno-ileal Peyer's patch
4 - Mid Jejuno-ileal Peyer's patch
5- Caudal Jejuno-ileal Peyer's patch
6 - Colic Peyer's patch

At three months age, 96% of the follicles were differentiated into cortex and medulla. The JPP follicles were short, broad and pear-shaped and had wide interfollicular areas while the JIPP follicles were long cylindrical with small triangular interfollicular areas. Some follicles showed invaginations on the domes (Fig. 14). However, some JIPP follicles showed similar shapes and broad interfollicular areas as JPP follicles. Colic Peyer's patches were cone-shaped and had prominent corona.

The follicles were more cellular than in the newborns, and the lamina propria of the villi and domes were also more populated with lymphocytes. In some follicles, the dome epithelium had goblet cells, which were more prominent on the villi epithelium. The follicles had the basic morphologic characters of dome, corona and interfollicular zone, but only 25% of the JPP and JIPP had typical germinal centres. All the colic Peyer's patches follicles had germinal centers. Mitotic cells were frequently observed in the periphery of the germinal centers. A caplike accumulation of small lymphocytes between the germinal centers and the domes formed the corona. Post-capillary high-endothelial venules in the interfollicular area were also observed. Tabulated results of the GALT histological parameters of 3 months old kids are presented in Table 4.

In the goats of 8 - 12 months age, the Peyer's patches follicles had distinct cortices and medulla, had germinal centers and their shapes were as described above for the newborn and 3 months old kids. The interfollicular areas were generally widest

Table 4 : Histological parameters of the GALT of fifteen three months old kids. (Means ± Standard error of the means )

| Peyer's patches No. | Parameters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 2 | 80 | 675±63 | 618±71 | 294±40 | 263±31 | 1.0±0.5 | 48±7 | 7.1±0.9 | 3.4±0.2 | 4.7±0.2 | 28±5 | 4.1±0.1 |
| 3 | 100 | 824±120 | 453±31 | 243±47 | 207±29 | 2.1±0.4 | 48±9 | 6.9±0.6 | 3.5±0.2 | 4.6±0.3 | 36±3 | 4.3±0.2 |
| 4 | 100 | 1063±122 | 392±18 | 159±17 | 165±16 | 1.2±0.5 | 22±3 | 6.6±0.2 | 3.4±0.1 | 4.6±0.2 | 17±2 | 4.2±0.2 |
| 5 | 100 | 964±62 | 469±23 | 317±52 | 289±48 | 2.3±1.0 | 46±13 | 6.5+0.2 | 3.5±0.1 | 4.9±0.3 | 19±4 | 4.7+0.2 |
| 6 | 100 | 807±81 | 560±61 | 269±20 | 213±46 | 1.6±0.9 | 97±56 | 8.0+0.3 | 3.4±0.1 | 4.6±0.1 | 60±33 | 4.4±0.1 |

Key to Table 4 :

Parameter No. :
1. Cortex-Medulla differentiation (%)
2. Depth of follicles (Microns)
3. Depth of follicles (Microns)
4. Interfollicular area breadth (Microns)
5. Dome size.(Microns)
6. Goblet cells per dome epithelium
7. Number of IEL per 100 dome epithelial cells
8. Diameter of large lymphocytes (lymphoblasts) in the follicle center (Microns)
9. Number of small lymphocytes in the follicles center (Microns)
10. Diameter of IEL of dome epithelium (Microns)
11. No. of IEL per 100 villi epithelial cells
12. Villi IEL diameter (Microns)

Peyer's Patch Number:
2 - Jejunal Peyer's patch
3 - Cranial Jejuno-ileal Peyer's patch
4 - Mid Jejuno-ileal Peyer's patch
5 - Caudal Jejuno-ileal Peyer's patch
6 - Colic Peyer's patch

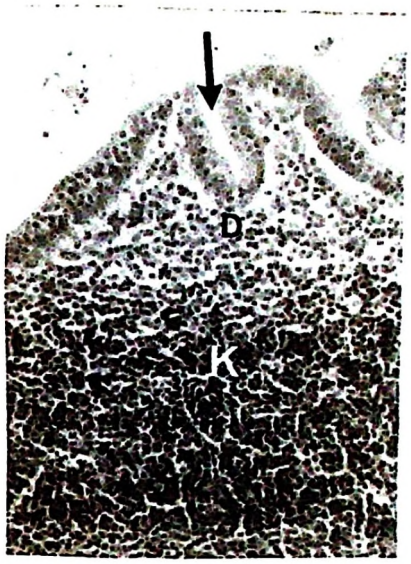


Fig. 14

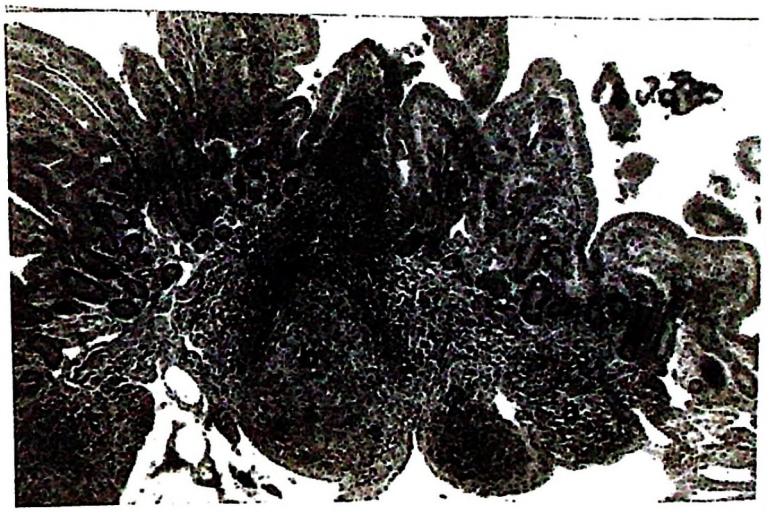

Fig. 15

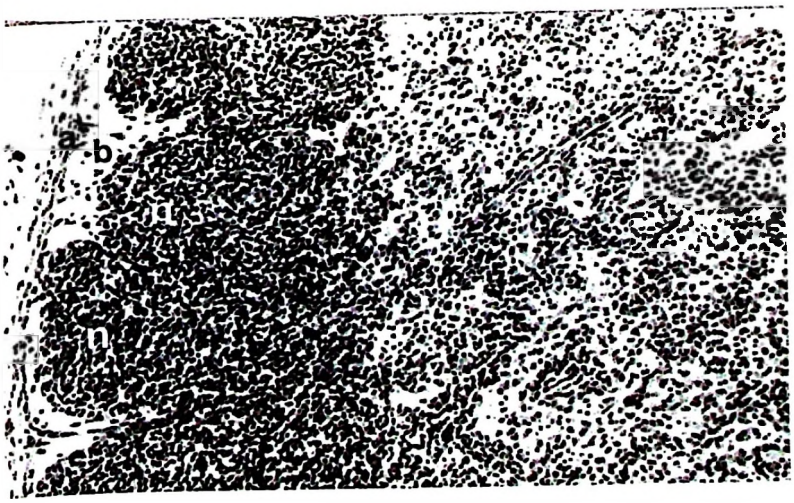


Fig. 16

Legends for Figures 14, 15 and 16

Figure 14: Invagination (arrow) on a dome epithelim in a 3 months old kid. D, dome; K, Corona, (H. & E. 125 X).

Figure 15 : Caudal JIPP section of a 8 - 12 months old goat. Atrophied follicles (a) near a large follicle (b). (H. & E. 50 X ).

Figure 16 : Colic lymph node of a newborn kid. Cortex is narrow, with primary lymphoid nodules (n). a, capsule; d, subcapsular sinus. (H. & E. 125 X ).

in the JPP. Some follicles in the JIPP were smaller in size in relation to adjacent follicles and had an increased infiltration with reticular cells (Fig. 15). These were in different stages of atrophy. In the interfollicular areas, postcapillary high endothelial venules were observed, and plasma cells were also observed in the lamina propria of the villi. Table 5 presents the histological parameters of the GALT of the 8 - 12 months old goats.

Figs. 17 and 18 (from columns 2 and 3 of Tables 3, 4 and 5) show the mean Peyer's patches follicles depth and breadth changes along the intestinal tract and with age. The figures show great variation in the follicles depth and breadth. Generally, the newborn kids had the smallest depth and breadth. However, it is interesting to note that the mid JIPP of the 8 - 12 months old goats had a depth which was lower than that of the newborn and 3 months old kids. Its breadth was also lower than that of 3 months old kids and about equal to that of newborn kids.

The mean of intraepithelial lymphocytes (IEL) number on the dome and on the villi change with age and along the intestinal tract are as shown in Figs. 19 and 20. The figures show a general increase of the IEL with age and a general trend of decrease of dome and villi IEL from cranial end of the intestine to caudal end in 8 - 12 months old goats. There was also a general decrease of dome IEL from cranial end to caudal end of the intestine in the newborn kids. Nevertheless, the mean number of IEL on the dome was always higher than on the villi.

Table 5 : Histological parameters of the GALT of fifteen goats aged 8 - 12 months (Means ± Standard error of the means )

| Peyer's patch No. | Parameters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 2 | 100 | 867±64 | 598±41 | 316±73 | 296±24 | - | 84±11 | 8.1±0.3 | 3.1±0.1 | 3.9±0.3 | 67±10 | 4.0±0.2 |
| 3 | 100 | 758±121 | 449±52 | 151±25 | 240±24 | 0.2±0.01 | 72±7 | 7.0±0.3 | 3.1±0.1 | 4.3±0.2 | 41±7 | 4.1±0.3 |
| 4 | 100 | 679±147 | 305±23 | 161±30 | 187±36 | 0.4±0.2 | 69±14 | 6.8±0.4 | 3.3±0.1 | 4.1±0.3 | 52±7 | 4.1±0.1 |
| 5 | 100 | 987±77 | 524±80 | 238±38 | 339±52 | 0.2±0.1 | 57±4 | 7.3±0.3 | 3.0±0.1 | 4.3±0.2 | 37±8 | 4.4±0.2 |
| 6 | 100 | 758±70 | 715±76 | 235±39 | 70±19 | - | 48±1 | 7.7±0.1 | 3.2±0.1 | 3.6±0.1 | 14±1 | 3.7±0.1 |



Key to Table 5 :

Parameter No. 1. Cortex-Medulla differentiation (%)
2. Depth of follicles (Microns)
3. Breadth of follicles (Microns)
4. Interfollicular area breadth (Microns)
5. Dome size (Microns)
6. Goblet cells per dome epithelium
7. Number of IEL per 100 dome epithelial cells
8. Diameter of large lymphocytes (lymphoblasts) in the follicle center (Microns)
9. Diameter of small lymphocytes in the follicles center(Microns)
10. Diameter of IEL of dome epithelium (Microns)
11. No. of IEL per 100 villi epithelial cells
12. Villi IEL diameter (Microns)

Peyer's patch Number: 2 - Jejunal Peyer's patch
3 - Cranial Jejuno-ileal Peyer's patch
4 - Mid Jejuno-ileal Peyer's patch
5. Caudal Jejuno-ileal Peyer's patch
6. Colic Peyer's patch

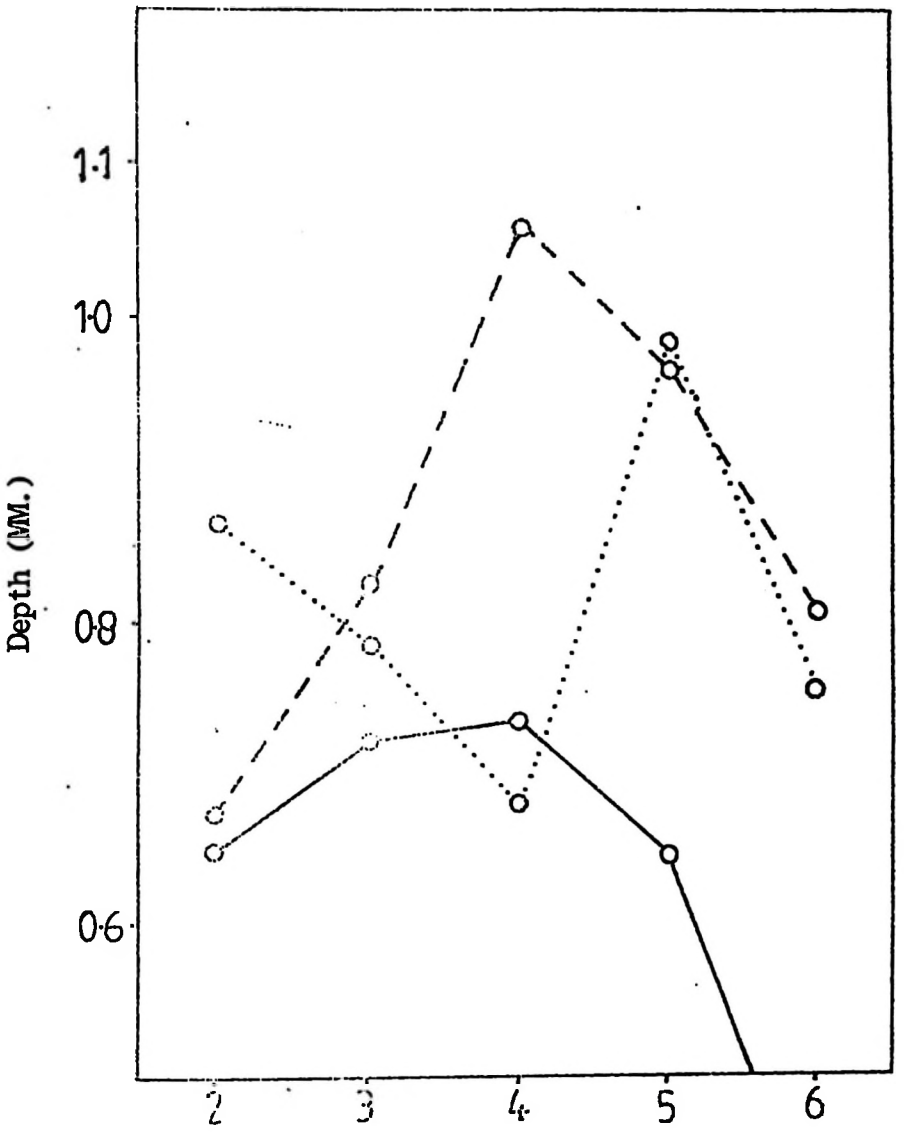


Fig. 17 : Mean depth of Peyer's patches follicles of newborn ( ——— ), 3 months old kids (- - - ) and 8 - 12 months old goats(.....)

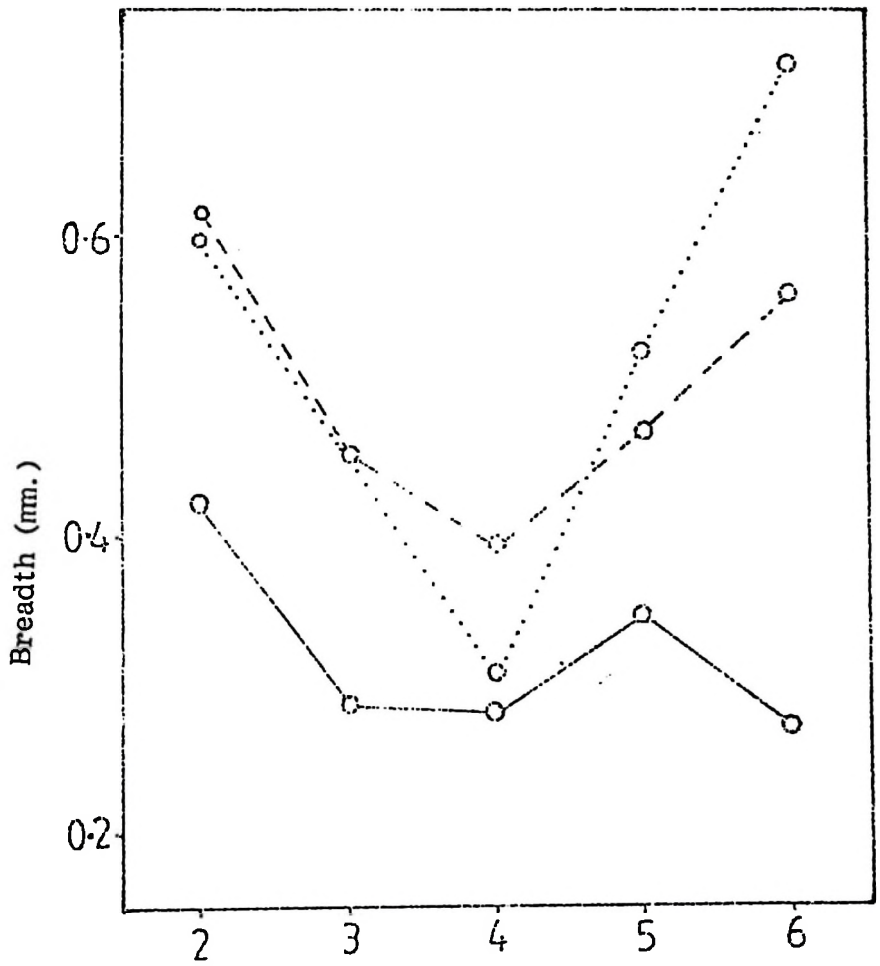


Fig. 18 : Mean breadth of Peyer's patches follicles of newborn (——), 3 months old kids (- - - ) and 8 - 12 months old goats (..... )

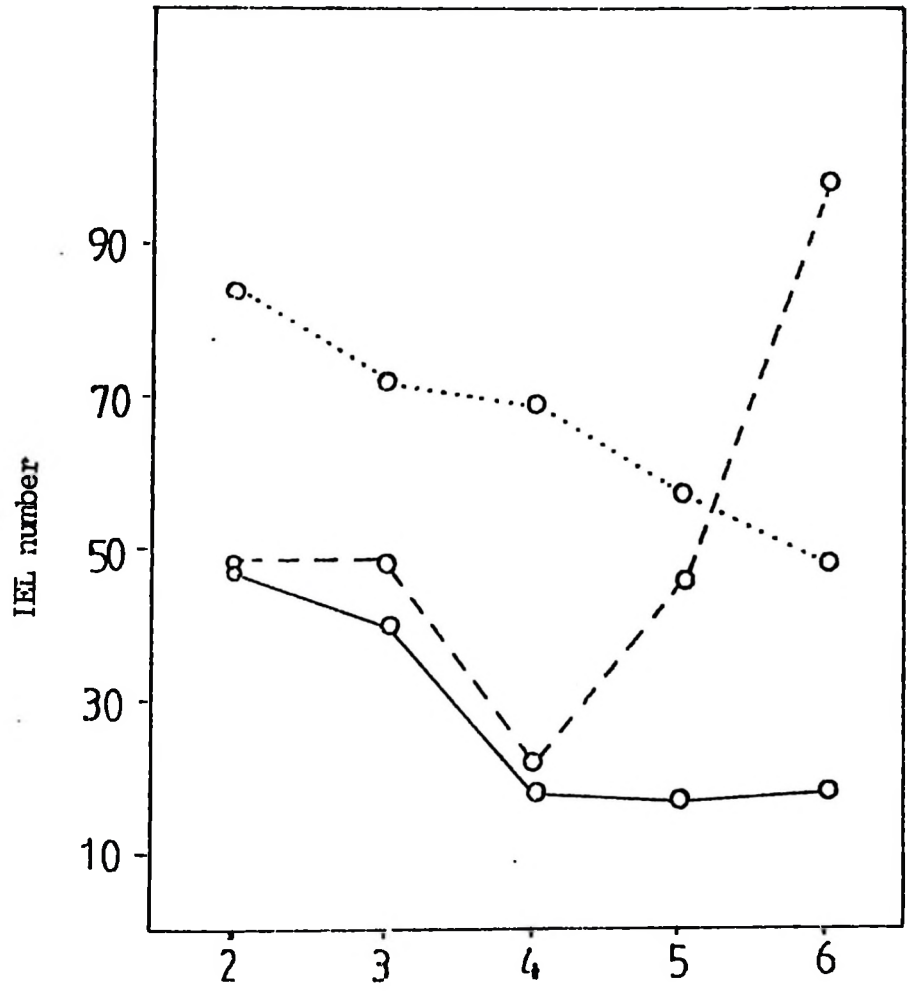


Fig. 19 : Mean dome IEL number of newborn (———), 3 months old kids (-----) and 8 - 12 months old goats (.....)

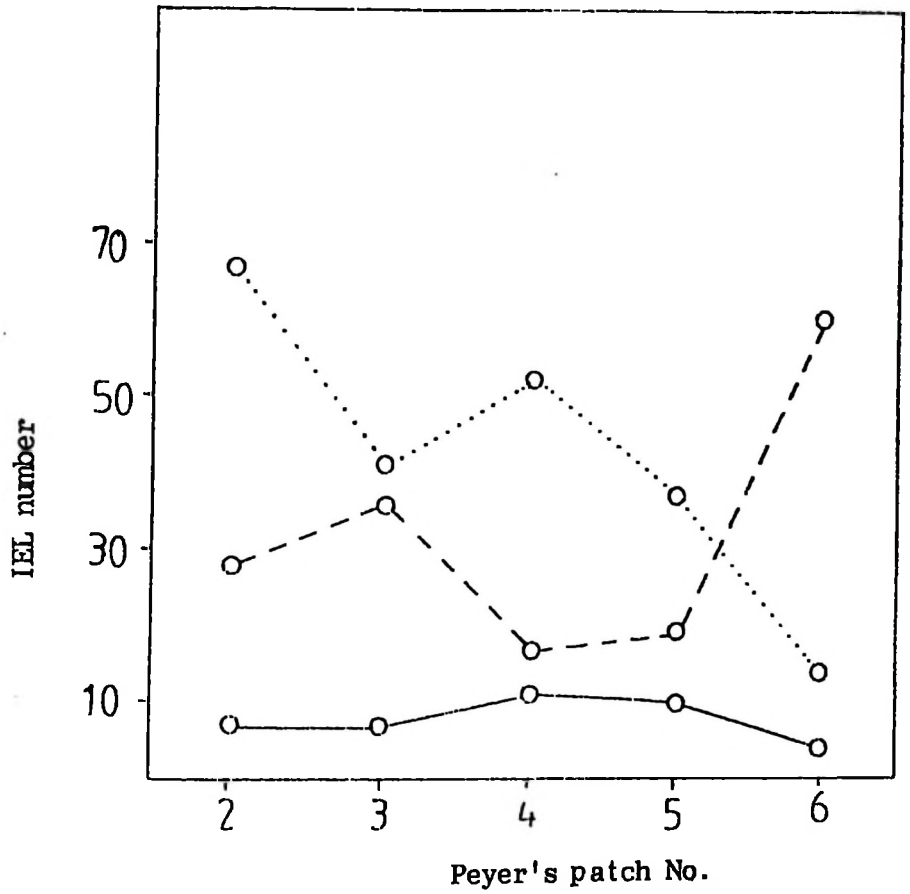


Fig. 20 : Mean villi IEL number of newborn (———), 3 months old kids (-----) and 8 - 12 months old goats (.....)

## 4.2 Postnatal gross and microscopic changes of the mesenteric lymph nodes

The gross morphological parameters of the mesenteric lymph nodes (MLN) are presented in Table 1. Their changes in weight and in their proportion to animals' body weight and age are presented in Figs. 21 and 22. The figures show a higher rate of weight increase of the MLN from newborn to 3 months age, and a lower rate of weight increase from three months to 8 - 12 months age. The proportion of MLN weight to body weight increased from birth to 3 months where it reached a peak, and from 3 months age the proportion decreased to the 8 - 12 months age. Figure 21 show that rates at which the jejunal and ileocecocolic lymph nodes increased in weight were higher from birth to three months age and the weight increase of the colic lymph nodes between the three ages was almost constant.

Apart from the presence of the connective tissue capsule and the cortex arrangement to medulla, there were marked differences in the structures of the lymph nodes of the three ages.

In the newborn kids, 53% of the nodes had distinct cortex and medulla, 40% were poorly differentiated and 7% were not. The lymph nodes of the 3 months and 8 - 12 months old goats were clearly differentiated into cortex and medulla.

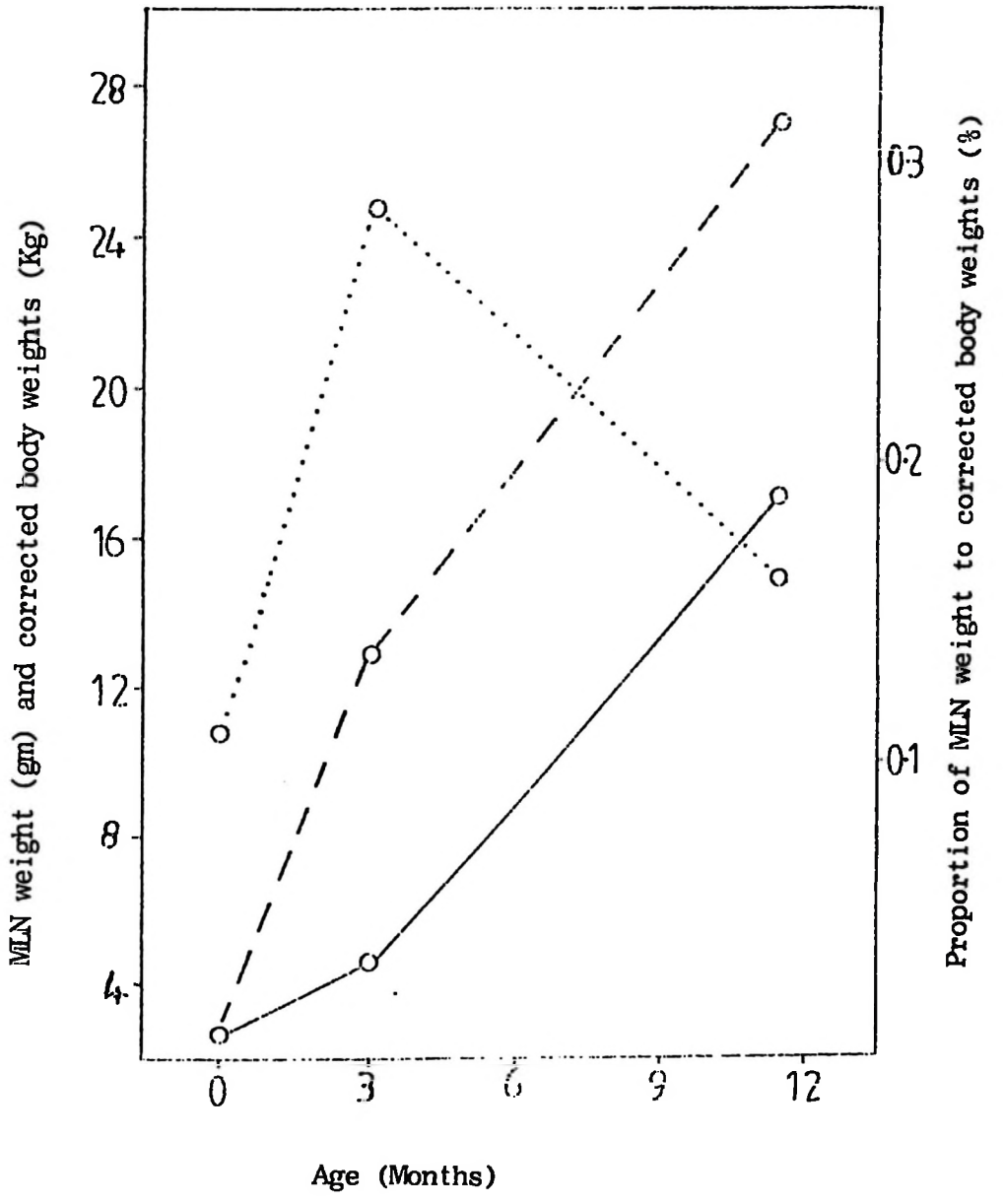


Fig. 21 : Postnatal changes in mean corrected body weights (———), MLN weights (------) and MLN-body weight proportions (.....)

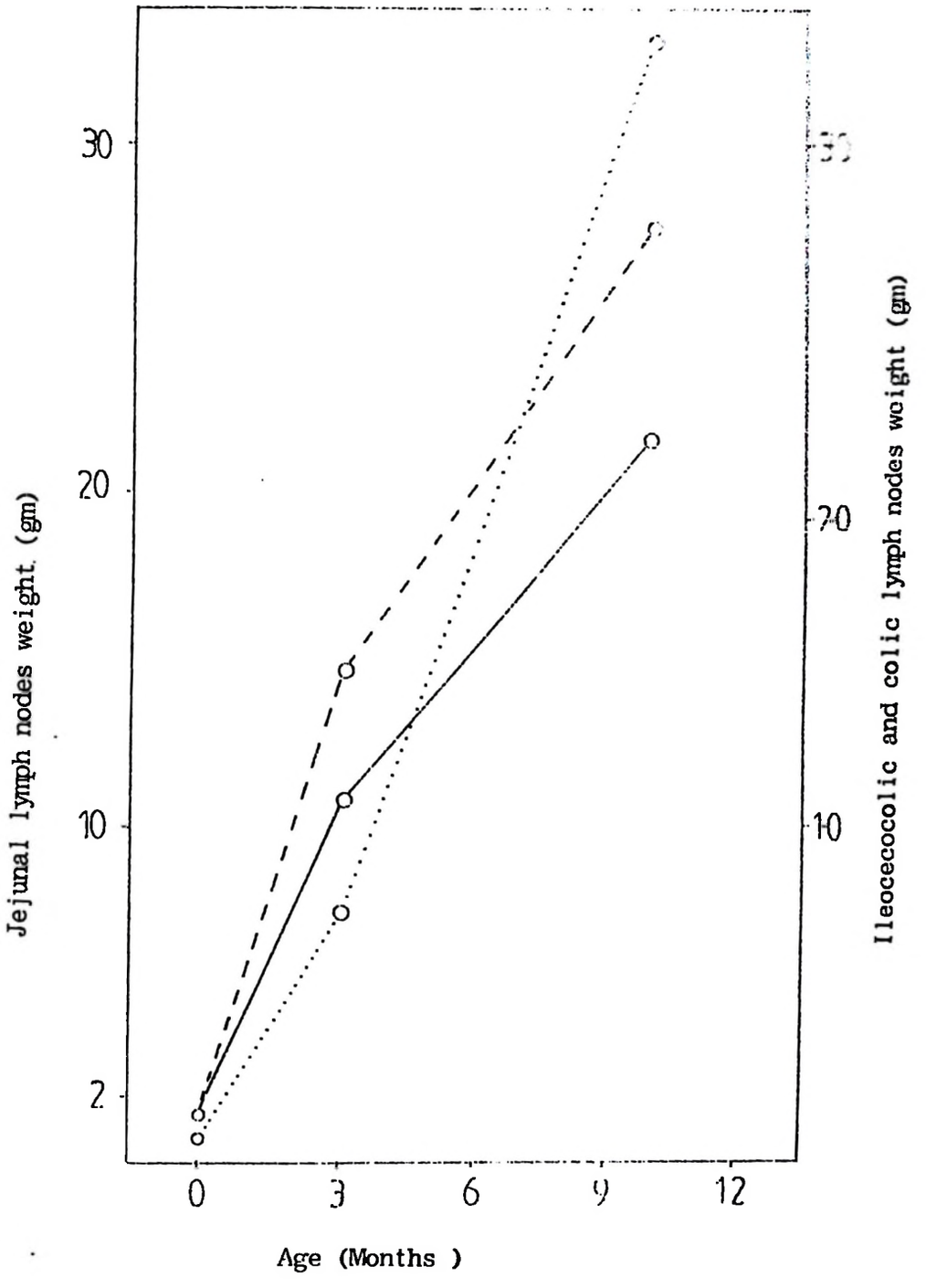


Fig. 22 : Postnatal weight increase of the jejunal (———), ileocecocolic (------) and colic (.....) lymph nodes

The medulla was in 84% of the newborn kids wider than cortex and 8% thinner and 8% about equal to cortex. In the three months old kids, the medulla of the nodes was in 71% wider than cortex, 6% were thinner than cortex and 23% about equal to cortex. The 8 - 12 old goats nodes showed almost equal percent of nodes with medulla wider than, narrower than or about equal to cortex.

The cortices of the newborn kids were small nodular or continuous thin layer (Fig. 16) while in the three months old kids, they were of large nodular type. In the 8 - 12 months old goats, the cortices were large nodular, small nodular or continuous thin layer.

Trabeculae were poorly developed in the newborn nodes, more so in the colic nodes, but were always observed in the other two age-groups.

Subcapsular and trabecular sinuses were poorly developed in the newborn and 3 months old kids. Medullary sinuses were always present in the jejunal and ileocecocolic lymph nodes of newborn kids, but were poorly developed or absent in 70% of colic lymph nodes. Medullary sinuses were always present in the other two age groups nodes. The sinuses were populated with lymphocytes, macrophages and reticular cells traversed the sinuses whose walls were lined by endothelial cells. Some of the sinuses from kids which had suckled were filled with light eosinophilic

material in which many lymphocytes were trapped, but near the hili the sinuses were clear (Fig. 23).

Secondary nodules were not observed in the newborn kids. However, primary nodules were observed in 80% of the newborn kids. In some kids which had suckled young secondary lymphoid nodules were observed in the cortex (Fig. 24).

In the 3 and 8 - 12 months old goats, secondary nodules were always present. These had a diameter of 200 - 500 microns, had a distinct large light center consisting nearly entirely of lymphoblasts. Mitoses were frequently observed, and dark zones of small lymphocytes on the capsular side of the germinal centres were observed.

Medullary nodules were observed in 7% of the newborn nodes, in 74% of 3 months old kids nodes and in all nodes of the 8 - 12 months old goats.

Plasma cells were not observed in the newborn lymph nodes. Post-capillary high-endothelial venules in the deeper cortex and in the cortico-medullary regions were observed in only 20% of the newborn kids lymph nodes (Fig. 25). They were always present in the other two age groups.

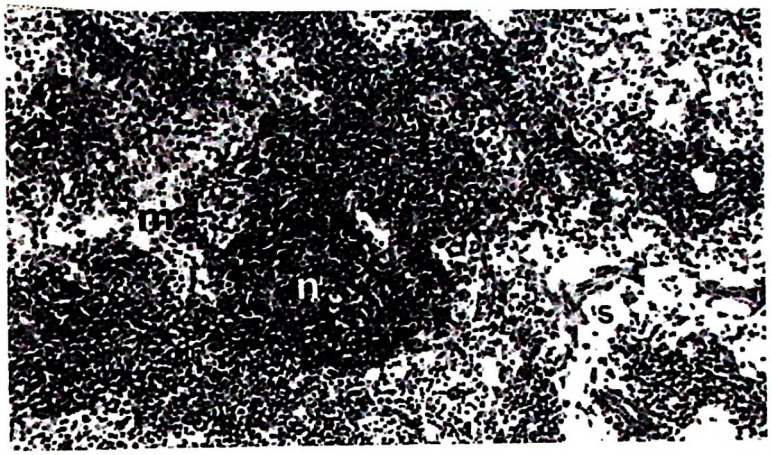


Fig. 23

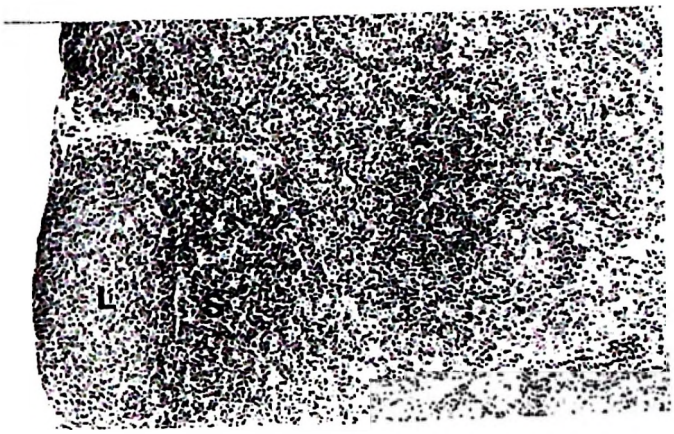


Fig. 24

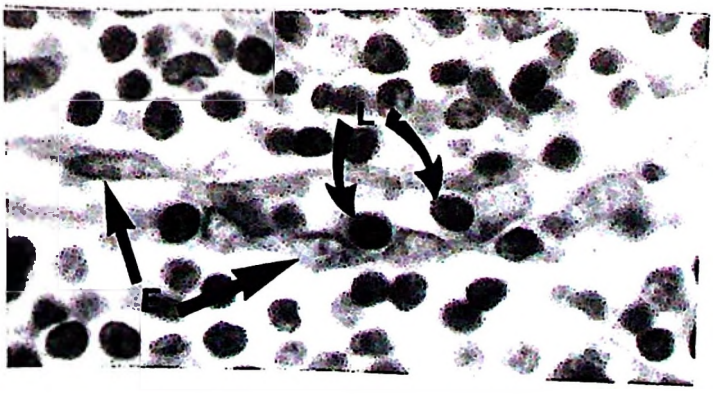

Fig. 25

Legends for Figures 23, 24 and 25

Figure 23 : Medulla of ileocecocolic lymph node of a newborn which had suckled. Medullary sinus (s) is clear and have reticular cells and few lymphocytes while (m) is filled with eosinophilic substance. n, medullary lymphoid nodule. (H. & E. 125 X).

Figure 24: Newborn kid colic lymph node cortex. The lympoid nodule has a zone of small lymphocytes (s) and a zone of large lymphocytes (L). (H. & E. 125 X).

Figure 25: A high-endothelial venule in paracortical region of ileocecocolic node of a newborn kid. E, endothelium; L, Lymphocytes migrating between endothelial cells, (H. & E. 1250 X).

## 4.3 Lymph drainage of the intestinal tract

Five groups of lymph nodes drained the intestinal tracts of goats.

a. Inn. pancreoticoduodenales- the group consisted of two to four nodes lying ventral to the pancreas. These drained the transverse colon, part of last centrifugal turn of ascending colon and duodenum. The efferents flowed into the intestinal trunk.

b. Inn. jejunales - this consisted of three to nine nodes of variable sizes lying in the mesentery between the last centrifugal coil of colon and jejunum. The group drained terminal duodenum, jejunum, last coil of centrifugal turn of colon, part of transverse colon and part of ileum. More than 75% of the jejuno-ileal Peyer's patch was drained by the group. In five animals, a node 10 - 18 cm. long was found. In three animals, a large bean-shaped node, 3 - 5 cm. long was found caudally in the chain and it drained a large part of the jejunal-ileal Peyer's patch.

c. Inn. ileocecocolici - in all the seven animals, the group consisted of a single node. Their shapes were variable, but mostly U-shaped. The nodes were located in the mesentery of the triangle formed by colon, cecum and ileum and they drained these structures.

d. Inn. colici - the group consisted of two to five nodes located on the right side and superficially or within the spiral colon. The group received afferent vessels from the colon coils.

e. Inn. mesenterici caudales - one to three small nodes located on the mesocolon or lying on the terminal descending colon made up the group. The nodes were closely related to the caudal mesenteric artery and received afferents from the descending colon and the efferents flowed into the lumbar trunk.

Efferents from the jejunal lymph nodes formed the jejunal trunk. However, efferent from the been-shaped nodes did not join the jejunal trunk. Instead, it joined efferent of ileocecocolic lymph nodes and the vessel formed joined the efferent from colic nodes to form the colic trunk (Fig. 26 ).

In three animals, efferent of one or two colic lymph nodes flowed into the ileocecocolic lymph node. Efferent from the latter then joined efferent from the rest of the colic lymph node (s) to form the colic trunk (Fig. 27). The jejunal and colic trunks confluenced to form the intestinal trunk which emptied directly into the cisterna chyli.

The cisterna chyli had variable shapes, sac-like, comma-shaped or round and were located either in the mesentery near the cranial mesenteric and celiac arteries origin or in the gap

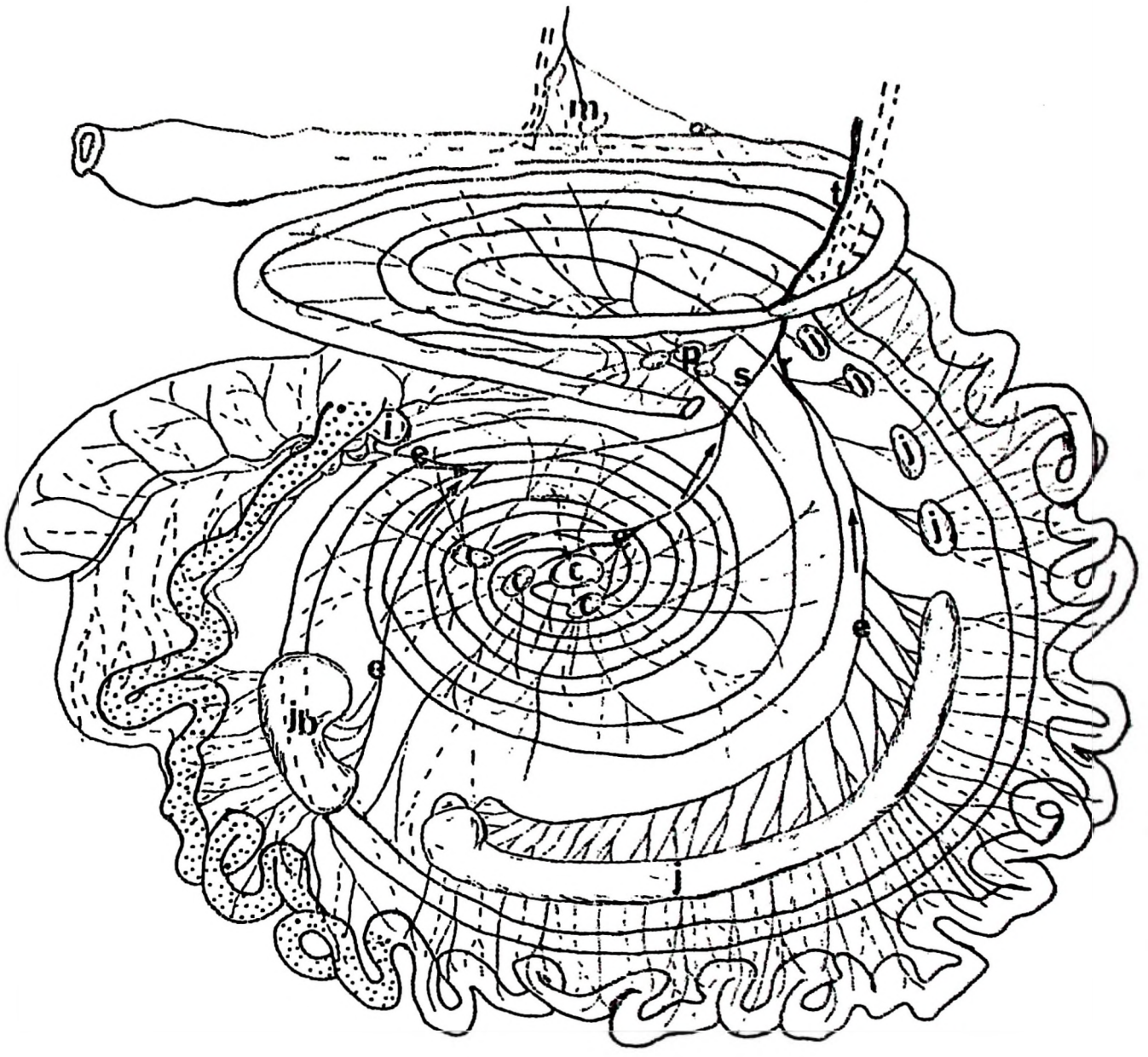


Fig. 26 : Lymph drainage of the intestinal tract of 3 months old kids. Efferent (e) from a bean-shaped nl. jejunalis (jb) join efferent from nl. ileocecocolicus (i). The vessel formed join efferent from nll. colici to form truncus colicus (s). Efferent from other nll. jejunales form truncus jejunalis (r), p, nll, pancreoticoduodenales; t, truncus intestinalis; m, nll. mesenterici caudales.

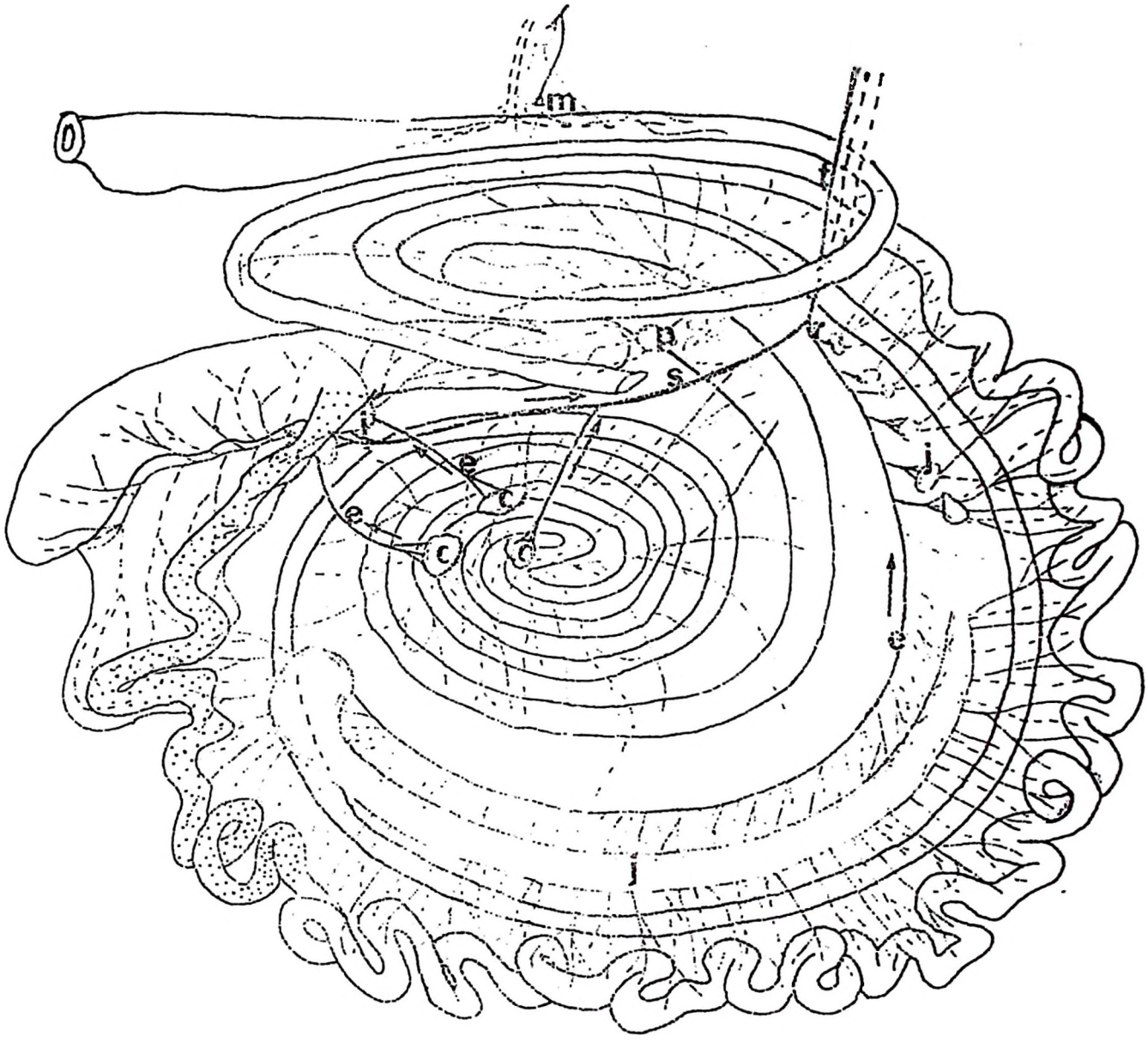


Fig. 27 : Lymph drainage of the intestinal tract of 3 months old kids. Efferent (e) from nll. jejunales (j) form truncus jejunalis (r), and efferents from two nll. colici (c) flow into nl. ileocecocolicus (i). Efferent from the latter join efferent of the other colic lymph node to form truncus colicus (s). Efferent from nll. pancreoti-coduodenales(p) join the truncus intestinalis (t). m. nll. mesenterici caudales.

between the right diaphragmatic muscular pillar and ventral to last thoracic or first lumbar vertebra. It lied on the left of the right kidney.

The thoracic duct passed through a gap in the right crura to enter the thoracic cavity where it lied on the right and dorso-lateral to the thoracic aorta. At intercostal spaces 4 or 5, the thoracic duct dipped ventrally and passed to the left of the aorta and right of the esophagus. The thoracic duct emptied into the left venous angle.

# CHAPTER 5

# DISCUSSION

## 5.1 The intestinal tract immune system at birth

Goat kids were born with an average of 35 Peyer's patches in the jejunum (Jejunal Peyer's Patches - JPP), one long Peyer's patch which started in the jejunum and terminated at the ileocecocolic junction (Jejuno-Ileal Peyer's Patchs - JIPP) and two patches in the colon. No patches were found in the duodenum. Peyer's patches are also not found in the duodenum in sheep and rabbits (Reynolds and Morris, 1983a; Sackman, 1981) while they are found in pig, dog and man (Chu et al., 1979b; Titkemeyer and Calhoun, 1955; Cornes, 1965). The number of discrete Peyer's patches in the jejunum was within the range reported in newborn lambs (Reynolds and Morris, 1983a). A single Peyer's patch which measured 0.8 m. was found in the terminal jejunum in the kids. A similar feature is found in lambs and pigs (Reynolds and Morris, 1983a; 1984; Chu et al., 1979a, 1979b) but not in man (Cornes, 1965). There is no report if this patch occurs in other domestic mammals.

At birth, about 17% of the intestine length was covered by Peyer's patches which is very similar to the finding in newborn lambs (Reynolds and Morris, 1983a). Since the intestinal tract is one of the major routes through which antigens gain entry into the animals body, and the Gut-Associated Lymphoid Tissue (GALT) forms the first line of defence which enterogenic antigens encounter, this high percentage of the patches on the intestine indicate a

reliable protection of the newborn.

Since the kids were sacrificed twelve hours after birth, there is therefore no doubt that the Peyer's patches, which were mature at birth, were formed during the prenatal period. Peyer's patches are also histologically mature at birth in sheep, cattle and dog (Reynolds and Morris, 1983a; Doughri et al., 1972; Bryant and Shifrine, 1972). In pigs mature patches are not observed until after birth (Chapman et al., 1974).

Interfollicular areas with the post-capillary high-endothelial venules (PC-HEV) were observed in the newborn kids (Fig. 13). The PC-HEV in the interfollicular areas represents the sites of lymphocyte recirculation. This finding implies that at least the avenues for lymphocytes recirculation are established before birth. In lambs, lymphocytes recirculation start in the fetal age (Cole and Morris, 1973; Reynolds, 1976).

The nature of the caprine placenta which is of epitheliochorial type makes the environment in which the fetus develops antigen-free both from outside antigens and from maternal immunoglobulins (Brambell, 1970). Therefore, the histological maturity of the Peyer's patches follicles observed in the newborn kids most likely had occurred in the absence of antigenic stimulus. However, there could have been stimulation from autogenous enterogenic antigens, but these have been shown not to exist at least in the sheep (Reynolds, 1980) which have the same type of placenta and equal

gestation period as the goat. Other factors than antigen must therefore have caused the lymphopoiesis in the Peyer' patches in the kids in a similar manner as in the sheep patches and the avian bursa of Fabricius.

The mesenteric lymph nodes (MLN) which are secondary lymphoid organs were immature at birth in the kids which had not suckled. MLN from normal fetal lambs are also immature at birth unless antigen is deliberately introduced into the gut, indicating that the intestinal tract is free of immunogenic materials (Reynolds, 1976, 1980). The findings in the goat indicate that the fetal gut was free of immunogenic materials as in the sheep.

Seven percent of the MLN were not differentiated into cortex and medulla, 40% were poorly differentiated and 53% were clearly differentiated. In newborn lambs, the MLN are differentiated into cortex and medulla (Cole and Morris, 1973). As there is an indication that the caprine and sheep fetal guts are free of immunogens, the degree of differentiation of the MLN at birth may be due to species difference. According to the degree of differentiation of the lymph nodes (Sugimura, 1962) the newborn lymph nodes were immature D III type.

Within twelve hours after birth and colostrum ingestion the MLN and Peyer's patches showed increased cellularity in the medullary and cortical regions. In some MLN of kids which had suckled, young secondary lymphoid nodules were also observed.

This indicated that an immunological reaction had taken place and shows the fast ability of the animals' immune system to react to enterogenous antigenic stimulation. As there [illegible] report on how long after birth and colostrum ingestion secondary lymphoid nodules appear in the MLN, it seems to occur within twelve hours in the caprine.

Ruminants are born with virtually no circulating immunoglobulins due to the nature of the placenta (Brambell, 1970). Maternal antibodies in the colostrum are postnatally transferred across the epithelium of the small intestine within the first 36 hours after birth (Simpson-Morgan and Smeaton, 1972). The colostral proteins are absorbed on the villi epithelial cells by pinocytosis and discharged into the lamina propria by exocytosis. From the lamina propria, the absorbed colostral globulins are transported to the blood circulatory system through the lymphatic system (Brambell, 1970).

Eosinophilic globules were observed in the small intestine villi epithelium, in the lamina propria and occasionally in the lacteals of the kids which had suckled. These had similar staining to the whey in the lumen of the intestine and most probably they were colostral proteins. Similar globules were observed in piglets of less than five days age (Chu et al., 1979a). The globules were not observed on the dome epithelium in the kids. Similar observation is reported in piglets (Chu et al., 1979a). These findings

indicate that colostral protein are absorbed by villi epithelium but not probably by the dome epithelium.

Epithelial cells of the villi of kids which had not suckled were columnar and had their nuclei near the apices (Fig. 12). In the kids which had suckled, also in the 3 months and 8 - 12 months old goats, the villi epithelial cells nuclei were located near the bases. It is therefore possible that, kids are born with immature villi epithelium which matures when the gut function of absorption is initiated. Whether it is the absorption per se or other unknown factors which are involved in eliciting this change is not known.

In some MLN, light eosinophilic mass was observed in the outer areas of the medullary sinuses. Most likely, the mass was colostral proteins on their way through the MLN, but had not reached the hili which appeared clear in such sections.

## 5.2 Microanatomy of the Peyer's patches

The caprine dome epithelium was columnar and had few goblet cells. It is not columnar in sheep (Reynolds and Morris, 1983a). Pigs dome epithelium also have few goblet cells (Chu et al., 1979a, 1979b) but they are lacking in calves, rabbits and chicken (Landsverk, 1981; Faulk et al., 1971; Waksman et al., 1973; Burns, 1982). So, there seem to be a species difference on the microanatomy of the dome epithelium.

In the three age groups, the microanatomy of the JIPP differed from that of JPP. The JIPP follicles were cylindrical or sac-shaped and were surrounded and separated by connective tissue capsule which surrounded the greater part of each follicle. The interfollicular areas were small and triangular-shaped. The JPP follicles were short, broad and pear-shaped and had large inter-follicular areas in which high-endothelial post-capillary venules were found. In most cases, the interfollicular areas of the JPP extended to the follicles bases. Similar observation are reported in sheep and calves (Reynolds and Morris, 1983a; Reynolds et al., 1985; Landsverk, 1984). The colic Peyer's patches (CPP) of the three age groups had cone-shaped follicles, prominent corona and interfollicular areas whose mean breadth was approximately between those of JIPP and JPP (Tables 3, 4 and 5 column 4 ). The rabbit has three types of GALT follicles (Waksman et al., 1973). The appendix of the rabbit has histological structure similar to the caprine JIPP, the Peyer's patches resembles the JPP and the sacculus rotundus resemble the CPP follicles.

The difference between the histology of the JIPP, JPP and CPP raise a possibility that these three types of Peyer's patches may also be different functionally. In the sheep, the ileal Peyer's patch (equivalent to the JIPP of goat) is the major site of B-lymphocytes production (Reynolds, 1976; Gerber, 1979; Miyasaka et al 1984b; Larsen and Landsverk, 1985) whereas the JPP is the major site for T-lymphocytes recirculation (Kagnoff, 1981; Larsen and Landsverk, 1985; Reynolds et al., 1985). Whether the caprine JIPP is also the major site of B-lymphocytes production is yet to be

determined. Large interfollicular areas with high-endothelial post-capillary venules were observed in the JPP. The JPP may therefore as in sheep serve as the major site for T-lymphocytes recirculation.

## 5.3 Change of the immune system of the intestinal tract with age

The mean number of Peyer's patches in the jejunum in the three age groups did not differ significantly ($P > 0.05$). However, the mean number was higher than that reported by Titkemeyer and Calhoun (1955). This may be because the authors did not process the intestine, which was done in this study. The processing in acetic acid followed by staining with polychrome methylene blue made the follicles more apparent and thus easier to count.

There was no significant increase with age in the mean number of Peyer's patches in goats. In man, there is a general tendency for the patches to increase in size and number with increasing age to puberty after which there is a decrease (Cornes, 1965).

There was no significant correlation between length of intestine and length of Peyer's patches in the three age groups. Nevertheless, the proportion of intestine on which Peyer's patches extended in both newborn and 3 months old kids (Fig. 7 ) was significantly higher ($P < 0.05$) than that of 8 - 12 months old goats. The intestinal tract may therefore be

better protected from birth to 3 month age than latter.

From three months to 8 - 12 months age, the proportion of intestine covered by Peyer's patches was on the decrease (Fig. 7). At 8 - 12 months age, the macroscopic evidence of the long JIPP had started to disappear. The gross atrophy was supported by histological observations on the depth and breadth of the JIPP sections (Figures 17 and 18). The JPP and CPP follicles did not show histological signs of atrophy. The mean depth of the cranial end JIPP follicles of the 8 - 12 months old goats was lower than that of 3 months old kids though not significantly ( $P > 0.05$). But, the mean depth of mid JIPP follicles was significantly lower ( $P < 0.05$)than that of 3 months old kids, and it was lower though not significantly than that of newborn kids. The mean breadth of mid JIPP follicles of the 8 - 12 months old goats was also significantly lower ( $P < 0.05$) than of 3 months old kids and insignificantly higher ( $P > 0.05$) than of newborn kids. These findings show that atrophy of the JIPP which was grossly visible, was more prominent histologically at the mid JIPP area and atrophy had likely started in the middle portion of the patch.

The life history of the caprine JIPP of prenatal maturation and postnatal involution place it in the same category as the thymus and the avian Bursa of Fabricius which are primary lymphoid organs. Similar to the Bursa of Fabricius, the JIPP is closely associated with the gut lumen. It is therefore possible that the JIPP is the caprine equivalent of the avian Bursa of Fabricius.

The rate of weight increase of the jejunal and ileocecocolic lymph nodes was higher from birth to 3 months age, but lower from 3 months to the 8 - 12 months age (Fig. 22). The rate of weight increase of the colic lymph nodes was almost constant. The difference could be due to their differences in drainage areas and sequentially differences in lymphocytes number inputs.

There is high lymphopoietic activity in the Peyer's patches during the first three months in sheep(Cole and Morris, 1973; Reynolds, 1976; 1980 ) and presumably the same occurs in goats too as many mitotic cells were observed in the follicles of 3 months old kids. The vast majority of the lymphocytes that leave the Peyer's patches in sheep do so via the lymph (Reynolds and Pabst, 1984; Binns et al., 1985). Very prominent lymphatics were observed from the caprine Peyer's patches and therefore most likely the vast majority of the lymphocytes that leave the Peyer's patches in this species also do so via the lymph. As the jejunal and ileocecocolic lymph nodes drained all the Peyer's patches, there could have therefore been a higher lymphocytes input into these two groups of nodes during the period when the lymphopoictic activity in the patches was high, hence the observed higher rates of weight increase up to the 3 months age.

From 3 months age, the proportion of intestine covered by Peyer's patches was on the decrease (Fig. 7) and the JIPP atrophy. There was, therefore, most probably a lowered lymphopoietic activity in the JIPP and consequently a reduced afferent lymphocytes input into the jejunal and ileocecocolic lymph nodes. This was reflected

by the observed lower weight increase rates of jejunal and ileocecocolic lymph nodes from 3 to 8 - 12 months age.

The observed almost constant rates of weight increase of the colic lymph nodes could be due to the fact that they did not drain any patch and could have had therefore low lymphocytes input throughout. Their weight increase could have been due to lymphocytic proliferation caused by continuous antigenic stimulation from the gut lumen, and hence the observed almost constant rate of weight increase.

Mesenteric lymph node-body weight ratio (Fig. 21 and Table 1) was significantly ($P < 0.05$) highest in the 3 months old kids followed by newborn kids. Similarly, in cattle, lymph node weight - body weight ratios are higher in young animals after which they decrease (Lubis et al., 1982). The mesenteric lymph nodes of 3 months old kids and 8 - 12 months old goats were histologically mature. According to Sugimura (1962) they were mature DIV type.

Dome and villi interepithelial lymphocytes (IEL) number showed a general increase with age (Figs. 19 and 20 ). A similar trend of IEL increase with age has been reported in piglets of up to one month age (Chu et al., 1979a) and in lambs of up to two months age (Reynolds, 1980). However, the mechanism leading to this increase is not understood. The development of IEL has been suggested not to be antigen - dependent as they are present in similar number in antigen free isografts of small intestine and in

normal intestine (Kagnoff, 1981). In contrast, Reynolds and Morris (1983b) observed fewer IEL in isolated ileal segments and suggested that the paucity was due to a reduced extravasation of these cells or was associated with reduced antigenic stimulation.

There was a general decrease of IEL number on the domes and on the villi from cranial end of the intestine to the caudal end in the 8 - 12 months old goats. A similar trend was observed in newborn kids dome IEL number. Chu et al. (1979b) reported a general decrease of IEL number from cranial end to caudal end of the intestine in piglets. As the cranial part of the intestinal tract comes first into contact with the antigen-laden ingesta, this finding supports the view that antigen exposure in the gut play a role in the population of the gut epithelium with lymphocytes. However, the influence of normal processes of digestion and absorption or age are also possible factors (Ferguson and Parrott, 1972; Reynolds and Morris, 1983b; 1984).

The high IEL number on the domes and villi in the anal ends of the small intestine and in the colon of the 3 months old kids could have been caused by subclinical worm/protozoa infestation. Nodular worm infestations is common in the areas where the kids were bought. Three kids of 3 months age were discarded due to nodular worm infestation during the study.

The mean number of IEL on the domes were significantly higher than on the villi (Tables 3, 4 and 5 columns 7 and 11). As was indicated above, the dome epithelium seemed not to participate in

colostral proteins absorption, a function which seemed to take place on the villi. The finding of higher IEL number on the dome is therefore of much interest. The higher presence of IEL on the dome epithelium suggests that the dome epithelium may play an active role in gut immune reactions, perhaps by providing a site for contact between the intestinal contents and the lymphoid system as suggested by other workers (Bockman and Cooper, 1973; Owen and Jones, 1974; Owen, 1977; Landsverk, 1981; Sackman, 1981; Chu and Liu, 1984). The dome invagination which was occasionally observed increase the dome surface area, hence increase the area for antigen contact with the lymphoid cells.

The mean size of the IEL (Tables 3, 4 and 5 columns 10 and 12) was higher than that of typical small lymphocytes which is similar to reports by Shimizu and Andrews (1967) and Kagnoff (1981).

## 5.4 Lymph drainage and flow from the intestinal tract

Results from the study of the lymph flow from the intestinal tract confirm the value of Evans blue stain mixed with natural egg albumen in the demonstration of lymph vessels in animals.

As seen in Figures 26 and 27 the pancreoticoduodenal, jejunal and colic lymph nodes drained the regional areas, while the ileocecocolic lymph nodes showed variations.

In three out of the seven goats, a bean-shaped jejunal lymph node being the most distal in the chain was observed. It drained a large part of the JIPP. The rest of the JIPP was drained by other jejunal lymph nodes and by the ileocecocolic lymph node. The CPP was drained by the the latter. Efferent from the bean-shaped jejunal nodes always joined the efferent from the ileocecocolic lymph node before confluencing with colic lymph nodes efferent to form the colic trunk. This flow pattern has not been reported before in goats.

Efferent from one or two colic lymph nodes in three goats flowed into the ileocecocolic lymph node (Fig. 27). This was not reported by Von Forstner (1973) while Schummer et al. (1981) states that the efferents from ileocecocolic lymph nodes either may flow into the colic lymph nodes or enter the intestinal trunk directly.

Inn. mesenterici caudales were always observed in this study. However, Von Forstner (1973) reported that they are inconstant in goats.

The intestinal trunk flowed directly into the cisterna chyli and the goats, therefore, did not have a visceral trunk. This is similar to a finding by Von Forstner (1973).

The single lymph node located in the mesentery between the ileum, colon and cecum drained these structures. As the nomenclature of the lymph nodes is based on their location and drainage areas, the node is therefore, In. ileocecocolicus as named by Von Forstner (1973). An equivalent group of nodes in goats has been named Inn. ileocolici (Schummer et al., 1981) and Inn. cecales (Tanudimadja and Ghoshal, 1975 ).

# CHAPTER 6

## CONCLUSIONS

The following conclusions were made from the study:

a. Kids were born with an average of 35 histologically mature discrete Peyer's patches in the jejunum (Jejunal Peyer's patches - JPP) and 1 long Peyer's patch extending from jejunum to ileocecocolic junction (Jejuno-ileal Peyer's patch - JPP) measuring about 0.8 m. in length. In addition, the colon had 2 Peyer's patches.

b. Mesenteric lymph nodes of newborn kids were immature and secondary lymphoid nodules appeared in the cortices of the nodes within twelve hours after birth and colostrum ingestion.

c. The average number of JPP in 3 months old kids and 8 - 12 months old goats was about 38 and 33 respectively and they both did not differ significantly ( $P > 0.05$) from the mean of the newborn kids. The JIPP measured about 1.4 m. and 1.5 m. in 3 months old kids and 8- 12 months old goats, respectively. In the three age groups, the JIPP were wider caudally than cranially. In the 3 months and 8 - 12 months old goats the colon had an average of 2 Peyer's patches. Peyer's patches were rarely found in the duodenum and in the cecum.

d. The proportion of intestine on which Peyer's patches extended was highest in 3 months old kids followed by newborns. Mesenteric lymph node-body weight ratio was highest in 3 months old kids. There was no significant correlation between

mesenteric lymph nodes weight and body weight, and between length of Peyer's patches and length of intestine.

e. Caprine Peyer's patches seem histologically to be differentiated into three types. JPP follicles where short, pear-shaped and had large interfollicular areas; JIPP follicles were cylindrical or sac-shaped and had small triangular interfollicular areas; and the follicles of the Peyer's patches of the colon (Colon Peyer's patches - CPP) were cone-shaped , and had prominent corona. These three architectural forms of follicles resembled the description of Peyer's patches, appendix and sacculus rotundus, respectively of rabbits. The three types of Peyer's patches had different life histories. JPP and CPP follicles showed no signs of atrophy with age, JIPP showed atrophy with age. Atrophy appeared to start at the middle part of the JIPP. Histological maturity at birth and atrophy with age of the JIPP resembled a primary lymphoid organ such as the thymus and the avian Bursa of Fabricius. JIPP of caprine may be the equivalent of the avain Bursa of Fabricius.

f. Intraepithelial lymphocytes (IEL) number on the dome and villi epithelium increased with age. There was a general trend of a decrease in the number of IEL on the domes and villi in the 8 - 12 months old goats and on the domes in the newborn kids from cranial end to caudal end of the intestinal tract. The size of the IEL was larger than that of typical small lymphocytes.

g. The rate of weight increase of the jejunal and ileocecocolic lymph nodes were higher from birth to 3 months age, but lower from 3 months to the 8 - 12 months age. The rate of weight increase of the colic lymph nodes was almost constant.

h. A bean-shaped node was found in the caudal end of the jejunal lymph nodes chain in three out of the seven goats. The node together with the ileocecocolic lymph node drained the JIPP. Efferent from the bean-shaped node always joined efferent from ileocecocolic lymph node before confluencing with the efferent of the colic lymph nodes to form the colic trunk.

i. Efferent of some colic lymph nodes drained into ileocecocolic nodes. Efferent from the latter in such cases joined the efferent from the other colic lymph node (s) to form the colic trunk.

j. Goats did not possess truncus visceralis since the truncus intestinalis emptied directly into the cisterna chyli.

## APPENDIX I

Ageing of goats (Schummer et al., 1979 )

| Teeth | Time of eruption |
|---|---|
| Di $\bar{1}$ | at birth |
| Di $\bar{2}$ | at birth |
| Di $\bar{3}$ | at birth |
| Di $\bar{4}$ | 1 - 3 weeks |
| Dp 2/2 | 3 months |
| Dp 3/3 | 3 months |
| Dp 4/4 | 3 months |
| M 1/1 | 5 - 6 months |
| M 2/2 | 8 - 10 months |
| M 3/3 | 18 - 24 months |

| | Time of replacement |
|---|---|
| I $\bar{1}$ | 15 months |
| I $\bar{2}$ | 21 months |
| I $\bar{3}$ | 27 months |
| I $\bar{4}$ | 36 months |
| P 2/2 | 17 - 20 months |
| P 3/3 | 17 - 20 months |
| P 4/4 | 17 - 20 months |

Holst and Denney (1980)

8 months - all deciduous present

8 - 12 months - $M_1$ present

12 - 14 months - $M_2$ visible

$I_1$, 2, 3 permanent replacement

| | | |
|---|---|---|
| | | $d^p$ replaced |
| 36 months | - | $I_4$ permanent |
| | - | $M_3$ fully erupted |

## APPENDIX II

Boundaries of ruminants' intestine (Habel, 1975; Schummer et al., 1979).

1. Duodenum - Pylorus to duodenojejunal flexure (where the duodenocolic ligament attaches it to the descending colon)

2. Jejunum - duodenojejunal flexure to ileocolic fold

3. Ileum - ileocolic fold attachment to cecocolic junction

4. Colon - ascending from cecocolic junction as proximal loop (centripetal and centrifugal coils) and distal loop; descending colon - from transverse colon to rectum; rectum - from the point where the descending colon enters the pelvic cavity.

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