DEVELOPMENTAL HISTOLOGY OF THE LYMPHOID TISSUE IN THE FETAL PIG

By

DONALD PAUL MERSCH

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

(Anatomy)

at the

UNIVERSITY OF WISCONSIN

1967

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to Dr. John W. Anderson who provided the initial stimulus for the development and growth of this project. His numerous suggestions about the content and organization of the material proved invaluable and without his corrective influence on many points of fact, phrasing, and theory, this paper could not have reached its final form.

The author is also appreciative of the courteous assistance extended by the Oscar Mayer Packing Company to facilitate procurement of the object of study.

To my dear wife who cheerfully typed and retyped the many drafts of this paper, often under the pressure of deadlines, I am also indebted.

Index

Introduction

Although the lymphoid system plays a central role in the development of immunological competence (Miller, '64), a review of the literature revealed that the amount of information on mammalian lymphoid development is scant, and that much of the descriptive literature concerning spleen, thymus, and lymph node development is on the pig, dating back to the early part of this century. (thymus - Bell, '05, Badertscher, '15; lymph nodes - Gulland, '94, Sabin, '05, '09, Heuer, '09; spleen - Sabin, '10, Thiel and Downey, '21; palatine tonsil - Levin, '30). The literature contains very little additional information on the development of lymphoid tissue from studies on other mammals. More recently experimental studies have been conducted on mammalian lymphoid tissue, shedding new light on the function of the thymus and the interaction of the components of the lymphoid system (cf. Auerbach, '65, for review).

In contrast to these earlier descriptive papers on a single lymphoid organ, the number of composite studies on the lymphoid system as a whole is quite restricted. Since it has long been known that there is a considerable variation in the time of development of the lymphoid organs in different species (Archer et al.

'64), a composite histological investigation into the sequential fetal development of all lymphoid organs in one species seemed appropriate. Through such a study, understanding of the general development of the lymphoid system could be achieved.

For the present investigation, the pig was selected as the object of study since; 1) the material could be obtained easily at all desired stages, 2) it has a relatively long gestation period (128 days), which facilitates a developmental study, 3) no composite histological investigation on the development of all the lymphoid tissues has been conducted on the fetal pig.

Special consideration was given to the following problems at all stages studied: 1) the earliest time lymphocytes could be identified, 2) a description of the cellular composition, 3) a brief description of the architecture including development of the arteries and, in the spleen, their lymphoid sheaths.

The nature of the present study is such as to add to, corroborate, and organize the existing information on the development of the lymphoid system in the pig, representative of mammals, on an organ system basis.

Materials and Methods

Embryos of the pig (Sus domesticus) were used as the object of study in this investigation. A series of embryos between 1.5 cm. in crown rump length (20 days gestation age) and 25 cm. (110 days), were collected and prepared for study.

The lymphoid tissues were examined at the following stages:

Crown rump length (cm.)	Age in days (Warwick '38, Keibel '97)
1.5	20
2.25	30
3.75	36
6.0	45
8.0	50
12.0	60
16.0	70
19.0	90
25.0	110

The gestation period of the pig is about 120 days, and a full term fetus is about 28 cm. long.

The tissue was obtained from the hog disassembly line at Oscar Mayer Packing Company. Samples of tonsil,

cervical thymus, spleen, mesenteric lymph nodes, and cecum with a segment of terminal ileum were taken from embryos immediately after removal from the uterus of the freshly killed hog. In most specimens the heart of the embryo was still beating, suggesting that destruction of cell detail because of post-mortem changes would be minimal. All samples were placed in Bouin's fixative within 3-4 minutes after the embryo was obtained from the uterus. Embryos up to 25 mm. in length were left whole and were placed directly in fixative. In those 25 mm. to 37 mm. slits were made in the body wall so as to allow the fixatives to penetrate more rapidly. In all later stages, the embryos were dissected and the tonsil, thymus, spleen, mesenteric lymph nodes and cecum were removed and placed directly in the fixing fluid to insure rapid and thorough fixation. A post-natal mesenteric lymph node was prepared and examined in a similar fashion.

The material was dehydrated in graded alcohols, cleared in chloroform, infiltrated in vacuo, and embedded in paraffin in the usual manner. Five micron sections, representative of the respective tissue, were used on all except the 20 and 30 day samples where 10 micron serial sections were mounted. A variety of stains was employed to provide the best evidence for the histology of the organs. Movat's trichrome stain (Movat '55)

was the most informative on all features, and served as a general stain, showing nuclei, cytoplasm, and connective tissue in excellent detail. Harris hematoxylin with a .25% eosin counter stain proved useful for cytoplasmic processes. Periodic acid-Schiff (PAS) (Lillie '65) demonstrated reticular and elastic fibers as well as megakaryocytes. PAS counter stained with hematoxylin showed relationship of cells to reticular fibers.

Observations

Thymus. In the 20 day embryo, the thymus was represented by a ventral outgrowth of epithelium from the third pharyngeal pouch (fig. 1). The epithelial cells had large, basophilic nuclei with dispersed chromatin. The nuclei were round to oval, and the cytoplasmic limits of the cells were undefined. The primordium was sharply set off from the surrounding mesenchymal cells (fig. 3).

By 30 days the primitive thymus had expanded in size and had migrated further from the pharyngeal pouch. It was easily located, since for part of its length it was slightly anterior and lateral to the larynx, internal carotid artery, and thyroid gland (fig. 2). The mesenchymal cells surrounding the primordium had now oriented in a circular array. The epithelial nature of the previous stage still predominated, and no cells resembling lymphocytes were apparent in the primordium or in the surrounding connective tissue. Mitotic figures were common.

At 36 days the primitive thymus was no longer a solid mass, but showed the beginnings of lobulation. A single layer of fibroblasts invested each lobule

(fig. 4). The nuclei of the cells making up the thymus varied considerably in size, but were remarkably similar in staining characteristics. The thymus was devoid of vessels at this stage. Lymphoid cells were not apparent in the mesenchyme surrounding the thymus, but a few cells within the thymus showed lymphoid characteristics (fig. 4).

The thymus of the 45 day embryo contained a large number of lymphocytes, and the connective tissue surrounding the thymus was also heavily populated with them. No differentiation into cortex or medulla was apparent (fig. 5). There was a further tendency for the thymus to form lobules.

The thymus at 50 days was considerably lobulated and contained large numbers of lymphoid cells, many of which were lymphoblasts. The connective tissue surrounding the thymus was also filled with local accumulations of lymphocytes (fig. 7). The parathyroid was found in the same connective tissue investment as the thymus (fig. 6). This thymus had partially differentiated into cortex and medulla; the medulla contained mostly large cells, with a slightly eosinophilic cytoplasm that resembled the earlier epithelial cells, and a few primitive Hassal's

corpuscles (fig. 11), while the cortex had a high content of basophilic cells which were lymphoid in appearance. The cortex contained four main cell types; large epithelial cells, small lymphocytes, larger lymphocytes, and reticular cells (fig. 7). A rich blood supply had developed.

At 60 days, the thymus was much larger and was completely divided into lobules by connective tissue septa. There was an obvious division into cortex and medulla; the medulla cells were large, less basophilic and less closely packed than the surrounding cortex (fig. 8). Lymphocytes and lymphoblasts were now much denser in the cortex, making the reticular cells less apparent (fig. 9). The septa of connective tissue no longer contained lymphocytes. Hassal's corpuscles were well developed and consisted of several epithelial cells enmeshed by reticular fibers (fig. 12). The vascularity had increased.

The further development of the thymus progressed from the basic pattern of the 60 day stage. As the mature fetal state was approached, there was a further increase in medulla, which formed the entire core of the older stages (fig. 10). The mature medulla was characterized by an increase in the number of Hassal's

corpuscles and lymphocytes, and a decrease in epithelial cells (fig. 13). As the thymus matured it became better vascularized. The cortex became more densely packed with lymphocytes, thus filling the meshwork of reticular fibers.

Spleen. Embryos of 20 days exhibited a swelling on the side of the dorsal mesogastrium, resulting from a dense accumulation of mesenchymal cells located just under the sufrace epithelium (fig. 14). Many mitotic figures were seen. The nuclei were oval, large and pale, and each generally contained a single nucleolus. The cytoplasm of this cell was very faint, and its limits were not clearly defined with the stains used.

The single layer of regularly arranged epithelial capsule cells was separated from the underlying mesenchyme by a faint basement membrane. These epithelial nuclei were round with a distinct nuclear membrane. The cytoplasmic limits of the capsular epithelial cells were also very indistinct.

By 30 days the spleen was more independent from the dorsal mesogastrium, but was still attached by a projection of cells; the primitive gastro-splenic ligament (fig. 15). The dense mesenchymal character of

the 20 day stage still predominated. However, the nuclei were more irregular in shape and the chromatin was somewhat less dispersed.

Vessels were first observed in the spleen at 30 days. These vessels were irregularly shaped channels with a PAS positive basement membrane. In a few channels the endothelium was distinct, but in most it was not obvious. The channels most likely represented narrow, branching capillary networks which were supplied by the splenic artery.

The capsular epithelium was more columnar, and the nuclei were located near the free surface. The basement membrane separating the capsule from the underlying mesenchyme was more distinct (fig. 15).

At 36 days the spleen was almost entirely free of its association with the dorsal mesogastrium except for a small mesenteric attachment (fig. 16). The dense mesenchymal mass characteristic of the spleen in previous stages now showed signs of change. The main cell type was still the mesenchymal cell; but both hemocytoblasts and nucleated erythrocytes (typical of mammalian embryos) were seen (fig. 17). The erythrocyte nucleus was hyperchromatic, and the nuclear membrane was indistinct. The cytoplasm was devoid of stainable

substance, but a distinct plasmalemma was present. In contrast to this, the hemocytoblast cell nucleus was much larger, and had clumped chromatin with some clear areas, so it was not as basophilic. The nuclear membrane was very distinct. The cytoplasm was almost unidentifiable, and no plasmalemma was seen (fig. 18).

The cells in the 36 day specimen were not so closely packed as in earlier stages, and a fine reticular network was apparent following PAS. The size and number of vascular clefts were increased, and some of the larger clefts had a primitive endothelial lining. The capsule cells had become more cuboidal in character. Minute projections of the capsular basement membrane into the parenchyma of the spleen were apparent on a PAS preparation.

The 45 day embryonic spleen was considerably advanced from earlier stages. The mesenchymal character of the earlier stages made the spleen appear entirely white on gross observation, but the 45 day embryonic spleen showed scattered red accumulations. On microscopic examination, these red areas were seen to be islands of erythroblastic cells distributed over the entire spleen (fig. 19). These islands consisted of 10-20 basophilic differentiating cells against a back-

ground of surrounding mesenchymal cells. There were 15-20 such islands in a typical section of the 45 day spleen (fig. 19). The cells represented a larger segment of the hemopoietic range than the previous stage (fig. 20).

Three large pools of mature erythrocytes appeared in areas where a connection between the vascular system and the primitive splenic sinuses had been established (fig. 19). These red cell infiltrated sinuses were lined by reticular endothelial cells which had differentiated from the earlier mesenchymal cells. This reticular network was more complex than previous stages with many typical sinuses; however, it was difficult to distinguish the sinuses from the smaller vascular channels.

The differentiating colonies of the 45 day specimen contained hemocytoblasts, basophilic erythroblasts and polychromatophilic erythroblasts, along with many normoblasts (fig. 20). This stage was also the first in which there appeared a few scattered megakaryocytes, with nuclei smaller than the typical adult megakaryocytes. These cells represented a transition from a mesenchymal cell to the mature megakaryocyte. Their cytoplasm stained red in the PAS and alcian blue in

Movat's which facilitated their identification.

Vascular channels were quite numerous. Most channels were not elaborate, consisting simply of endothelium (fig. 20).

The capsule projected into the spleen proper and occasionally a smooth muscle nucleus was seen in these projections; but no elastic fibers were identified. The capsule cells continued to become more flattened and squamous.

The 50 day specimen showed an increased number of mature erythrocytes and erythrocytic precursor cells. The mature erythrocytes were uniformly distributed throughout the spleen, but the accumulations of erythroblastic cells were still clumped into colonies or islands as noticed in earlier stages (fig. 21). A large number of proerythroblasts were present at this age (fig. 22).

Lymphocytes were first noticed at this stage, and there was a definite tendency for the lymphocytes to aggregate near or around vascular channels, thus representing the first distinct white pulp (fig. 23). The vascular channels had developed smooth muscle cells and elastic fibers in their lining. There was a positive correlation between the development of the

artery and the number of lymphocytes around it. The mature lymphocytes around the vessels allowed a clear comparison between the lymphocyte and the surrounding erythroypoetic cells (fig. 23).

The structural elements of the 50 day spleen showed the expected increase in smooth muscle cells and fiber elements. The reticular fibers were coarser and the sinusoids expanded (fig. 24).

At 60 days erythropoiesis was no longer confined to islands, but rather the whole spleen was involved (fig. 25). All erythropoietic cell types were present in large numbers. Small areas of lymphocytic concentration were enmeshed in the reticular fibers surrounding the arteries, but they were often hard to identify because of the erythropoiesis (fig. 25). Mature megakaryocytes were increasingly apparent and their typical large size and multilobate nuclei made their identification simple.

The vessels were well developed and began to demonstrate the mature features of the postnatal animal. The trabeculae were distinct and contained more smooth muscle cells with many elastic fibers. The trabeculae projected half way into the spleen, and thereby partially partitioned the spleen into compartments.

The main difference in the 70 day spleen in comparison to the 60 day spleen was that the islands of white pulp were more distinct in the older animal (fig. 26 c.f. fig. 25). The white pulp nodules were dispersed around vascular channels. These nodules contained a distinctive organized reticulum that appeared continuous with the tunica adventitia of the vessels. The organ was still intensely erythropoietic and the usual cell types were present in the red pulp, which occupied most of the organ (fig. 26).

In the 90 day specimen the massive erythropoiesis characteristic of the 60 and 70 day spleen had subsided and now there was a considerable increase in white pulp and a corresponding decrease in erythropoietic red pulp. Many central arteries were present (fig. 27).

The only major difference between the 90 and the 110 day stage was the further increase in white pulp in the older spleen (fig. 28). Three types of pulp were present; 1) white pulp, without germinal centers, 2) red pulp with active erythropoiesis, and 3) red pulp with a high reticular cell content and no erythropoiesis (fig. 29).

Trabeculae and central arteries were easily identified. An occasional pulp artery was seen, but

our non-perfused material did not lend itself to a study of the smaller vascular channels. The capsule consisted of an outer layer of squamous cells with mixed collagen and elastic fibers (fig. 30).

Mesenteric lymph nodes. The mesentery of the 20 and 30 day embryos consisted of a large number of undifferentiated mesenchymal cells with no other free cells apparent. Many primitive vascular channels penetrated the mesentery. Differentiation brought the presence of arteries, veins and plexuses of lymph ducts by 45 days (fig. 31), with scattered lymphocytes in the connective tissue; but no organized lymphoid tissue was seen until the 60 day stage, when an area in the mesentery showed lymph node characteristics (fig. 33). The node was long, narrow and rod shaped, and appeared grossly as a white oblong area in the intestinal mesentery. It developed adjacent to an extensive glomus which extended from the node to the gut. Some of the mesenchymal cells of the mesentery had differentiated into reticular cells, making up the core of the primitive node. These cells had oval, large nuclei with somewhat clumped chromatin and single nucleoli. The cytoplasm of the cell was very faint, and tapered to fine filamentous projections that contacted surrounding cells. A few small lymph-

ocytes and lymphoblasts were sparsely dispersed in this framework of reticular cells. This dense core of reticular cells was occasionally permeated by clear sinusoidal areas, especially under the capsule and in the center of the node. No nodules were apparent. A thin, loose capsule surrounded the primitive node and separated it from the surrounding mesentery (fig. 34).

At 70 days there were fewer primitive reticular cells making up the framework of the node, and a striking increase in the number of lymphocytes, which were starting to aggregate into nodules (fig. 35). Small lymphocytes and lymphoblasts were apparent in these nodules (fig. 36).

The 90 day node showed little change. There was a further increase in the number of lymphocytes, and continued organization into nodules. A large subcapsular sinus occupied much of the periphery of the node (fig. 37, 38).

At 110 days the node was well partitioned by trabeculae, and the whole node was now organized into closely packed nodules with medullary cords and sinuses (fig. 39). The coarse network of reticular fibers in the sinuses held a wide variety of cells in its meshes. The nodules had a fine reticular framework in which were

held the densely packed lymphocytes (fig. 40).

At one day post natum, germinal centers were seen in some but not all nodules. The sinuses were obscured by the presence of large numbers of cells (fig. 41). The germinal centers contained large lymphoblast cells and the surrounding zones were packed with many small lymphocytes (fig. 42).

Palatine Tonsil. At 20 days the palatine processes had not begun to protrude from the maxillary process. By 30 days the lateral palatine process projected from each maxillary process of the upper jaw toward the midline, and the rostral region had fused; but caudally, the tongue was elevated between the two lateral palatine processes. The stratified squamous, non-keratinized epithelium of the palate showed no invasion of lymphocytes, nor were crypts present. In the 36 day embryo fusion of the palatine processes extended further caudally. The epithelium of the soft palate still showed no signs of lymphoid infiltration, and it was sharply delineated from the underlying connective tissue.

At 45 days the palate showed some unevenness in the epithelial surface. The underlying mesenchyme had

become vascularized, but no lymphocytes occurred around the vessels. The 50 day specimen showed a few invaginations of the surface epithelium on the soft palate, producing crypts and glands, but again no lymphocytes were seen around these invaginations.

At 60 days the first tonsilar tissue was noticed. Numerous nodules of lymphocytes had accumulated in the lamina propria of the soft palate under the stratified squamous epithelium (fig. 43). The nodules produced undulations in the overlying epithelium. The epithelium over the apices of nodules showed histological changes portending the later formation of crypts (fig. 43). The submucosa of the soft palate was quite well vascularized, and many granulocytes could be seen near the vessels (fig. 44). Some lymphocytes were dispersed between the subepithelial nodules, but most of them were densely packed in the nodules (fig. 46). A fine reticulum of PAS-positive fibers provided the framework for the nodule. Most of the fibers surrounded the base of the nodule, thus partially separating it from the underlying submucosa and adjacent nodules. A fine, PAS-positive basement membrane delineated the nodule from the overlying epithelium (fig. 45). Mucous glands were numerous in the soft palate but none were seen in the

tonsilar area. A deep, anterior, non-lymphoid crypt separated the tonsil from the rest of the palate. The posterior boundary of the tonsil was not abrupt, but rather the lymphoid nodules diminished in number and size until they could no longer be recognized.

By 70 days numerous epithelial crypts had invaginated, carrying the underlying lymphoid tissue deeper into the lamina propria (fig. 47, cf. fig. 43). Thus the lymphoid tissue had come to lie close under the epithelium of the crypts, while it was separated from the surface epithelium by a layer of cellular connective tissue. Occasionally lymphocytes were seen invading the epithelium. The nodules were sometimes fused, and sometimes separated by loose connective tissue infiltrated with lymphocytes. Many vascular channels were located below the tonsilar tissue in the submucosa. An occasional lymphatic was filled with lymphocytes, suggesting the tonsil may have been producing the lymphocytes for export. Large numbers of granular leukocytes were localized at the base of the tonsilar tissue in this vascular area (fig. 48).

In the 90 day specimen, the lymphocytes were less dispersed and more clumped into nodules around the base of the crypts, but the basic pattern of the 70 day

tonsil remained. Again, large numbers of free leukocytes were seen in the connective tissue surrounding the tonsil.

The 110 day stage showed no new features. The vascularization of the submucosa was highly developed and many small arteries and veins were seen. The lymphoid follicles in the tonsil of the 110 day mature fetus consisted of compact lymphocytes organized into nodules. At no stage were germinal centers present.

Peyer's Patches. Part of the terminal ileum was removed with each sample of caecum, in an attempt to study Peyer's patches. Developing Peyer's patches were first demonstrated in the 70 day specimen, consisting of aggregated lymphocytes in the lamina propria between bases of the intestinal villi. Only six or seven nodules were observed on a typical section (fig. 49). The nodules occasionally extended through the muscularis mucosae into the underlying sub mucosa. The nuclei of the cells in the nodule were typical of small lymphocytes (fig. 50). A basement membrane separated the epithelium of the intestine from the underlying lymph nodules in the lamina propria.

At 90 days the nodules, similar in histology to those at 70 days, extended further along the ileum.

By 110 days loose lymphatic tissue surrounded the lamina propria of the terminal ileum, and scattered throughout the loose network of lymphocytes were many aggregations of cells into nodules. This Peyer's patch was more elaborate in comparison to the 70 and 90 day stage and extended the entire length of the segment of the ileum studied. It contained many more nodules and more internodular lymphocytes than earlier stages (fig. 51).

Cecum. An extensive examination was made of longitudinal and cross sections of the cecum at all stages. It was concluded that at no prenatal age was there any organized lymphoid tissue in the lamina propria of the developing cecum. Figure 53 shows the histological composition of the 110 day fetus demonstrating that the cecum of the mature fetus had developed no lymphoid nodules. The only lymphocytes present in the cecum were those located in lymphatic channels (fig. 54).

Discussion

Table I summarizes the findings of the present study and shows that, except for germinal centers, the lymphoid system of the pig is anatomically well developed before birth. This conclusion can be compared with several isolated observations on the fetal pig lymphoid system recorded in the past. Although none of the earlier studies were concerned with the development of the lymphoid system as a whole, a composite picture can be obtained from them; and when this is extended by the present study, a summary of lymphoid system development in the fetal pig can be made.

The histogenesis of the developing pig thymus has been studied by Bell ('05). He reported that at 3.7 cm. (35 days), lymphoid transformation had begun in the thymus, and three types of cells developed from the cellular reticulum formed from the epithelium of the third pharyngeal pouch. One of these cells was a lymphoblast. He concluded that few mitoses occur at this time and no blood vessels are present inside the anlage. The lymphoblasts gradually break loose from the cellular reticulum and move into its spaces and form lymphocytes. By 4.5 cm. (40 days), lymphoblasts are more numerous. He also saw a few lymphocytes in the thymus at this stage, but

Table I

Age in days	Thymus	Spleen	Mesenteric lymph nodes	Tonsil	Peyer's Patch
less than 30	epithelial	mesenchymal			
36	lymphocytes in thymus	hemocytoblasts and nucleated erythrocytes			
45	lymphocytes in connective tissues surrounding the thymus, vascularization begins.	complete erythropoietic lineage, primitive vessels	lymph duct plexus		
50	Hassal's corpuscles cortex and medulla	lymphocytes, red pulp	increased lymph plexus		
60	mature fetal pattern established	increased erythropoiesis and white pulp	early node formed with lymphocytes	lymphocytes in nodules of soft palate	
70		all red pulp is erythropoietic	lymphocytes form nodules, sinuses appear	crypts form adult pattern	lymphocytes in loose nodules
90		erythropoiesis starts to subside, incressed white pulp	more nodules develop		increase of nodules along ileum
110		white pulp, erythropoietic, and non-erythropoietic red pulp	nodules, meduallary cord and sinus present		extensive nodules along entire terminal ileum

noted that there were no lymphocytes in the connective tissue around the thymus or in the blood. Lymphocytes did not appear in the connective tissue around the thymus until shortly after they were formed from the lymphoblasts in the thymus. This is consistent with our findings (fig. 6). At 7 cm. (48 days), a few lymphocytes were noticed outside the thymus. A great many nuclei were observed in mitosis and blood vessels were numerous. At 8.5 cm. (51 days), large numbers of lymphocytes were described both in the thymus and the surrounding area. The differentiation into cortex and medulla had occurred by this stage. Blood vessels were distributed to all parts of the organ, but were few in number. By 9.5 cm. (54 days), the medulla had expanded and frequent Hassal's corpuscles were observed. At 14 cm. (65 days), and later stages, the formation of lymphocytes was still in progress.

The histology of the developing thymus has also been discussed by Badertscher ('15). He found that in the 2.3 cm. (31 day) embryo, the thymus is a purely epithelial structure. He also believed that the lymphocytes, first present in the thymus, were all large lymphocytes, and he believed they migrated into the thymus from the mesenchyme, since lymphocytes were found scattered throughout the mesenchyme near the

blood vessels about the thymus, before they were present in the thymus. This is in direct contradiction to the present observations (fig. 4) and those of Bell ('06). Badertscher went on to state that at 2.6 cm. (32 days), lobules were beginning to appear; and at 3 cm. (33 days), blood vessels were numerous in the connective tissue septa penetrating the thymus, but none were noticed in the lobules. Large lymphocytes were present in small numbers in the thymus at 3 cm. (33 days). The nuclei of the lymphocytes were slightly smaller than the epithelial nuclei, and this allowed their separation from the epithelial cells. At 3.7 cm. (36 days), Bardertscher could see the lymphocytes pierce the thymus from the mesenchyme and thought he could detect, ". . . a lymphocyte some distance from the periphery (of the lobule) with a trail leading to the surface of the lobule marking the path that the active lymphocyte took in its migration from the place of entrance". From the illustration he provided to demonstrate this migration, it would seem likely that he could have easily mistaken the "trail", simply for an area of the thymus devoid of nuclei, since the area had the same appearance as the general cytoplasm of the thymic epithelium. At 4.2 cm. (38 days), many large and small lymphocytes characterized the thymus, but no blood vessels were present. At 6.5 cm. (46 days),

the medulla appeared. Soon after the medulla developed, the epithelial nuclei contained in it started to transform into Hassal's corpuscles. Some of the epithelial cells also formed the reticulum of the gland. Large numbers of lymphocytes were seen over the entire thymus and the surrounding connective tissue. From 8.5 cm. (51 days) on, the size of the lobules increases and they were seen to contain all types of lymphocytes. By referring to Table I it can be seen that the observations of Bell and, except for his observations regarding the origin of the lymphocytes, Badertscher are consistent with the findings of this investigation.

An excellent study on the development of mammalian spleen has been conducted, and for the major part of this study, the embryonic pig was used (Theil and Downey, '21). The sequence of development for the pig spleen was seen to be similar for the other mammals studied; rat, gopher, guinea-pig, and sheep. It was observed that as early as 3 - 4 cm. (35-38 days), hemocytoblasts were seen differentiated from mesenchymal cells in no specific location and in so connection with the vascular system (cf. fig. 16). Between 6 - 7 cm. (45-48 days), red pulp formed from the mesenchyme. This pulp first formed in restricted pools in the mesenchyme, but these pools soon expanded and by 50 days almost the entire spleen was red

pulp (cf. fig. 19). At 6 cm. (45 days), erythrocytes were produced in the spleen proper by differentiation of hemocytoblasts: prior to this time, the nucleated erythrocytes present in the spleen were of extrasplenic origin (yolk sac, liver). At. 7 cm. (48 days), lymphocytes were observed around arteries (cf. fig. 23). The small lymphocytes were thought to transform directly from mesenchyme and not pass through the hemocytoblast stage. By 9 - 12 cm. (55-60 days), intense erythropoiesis was observed, and by 17 cm. (76 days), the whole organ was erythropoietic. By 17.5 cm. (80 days), the erythropoiesis began to subside. Most mesenchyme of the original splenic mass was thus used up in erythrocyte production. The mesenchyme which was not used in erythropoiesis remained fixed and differentiated into a loose network of reticulum.

Information regarding the development of the circulation in the early spleen, by injection methods (Sabin, '10), shows that between 3 - 7.5 cm. (34-48 days), the splenic circulation consists of a capillary network, and that between 7.5 - 10 cm. (48-55 days), this network gives way to the development of arteries and veins. In embryos larger than 10 cm. (55 days), the vessels are adult in type.

Since the lymph nodes develop at different times in different areas of the body, (Sabin, '09) a general understanding of the course of development of any node is appropriate so we can then relate this general development to the development of mesenteric lymph node in the pig.

Gulland ('94) conducted a developmental study of the lymph nodes in a variety of mammals; sheep, rabbit, mouse, human, guinea-pig, and rat. He found that lymphatic vessels appeared before any trace of lymphatic nodes were found. The lymphatic vessels were described as arising by a dilatation of pre-existing spaced hollowed out in the mesenchyme in the line of the greatest lymph flow.

The lymph vessel plexuses thus formed do not constitute the node proper, but contribute to the peripheral sinus of the node, and others as the afferent and efferent vessels. The node proper arises from nodules of connective tissue in the mesentery which the lymph ducts, derived from the mesentery, surround. The nuclei of the connective tissue between the ducts become more numerous. He felt that the leucocytes were brought into the node by the blood since they do not appear in the node until they have long been present in the blood and about the thymus. In his view, the vast majority of leucocytes enter from

the blood vessels that supply the node. The leucocytes are then caught in the meshes of the developing node, and divide by mitosis.

Sabin ('05) studied the development of the lymph nodes in the pig with injection methods. She concluded, in contrast to Gulland, that the lymphatic vessels originate from the veins and not from mesenthymal spaces. Her description of the general development of the node was similar to the previous description of Gulland. Sabin found that the lymphatics bud off from the veins and then expand into a lymph heart a short distance from the vein. In amphibians, this lymph heart is a pulsating structure with muscle fibers in its walls, but in higher evolutionary forms, it is a thin walled non-muscular, non-pulsating sac. Lymphatic ducts continue to bud off this lymph heart, spreading peripherally to supply a given region. The lymph nodes develop along this plexus of ducts. The connective tissue surrounding the lymphatic plexus contains blood capillaries which later proliferate and form tufts of capillaries surrounded by connective tissue. Sabin observed ('05) the transition between the connective tissue cells and the lymphocytes, thus assuming a mesenchymal origin. As mentioned earlier, Gulland ('94) also observed the occurence of lymphocytes in clumps around these capillaries, but he concluded that they were

filtered from the blood stream. Sabin further observed the blood capillaries and connective tissue increase in the center of the node, thereby making it necessary for retrogression or destruction of some of the lymph ducts. The artery then develops follicles of lymphocytes around it, and a "germinal center" develops. She defines a "germinal center" as a tuft of lymphocytes around capillaries, and not by the current definition. The follicle is divided into germ center and lymph cord (medullary cord). The connective tissue bridges between the closely packed lymph ducts around the border of the follicle becomes slender, and thereby creates peripheral sinuses. Reticular fibers develop subsequentially in these connective tissue bridges between the sinuses. The sinuses begin to grow down into the nodes between the follicles, and connective tissue between the sinuses also pushes in and thereby forms trabeculae. The lymphocytes wander out from the enclosed nodules and fill up the lymph cords. The nodes can increase in size by the invasion of more ducts from a lymph plexus while there is no definite capsule, but after the capsule is formed late in development the node can only increase in size by joining with adjacent nodes.

Sabin extended her findings on lymph node development in the pig by studying serial sections of the Mall

Collection of human embryos and correlating her findings as to the method of development of lymph nodes in the pig with the human (Sabin, '09).

The observations of this investigation correlate well with the stages described by Gulland and Sabin, but no data were obtained as to the origin of the nodes. The early stage of node development characterized by a lymph duct plexus is illustrated in our material in figure 31 and figure 32. The next stage of development demonstrating an expansion of the connective tissue between the ducts is shown in figure 33 and figure 34. Nodular (fig. 35 and 36) and medullary cord stages (fig. 39 and 40), mentioned by Sabin and Gulland were also seen in our preparations.

Heuer ('09) found that the lymphatic system of the intestine in the pig develops fairly late in the embryonic state. From 4 - 10 cm. (38-55 days), the entire mesentery of the gut was reported filled with an abundant plexus of ducts. This type of plexus was also observed in the present study (fig. 31). He also noted the long line of single mesenteric nodes, characteristic of the pig at the root of the mesentery, and he agreed with Sabin's earlier description of the origin of the lymphatic system. It seems strange that he makes no mention of the extensive glomus that extends from the

node, at the base of the mesentery, to the gut. A portion of this structure is seen in figure 33.

Our observations on the tonsil are in accord with the only other study of the pig tonsil in the literature (Levin, '30). Levin first found tonsilar tissue at 9 cm. (52 days) in the soft palate, and by 10 cm. (55 days), small nodules of mesenchyme were apparent with lymphocytes around the underlying blood vessels. Lymphocytes soon fill the mesenteric nodules at 12 cm. (60 days). Crypts were then formed from 14.2 cm (65 days), with a corresponding elaboration of the lymphoid nodules to give a mature fetal tonsil by 16 cm. (70 days).

Apparently no previous developmental studies on Peyer's patches and cecum in the fetal pig exist in the literature.

The developmental sequence of the thymus spleen and mesenteric lymph node in the fetal pig has been described by Pestana et al. ('65). The method of fixation they employed, and the resulting histology, left something to be desired, but the information obtained is useful. They report the first evidence of lymphoid tissue in the thymus of embryos of 4 - 5 cm. (38-42 days). The spleen and lymph nodes could not be identified at this stage. We did not observe the lymph nodes at this stage, but it is unusual that the spleen was not found since it is quite large and could be dissected grossly (fig. 16).

Differentiation of the thymus into cortex and medulla was reported at 6 - 7 cm. (45-48 days). The spleen was identified at 6 - 7 cm. (45-48 days), but lymph nodes were not found in the mesentery. Mesenteric lymph nodes were first noticed between 9 - 10 cm. (52-54 days). The thymus also developed into cortex and medulla with some Hassal's corpuscles at this time. The thymus appeared to be fully developed by 12 cm. (60 days), and the spleen, at this time, showed the early development of red and white pulp. Red pulp was obvious in this study by 50 days (fig. 21). Small lymphocytes were seen in the mesenteric lymph node between 13 - 14 cm. (62-64 days), and at 17 - 18 cm. (79-84 days), follicular arrangement was noted.

The morphological - embryological approach employed in this study of sequential staged material is inadequate to determine the exact origin of cells. We have, however determined the general morphology of the lymphoid organs at various times during their development. It is now obvious that the period between 30 and 50 days is critical in the development of thymus and spleen, for it is during this time that the epithelial thymic primordium and the mesenchymal splenic primordium undergo differentiation into cell types which populate the adult. The appearance of lymphocytes in the mesenteric lymph node of the gut occurs between 50 and 60 days of development. This is

also the time when lymphocytes appear in the palatine tonsil. It appears that Peyer's patches accumulate lymphocytes between 60 and 90 days of development. If a cytological study of close sequentially staged well-prepared material were undertaken during these critical times, it could perhaps yield detailed information concerning cellular origin and differentiation in these respective tissues.

A further continuation of this study might include a closer examination of the early thymus, for it was observed that the lymphocytes in the connective tissue surrounding the thymus primordium at 45 - 60 days appeared to group around arterioles in that region. No record was found of a thorough investigation into the development of the vascular supply of the thymus. If the thymus is, in fact, seeding lymphocytes to other lymphoid organs which have no lymphocytes prior to 45 days, a study of the vascular supply in the developing thymus seems warranted, for it is probably through the vascular channels that the seeded cells migrate. It is possible that the lymphocytes seen in the connective tissue of the thymus are migrating to the vascular elements observed and, via the circulatory system, infiltrating other lymphoid organs. To provide information on this hypothesis, it would be necessary

to do an exacting study of the circulatory arrangement surrounding the early thymus with injection and perfusion methods.

Beard, who studied the thymus of the rat, stated in 1900 that the first lymphocytes of the body appeared within the thymus-epithelium, and that they are found here before they are seen in the mesoderm or in the blood. This is one of the earliest observations regarding the epithelial origin of lymphocytes. He regarded the thymic leukocytes as the apparent source of all the leukocytes of the body. He assumed that the parent leukocytes infiltrate the blood and thereby the other parts of the body. The problem of the epithelial origin of the lymphocytes in the thymus has been debated in the literature since these early observations. The mesenchymal origin of the lymphocytes has received support from among others. Norris ('38), Godwin ('39), Klapper ('46), and, as we have seen, Badertscher ('15). Proponents of the epithelial origin are, among, others, Beard ('00) and Bell ('06). Recently this issue has received new attention from Ackerman and Knouff ('65) in the thymus of the embryonic hamster. They concluded that the lymphoblastic and lymphocytic elements appearing in the embryonic thymus of the hamster are of epithelial rather than mesenchymal derivation, based on the following

observations: 1) the absence of lymphocytes, lymphoblasts, and hemopoietic activity in the connective tissue surrounding the embryonic thymus before and during the period of initial lymphoblastic formation; 2) the presence of a continuous basement membrane surrounding the developing thymus; 3) absence of cells passing through the basement membrane during this phase of development ; 4) the absence of vascularity or vascular invasion of the thymus until after the appearance of lymphoblasts in the thymic parenchyma; 5) the demonstration of a sequential series of morphological transitions between undifferentiated epithelial cells and lymphoblasts and 6) the subsequent homoplastic proliferation and maturation of the lymphocytic elements from lymphoblasts in the developing thymus.

The observations of the present investigation are consistent with the theory of the epithelial origin of lymphocytes in the thymus of the embryonic pig since; 1) lymphocytes were seen in the developing thymus prior to the time they were seen in the connective tissue surrounding the thymus (fig. 4); 2) the thymic primordium appears to be incapsulated by connective tissue elements (fig. 3, 4, 5) (To further substantiate this connective tissue sheath, a series of closely staged PAS preparations would be necessary); 3) a large number of mitotic figures were seen in the thymus prior to the appearance of

lymphocytes outside the thymus. On comparison with results of Ackerman ('65) in the embryonic hamster, the similarity of the above observations with theirs will be obvious. Since our material was not closely staged, some of his other conclusions can not be substantiated.

In a recent abstract, Ackerman ('67) suggests that the thymus is not, in fact, the first lymphoid organ to develop in the cat, but that the cervical and para-aortic lymph nodes are populated with lymphocytes prior to the time they appear in the thymus. Until photographic evidence of these observations is published, it is difficult to assess their sig nificance.

Auerbach has proved much experimental information on the epithelial origin of the thymic cells. If the mouse thymus rudiment is dissected, and isolated, and then grown as a graft in the anterior chamber of the eye of the adult mouse (Auerbach, '61) or in tissue culture (Auerbach, '64a), it will develop into a mature lymphoid organ with all the cells common to the thymus; large, medium, and small lymphocytes, epithelial cells, and reticular cells. However, if the thymic rudiment is separated into its epithelial and mesenchymal portions by trypsin, and these grown in tissue culture separately, no further development takes place (Auerbach, '61). After the thymic epithelium is recombined with undifferentiated

mesenchyme, from a variety of places, the necessary stimulus for differentiation is provided and the rudiment continues to develop. When a millipore filter membrane is placed between the epithelial and mesenchymal components of the embryonic thymus, morphogenesis continues (Auerbach, '61). This could suggest a hormonal type interaction between the epithelial and mesenchymal components if the filter prevents cellular migration. The mesenchyme would thus provide the stimulus for the development of the epithelial cells into more mature types.

When the thymus rudiment was developing in the eye chamber, it was conceivable that cells from the circulation might be entering the developing organ. To provide information on this question, Auerbach ('64b) grafted developing thymus rudiments into adult animals previously irradiated with lethal or sublethal doses of irradiation, into animals previously thymectomized, or into thymectomized as well as irradiated before grafting. The morphogenesis of all the grafted rudiments was similar so it was concluded that the thymus produces its lymphocytes by differentiation from the epithelial rudiment. Auerbach has concluded from these and other experiments that the thymic epithelium is the source of the thymic lymphocytes and that the

thymic cells serve to seed out to the other lymphoid organs.

Archer et al. ('64) studied the developmental biology of the lymphoid tissue in the fetal and new born rabbit. They found that normal lymphoid tissue development in the rabbit starts with the thymus, followed by the spleen and gut, and finally the peripheral lymph nodes; it involves essentially a peripheralization. The thymus of the rabbit was lymphoid well before birth. This development in the spleen occurred at a variable time,ranging from four days before birth to a few days after. In neonatally thymectomized rabbits the number of lymphocytes in circulating blood, spleen, and peripheral lymph nodes was reduced during the first weeks of life, compared to normal animals. At 7 to 10 weeks of age, however, these animals showed lymphoid development of the spleen and nodes and a rise in circulating lymphocytes. The appendix apparently developed normally in neonatally thymectomized rabbits, suggesting a degree of independence of the thymus and perhaps a role as a central lymphoid tissue in the delayed maturation of the lymphoid tissues in the animals lacking a thymus. Further support for thymus-like function of the appendix came from histologic comparison of this organ at various

stages of development with the bursa of Fabricus of the chicken, an organ known to function as a central lymphoid tissue (Aspinall et al., '63). Similarities were noted both during development and at maturity. Appendectomy carried out together with thymectomy in neonatal period interfered with the apparent recovery of the peripheral lymphoid tissue from the effects of thymectomy, as observed at nine weeks. Such animals were also very deficient in their antibody response to primary antigenic stimulation with bovine y-globulin.

Our investigation of the lymphoid elements in the developing pig cecum demonstrated clearly that no aggregations of lymphocytes exist in the prenatal cecum. If, in fact, the cecum of the rabbit could serve as an essential seeding organ for that species, other species, for example, the pig, must either be lacking this function, or have developed another operational mechanism to assume the proposed seeding function of the cecum in the rabbit. An observation of the post-natal pig cecum is necessary to carry this argument further.

Table II summarizes the time of first appearance of lymphocytes in different organs of the chicken and various mammals. It makes apparent why neonatal thymectomy (or bursectomy, in the chicken) will result

Table II

Species	Gestation period in	Days at which lymphocytes first appear in: Thymus	Spleen	Lymph nodes	Tonsil	Peyer's Patch	Cecum
Pig	128	37-39	45-48	50-53	53-55	62-64	never
Rabbit (Archer, '64	32	20	28-32	36		36	36
Dog (Kelly, '63	60-63	25	54	48		61	
Mouse	20	prenatal	2-3 postnatal	→	→	→	→
Rat	21	prenatal	postnatal	→	→	→	→
Chicken	21	prenatal (Bursa - 16)	postnatal	→	→	→	→

in "wasting disease" in some animals (mouse - Miller, '61, rat - Arnason et al., '62, chicken - Papermaster, '62) but not others (dog - Kelly, '63, pig - Pestana, '65b).

Summary

The thymus of the pig develops as an epithelial outgrowth of the third pharyngeal pouch. It is composed entirely of epithelial cells until about 36 days, when lymphoid cells are apparent in the gland. By 45 days, lobulation begins, the lymphocytes in the thymus proliferate, and numerous lymphocytes appear in the connective tissue septa between the lobules. Between 46 - 49 days, an early appearance of cortex and medulla is noted, and about 50 days, Hassal's corpuscles appear in the medulla region. The cortex becomes densely packed with lymphocytes, and the medulla is well developed to bring about the mature fetal pattern at an early embryonic age of around 60 days.

The spleen develops in the dorsal mesogastrium, and until 30 days, consists of a dense mesenchymal rudiment. Between 30 - 36 days, the blood forming activity begins and the mesenchymal cells characteristic of the early spleen differentiate into hemocytoblasts which in turn form primitive erythrocytes. At 36 days, a few scattered erythropoietic cells primarily nucleated erythrocytes and hemocytoblasts are seen. These cells expand in number, and by 45 days, islands of erythropoietic activity with the full range of erythroblastic cells

were seen scattered over the entire spleen.

The tendency for lymphocytes to accumulate around vessels is pronounced by 50 days. At 60 days, the spleen is primarily an erythropoietic organ with the erythropoiesis blanketed by small areas of lymphocytic concentration around the arteries, thus the greater part of the organ is erythropoietic red pulp. From 70 days to the postnatal state, there is an increase in the amount of white pulp which concentrates around the arteries to form lymphoid sheaths.

The mesenteric lymph node follows the general development described by Sabin ('05) and Gulland ('94). At 45 days, a plexus of lymphatic ducts was present in the mesentery, but lymph nodes were not apparent until 60 days. The primitive node consisted of a core of reticular cells with a few lymphocytes and lymphoblasts dispersed among them, but no nodules were present. Numerous sinusoidal areas were noted. By 70 days, there was an expansion of lymphocytes which started to aggregate into nodules. The 90 and 110 day stages showed a continual expansion in the number of lymphocytes and the number of nodules, medullary cords, and sinuses.

Lymphoid aggregations in the soft palate are first seen at 55 days. The palatine tonsil consisted of a few small nodules of lymphocytes in the lamina propria

directly under the squamous epithelium. No crypts were seen. At 70 days, numerous epithelial crypts had invaginated carrying the underlying lymphoid tissue deeper into the lamina propria to produce the mature fetal pattern.

No organized lymphoid tissue exists in the cecum of the fetal pig.

A brief discussion to correlate the findings of this investigation with the theory of the epithelial origin of the lymphocytes was conducted.

Bibliography

Ackerman, G. A., and R. A. Knouff 1965 The epithelial origin of the lymphocytes in the thymus of the embryonic hamster. Anat. Rec. 152: 35-53.

Ackerman, G. A. 1967 Developmental relationships between the appearence of lymphocytes and lymphopoietic activity in the thymus and lymph nodes of fetal cats. Anat. Rec. 157:

Archer, O. K., D. E. R. Sutherland and R. A. Good 1964 The developmental biology of the lymphoid tissue in the rabbit Lab. Invest. 13: 259-271.

Arnason, B. G., B. D. Jankovic, B. H. Waksman, and C. Wennersten 1962 Role of the thymus in immune reactions in rats. II. Suppressive effect of thymectomy at birth on reactions of delayed (cellular) hypersensitivity and circulating small lymphocyte. J. Exp. Med. 116: 177-191.

Aspinall, R. L., R. K. Meyer, M. A. Graetzer, and H. R. Wolfe 1963 Effect of thymectomy and bursectomy on the survival of skin homografts in chickens. J. Immun. 90: 872-885.

Auerbach, R. 1961 Experimental analysis of the origin of cell types in the development of mouse thymus. Dev. Biol. 3: 336-354.

_____. 1964a Experimental analysis of mouse thymus and spleen morphogenesis. In: The Thymus in Immunobiology. R. A. Good and A. Gabrielsen, Eds. New York: Harper and Row.

_____. 1964b On the function of the embryonic thymus. Wistar Inst. Symp. Monograph II: 1-8.

_____. 1965 Experimental analysis of lymphoid differentiation in the mammalian thymus and spleen. In: Organogenesis. DeHarr and Ursprung, Eds. Holt, Reinehart and Winston 539-557.

Badertscher, J. A. 1915 The development of the thymus in the pig. II. Histogenesis. Am. J. Anat. 17: 437-493.

Beard, J. 1900 The source of leukocytes and the true function of the thymus. Anat. Anz. 18: 561-573.

Bell, E. T. 1906 The development of the thymus. Am. J. Anat. 5: 29-62.

Godwin, Melvin Crawford 1939 The mammalian thymus. IV. The development in the dog. Am. J. Anat. 64: 165-192.

Gulland, G. A. 1894 The development of the lymphatic glands. J. Path. and Bact. 2: 447-485.

Heuer, G. 1909 The development of the lymphatics in the small intestine of the pig. Am.J. Anat. 9: 93-118.

Keibel, F. 1897 Normentafel zur entwicklungs-geschiehte des schweines. (Sus scrofa domesticus) Pub. Verlag von Gustav Fisher.

Kelly, W. D. 1963 The thymus and lymphoid morpho-genesis in the dog. Fed. Proc. 22: 600.

Klapper, Clarence 1946 Morphogenesis of the thymus. Am. J. Anat. 78: 139-179.

Levin, P. M. 1930 The development of the tonsil of the domestic pig. Anat. Rec. 45: 189-201.

Lillie, R. D. 1965 Histopathologic technic and practical histochemistry. 3rd ed. McGraw-Hill, New York.

Miller, J. F. A. P. 1961 Immunological function of the thymus. Lancet 2: 748-759.

Movat, Henry Z. 1955 A. M. A. Archives of Pathology. 60: 289-295.

Norris, E. H. 1938 The morphogenesis and histo-genesis of the thymus gland in man: In which the origin of the Hassal's corpuscles of the human thymus is discovered. Carnegie Contrib. Embryol. 27: 191-208.

Papermaster, B. W., D. I. Friedman, and R. A. Good 1962 Relationship of the Bursa of Fabricius to immunologic responsiveness and homograft immunity in the chicken. Proc. Soc. Exp. Biol. Med. 110: 62-76.

Pestana, Carlos, G. A. Hallenbeck, and R. G. Shorter 1965a Development of lymphoid tissues in the fetal pig. J. Surg. Res. 5: 471-475.

_____. 1965b Thymectomy in newborn pigs. J. Surg. Res. 5: 306-317.

Sabin, R. F. 1905 The development of lymphatic nodes in the pig and their relation to the lymph hearts. Am. J. Anat. 4: 355-389.

_____. 1909 The lymphatic system in human embryos, with a consideration of the morphology of the system as a whole. Am. J. Anat. 9: 43-91.

_____. 1910 The development of the spleen. In: Keibel and Mall Manual of Human Embryology. 2: 745-751.

Thiel, G. A. and H. Downey 1921 The development of the mammalian spleen with special reference to its hemapoietic activity. Am. J. Anat. 28: 279-339.

Warwick, B. L. 1938 Prenatal growth of swine. J. Morph. and Physiol. 46: 59-84.

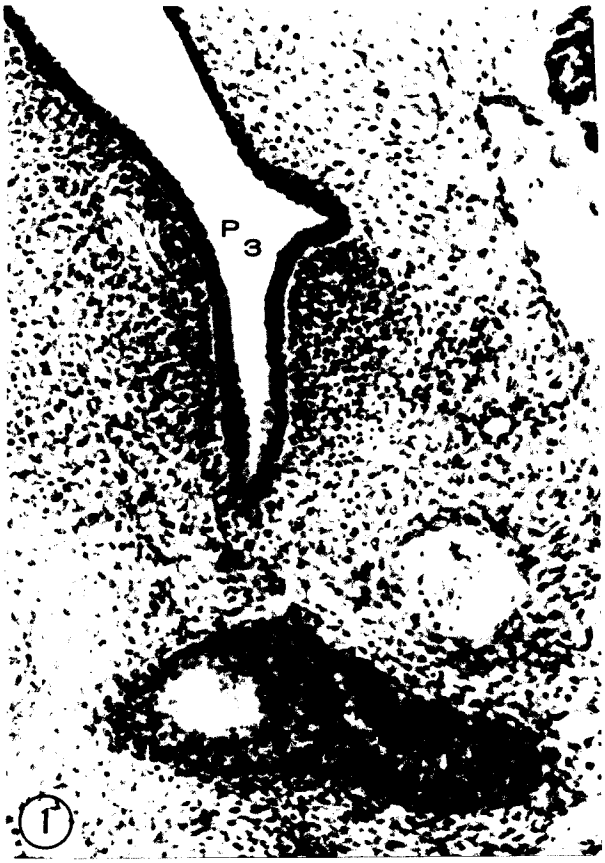
Plate I

Fig. 1. Cross section through the 3rd pharyngeal pouch (P_3) region of the 20 day pig embryo showing the thymus primordium invaginating from the epithelium of the 3rd pharyngeal pouch. Hematoxylin - eosin, X 170.

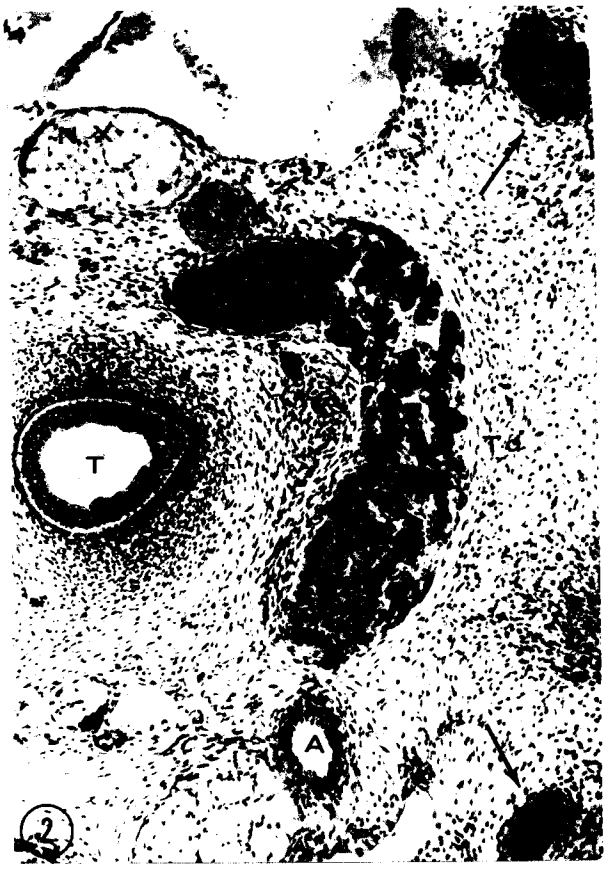
Fig. 2. Cross section through the upper neck region of the 30 day pig embryo showing the thymus primordium (arrows), located anterior and lateral to the thyroid (Td), trachea (T), and internal carotid artery (A). The 10th nerve (N.X) is also shown. Hematoxylin - eosin, X 63.

Fig. 3. High power magnification of the thymus primordium as seen in fig. 1. The lumen (P) of the 3rd pharyngeal pouch is seen surrounded by the entirely epithelioid primitive thymus. The primordium appears encapsulated by connective tissue fibroblasts (arrows). No lymphoid cells appear in the connective tissue around the thymus. Hematoxylin - eosin, X 680.

Fig. 4. 36 day thymus. The thymus shows early lobulation with a connective tissue investment. A few cells (arrows) show lymphocytic characteristics. Note the absence of lymphocytes in the surrounding connective tissue. Hematoxylin - eosin, X 430.



1



2

3

4

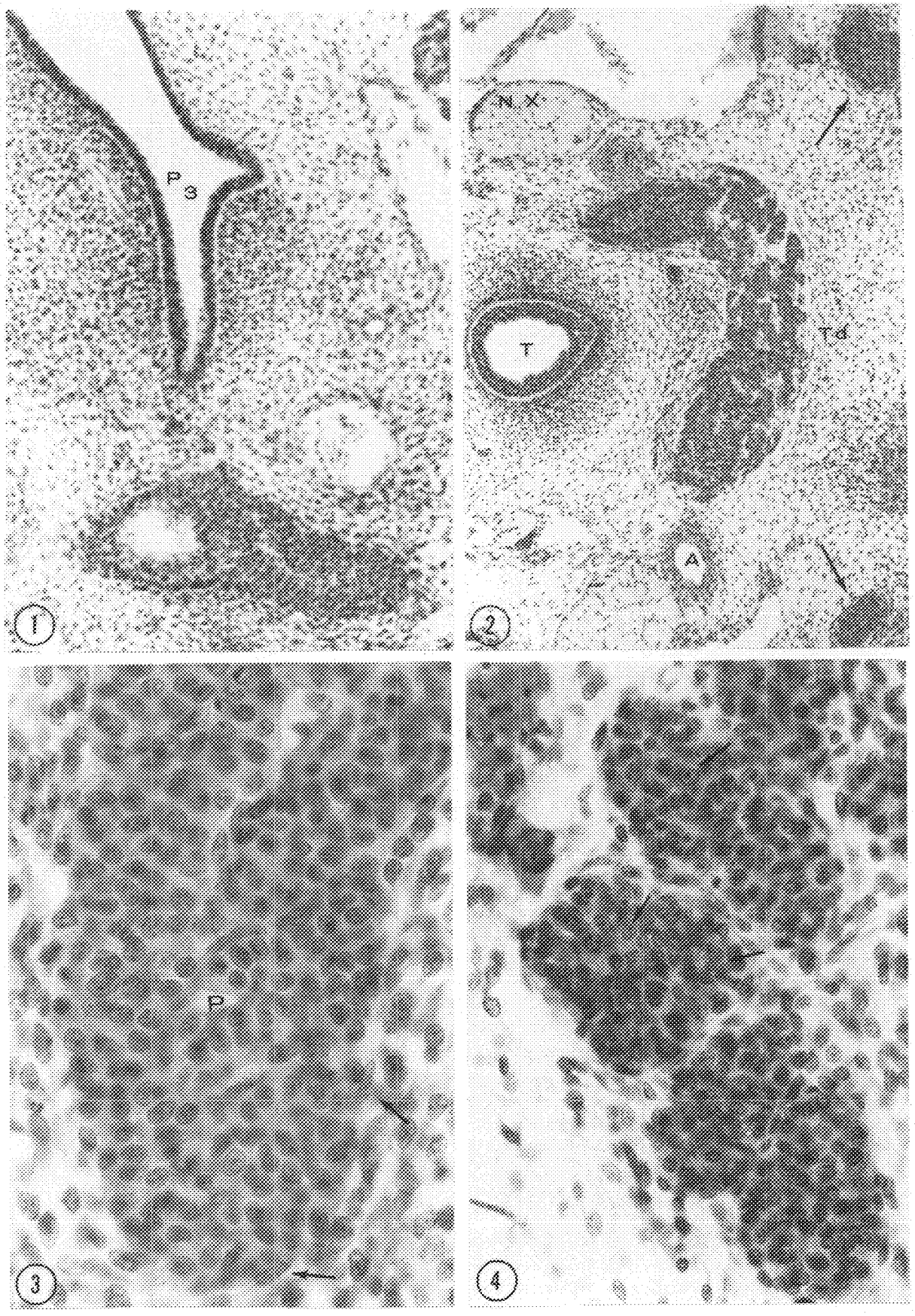
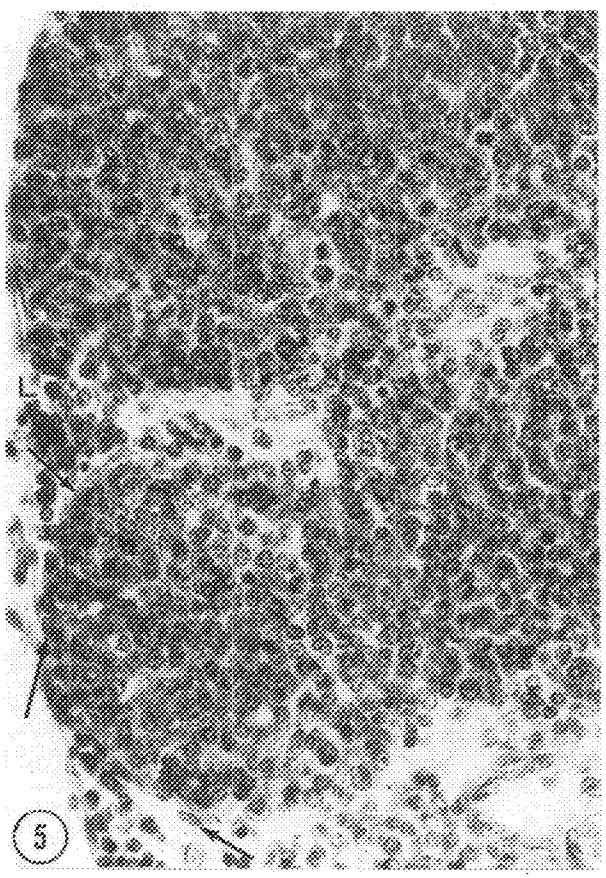

P3
1
NX
T
Td
A
2
P
3
4

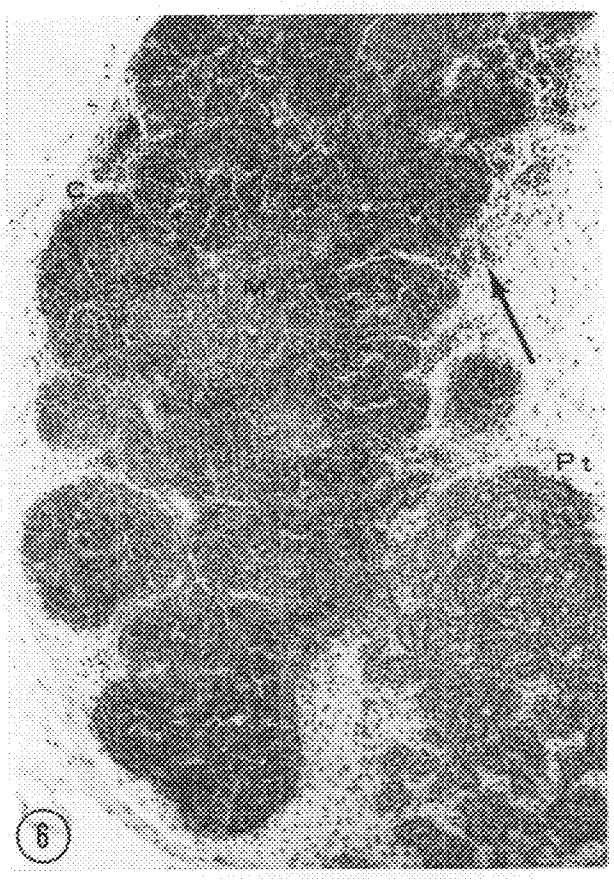
Plate II

Fig. 5. 45 day thymus. The thymus cells have organized into distinct lobules with a thin connective tissue capsule (arrows). Large lymphocytes are apparent in the thymus and many other lymphocytes (L) fill the connective tissue septa between the lobules. There is no separation into cortex and medulla. Hematoxylin - eosin, X 430.

Fig. 6. 50 day thymus. Early differentiation into cortex (C) and medulla (M) is apparent. There are large accumulations of lymphocytes in the connective tissue surrounding the thymus (arrow). The parathyroid (Pt) is developing adjacent to the thymus. Hematoxylin - eosin, X 110.

Fig. 7. Higher magnification of fig. 6, showing the cortex, (C), filled with lymphocytes, medulla (M), with fewer, large cells, and lymphocytes (L) in the connective tissue septa between two lobules. 50 day thymus. Hematoxylin - eosin, X 430.


L
5


C
Pt
6

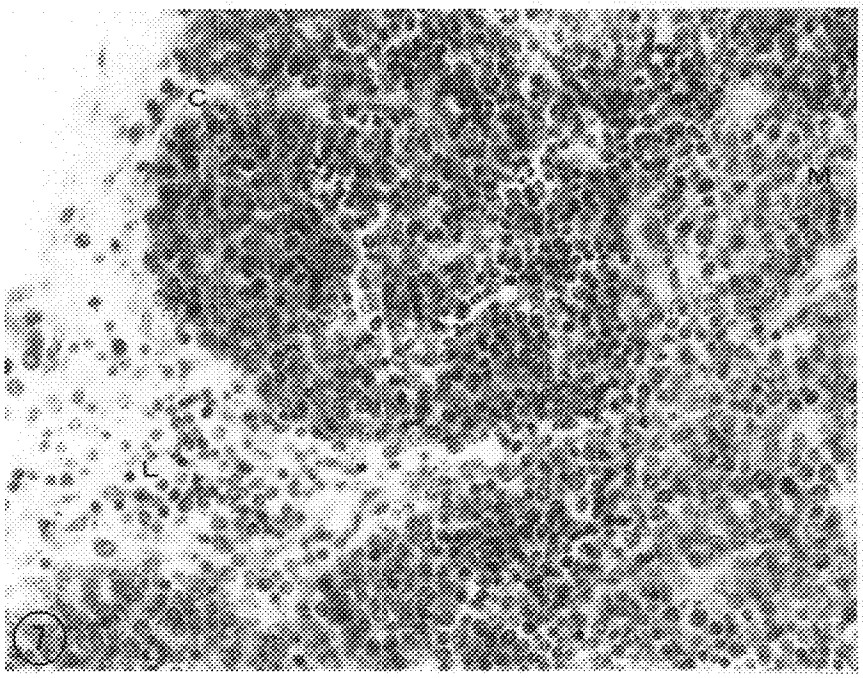
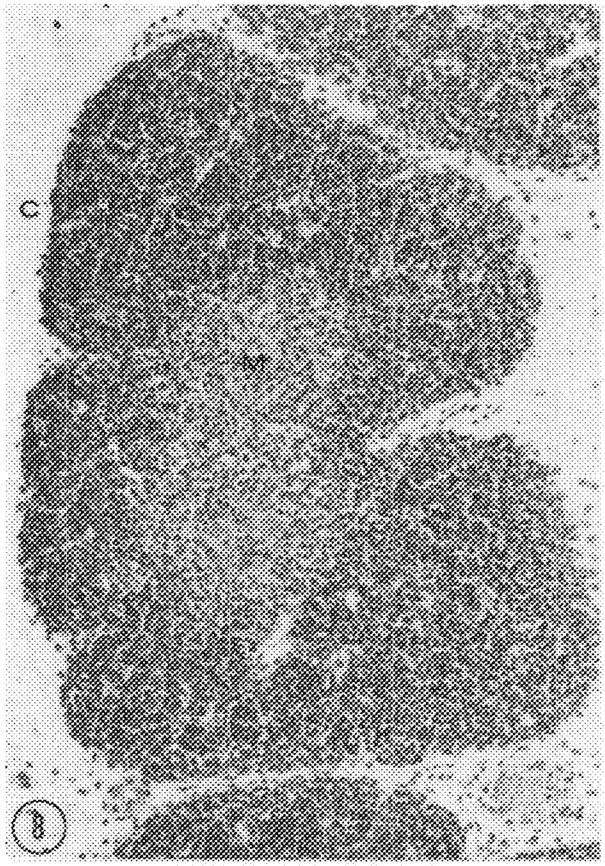

C
L
7

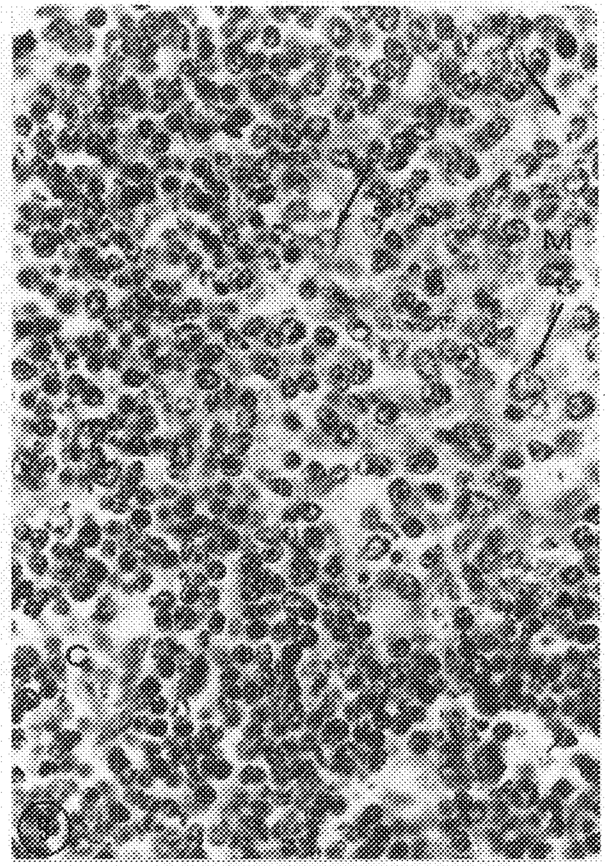
Plate III

Fig. 8. 60 day thymus. The mature fetal pattern (fig. 10.) is established by 60 days. Note the distinct cortex and medulla, and the absence of lymphocytes in the connective tissue septa surrounding the lobule. Movat's, X 170.

Fig. 9. Higher magnification of fig. 8. shows the cortex (C) filled with lymphocytes of all sizes and the medulla (M) with fewer lymphocytes and many epithelial cells (arrows). 60 day thymus. Movat's, X 430.

Fig. 10. 110 day thymus. The continued growth of the 60 day thymus pattern (fig. 8.) produces the multilobulated 110 day thymus with an extensive medulla. Movat's, X 43.


C
M
8


M
C

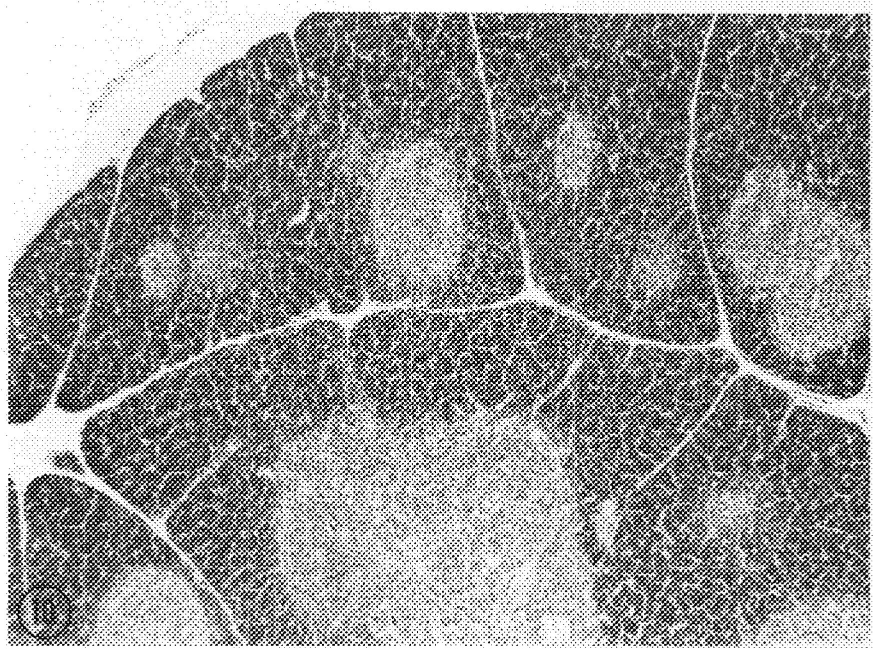

10

Plate IV

Fig. 11. 50 day thymus. Medulla showing a primitive Hassal's corpuscle (H) with numerous epithelial cells (arrows) around it. Lymphocytes are also present. Hematoxylin - eosin, X 1700.

Fig. 12. 60 day thymus medulla showing a more advanced Hassal's corpuscle (cf. fig. 11.) containing five degenerating epithelial cells bounded by fine reticular fibers (arrows). The surrounding lymphocytes are slightly out of focus to demonstrate the degenerating nuclei of Hassal's corpuscles to best advantage. Movat's, X 1700.

Fig. 13. 110 day medulla. A mature Hassal's corpuscle is seen with complete degeneration of the epithelial nuclei into fibrous elements. Epithelial cells are not apparent in the surrounding medulla, but large numbers of lymphocytes are present. Movat's, X 680.

Fig. 14. Cross section through the stomach region of a 20 day pig embryo. The spleen primordium (Sp) is seen in the dorsal mesogastrium (Dm) adjacent to the stomach (St). The splenic cells are of uniform mesenchymal character. Movat's, X 270.

H
11

13

Sp
Drm
St
14

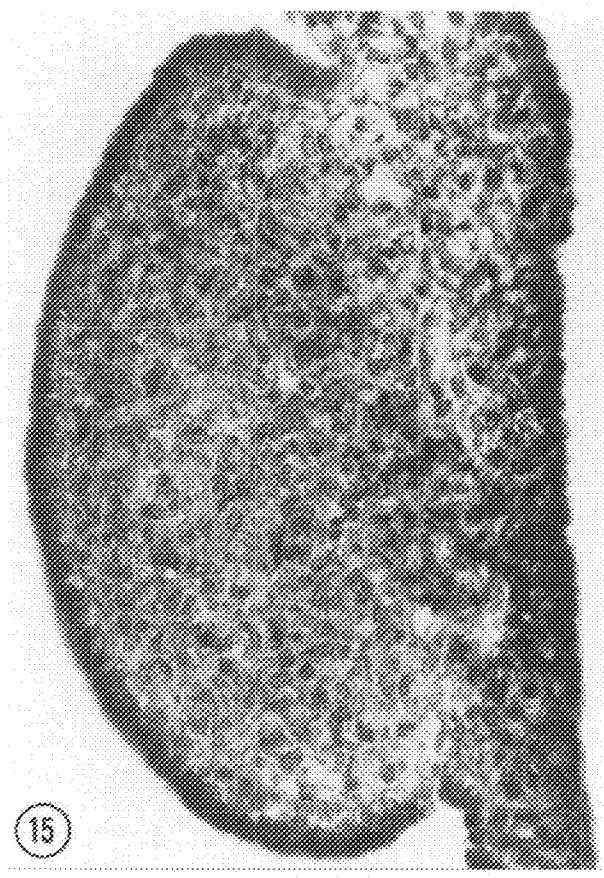
Plate V

Fig. 15. 30 day spleen. The spleen is attached to the dorsal mesogastrium by a thin projection of cells. A distinct capsule of peritoneum covers the splenic mass of undifferentiated mesenchymal cells. Movat's, X 430.

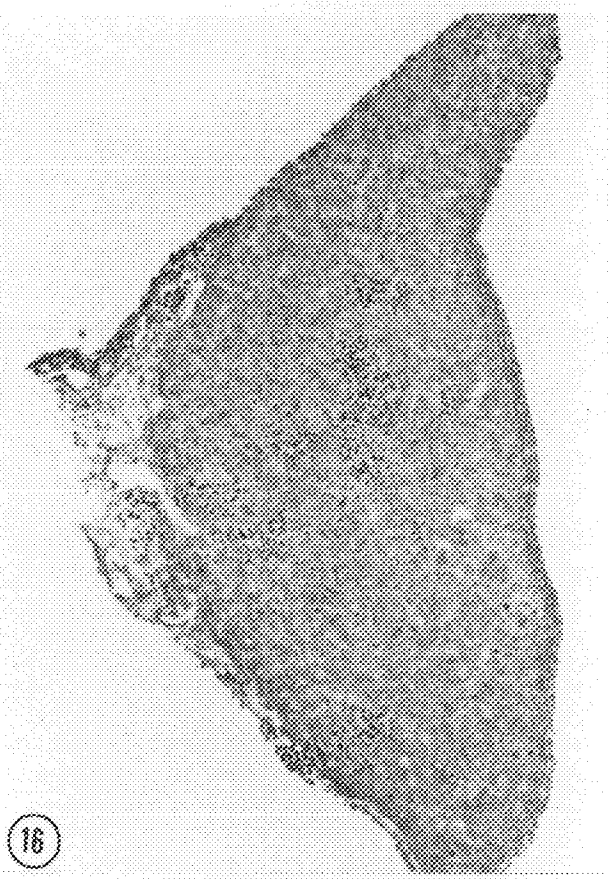
Fig. 16. 36 day spleen. The spleen is free from the dorsal mesogastrium. Differentiated cells are dispersed in the mesenchymal splenic mass. PAS - Hematoxylin, X 170.

Fig. 17. Higher magnification of fig. 16 shows the cell types present in the 36 day spleen. Against the mesenchymal background can be seen: 1) nucleated erythrocytes, 2) polychromatophilic erythroblasts, 3) hemocytoblasts and 4) cells intermediate between mesenchymal cells and hemocytoblasts. PAS - Hemotoxylin, X 430.

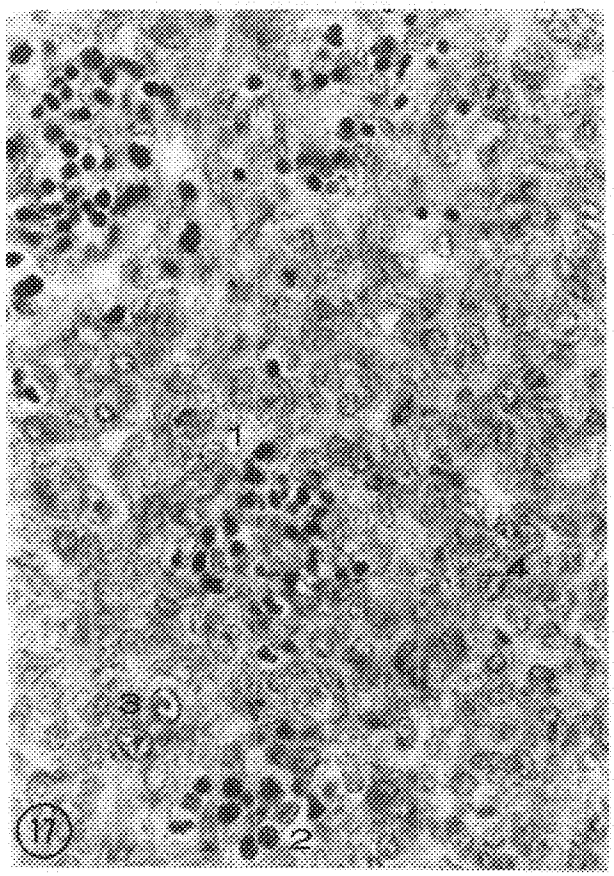
Fig. 18. Oil immersion of 36 day spleen. Two hemocytoblasts (arrow) are seen, along with mesenchymal cells and nucleated erythrocytes. Movat's, X 1700.



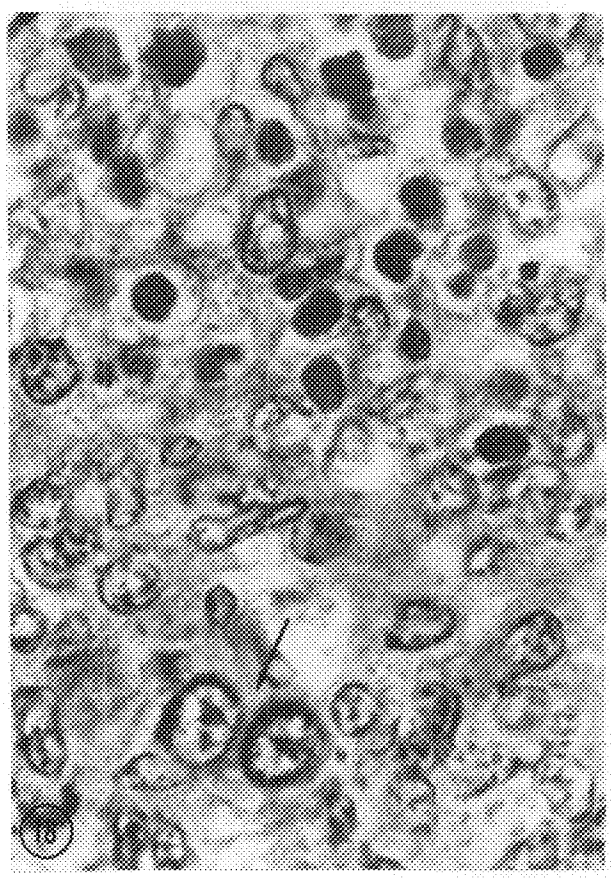
15



16



17



18

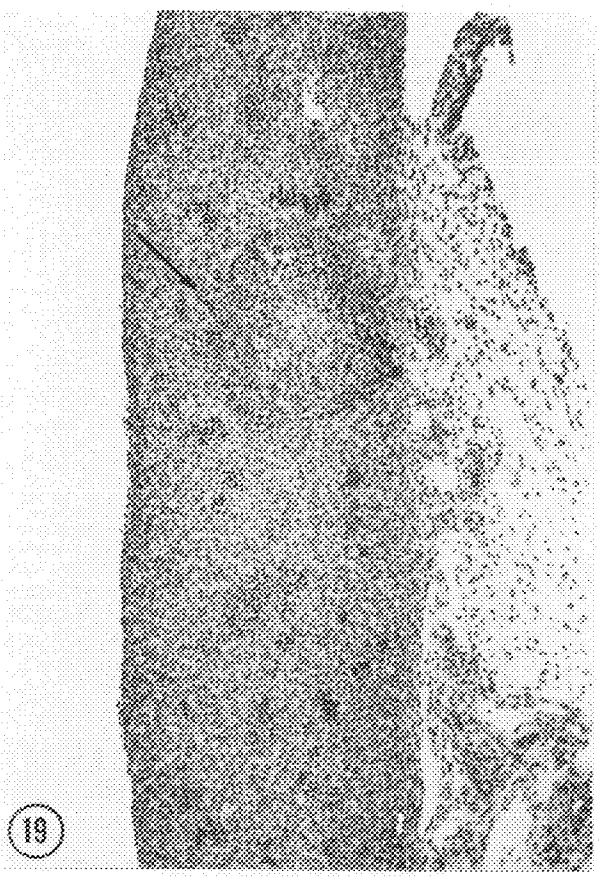
Plate VI

Fig. 19. 45 day spleen. Islands of differentiating cells are scattered through the spleen which is still primarily mesenchymal in character. The vascular system has connected with the early splenic sinuses to form a pool of red pulp (arrow). Movat's, X 110.

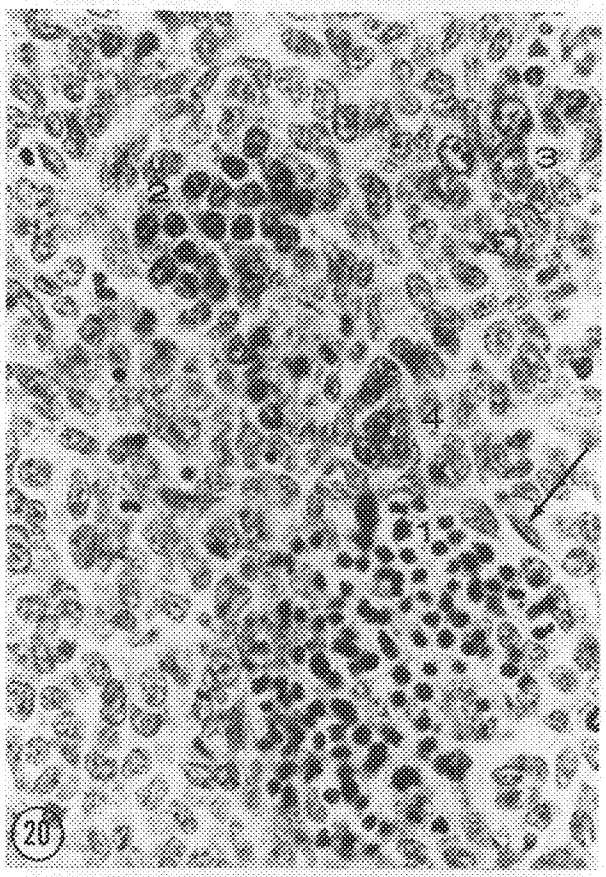
Fig. 20. Higher magnification of the 45 day spleen demonstrating the cell types present. Interspersed between the mesenchymal cells are; 1) nucleated erythrocytes, 2) polychromatophylic erythroblasts, 3) hemocytoblasts, and 4) cells intermediate between mesenchymal cells and hemocytoblasts. A vascular channel with a single cell endothelium is also apparent (arrow). Movat's, X 680.

Fig. 21. 50 day spleen. Erythropoiesis has increased (cf. fig. 19.) to the point where the entire spleen is one mass of red pulp. Movat's, X 170.

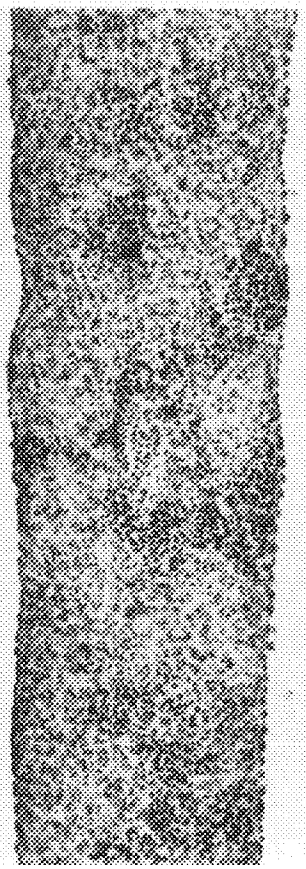
Fig. 22. Higher magnification of the erythropoietic components in fig. 21. Numerous non-nucleated erythrocytes (arrow 1) and reticular cells (arrow 2) are also apparent. Little mesenchyme remains. 50 day spleen. Movat's, X 430.



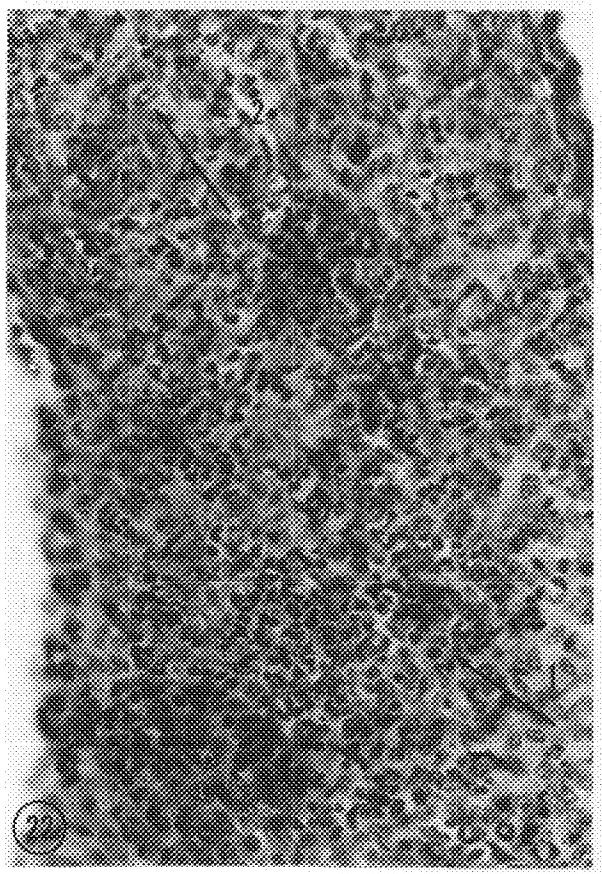
19



20



21



22

Plate VII

Fig. 23. Oil immersion of 50 day spleen. Lymphocytes (arrows) appear around an early vascular channel (V). Hemocytoblasts, nucleated erythrocytes, and reticular cells are also shown. Movat's, X 1700.

Fig. 24. 50 day spleen. The reticular elements are demonstrated. PAS, X 430.

Fig. 25. 60 day spleen. Erythropoietic activity is spread over the entire spleen. Accumulations of lymphocytes around vessels (arrows) form early white pulp. Trabeculae are seen in the lower corners. Movat's, X 110.

Fig. 26. 70 day spleen. Erythropoiesis and the formation of white pulp (arrows) are further developed in the 70 day spleen. Movat's, X 170.

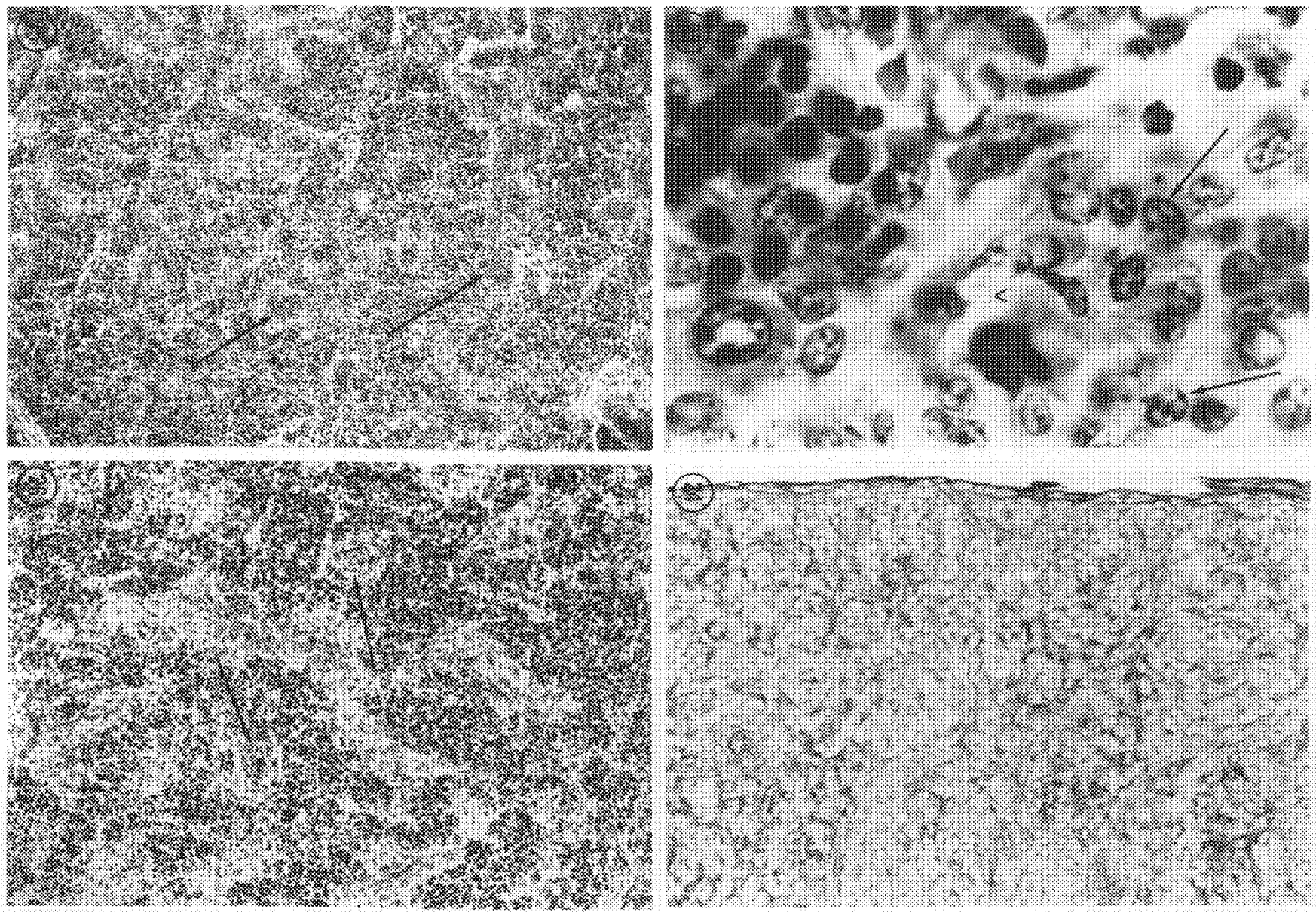


Plate VIII

Fig. 27. 90 day spleen. Erythropoiesis is at its maximum. The amount of white pulp around the vessels has expanded. Trabeculae are also apparent. Movat's, X 110.

Fig. 28. 110 day spleen. The over-all distribution of pulp is shown. Movat's, X 110.

Fig. 29. Higher magnification of the 110 day spleen showing the three types of pulp in the mature fetal spleen. The upper portion of the figure shows white pulp around two central arteries. In the middle section, non-erythropoietic red pulp is seen with numerous reticular cells (arrow). The lower portion of the figure demonstrates erythropoietic red pulp. Movat's, X 430.

Fig. 30. 110 day spleen. The distribution of reticular fibers is apparent. The erythropoietic red pulp (arrow) has a lower content of reticular fibers than does the non-erythropoietic red pulp. PAS, X 110.

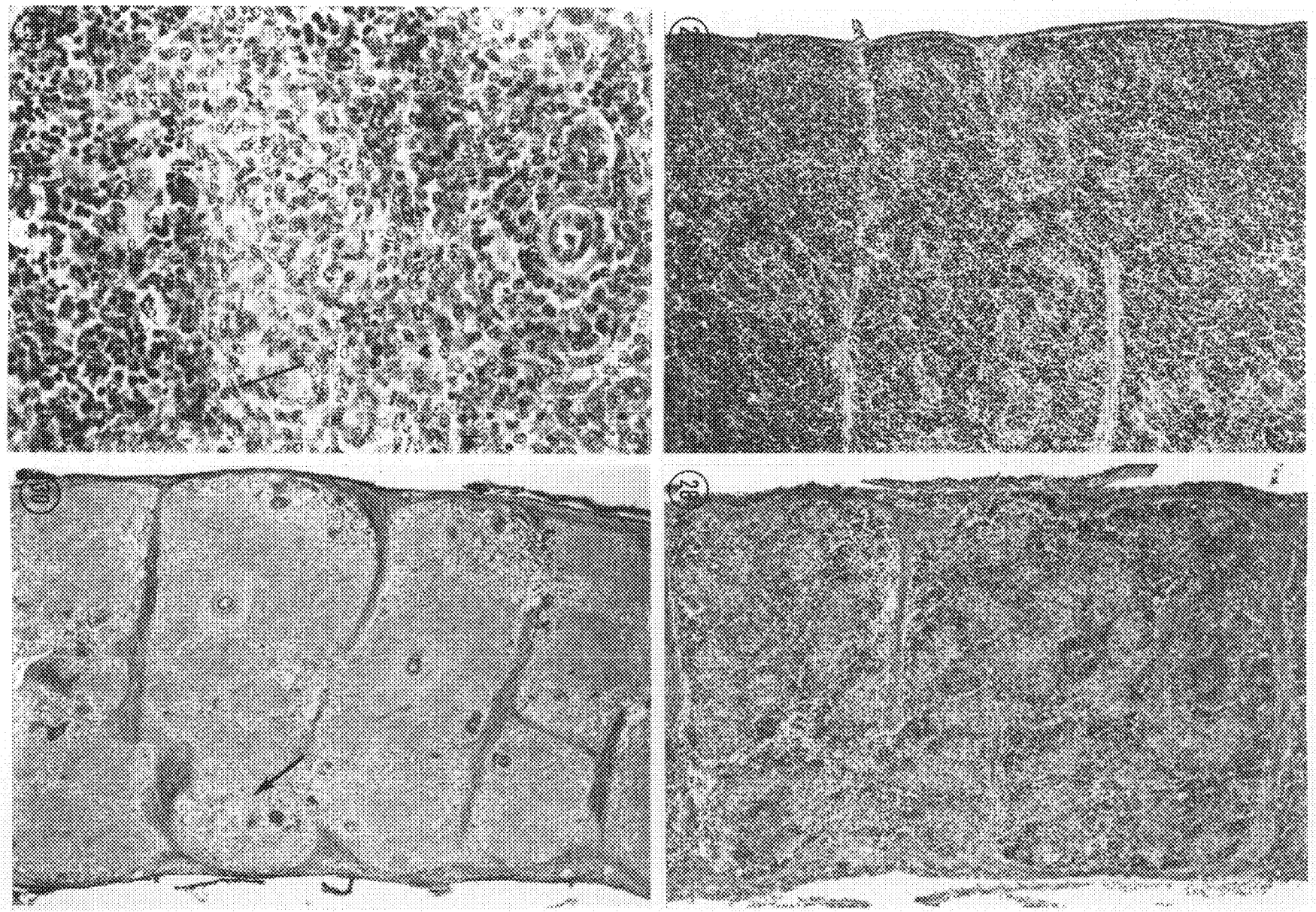


Plate IX

Fig. 31. Mesentery of the 45 day embryo. A plexus of lymphatic ducts (D) is seen in the mesenchyme of the mesentery. Movat's, X 430.

Fig. 32. 50 day mesentery. Nodes have not yet formed, but the lymphatic ducts have proliferated (arrows). Movat's, X 110.

Fig. 33. 60 day mesenteric lymph node. A primitive node (N) has developed in the mesentery (M) adjacent to a large glomus (G). A subcapsular sinus is apparent (arrow). Movat's, X 110.

Fig. 34. Higher magnification of a region under the subcapsular sinus of fig. 33. The sinus (S) is lined by reticular cells (arrow 1). Further into the node, occasional large lymphocytes (arrow 2) are seen dispersed in the reticular cell core. Movat's, X 430.

31

32

33

34

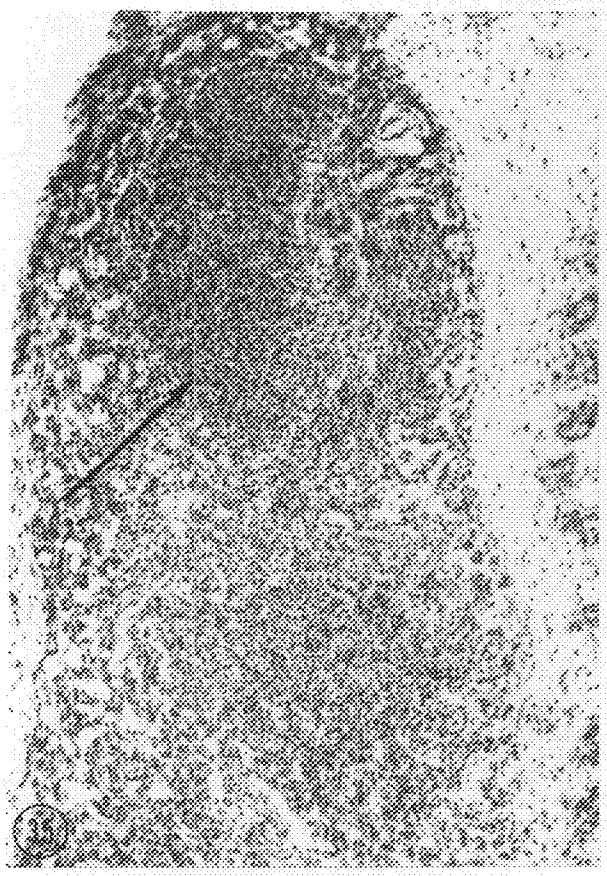
Plate X

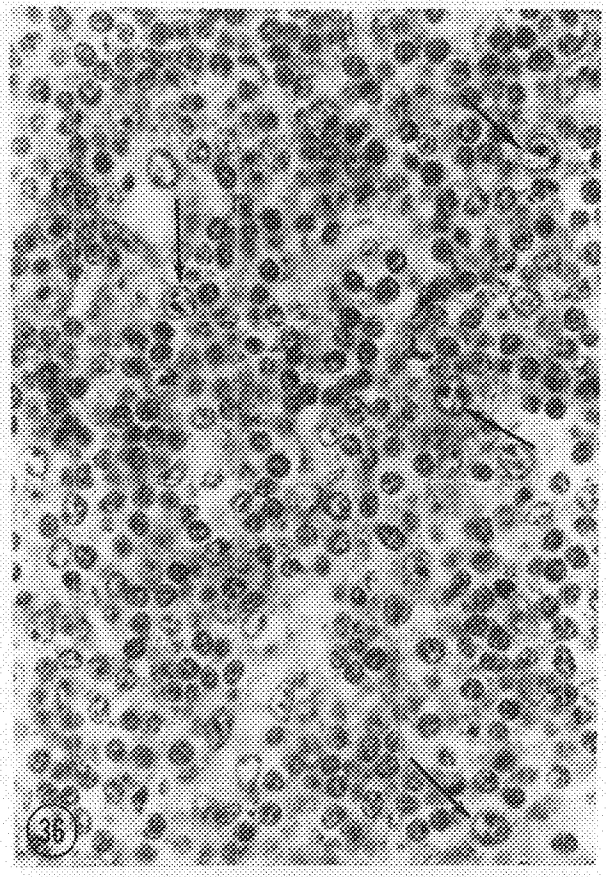
Fig. 35. 70 day mesenteric lymph node. An accumulation of lymphocytes is apparent in the node (arrow). Movat's, X 110.

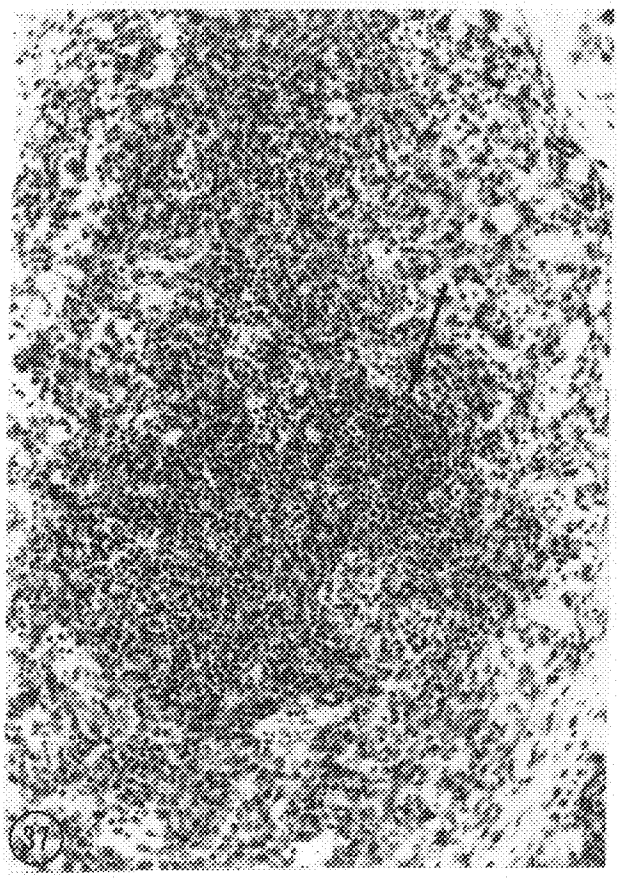
Fig. 36. Higher magnification of the region at the tip of the arrow in fig. 35. A number of small lymphocytes and several large lymphocytes (arrows) are observed in the reticular framework of the node. 70 day node. Movat's, X 430.

Fig. 37. 90 day mesenteric lymph node. A larger accumulation of lymphocytes forms a primitive nodule in the node (arrow). Movat's, X 170.

Fig. 38. Higher magnification of fig. 37. near the tip of the arrow. There are fewer large lymphocytes in this node (cf. fig. 36.). A primitive capsule is found around the accumulation of cells (arrow). A sinusoidal area with many reticular cells is shown in the upper area of the fig. Movat's, X 680.







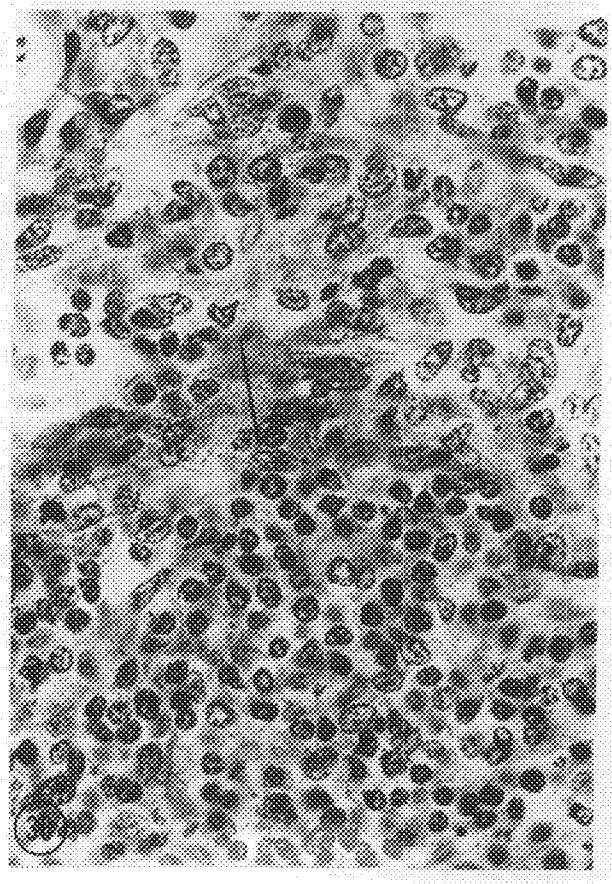


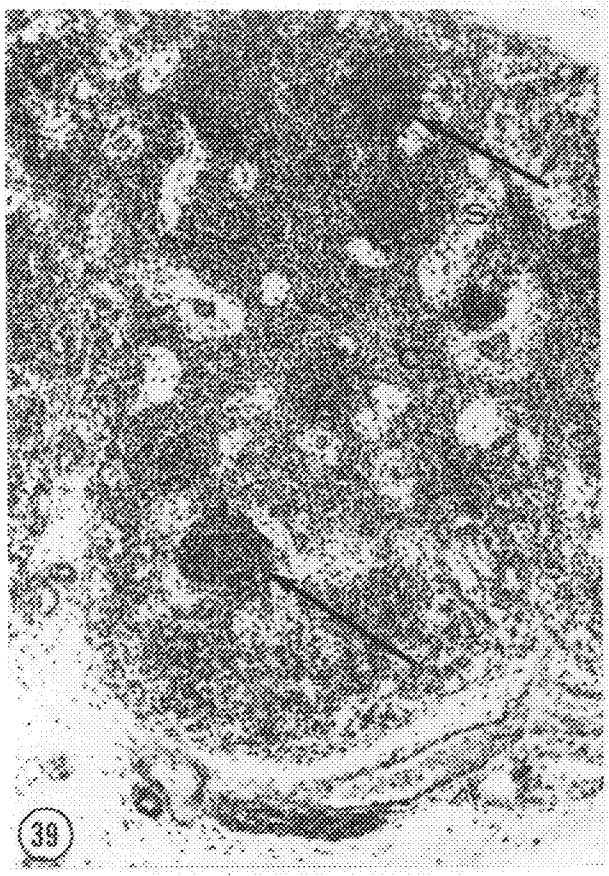
Plate XI

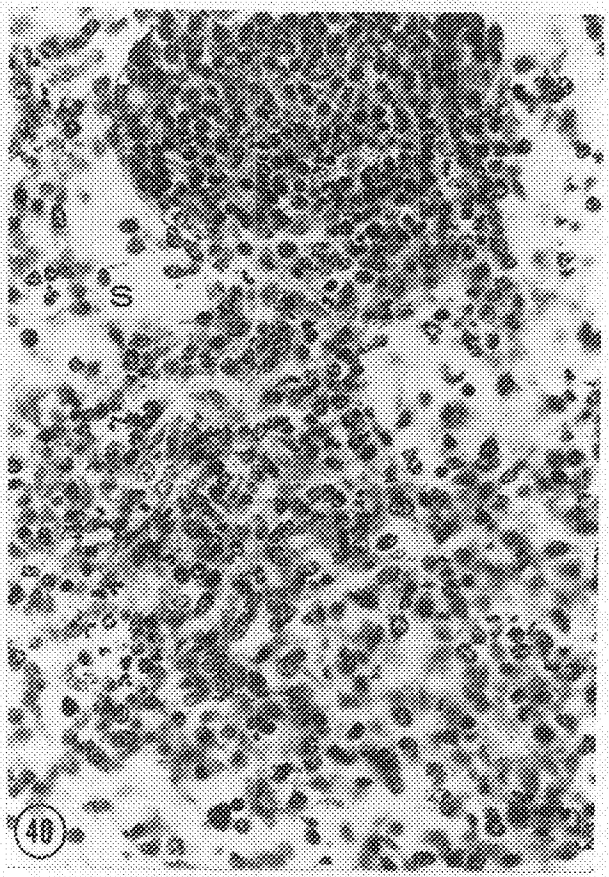
Fig. 39. 110 day mesenteric lymph node. Numerous nodules occur through out the node (arrows), as well as medullary cords (C) and sinuses (S). Movat's, X 110.

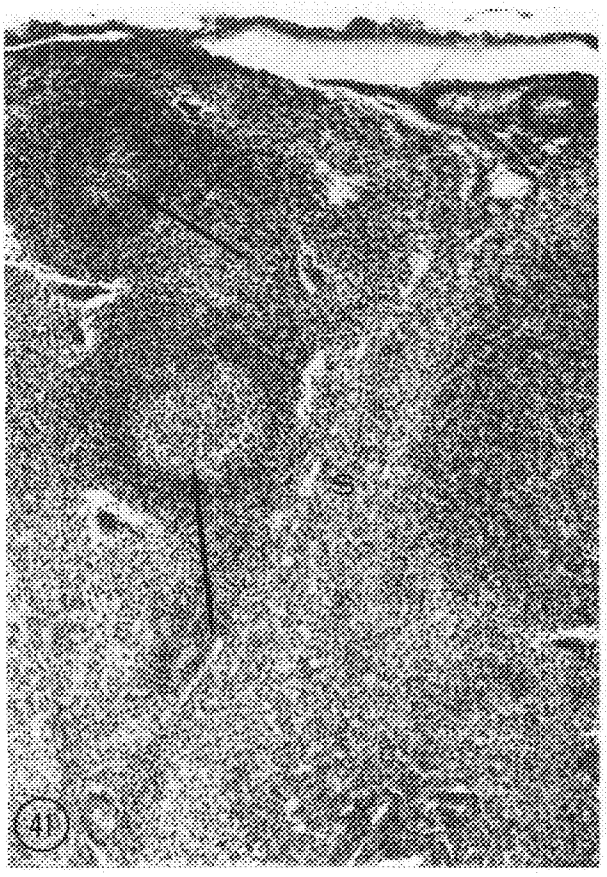
Fig. 40. Higher magnification of fig. 39. at the tip of the lower arrow. The cells of the nodule are small lymphocytes, (upper portion of fig. 40.) with sinusoidal reticular cells surrounding them (S). 110 day node. Movat's, X 430.

Fig. 41. Postnatal mesenteric lymph node. The nodule shows germinal centers (arrows). The medullary sinuses are filled with cells (cf. M, fig. 39.). Movat's, X 110.

Fig. 42. Higher magnification of fig. 41. showing the lymphoblasts of the germinal center (GC) and the small lymphocytes of the marginal zone (arrow). Movat's, X 430.


39


S
40


41

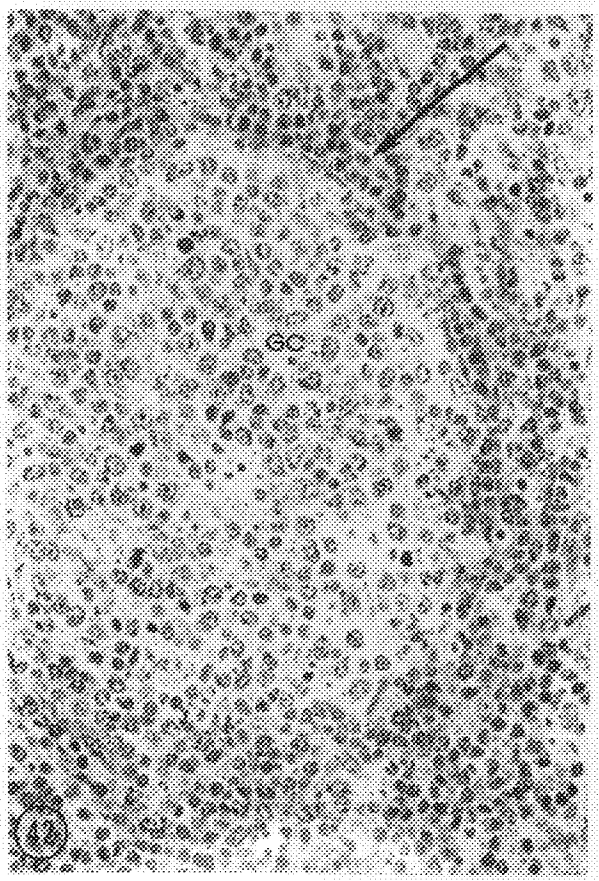

GC
42

Plate XII

Fig. 43. Section through the 60 day soft palate showing the palatine tonsil. Nodules of lymphocytes appear in the lamina propria under the stratified squamous epithelium of the pharynx (P). Hematoxylin - eosin, X 170.

Fig. 44. Higher magnification of a nodule from fig. 43. The nodule is composed of densely packed lymphocytes. Reticular cells surround the base of the nodule (arrows). 60 day palatine tonsil. Hematoxylin - eosin, X 430.

Fig. 45. 60 day palatine tonsil. The reticular elements encapsulate the outer border of the nodule (arrows). PAS, X 430.

Fig. 46. 60 day palatine tonsil. The area underlying the tonsil nodule contains many vascular channels (V) and a large number of granulocytes (arrow). Some lymphocytes (L) at the base of the nodule are also shown. Hematoxylin - eosin, X 430.

P
43

44

45

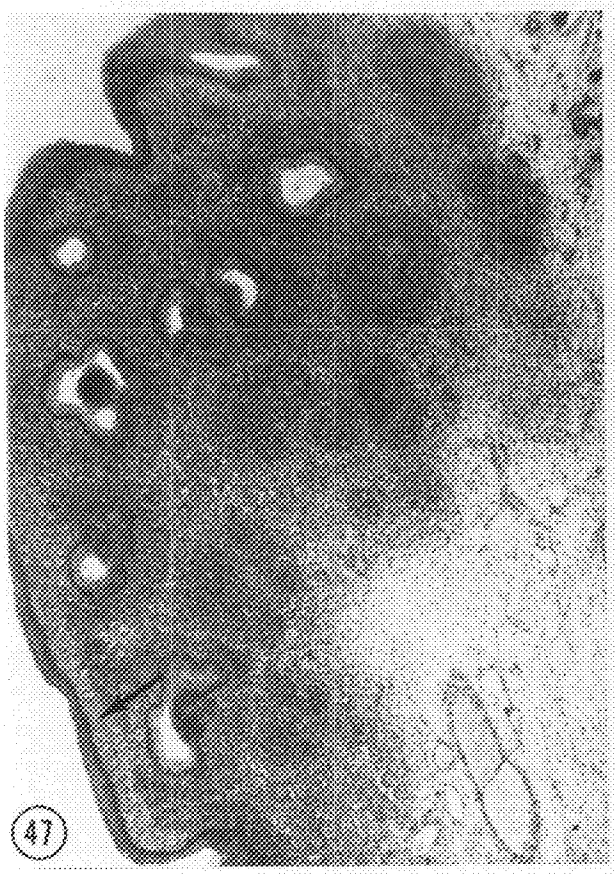
46

Plate XIII

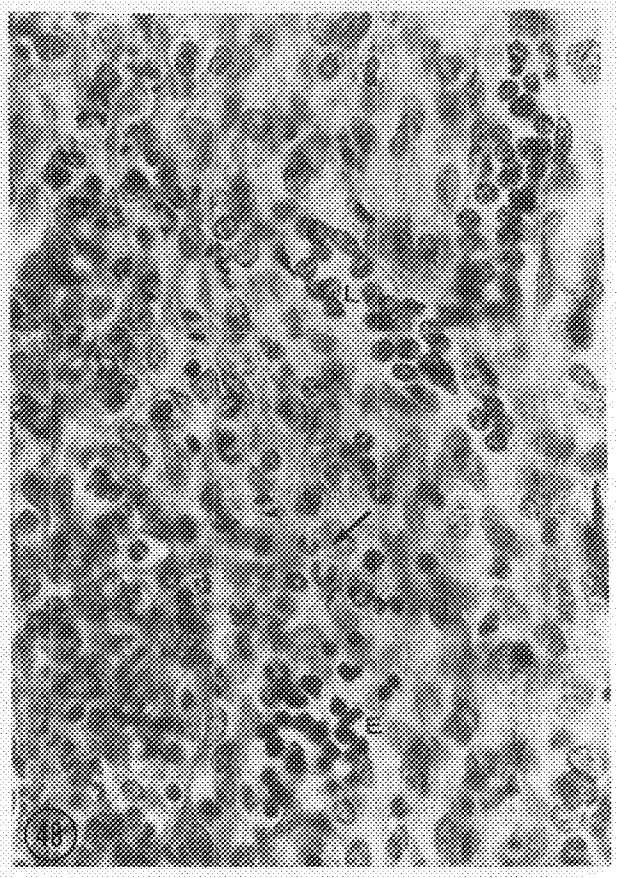
Fig. 47. 70 day palatine tonsil. Numerous crypts lined with lymphocytes comprise the tonsil (oblique section). Hematoxylin - eosin, X 43.

Fig. 48. Area underlying the 70 day tonsil, demonstrating lymphocytes (L) in lymphatics, nucleated erythrocytes (E), and granulocytes (arrow). Hematoxylin - eosin, X 430.

Fig. 49. 70 day Peyer's patch. Aggregations of lymphocytes (arrow) are located in the lamina propria at the base of the intestinal villus. Hematoxylin - eosin, X 170.



47



48

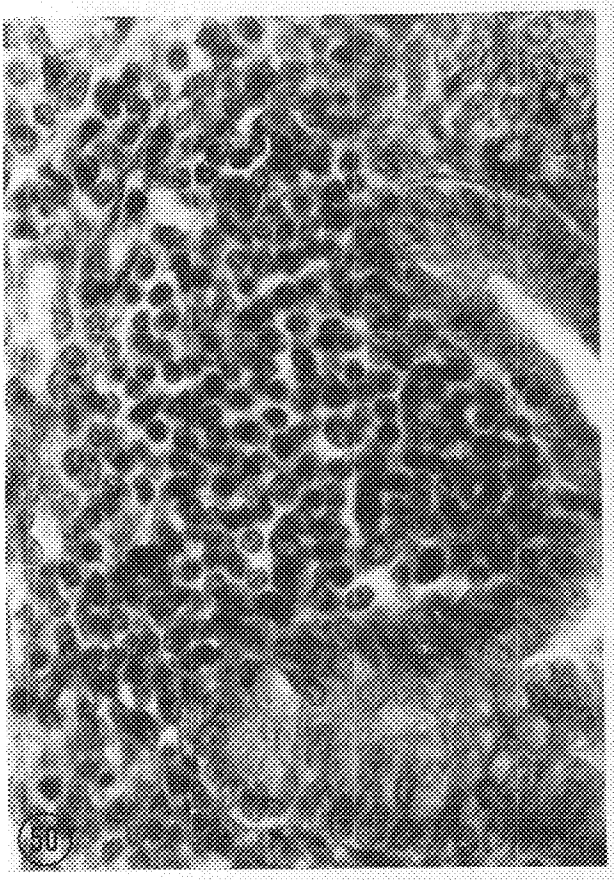
49

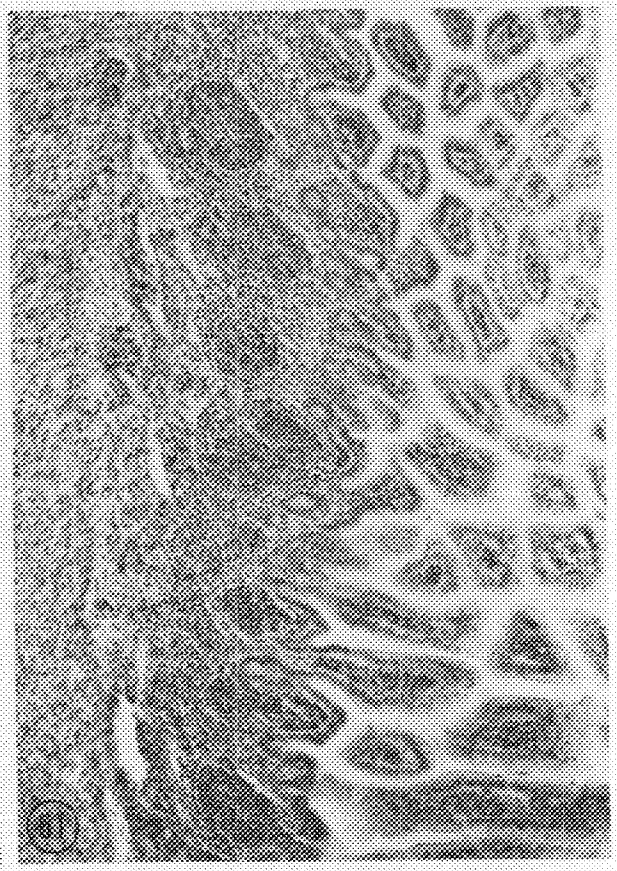
Plate XIV

Fig. 50. Higher magnification of the Peyer's patch in fig. 49. Lymphocytes are loosely packed into the nodule. 70 day Peyer's patch. Hematoxylin - eosin, X 680.

Fig. 51. 110 day Peyer's patch. Nodules of lymphocytes extend along the entire terminal ileum. Hematoxylin - eosin, X 110.

Fig. 52. Higher magnification of fig. 51. Lymphocytes and lymphoblasts (arrows) are seen in the mature fetal Peyer's patch nodule. Hematoxylin - eosin, X 680.


50


51

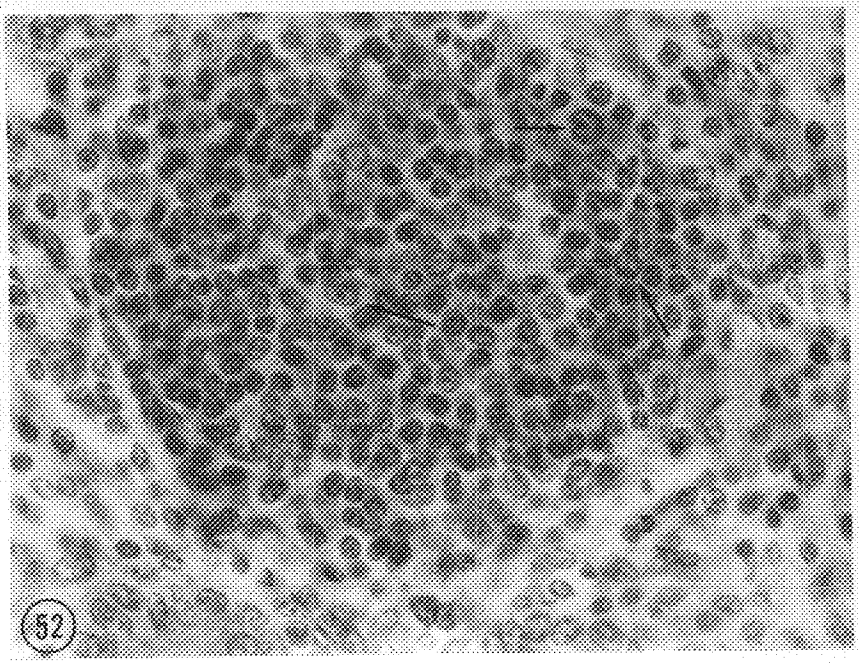
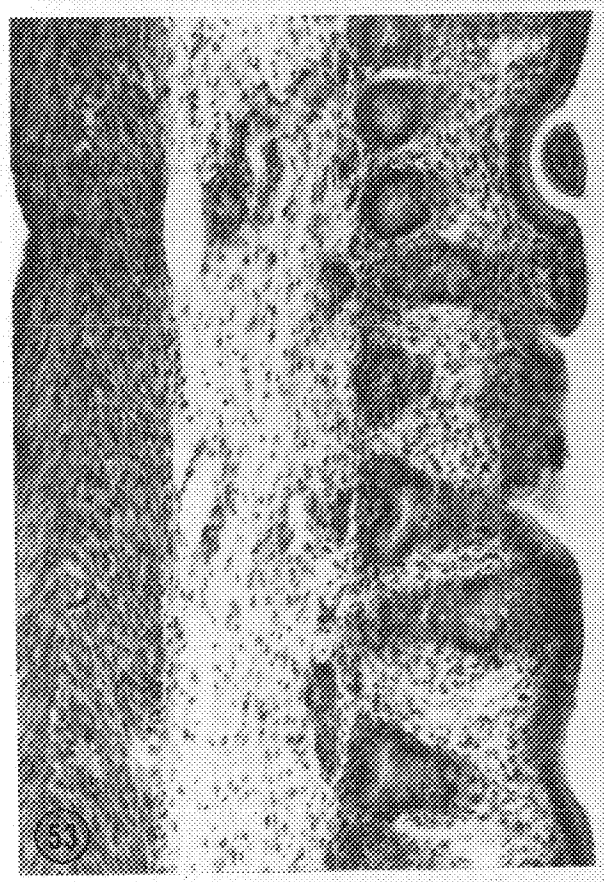

52

Plate XV

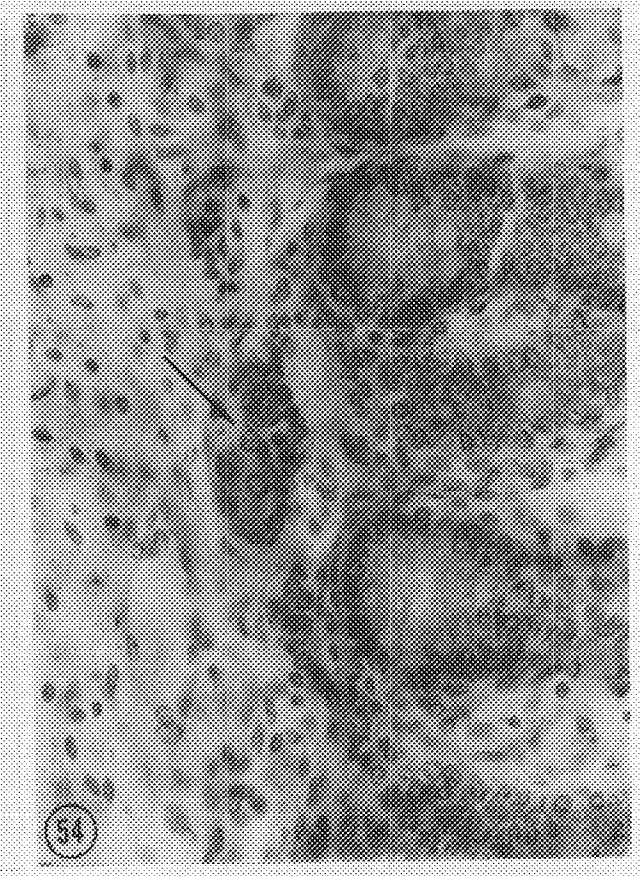
Fig. 53. 110 day cecum. No organized lymphoid tissue exists in the lamina propria of the mature fetal cecum. Hematoxylin - eosin, X 170.

Fig. 54. Higher magnification of a 110 day cecum showing that the only lymphocytes apparent in the lamina propria are in lymphatic channels (arrow). Hematoxylin - eosin, X 430.

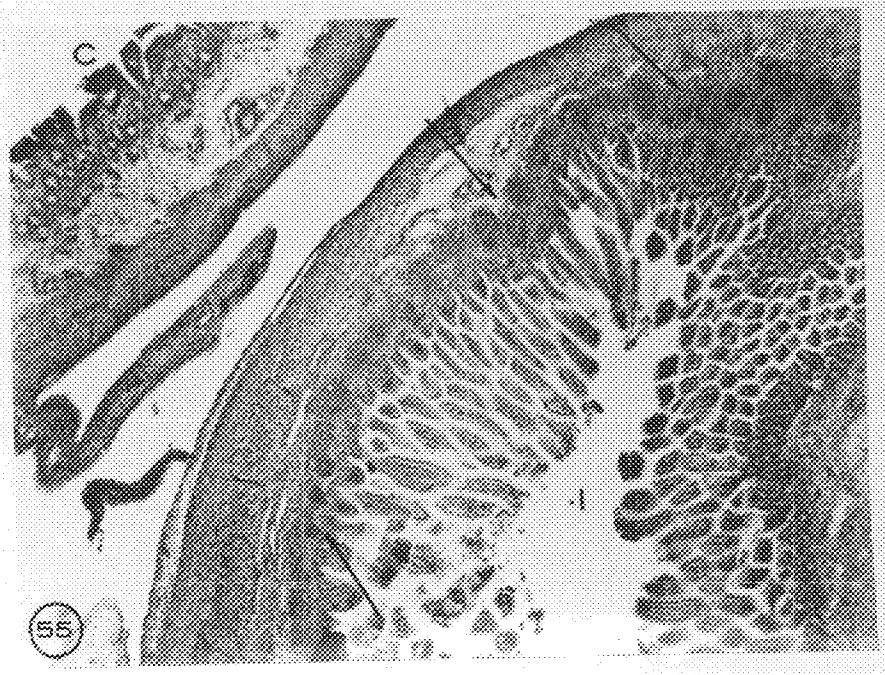
Fig. 55. 110 day Peyer's patch and cecum. The extensive Peyer's patch (arrows) of the terminal ileum (I) is shown in contrast to the cecum (C) which is devoid of lymphoid tissue. Hematoxylin - eosin, X 43.



53



54



55

APPROVED: John W. Anderson

Associate Professor of Anatomy

DATE August 10, 1967